

## Coronavirus Pandemic

# Investigation of Viral Load Cycle Threshold Values in Patients with SARS-CoV-2 Associated Pneumonia with Real-Time PCR Method

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### Abstract

**Introduction:** In this study, we aimed investigate the relationship of SARS-CoV-2 viral load cycle threshold (Ct) values with pneumonia.

**Methodology:** A total of 158 patients in whom SARS-CoV-2 was confirmed in upper respiratory tract (URT) samples with molecular method and who had computed tomography (CT) of the chest, between April 2020 and June 2020 were included in this retrospective cross-sectional study.

**Results:** Mean age of 158 PCR positive patients was  $45.22 \pm 17.89$  and 60.8% of them were male. Pneumonia was detected in 40.5% of the patients on their chest CT. A weak but significant correlation was found between SARS-CoV-2 Ct value detected with PCR in analysis of oropharyngeal/ nasopharyngeal (OP/NP) samples and chest CT score (Pearson's  $r: 0.197, p = 0.01$ ). No correlation was found between the first detected viral load Ct value and age, gender and mortality. There was no significant correlation between chest CT score and mortality. While the areas remaining under ROC curve for Ct value in analysis of OP/NP samples in prediction of chest CT score  $\geq 1, \geq 5$  and  $\geq 10$  were 0.564, 0.640 and 0.703 respectively.

**Conclusions:** We found that the amount of SARS-CoV-2 viral load (inverse relationship with Ct) detected in OP/NP samples of patients with COVID-19 pneumonia did not reflect the increasing severity of pulmonary lesions on chest CT. Although primary target of SARS-CoV-2 is all epithelial cells of the respiratory tract we believe studies comparing viral loads in lower respiratory tract samples are needed to determine the severity of pulmonary disease.

**Key words:** SARS-CoV-2; pneumonia; viral load; real-time PCR; rt-PCR.

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### Introduction

Human coronavirus (HCoV) is a zoonosis mainly associated with respiratory tract infections in humans [1]. Only two types of HCoV had been known until 2003: HCoV-229E and HCoV-OC43. At the time, two new HCoV types, HCoV-NL63 and HCoV-HKU1, were identified after SARS-CoV-associated outbreak called severe acute respiratory tract syndrome (SARS). SARS in 2003 and Middle East Respiratory Syndrome (MERS) outbreaks in 2012 revealed that HCoV could

cause life-threatening infections [2,3]. Finally, the infection called severe acute respiratory tract infection 19 (COVID-19) that emerged in Wuhan, China in early December 2019 is caused by SARS-CoV-2 which is an enveloped positive-sense single-stranded RNA virus belonging to genus *Betacoronavirus* from *Orthocoronavirinae* subfamily of *Coronaviridae* family [4]. When the cases rapidly spread in China and then in the rest of the world, COVID-19 was reported as an internationally significant public health

emergency on the 30th of January, 2020 and declared a global pandemic on the 10th of March, 2020 by the World Health Organization [5]. Person-to-person transmission primarily occurs through direct contact or droplets spread from an infected person through coughing or sneezing [6]. Clinical spectrum of COVID-19 is quite extensive and most of infected individuals have only a mild or subclinical disease especially in early stage of the disease [7]. Severity and mortality rate of the disease are also associated with comorbidities such as advanced age, diabetes, hypertension and cardiovascular diseases [8]. The diagnosis of COVID-19 is based on real-time PCR testing of oropharyngeal / nasopharyngeal (OP/NP) swabs samples [9]. High viral loads have been showed in upper and lower respiratory tracts of COVID-19 patients within 5 and 6 days after the symptoms occur [10,11]. Collecting OP/NP swab samples is recommended for screening or diagnosis generally in early period of the infection [9,12]. However, OP/NP samples can miss early infection [9]. Imaging is considered the first-line diagnostic method in suspected cases and is useful in monitoring changes during treatment. It also has the potential to identify individuals with a high suspicion of COVID-19 who have a negative RT-PCR test result [13]. The imaging findings of SARS-CoV-2 pneumonia are similar to those of other viral pneumonias [14]. Pulmonary lesions can be detected before the onset of symptoms, and up to day 14 (mean 4 days) thereafter [15,16]. In this study, we aimed to investigate whether there is a correlation between the viral load level first detected in OP/NP samples and the severity of pulmonary lesion determined in simultaneous chest CT in PCR-confirmed COVID-19 cases.

## Methodology

### *Study design*

The population of the study consisted of 158 pediatric and adult patients who were followed up and treated in Ankara Gülhane Training and Research Hospital COVID-19 outpatient clinic, COVID-19 clinics and Intensive Care Units between April 1 2020 and July 30 2020 in Turkey. A total of 158 patients with SARS-CoV-2 detected by PCR and simultaneous chest CT were included in the study. The inclusion criteria of the patients in this study were positive detection of SARS-CoV-2 by PCR and simultaneous chest CT at the time of first admission to the hospital. There is no age limit for patient selection. If there is more than one molecular test result of the same patient, the first test result that he applied to the hospital was evaluated.

Patients with negative results in molecular diagnostic tests were excluded from the study.

### *Molecular Analysis*

Clinical samples transferred to the “Bio-Speedy® COVID-19 Transfer Tube (BS-NA111)” (vNAT, Bioeksen, Istanbul, Turkey) were sent to the COVID-19 laboratory. Nucleic acid extraction from OP/NP samples was performed with 5 minutes incubation in vNAT (Bioeksen, Istanbul, Turkey).

The product obtained by extraction was added to the PCR reaction mix (Bio-Speedy COVID-19 RT-qPCR Detection Kit) (Bioeksen, Istanbul, Turkey). Amplification was performed on the Bio-Rad CFX96 Touch™ (Bio-Rad, USA) instrument. Analytical sensitivity of the kit is 99.4% and specificity is 99.0%.

### *Assessment of the results*

Results were assessed according to recommendations of the kit manufacturer. Real-time PCR Ct value is the value representing the first PCR cycle higher than the lowest level of fluorescent signal for target (i.e. viral RNA). Ct values are inversely proportional to the viral load level in host, and provide semi-quantitative assessment of viral load, thus, low Ct value shows high viral load or vice versa [17]. For SARS-CoV-2 RNA, Ct value of < 40 was defined as positive.

### *Chest CT*

Lesion descriptions of pneumonic infiltration were done according to nomenclature stated by Fleischner Society [18,19]. Presence and distribution of active infiltration in each patient were recorded. The distribution of infiltration was recorded as predominantly subpleural (mainly involving peripheral one-third of the lung), random (without predilection for subpleural or central regions) or diffuse (diffuse involvement relative to lung segments) [20]. CT scoring, a semi-quantitative assessment method, was used to measure the severity of pulmonary infiltration of COVID-19 [20]. A total of 5 lobes were evaluated in both lungs. Firstly, the extent of lesions in each lobe was visually estimated and the scores of 0 (none), 1 (affecting less than 25% of the lobe), 2 (affecting 26-50% of the lobe), 3 (affecting 51-75% of the lobe) and 4 (affecting more than 75% of the lobe) were given. Secondly, a total score was obtained by summing the scores of 5 lobes. The score for each patient was between 0 and 20. All CT images on chest imaging were assessed by a radiologist (F.C) with more than 10 years of experience. Disagreements on reader's perception

were resolved with consensus. Ethical approvals were obtained from Ethical Committee of the University of Health Sciences, Gulhane Training and Research Hospital (reference number: 2020/12/268).

### Statistical analysis

Data analysis was assessed with SPSS 25 (SPSS Inc., Chicago, IL, USA) software program. Visual methods (histogram and probability plots) and Kolmogorov-Smirnov test were used to investigate whether the variables were normally distributed or not. The variables were compared using independent samples T test, Mann-Whitney U test or one-way analysis of variance test Pearson correlation analysis was used to analyze the relationship between PCR Ct value and age, gender, mortality and chest CT score, as well as the relationship between chest CT score and mortality. Sensitivity, specificity values were investigated by determining the significant cut-off values of the test by receiver operating characteristic (ROC) curve analysis. P value of less than 0.05 was considered statistically significant.

### Results

Mean age of 158 SARS-CoV-2 PCR positive patients was  $45.22 \pm 17.89$  (age range: 4-99) and 60.8% of them were male. The mean age of 158 SARS-CoV-2 PCR positive patients was  $45.22 \pm 17.89$  years (age range: 4-99), of which 60.8% were male. The mean age of male patients was  $42.25 \pm 17.36$  years, which was

lower than female patients ( $p = 0.009$ ). Only three of the patients were under the age of 18. Most of the patients (71.5%) were hospitalized in wards. The mean Ct value of outpatients was  $26.76 \pm 5.10$  and  $27.53 \pm 4.65$  in inpatients. Death was observed in 2.5% (4/158) of the cases. Mortality was 1.6% (1/62) in women and 3.1% (3/96) in men ( $p = 0.56$ ). SARS-CoV-2 Ct values of the patients who survived and died were  $27.33 \pm 4.78$  and  $26.71 \pm 4.73$ , respectively ( $p = 0.81$ ). There was no significant correlation between Ct values and mortality (pearson r: -0.021,  $p = 0.80$ ). Patients were divided into five groups according to their age, as < 30, 30-39, 40-49, 50-59,  $\geq 60$  years. SARS-CoV-2 Ct values in these age groups were  $27.72 \pm 4.15$ ,  $26.44 \pm 5.73$ ,  $28.51 \pm 5.11$ ,  $27.82 \pm 4.42$ ,  $25.79 \pm 4.54$ , respectively ( $p = 0.31$ ). Pneumonia (chest CT score = 0-20) was detected in 40.5% (64/158) of the SARS-CoV-2 positive patients on their chest CT. The proportions of men and women in whom pneumonia was detected were 34.4% (33/96) and 50% (31/62) respectively ( $p = 0.05$ ). Only five of outpatients had pneumonia and all of these five outpatients were male. Pneumonia was mostly detected in the age group of 50-59 (28.1%) ( $p = 0.69$ ). Mean ages of patients in whom pneumonia was detected or in whom pneumonia was not detected were  $46.67 \pm 17.15$  and  $44.23 \pm 18.39$  respectively ( $p = 0.40$ ). Demographic, radiological and clinical data of the patients were presented in Table 1. In the analysis of OP/NP samples of patients with and without pneumonia, SARS-CoV-2 Ct values were  $28.03 \pm 4.44$

**Table 1.** Demographic, radiological and clinical data of the patients.

	Male (n = 96)	Female (n = 62)	Total (n = 158)	p value
Age (years)*	$42.25 \pm 17.36$	$49.82 \pm 17.85$	$45.22 \pm 17.89$	0.009
Status‡				
-Ambulatory	45 (46.9)	0 (0)	45 (28.5)	
-Clinical follow-up	51 (53.1)	59 (95.2)	110 (69.6)	< 0.001
-Follow up in intensive care unit	0 (0)	3 (4.8)	3 (1.9)	
Mortality	3 (3.1)	1 (1.6)	4 (2.5)	0.56
Pneumonia	33 (34.4)	31 (50.0)	64 (40.5)	0.05
Status of pneumonia patients§				
-Ambulatory	5 (15.2)	0	5 (7.8)	
-Clinical follow-up	28 (84.8)	29 (93.5)	57 (89.1)	0.03
-Follow up in intensive care unit	0	2 (6.5)	2 (3.1)	
Chest CT score‡				
0	63 (65.6)	31 (50)	94 (59.5)	
1-5	17 (17.7)	17 (27.4)	34 (21.5)	0.16
6-10	14 (14.6)	10 (16.1)	24 (15.2)	
11-20	2 (2.1)	4 (6.5)	6 (3.8)	
Pneumonia (Age groups)§				
< 30	9 (27.3)	4 (12.9)	13 (20.3)	
30-39	6 (18.2)	2 (6.5)	8 (12.5)	
40-49	3 (9.1)	9 (29)	12 (18.8)	0.69
50-59	10 (30.3)	8 (25.8)	18 (28.1)	
$\geq 60$	5 (15.2)	8 (25.8)	13 (20.3)	

\*Independent Samples Test, ‡ Pearson's chi-squared test, and § p value show the results of total patient group analysis.

**Table 2.** Distribution of mean cycle threshold (Ct) values in chest CT different score ranges.

Chest CT score	Cycle threshold ( Ct) values				
	Mean±Std Deviation	95% CI for Mean	Min	Max	P value
0	26.82 ± 4.94	25.80-27.80	17.32	37.55	0.41
1-5	27.13 ± 4.02	25.72-28.53	19.26	38.86	
6-10	29.02 ± 5.06	26.88-31.16	17.29	39.01	
11-20	28.98 ± 2.58	26.27-31.70	25.01	31.48	

CT: computed tomography; Std: Standard; Min: minimum; Max: maximum.

and  $26.82 \pm 4.94$ , respectively ( $p = 0.11$ ). Chest CT score was “0” in 59.5% of the cases. The CT score of the cases was 1-5 in 21.5%, 6-10 in 15.2%, 11-20 in 3.8%, 1-5 in 21.5%. While the mean CT score score was  $0.76 \pm 2.89$  in the outpatient group, it was  $3.02 \pm 3.87$  in the hospitalized group ( $p = 0.001$ ). The mean Ct values of 0, 1-5, 6-10, and 11-20 chest CT scores were  $26.82 \pm 4.94$ ,  $27.13 \pm 4.02$ ,  $29.02 \pm 5.06$ , and  $28.98 \pm 2.58$ , respectively ( $p = 0.41$ ) (Table 2). A weak but significant correlation was found between SARS-CoV-2 Ct value detected with PCR in OP/NP samples and chest CT score (pearson’s r: 0.197,  $p = 0.01$ ). The chest CT score was not associated with age, gender, or mortality (pearson’s r: -0.067,  $p = 0.40$ , pearson’s r: 0.124,  $p = 0.12$ , pearson’s r: -0.005,  $p = 0.95$ , respectively). Chest CT is one of the reference diagnostic methods in the diagnosis of COVID-19. The usability of SARS-CoV-2 Ct values to predict the chest CT score was investigated by ROC analysis. The area under the ROC curve was 0.564, 0.640 and 0.703, and the optimal Ct cut-off values were 25.11, 28.66 and 30.88, respectively. The performance of SARS-CoV-2 Ct values in predicting Chest CT score  $\geq 1$ ,  $\geq 5$  and  $\geq 10$  by ROC analysis is given in Table 3.

**Discussion**

Evidence from the SARS-CoV outbreak in 2002 showed that higher viral load was associated with worse prognosis. The SARS-CoV-2 pandemic differs from the SARS-CoV epidemic in many ways [21].

The results of SARS-CoV-2 RT-PCR diagnostic tests are interpreted based on Ct. Ct values are not reported while the test result is reported as positive or negative. The utility of Ct values, an indicator of viral load, in patient management is still unclear. The correlation of SARS-CoV-2 Ct values with clinical biomarkers, mortality and disease severity has aroused interest for researchers. Although these studies are

limited in number, they are available in the literature. In this study, which included outpatient and hospitalized COVID-19 patients, we evaluated the relationship between SARS-CoV-2 Ct values detected by RT-PCR in OP/NP samples at first diagnosis and chest CT score. We aimed to investigate whether it could predict the severity of pneumonic inflammation in the lung with the data we obtained.

In this study, mean Ct values in outpatients and inpatients were  $26.76 \pm 5.10$  and  $27.53 \pm 4.65$ , respectively, and were not statistically significant. In a similar study, Yagci et al. [22] reported the median Ct value of SARS-CoV-2 RNA as 28.16 (IQR: 24.5-31.6) for hospitalized patients and 26.77 (IQR: 23.1-29.7) for outpatients in samples collected from 730 patients.

Studies on the correlation between SARS-CoV-2 Ct values and mortality are very limited. In one of these studies, Huang et al. [23] reported that Ct values were lower in patients who died in hospital in their study involving 308 cases. Fa’ico-Filho et al. [24] in their study with a case series of 875 patients, they found the Ct values of the patients who survived and those who died to be 27 and 21, respectively. In this statistically significant study, they established a linear relationship between viral load and mortality. Yagci et al. [22] on the other hand, found the median Ct value of 27.8 and 29.2 in hospitalized survivors and deceased patients, respectively, and did not find a statistically significant difference. In this study, the SARS-COV-2 viral load Ct value detected at the first admission was found to be  $27.33 \pm 4.78$  and  $26.71 \pm 4.73$  in surviving and deceased subjects (n = 4), respectively ( $p = 0.81$ ). No significant correlation was found between Ct values and mortality. It was considered that the small number of cases may affect the interpretation of the results.

Chest CT, on the other hand, has a potential role in the diagnosis, follow-up and prognosis of COVID-19 [25]. In this study, pneumonia was detected in chest CT

**Table 3.** Adequacy of SARS CoV-2 cycle threshold (Ct) values in predicting pneumonia (according to the chest CT pneumonia scoring method).

Category*	Cut-off value (Ct)	Sn,%	Sp,%	AUC	95%CI	p value
$\geq 1$	25.11	79.7	39.4	0.564	0.474-0.653	0.18
$\geq 5$	28.66	57.9	68.3	0.640	0.542-0.738	0.009
$\geq 10$	30.88	55.6	79.9	0.703	0.561-0.844	0.04

\*: Chest CT pneumonia score; AUC: area under the curve; Sn: sensitivity; Sp: specificity; CI: Confidence Interval.

scans in 40.5% (64/158) of the patients at the time of initial diagnosis. The mean of SARS-CoV-2 Ct was found to be low (inversely associated with viral load) in patients without pneumonia (59.5%). As CT scores increased, Ct values increased in OP/NP samples. OP/NP swabs have become the most common type of specimen for the diagnosis of viral pneumonia [26,27]. However, detection of virus in the upper respiratory tract may reflect an upper respiratory tract infection, asymptomatic or a previous infection [27-29]. Therefore, low Ct values of SARS-CoV-2 in upper respiratory tract OP/NP swabs may not reflect the presence of pneumonia. On the other hand, lung involvement may be severe at high Ct values. Ct values as an indicator of viral load have been reported to determine disease severity but have not been widely used [30]. Because, depending on the volume of the sample and the inability to standardize the sample, the virus load in the sample taken and the Ct value obtained accordingly may be misleading. However, the use of Ct value is considered to be beneficial for SARS-CoV-2 case management [31]. Zhao *et al.* [32] viral load quantitation results detected in nasopharyngeal swab specimens of nine patients who tested positive for SARS-CoV-2 could not correlate with severity of lung lesions, number of segments, and severity of lung lesions on CT. In the CT image, the upper airway viral load was only 256 copies/ml in cases with high infiltration scores, while the viral load was as high as 80,800 copies/ml in very few cases with ground glass opacity. The results revealed that viral loads in nasopharyngeal swabs couldn't predict the severity of lung lesions by CT in COVID-19 cases. This study by Zhao *et al.* is considered to be the first study with analyze the link between viral loads in URT and the severity of lung lesions in COVID-19 with CT. Yagci *et al.* [22] evaluated the thoracic CT score of 730 patients with SARS-CoV-2 positive in their study in Istanbul, Turkey. Researchers found an inverse correlation between nasopharyngeal viral load and severity score of chest CT at presentation in both outpatients and inpatients. The results obtained in our study were similar to those of Zhao *et al.* and Yagci *et al.* Peiris *et al.* [33] reported in their study of the SARS infection outbreak in 2003 that the worsening course of SARS infection from the second week of illness was paradoxically associated with sero-conversion and a decrease in viral load. It has been explained that the diffuse bilateral shadowing of the lung in these patients may be related to indirect immune-mediated damage in this phase rather than direct viral infection in the first week [33-35]. Although only the viral load at the time

of admission was evaluated in this study, it is considered that the worsening in the chest CT score and the decrease in the viral load in the late admitted patients support the above mentioned inverse ratio.

In this study, the performance of SARS-CoV-2 viral load Ct value in predicting the extent of lung involvement was evaluated by ROC curve analysis. It was determined that as the chest CT imaging scores increased, the Ct values increased. The fact that there is an inverse relationship between the amount of viral load and Ct values supports that URT virus load is not related to the severity of lung lesions. There are several limitations in the study. The fact that the study was single-centered and the number of patients was limited prevents the generalization of the study findings. It is considered that more reliable data will be obtained with a larger sample and multi-centre studies. Also, in this study, SARS-CoV-2 positive samples included only OP/NP samples. Therefore, examination with lower respiratory tract samples and comparison with viral load in these samples could not be made. Finally, since SARS-CoV-2 is an infection with pneumonic infiltration, its relationship with other respiratory bacterial and viral agents that cause the same symptoms should be investigated.

## Conclusions

Based on the amount of SARS-CoV-2 viral load detected in the OP/NP samples of patients with COVID-19 pneumonia, in our study to determine the severity of pulmonary lesions on lung CT, no relationship could be established between these two parameters. This result suggests that the viral load in the sample and the resulting Ct value are sometimes misleading due to the volume of the OP/NP samples and the inability to standardize the sampling site. Since SARS-CoV-2 mainly causes pneumonia, we think it would be more useful to investigate viral loads in lower respiratory tract samples to determine the severity of lung disease. On the other hand, performing studies comparing both upper and lower respiratory tract samples can provide a healthier evaluation to determine the severity of pulmonary lesion in lung CT.

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