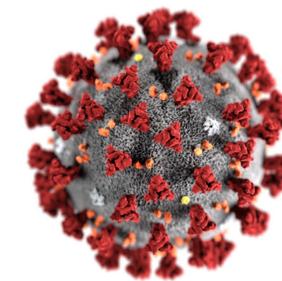


# Can the Cycle Threshold Values of a COVID-19 PCR Test Predict Severity of Infection?

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## Abstract

COVID-19 is a viral infectious disease caused by SARS-CoV-2. The gold standard for diagnosis is nucleic acid amplification testing of three viral genes using the Polymerase Chain Reaction (PCR) test. The number of amplification cycles it takes for the target viral gene to reach a threshold level is called Ct value. Thus, the amount of virus in a sample (viral load) is inversely related to the Ct value. This retrospective study investigated whether Ct values can be used as predictors of COVID-19 patient outcomes. Previous studies that researched this topic did not reach a consensus. The goal of this study is to confirm or deny if a correlation exists between Ct values and the patient's severity of infection. Ct values from 150 COVID-19 patients tested at NYU Winthrop Hospital using the Cepheid Gene Xpert Xpress SARS-CoV-2 assay were collected. Their medical records were accessed to view the severity of the infection, which was categorized based on a 6-point ordinal scale. The SOI ranks range from patients that were well enough to be discharged from the emergency room, to those requiring oxygen support, and lastly mortality. A correlation analysis evaluated the relationship between the SOI ranks and their respective Ct values. Although there seems to be a negative trend, the data analysis show that there is no significant correlation between Ct values and COVID-19 infection severity.

## Introduction

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded RNA virus and member of the Coronaviridae family that emerged in December 2019 from Wuhan, China.
- Symptoms vary between Coronavirus Disease 2019 (COVID-19) positive individuals. Some remain asymptomatic, while moderate cases experience cough, fever, and mild pneumonia. More severe cases involve respiratory failure, organ dysfunction, and death.
- The gold standard for diagnosis is with nucleic acid amplification testing, and one major platform being used is the Cepheid Xpert Xpress SARS-CoV-2 assay.
- The three targets detected by this system include the envelope (E), nucleocapsid (N2), and RNA-dependent RNA polymerase (RdRp) genes. A cycle threshold value is reported for each target.
- Ct values describe the number of amplification cycles it takes for the target gene to pass the threshold level and is inversely related to viral load.
- If Ct values correlate to patient's severity of infection, it can provide a way to predict the prognosis of a patient and determine treatment options faster.

## Methods

- Samples include COVID-19 positive patients tested at NYU Winthrop Hospital using the Gene Xpert Xpress SARS-CoV-2 assay. The sample source was nasopharyngeal swabs stored in a viral transport medium.
- Results from 928 COVID-19 positive patients were collected from April 1<sup>st</sup> to April 30<sup>th</sup>, 2020 and recorded on a spreadsheet. A random number generator selected patients from this spreadsheet to use for the study.
- From the patients selected, the N2 viral gene Ct value was recorded for 150 patients, 50 from each Ct value range, including low (<27), medium (27-32), and high (>32).
- The patient's outcomes were assessed based on 6 SOI ranks.
  - 1- Not hospitalized, discharged from ER
  - 2- Hospitalized, not requiring supplemental oxygen
  - 3- Hospitalized, requiring supplemental oxygen
  - 4- Hospitalized, requiring nasal high-flow oxygen therapy
  - 5- Hospitalized, requiring invasive mechanical ventilation
  - 6- Death
- IRB approval was given to carry out this retrospective study. The IRB ID is IRB2021-00117.
- Data analysis involved creation of a scatter plot and statistical measurements using Microsoft Excel software to determine if a correlation exists between Ct values and COVID-19 infection severity.

## Results

The Ct values and outcomes of 150 COVID-19 patients at NYU Winthrop Hospital were analyzed to assess whether a correlation exists between Ct values and severity of infection.

SOI Ranks	Low Ct Value High Viral Load		Medium Ct Value Medium Viral Load		High Ct Value Low Viral Load	
	Number of Patients	Percent	Number of Patients	Percent	Number of Patients	Percent
1	4	8%	1	2%	7	14%
2	4	8%	1	2%	5	10%
3	16	32%	25	50%	23	46%
4	7	14%	7	14%	9	18%
5	4	8%	6	12%	1	2%
6	15	30%	10	20%	5	10%
	n=50		n=50		n=50	

Table 1: Number of patients in each Ct value subgroup that fall into the different SOI ranks.

Note: SOI ranks: 1- Not hospitalized, discharged from ER; 2- Hospitalized, not requiring supplemental oxygen; 3- Hospitalized, requiring supplemental oxygen; 4- Hospitalized, requiring nasal high-flow oxygen therapy; 5- Hospitalized, requiring invasive mechanical ventilation; 6- Death

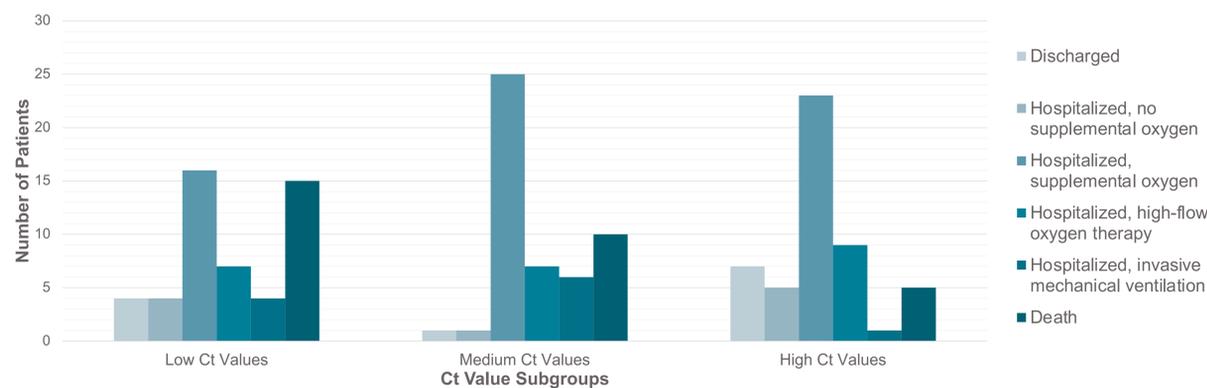


Figure 1: Number of patients in each Ct value subgroup that fall into the different SOI ranks.

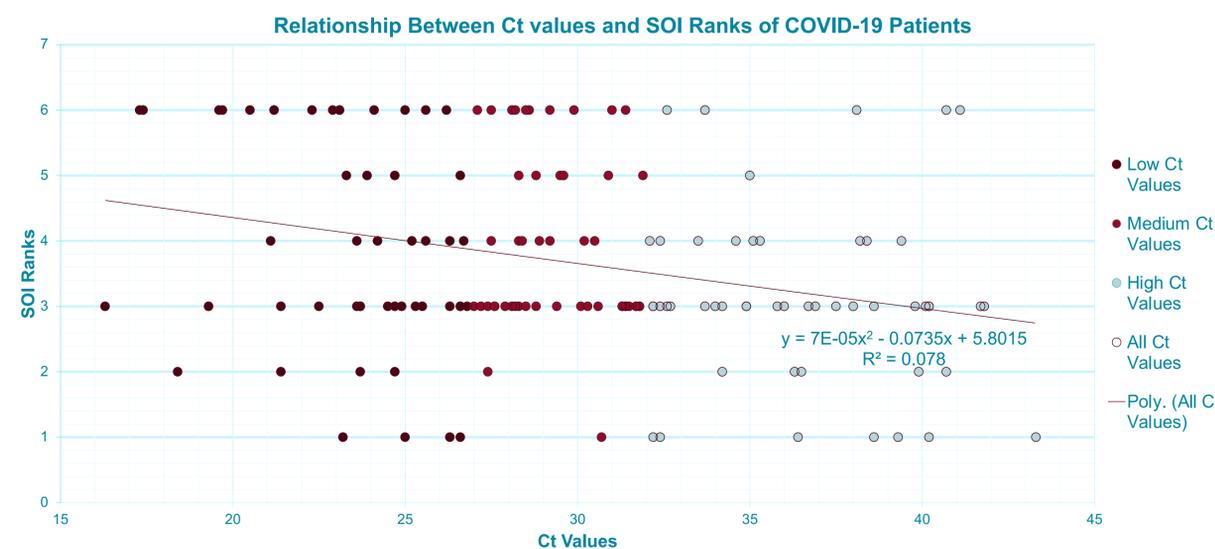


Figure 2: A scatter plot of Ct values and SOI ranks for data collected from 150 patients. A line of best fit is shown to express the relationship between the two variables.

The median SOI rank for each Ct value range:

- Low Ct Values: 4 Hospitalized, requiring nasal high flow oxygen therapy
- Medium Ct Values: 3 Hospitalized, requiring supplemental oxygen
- High Ct Values: 3 Hospitalized, requiring supplemental oxygen

The correlation coefficient between the Ct values and SOI ranks of the 150 patients:

- 0.2792907

Data analysis. Includes descriptive statistics on the Ct values and SOI ranks of all the patients.

## Conclusions

- The outcome of this retrospective study indicates that there is no significant correlation between Ct values and severity of infection in COVID-19 patients.
- The correlation coefficient is a negative value, which would suggest that there is an inverse relationship between the two variables. This means that the higher the Ct value the lower the SOI rank and vice versa, but since the value is close to 0 this correlation is seen as weak, near negligible.
- All 3 Ct value subgroups had the most patients fall into the 3<sup>rd</sup> SOI rank. 64 out of 150 patients were hospitalized and required some form of supplemental oxygen.
- The results suggest that Ct values:
  - Are not good predictors of patient's prognosis.
  - Do not aid with better understanding of the patient's condition.
  - Should not be used to provide insight onto which treatment options would enhance patient care.

## Discussion

Prior studies looking into this correlation indicated that patients Ct values were significantly lower in severe cases compared to mild cases, while other studies demonstrated that the viral load detected in asymptomatic patients were comparable to symptomatic patients. One stated that patients with mild symptoms would have their viral load drop dramatically after one week, but more severe cases would have high viral loads after one week of infection.

This retrospective study did not prove that a correlation exists between viral load and severity of infection in COVID-19 patients. The results were not unexpected because severe COVID-19 pathology is mostly due to a hyperinflammatory reaction to SARS-CoV-2 infection.

Although, there was not a significant correlation between the SOI ranks and Ct values of COVID-19 patients, the 6<sup>th</sup> and more severe SOI rank (death) alone showed a correlation with Ct values. 50% of patients who died (15/30) had a low Ct value at the time of hospitalization, while 33% (10/30) had medium Ct values, and 17% (5/30) had high Ct values

## Limitations

Other considerations to improve this study include:

- Increasing the sample size. Considering that this study only incorporated 150 patient samples, it is possible that a larger sample size could increase precision and provide a more accurate assessment on the relationship between the two parameters.
- Following the Ct values of the patients throughout their hospital stay. If a patient goes through multiple SOI ranks, the researcher should assess if the Ct value fluctuates during these times.
- Rather than looking at the 6 different symptom groups, it may be beneficial to focus on one specific rank, such as patient deaths.

The results of this study concurred with the previous studies that suggested that Ct values and viral loads of COVID-19 patient do not correspond with their infection severity. Further studies should be performed to fully prove if there is a relationship between the two variables.

## Acknowledgement

We thank NYU Winthrop Hospital for giving us access to their data and making this retrospective research possible.