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Authors

Camargo, Jose F
Lin, Rick Y
Komanduri, Krishna V

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SHORT COMMUNICATION

Lack of correlation between the SARS-CoV-2 cycle threshold (C_t) value and clinical outcomes in patients with COVID-19

Jose F. Camargo¹  | Rick Y. Lin²  | Krishna V. Komanduri³

¹Division of Infectious Diseases, University of Miami Miller School of Medicine, Miami, Florida, USA

²Department of Medicine, University of Miami Miller School of Medicine, Miami, Florida, USA

³Division of Transplantation and Cellular Therapy, Sylvester Comprehensive Cancer Center, Miami, Florida, USA

Correspondence

Jose F. Camargo, MD, Division of Infectious Diseases, University of Miami Miller School of Medicine, 1120 NW 14th St, Miami, FL 33136.

Email: jfc31@med.miami.edu

Abstract

Problem: The utility of the polymerase chain reaction (PCR) cycle threshold (C_t) values in the management of patients with coronavirus disease 2019 (COVID-19) remains controversial.

Methods: We assessed the correlation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) C_t values in nasopharyngeal swab samples with the oxygen requirements at the time of sample collection. Specimens were tested with the Simplexa PCR platform, which targets the SARS-CoV-2 *ORF1ab* and *S* genes.

Results: We identified 23 COVID-19 patients with 49 C_t values available. While C_t values from *ORF1ab* and *S* genes were highly correlated for a given specimen, there was no correlation between C_t values for any of these target genes and the oxygen requirements of the patient at the time of sample collection. We found no differences in the initial nor the nadir C_t values between survivors and non-survivors or mild/moderate versus severe/critical illness at the maximum point of illness.

Conclusion: SARS-CoV-2 C_t values have limited value in the management of COVID-19.

KEYWORDS

COVID-19, hematopoietic cell transplantation, oxygen, SARS-CoV-2/ C_t value

1 | INTRODUCTION

The cycle threshold (C_t) in reverse transcription polymerase chain reaction (RT-PCR) refers to the number of cycles needed to amplify viral RNA to reach a detectable level. C_t values are inversely correlated with the viral load and the ability to grow severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) *in vitro*;^{1–3} the probability of isolating SARS-CoV-2 in cell culture is 77, 24, and less than 3% for clinical specimens with C_t values between 10 and 20, 20–30, and 30–40, respectively.^{1,2,4} The C_t value-based estimates of viral load have been used to predict disease progression, infer transmissibility and differentiate active viral replication from prolonged virus shedding.⁵ At our transplant center, for example, we have used the C_t value in a case-by-case basis to clear transplant candidates to undergo urgently needed conditioning/lymphodepleting therapies under

the assumption that curative transplantation benefits outweigh the potential risks of COVID-19 in those with a high C_t value (e.g., >35), symptom resolution and are greater than 3–4 weeks from diagnosis. We have also used low C_t values in patients with a compatible clinical syndrome (e.g., worsening respiratory status) to guide clinical decisions such as administering a second course of remdesivir. However, definitive data to support the predictive value of C_t values in these situations are lacking.⁵ C_t values generated by the currently available qualitative PCR assays do not reliably correspond to specific RNA concentrations and are not consistent across platforms. In addition, C_t values from viral RNA can vary depending on the method of specimen collection, specimen source, transport, and the time from infection to collection to analysis.⁶ Current guidance from the Centers for Disease Control and Prevention recommends against the use of C_t values in the clinical setting.

2 | METHODS

To further explore the clinical utility of C_t values, we assessed the correlation of SARS-CoV-2 C_t values in nasopharyngeal swab samples with the oxygen requirements at the time of sample collection for PCR testing, in a cohort of hematological patients with COVID-19 managed at a large academic center. We identified 23 patients with COVID-19 with a total of 49 C_t values available (Supplementary Table 1). Specimens were tested with The DiaSorin Molecular Simplexa COVID-19 Direct real-time RT-PCR assay, which has a C_t cutoff less than 40 and targets the SARS-CoV-2 *ORF1ab* (encoding 16 nonstructural proteins) and *S* (encoding the structural spike glycoprotein) genes. Data were summarized using descriptive statistics. Mann-Whitney or Kruskal-Wallis tests were used where appropriate. All tests were two-sided and $p < .05$ was considered statistically significant. Statistical analyses were performed using GraphPad Prism Software, Inc, version 7.03.

3 | RESULTS

Patients underwent a median of two PCR tests (range, 1–7) with C_t value available. While C_t values from *ORF1ab* and *S* genes were highly correlated for a given specimen (Figure 1A), there was no apparent correlation between C_t values for any of these SARS-CoV-2 target genes and the oxygen requirements of the patient at the time of sample collection (Figure 1B,C). When plotting C_t values by the fraction of inspired oxygen (FiO_2) strata (0.21 vs. 0.24–0.28 vs. >0.4 ; Figure 1D,G) or simply by room air versus any oxygen supplementation (Figure 1E,H) there was no difference in the SARS-CoV-2 C_t values by oxygen requirements at the time of PCR testing. The trajectory of the C_t values over time is shown in Figure 1F,I.

We next compared the initial SARS-CoV-2 C_t value (i.e., on the PCR obtained at the time of COVID-19 diagnosis) by survival, disease severity and need for admission (Figure 2A,B) and observed no significant

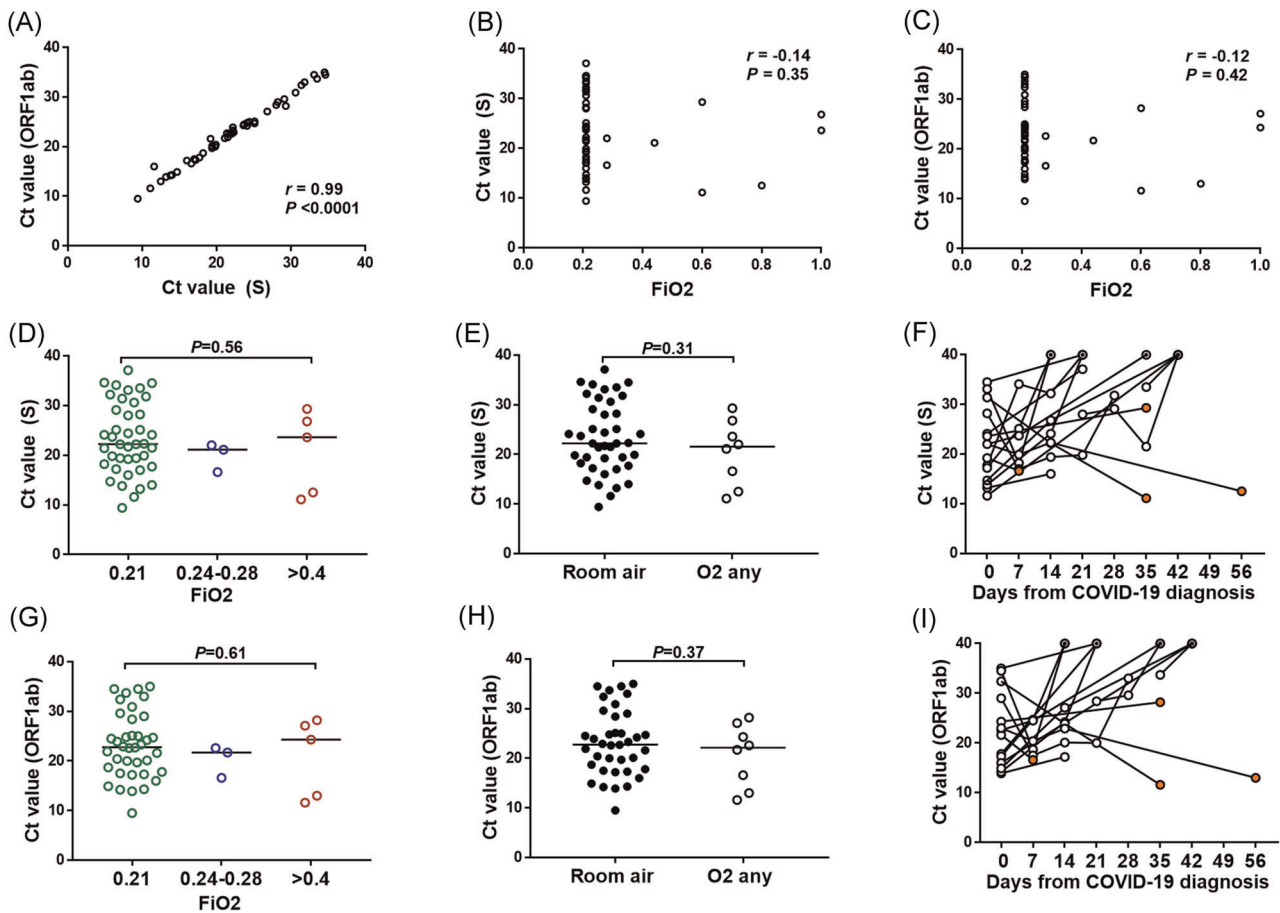


FIGURE 1 Lack of correlation between SARS-CoV-2 PCR cycle threshold (C_t) values and oxygen requirements at the time of PCR testing. (A–C) Spearman correlation between *S* and *ORF1ab* gene targets on the same clinical specimen (A), and between each SARS-CoV-2 gene target and the recorded fraction of inspired oxygen (FiO_2) (B–C) at the time of sample collection for PCR testing. (D–I) Distribution of SARS-CoV-2 C_t values across requirements of FiO_2 strata at the time of PCR testing for *S* (D–E) and *ORF1ab* (G–H) genes. Bars corresponds to the median C_t value. p value was calculated using Mann-Whitney or Kruskal-Wallis tests where appropriate for comparison between groups. Trajectory of C_t values over time for patients with 2 or more PCR swabs available (F, I). All negative values were given a value of 40 indicated with circled dot. Three patients with negative PCR documented on routine testing obtained more than 6 weeks from previous positive test were considered outliers and not included in the figure. Orange circles correspond to the last C_t value available if patient is deceased. PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

difference in the initial C_t values for the SARS-CoV-2 gene target tested between survivors and non survivors, or mild/moderate versus severe/critical disease at the maximum point of illness. Patients who required hospital admission had lower initial C_t values than those who did not (Figure 2A,B).

We also performed analysis using the lowest C_t value available (as a surrogate marker of the peak viral load) for a given patient and found no differences in the nadir C_t values between survivors and nonsurvivors or mild/moderate versus severe/critical illness at the maximum point of illness (Figure 2C,D). The group of patients who required admission to the hospital due to COVID-19, however, exhibited lower C_t values than those managed in the outpatient setting (Figure 2C,D).

4 | DISCUSSION

Admission SARS-CoV-2 viral load among hospitalized patients with COVID-19 independently correlates with the risk of intubation and in-hospital mortality.^{7,8} Those with C_t values less than 25 (i.e., high viral load) had a three-fold increased risk of intubation and five to six-fold increased risk of mortality.^{7,8} C_t value has been also used to assess response to antiviral therapy.⁹ However, several studies have shown that nasopharyngeal SARS-CoV-2 C_t values are not associated with COVID-19 severity and do not support a predictive role for the C_t value in the clinical setting.¹⁰⁻¹⁴ For example, in a report that included a total of 414 throat swabs collected from 94 patients, there was no obvious difference

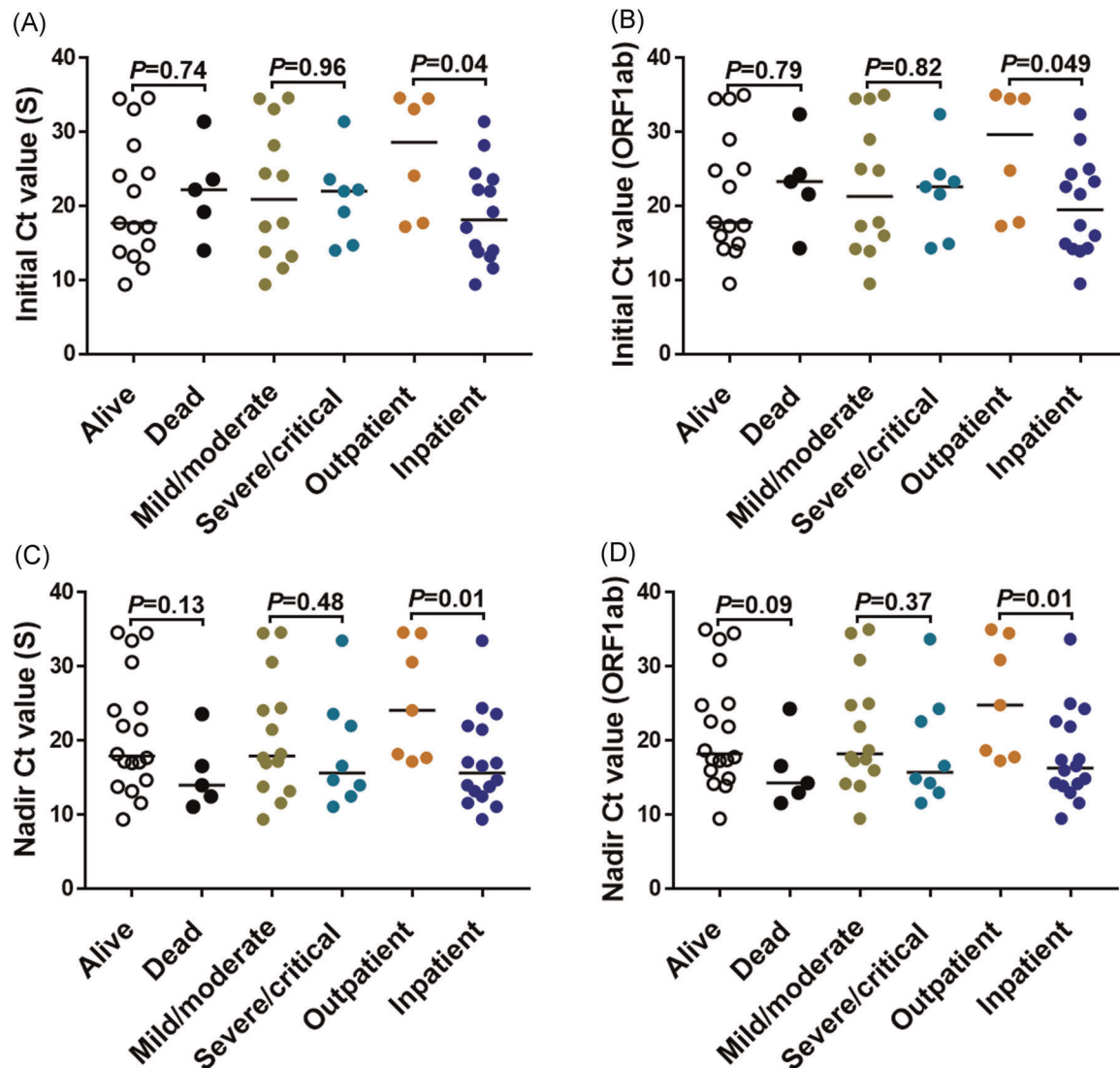


FIGURE 2 Lower SARS-CoV-2 PCR C_t value among COVID-19 patients requiring hospital admission. Initial (A–B) and nadir (C–D) SARS-CoV-2 C_t values by clinical outcome are shown for the gene targets specified in the y-axis. COVID-19 severity was defined as follows: mild (the clinical symptoms are mild and no pneumonia manifestations can be found in imaging); moderate (pneumonia manifestations on imaging); severe (tachypnea with respiratory rate >30 per minute, hypoxia requiring $FiO_2 > 0.4$ to attain $SpO_2 > 94\%$, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen [PaO_2/FiO_2] <300 mmHg, and/or lung infiltrates >50%); and critical (respiratory failure requiring mechanical ventilation, septic shock and/or multiorgan failure). Data for maximum COVID-19 severity missing in one patient admitted at an outside hospital. p value was calculated using Mann–Whitney for comparison between groups. COVID-19, coronavirus disease 2019; C_t , cycle threshold; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

in viral loads across disease severity.¹⁰ Another study showed similar initial and peak viral loads between patients with mild and those with severe COVID-19.¹¹ In addition, SARS-CoV-2 C_t values from asymptomatic patients are similar to those in symptomatic patients,¹²⁻¹⁴ which might account for the high rates of asymptomatic transmission.¹⁵

Here we further explored the clinical utility of C_t values and observed no correlation between C_t values from the nasopharyngeal PCR samples and disease severity as measured by the oxygen requirements. Although C_t values were significantly lower among patients requiring hospital admission, we observed no differences in the initial and nadir C_t values when segregating the cohort by COVID-19 severity or mortality outcomes.

Our study has limitations including small sample size, and that observations obtained with the *ORF1ab* and *S* target genes using the Simplexa PCR platform cannot be extrapolated to other target genes and/or platforms. Likewise, our cohort consisted of hematological patients and validation of our findings in other patient populations requires further study. Despite such limitations, to our knowledge, this is the first study assessing the correlation between C_t values and FiO_2 requirements at the time of PCR testing. We conclude that SARS-CoV-2 C_t values have limited value in the management of hospitalized patients with COVID-19.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

All authors contributed to study design, data analysis, and writing of manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Jose F. Camargo  <https://orcid.org/0000-0001-9584-5011>

Rick Y. Lin  <http://orcid.org/0000-0001-6226-0375>

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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