ORIGINAL RESEARCH

Prognostic Value of "Cycle Threshold" in Confirmed COVID-19 Patients

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Abstract

Objective: To study the correlation between the cycle threshold (CT) of reverse transcription–polymerase chain reaction (RT–PCR) test in confirmed COVID-19 patients and the severity of disease.

Background: RT–PCR test is a standard method for the diagnosis of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infections. This test is based upon the amplification of the fluorescent signal. The number of cycles that the fluorescent signal undergoes to reach the threshold is called "cycle threshold." It is inversely related to the nucleic acid content of the sample.

Patients and methods: This is a single-centered, retrospective observational study. We have included a total of 192 patients. SARS-CoV-2 infection was confirmed by the RT–PCR test. Entire data have been collected from the electronic medical records.
The primary outcome was 28-day mortality, whereas the secondary outcomes were intensive care unit (ICU) admission, invasive ventilation, acute kidney injury, renal replacement therapy (RRT), shock, and COVID-19 reporting and data system (CO-RADS) score on high-resolution computed tomography of the chest, total length of stay in the hospital, and the number of ICU days and ventilator days.

Results: We have calculated the mean CT value for all groups and calculated the *p*-value for statistical significance. For the total length of stay in the hospital and the number of ICU days and ventilator days, we applied the Pearson correlation coefficient.
The *p*-value was statistically significant for mortality, ICU admission, and shock groups. The CT values and the length of ICU stay were inversely correlated with the statistically significant *p*-value.

Conclusion: Low CT value is associated with increased ICU admission, high mortality, shock, and increased length of ICU stay.

Keywords: COVID-19, Cycle threshold, RT-PCR, Viral load.

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Introduction

During this COVID-19 pandemic worldwide, more than 20 million people got infected and more than 7.5 lakh deaths have happened. Sometimes as critical care physicians, we have seen an unexpected and rapid deterioration of recovering patients because of the cytokine storm. In this scenario, having a variable to predict the severity of the disease is helpful to prognosticate the family sometimes to make tailored decisions.

This novel coronavirus is an enveloped particle—containing single-stranded RNA. Diameter is 60–140 nm, with distinctive spikes ranging from 9–12 nm. The symptoms range from mild fever and dry cough to severe acute respiratory distress syndrome and multiorgan failure.

The angiotensin-converting enzyme 2 (ACE2) receptor and the type 2 transmembrane serine protease will facilitate virus entry into host cells.[1] Host target cells, especially type II alveolar epithelial cells, contain these two receptors.[2] The virus infects both epithelial cells and endothelial cells. Inflammatory cascade will be activated on infected sites, resulting in thickening of alveolar membrane and infiltration of alveolar spaces with monocytes and macrophages.[3] This entire process results in ground-glass opacities on the high-resolution computed tomography (HRCT) of the chest.

In severe infections, there will be activation of the coagulation system and consumption of clotting factors results in diffuse intravascular coagulation.[4]

RT–PCR test is the standard method for the diagnosis of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infections.[5] This test is based upon the amplification of the fluorescent signal.[6] The number of cycles that the fluorescent signals undergo to reach the threshold is called "cycle threshold(CT)."[7] It is inversely related to the nucleic acid content of the sample.[8] Based on this concept, we are considering CT values as a surrogate marker of viral load. If CT is high, viral load is less and if CT is low, viral load is more.[5] As per the Indian Council of Medical Research (ICMR) instructions on the test, CT less than 35 is considered positive and more than 35 is considered negative.

As per available evidence over previous SARS-CoV-2 infection, the severity of the disease is inversely related to the viral load.[9]

Study Design and Methods

This is a single-centered, retrospective observational study done at Virinchi Hospital, Hyderabad (Telangana, India). We have included a total of 192 patients of age more than 18 years, who required hospital admissions between June 4, 2020, and July 23, 2020.

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SARS-CoV-2 infection was confirmed by RT–PCR test done on the nasopharyngeal swabs of all patients. CT of all positive patients was available, but CT of negative patients was not available. Our criteria for admitting patients were hypoxia, breathlessness, oxygen requirement, and unstable hemodynamics. We were not admitting patients who were not requiring oxygen.

All admitted patients have received hydroxychloroquine (HCQ), azithromycin, steroids, and therapeutic anticoagulation. We were monitoring QT intervals along with daily electrocardiograms. If increasing, HCQ and azithromycin were discontinued. Patients requiring oxygen more than 10 liters/minute, noninvasive ventilation (NIV), high-flow nasal cannula (HFNC), mechanical ventilation, and vasopressors were shifted to the intensive care unit (ICU). For all patients, HRCT chest was done at the time of admission and the COVID-19 reporting and data system (CO-RADS) score was being reported by the radiologist. For patients in whom oxygen requirement was increasing, remdesivir was started and tocilizumab was given as per interleukin 6 (IL-6) levels.

Written informed consent was waived off by the ethics committee as it is a noninterventional study, purely based upon data from the electronic medical records.

The entire data have been collected from the electronic medical records that are being used throughout the hospital. Demographic data, history, course in the hospital, laboratory tests, and radiology reports have been collected. Any gaps in history, course, and interventions were clarified by discussing with the treating physician personally.

The primary outcome was 28-day mortality whereas the secondary outcomes were total length of stay in the hospital, the number of ICU days and ventilator days, acute kidney injury (AKI), renal replacement therapy, shock, and the CO-RADS score. AKI was diagnosed based on serum creatinine.

We applied the Pearson correlation coefficient for continuous variables, like total length of stay in the hospital, ICU days, and ventilator days. These continuous variables are represented with a scatter plot using Cartesian coordinates.

For other outcomes, we divided them into two groups, calculated the mean value of CT for each group, and applied standard deviation (SD). These mean values were compared with the help of an unpaired *t*-test. We have calculated *p*-value for statistical significance for each variable. Mean values of each group were represented with the help of bar diagrams.

Results

A total of 192 laboratory-confirmed SARS-CoV-2 patients got admitted to our center between June 4, 2020, and July 23, 2020. Among them, 26 (13.54%) patients were dead and 166 (86.45%) patients got discharged. The mean CT of nonsurvivors was 23.43 (SD, 4.87). The mean CT of survivors was 25.93 (SD, 4.46) (Fig. 1). The *p*-value was 0.01, statistically significant. So, low CT was associated with higher mortality.

Forty-nine (25.5%) patients required ICU admission and 143 (74.4%) patients were managed in general wards. The mean CT of patients who required ICU admission was 24.1 (SD, 4.65) and the mean CT of patients who did not require ICU admission was 26.1 (SD, 4.47) (Fig. 2). The *p*-value was 0.009, so statistically significant. Patients with low CT values do not require ICU admission.

Twenty-six (13.54%) patients required invasive ventilation and 166 (86.45%) patients did not require invasive ventilation. The mean CT of ventilated patients was 24.1 (SD, 3.71). The mean CT of nonventilated patients was 25.8 (SD, 4.68) (Fig. 3). The *p*-value was

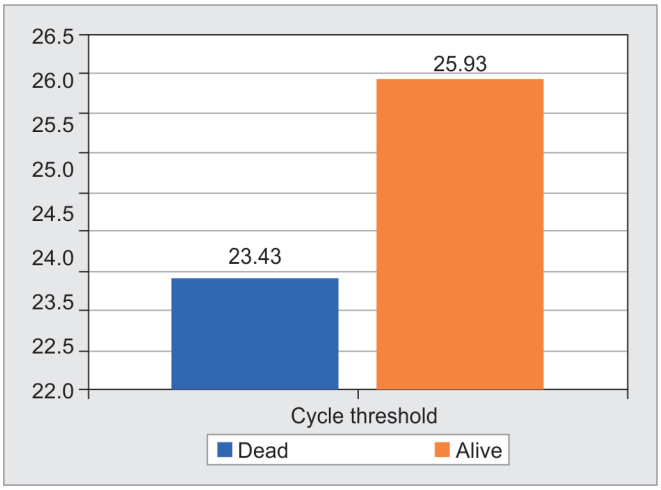

Fig. 1: Bar diagram showing the mean cycle threshold of nonsurvivors and survivors

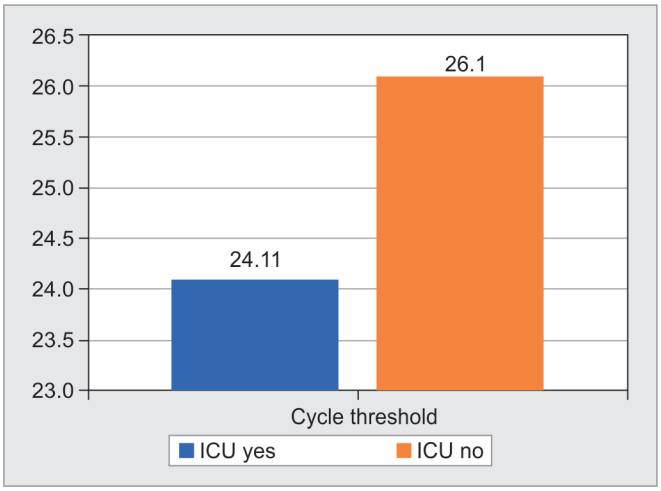

Fig. 2: Bar diagram showing the mean cycle threshold of patients who required ICU admission and who did not require ICU admission

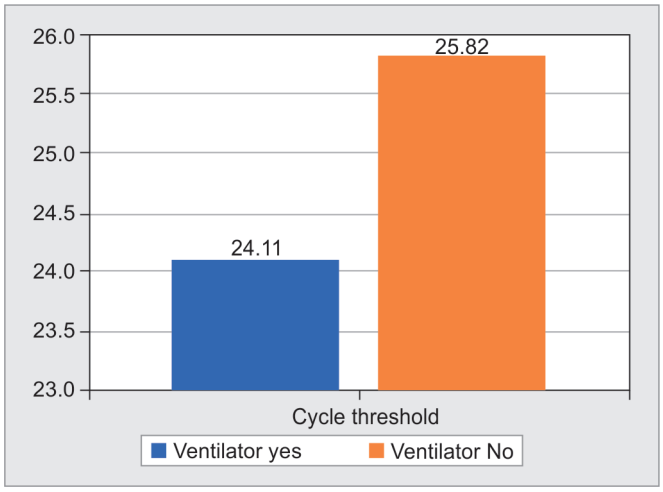

Fig. 3: Bar diagram showing the mean cycle threshold of patients who required invasive ventilation support and who did not require invasive ventilation support

0.077; it is not statistically significant. So, the CT cannot predict the requirement of invasive ventilation.

Fifty-six (29.16%) patients developed AKI. In 136 (70.83%), renal function was not affected. The mean CT of patients who had AKI was 25.83 (SD, 4.77) and the mean CT of patients who did not develop AKI was 25.49 (SD, 4.53 [Fig. 4]). The *p*-value was 0.638, statistically not significant. So, the CT value cannot predict the incidence of AKI.

Twenty-four (12.5%) patients developed shock and 168 (87.5%) patients did not develop shock. The mean CT of patients who had a hemodynamic instability was 23.18 (SD, 4.93) and the mean CT of patients who did not have a hemodynamic instability was 25.93 (SD, 4.45) (Fig. 5). The *p*-value was 0.006, statistically significant. So, the CT can predict the incidence of a shock.

One hundred seventy-one (89%) patients had HRCT chest CO-RADS score of 4 and 5 (labeled as confirmed cases) and 21 (10.9%) patients had CO-RADS score of less than 4 (labeled as suspected cases). The mean CT of radiologically confirmed patients was 25.7 (SD, 4.5) and the mean CT of suspected patients was 24.6 (SD, 5.2) (Fig. 6). The *p*-value was 0.319. So, the radiological score or the findings do not depend on the CT value.

Pearson correlation coefficient was calculated to see the relationship between CT value and length of stay in the hospital and the number of ICU days and ventilator days.

The Pearson correlation coefficient for the length of stay in the hospital was −0.105, negative correlation (Fig. 7). But the *p*-value was 0.146, statistically not significant.

The Pearson correlation coefficient value for the length of ICU stay was −0.213, negative correlation (nonlinear) (Fig. 8). The *p*-value was 0.003, statistically significant. So, the number of ICU days is inversely related to the CT value.

The Pearson correlation coefficient value for the number of ventilator days was −0.007, negative correlation (Fig. 9). But the *p*-value was 0.291, statistically not significant.

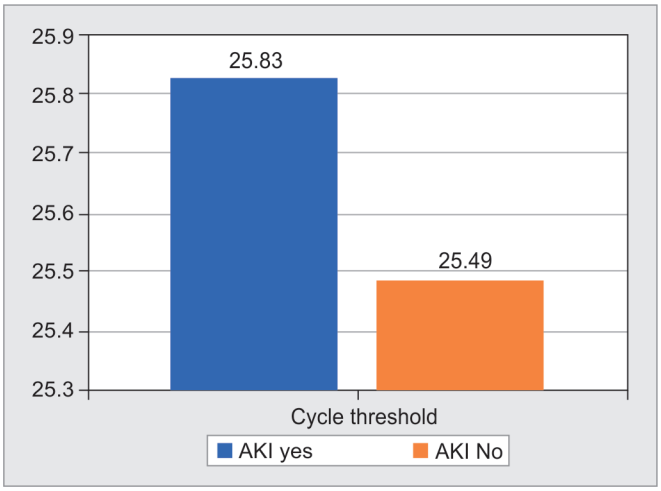

Fig. 4: Bar diagram showing the mean cycle threshold of patients who developed acute kidney injury during hospital course and who did not develop acute kidney injury during hospital course

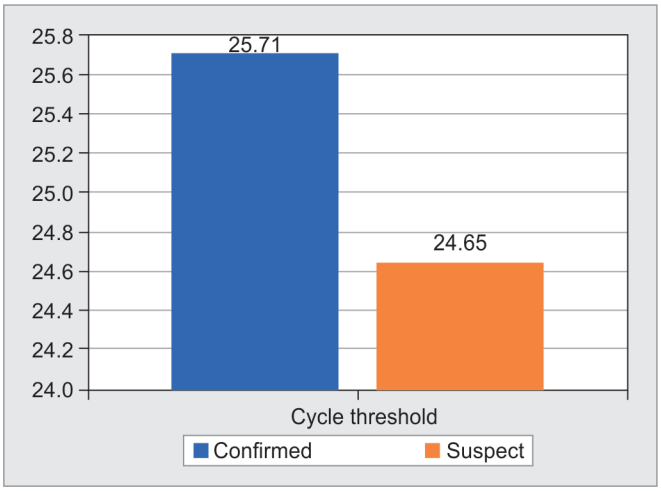

Fig. 6: Bar diagram showing mean cycle threshold of patients whose HRCT chest showed confirmatory COVID changes and who did not have typical COVID changes on HRCT chest

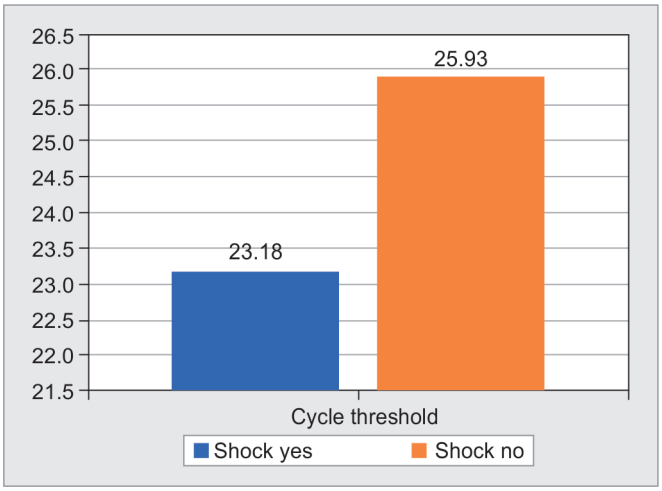

Fig. 5: Bar diagram showing the mean cycle threshold of patients who had hemodynamic instability and who did not have hemodynamic instability

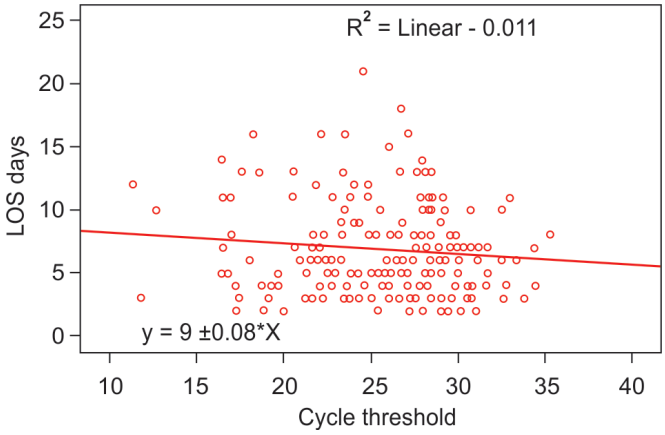

Fig. 7: Pearson correlation curve between cycle threshold and length of stay (LOS) in the hospital

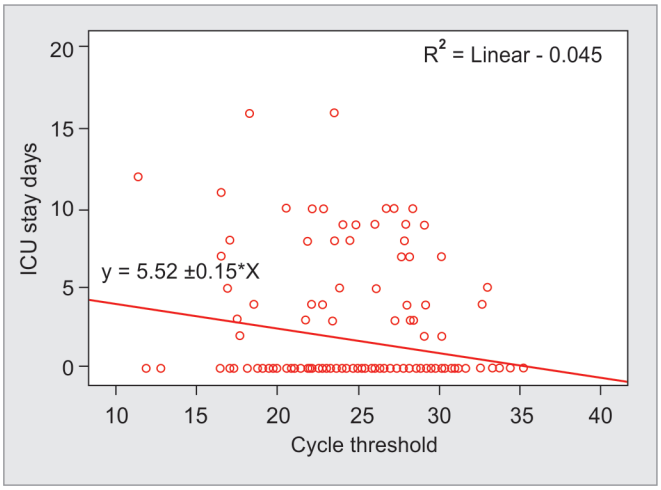

Fig. 8: Pearson correlation curve between cycle threshold and length of stay in the intensive care unit

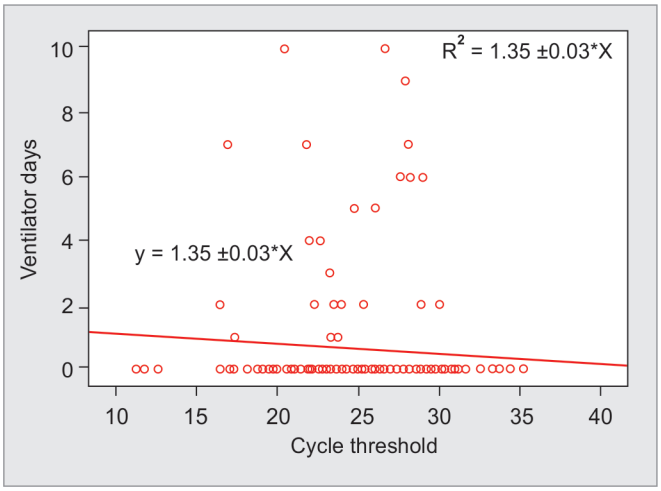

Fig. 9: Pearson correlation curve between cycle threshold and number of ventilator days

Discussion

In this single-centered, retrospective observational study, we have included 192 hospitalized patients. SARS-CoV-2 infection was confirmed with real-time RT–PCR test, which is a standard method of molecular diagnostic test. For all patients, both nasopharyngeal and oropharyngeal swabs were collected. The yield of the nasopharyngeal swabs is better than the oropharyngeal swabs.[5]

All patients have received standard treatments, like HCQ, azithromycin, steroids, and therapeutic anticoagulation. If oxygen requirement was increasing, we started them on remdesivir. If IL-6 was in an increasing trend with clinical worsening, then tocilizumab was given.

The mean age of the population was 51.6 years (SD, 13.3) and 139 (72%) patients have comorbid conditions like diabetes mellitus, hypertension, coronary artery disease, and chronic kidney disease.

In this study, we have proved that low CT is associated with increased mortality, increased ICU stay, and increased incidence of shock. There is a definitive negative correlation between the length of ICU stay and the CT value.

Previous studies have proved that biomarkers like C-reactive protein (CRP), lactate dehydrogenase (LDH), and IL-6 are the bad prognostic markers.[10,11] Another study has proved that lymphopenia is also one of the bad prognostic markers.[12] Lymphopenia is because of ACE2 receptor on lymphocytes[12,1] which is a direct target for the virus and destruction of lymphatic organs, inflammatory markers can cause apoptosis of lymphocytes[12,13], and metabolic derangements like hyperlactatemia can destroy lymphocytes.[12,14]

One retrospective study showed that persistent high viral load is associated with more severe diseases.[15] But in this study, the severity grading was based upon the respiratory mechanics and oxygenation.

Conclusion

We can consider CT as one of the prognostic variables along with other biomarkers. Low CT is associated with increased ICU admission, high mortality, shock, and increased length of ICU stay. Further research on large group can confirm our results.

Limitations

Single-center-based study.
Only admission CT has been documented.

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