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RESEARCH ARTICLE

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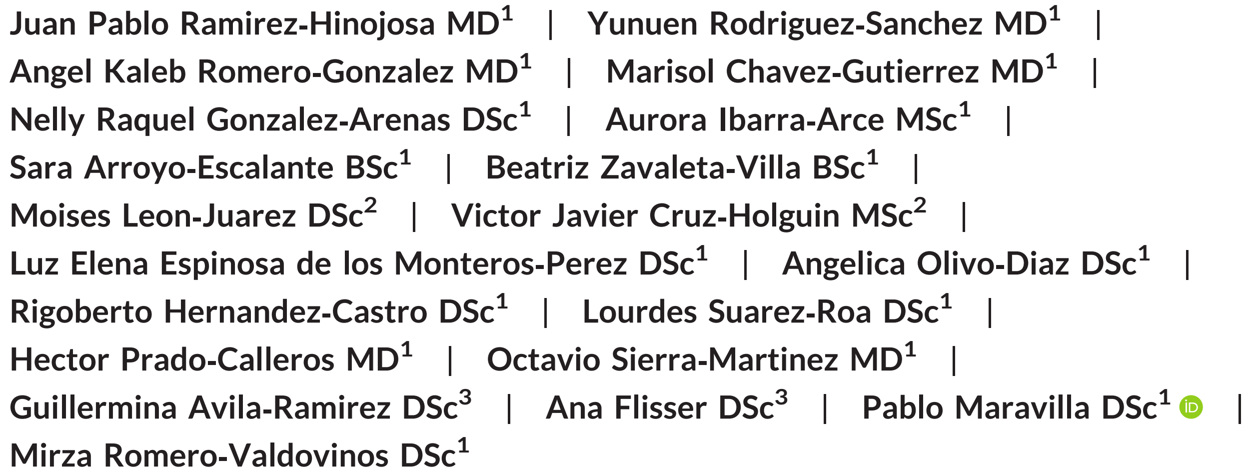
Association between cycle threshold (C_t) values and clinical and laboratory data in inpatients with COVID-19 and asymptomatic health workers



Juan Pablo Ramirez-Hinojosa MD[1] | Yunuen Rodriguez-Sanchez MD[1] | Angel Kaleb Romero-Gonzalez MD[1] | Marisol Chavez-Gutierrez MD[1] | Nelly Raquel Gonzalez-Arenas DSc[1] | Aurora Ibarra-Arce MSc[1] | Sara Arroyo-Escalante BSc[1] | Beatriz Zavaleta-Villa BSc[1] | Moises Leon-Juarez DSc[2] | Victor Javier Cruz-Holguin MSc[2] | Luz Elena Espinosa de los Monteros-Perez DSc[1] | Angelica Olivo-Diaz DSc[1] | Rigoberto Hernandez-Castro DSc[1] | Lourdes Suarez-Roa DSc[1] | Hector Prado-Calleros MD[1] | Octavio Sierra-Martinez MD[1] | Guillermina Avila-Ramirez DSc[3] | Ana Flisser DSc[3] | Pablo Maravilla DSc[1] | Mirza Romero-Valdovinos DSc[1]

[1]División de Parasitología, Hospital General "Dr. Manuel Gea Gonzalez", Mexico City, Mexico

[2]Departamento de Inmunobioquimica, Instituto Nacional de Perinatologia, Mexico City, Mexico

[3]Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autonoma de Mexico (UNAM), Mexico City, Mexico

Correspondence
Pablo Maravilla and Mirza Romero-Valdovinos, División de Parasitología, Hospital General "Dr. Manuel Gea Gonzalez", Calzada de Tlalpan, 4800, Mexico City 14080, Mexico.
Email: maravillap@yahoo.com and mirzagrv@yahoo.com

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Abstract

In-house assays for the diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by quantitative reverse-transcription polymerase chain reaction (qRT-PCR), are feasible alternatives, particularly in developing countries. Cycle threshold (C_t) values obtained by qRT-PCR were compared with clinical and laboratory data from saliva of inpatients with COVID-19 and asymptomatic health workers (AHW) were studied. Saliva specimens from 58 inpatients confirmed by qRT-PCR for SARS-CoV-2 using nasopharyngeal specimens, and 105 AHW were studied by qRT-PCR using three sets of primers for the *N* (*N*1, *N*2, and *N*3) gene of SARS-CoV-2, according to the CDC Diagnostic Panel protocol, showing a positivity of 88% for inpatients and 8% for AHW. Bivariate analysis revealed an association between C_t < 38.0 values for *N*2 and mechanical ventilation assistance among patients ($p = .013$). In addition, values of aspartate-transaminase, lactate dehydrogenase, and ferritin showed significant correlations with C_t values of *N*1 and *N*3 genes in inpatients. Therefore, our results show that C_t values correlate with some relevant clinical data for inpatients with COVID-19.

KEYWORDS

COVID-19, cycle threshold (C_t), saliva, SARS-CoV-2

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1 | INTRODUCTION

Infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19), which has emerged as a global health problem and generated an imminent economic crisis. The World Health Organization (WHO) compiles evidence regarding the transmission of SARS-CoV-2 through direct or indirect contact with infected people that release the virus through body secretions, such as saliva and respiratory secretions or droplets, which are expelled when a person coughs, sneezes, talks or sings by WHO.[1] SARS-CoV and Middle East respiratory syndrome coronavirus RNA can be detected in saliva,[2,3] even before lung damage appear,[2] and saliva has a concordance rate greater than 90% with nasopharyngeal samples in the detection of respiratory viruses.[3] Currently, molecular assays that use self-collected saliva samples are widely available at Rutgers The State University of New Jersey (https://www.rutgers.edu/news/new-rutgers-saliva-test-coronavirus-gets-fda-approval).[4]

SARS-CoV-2 uses the angiotensin-converting enzyme 2 receptor to enter cells.[5,6] An in silico study revealed that the expression of this enzyme was higher in minor salivary glands than in the lung.[6] An early study of serial self-collected saliva by patients infected with SARS-CoV-2 showed that 92% (11/12) of saliva samples were positive for the virus. The patients' viral loads were monitored and generally showed a declining trend, leading authors to conclude that saliva sampling could be a promising noninvasive method for diagnosis, monitoring, and control in patients with SARS-CoV-2.[3,7,8] An Italian study performed with 25 patients with COVID-19 concluded that saliva is a reliable carrier for detecting SARS-CoV-2.[9] A study performed with 70 inpatients with COVID-19 showed that saliva specimens and nasopharyngeal swabs have similar sensitivity during the course of hospitalization in the detection of SARS-CoV-2 using a primer set from the Centers for Disease Control and Prevention (https://www.fda.gov/media/134919/download).[10]

The success of PCR (qRT-PCR) relies on the amplification of tiny amounts of viral genetic material in a sample. A variety of RNA target genes and protocols are now available at World Health Organization (https://apps.who.int/iris/handle/10665/331329),[11] most of which target 1 or more of the envelope (*env*), nucleocapsid (*N*), spike (*S*), RNA-dependent RNA polymerase (*RdRp*), and *ORF1b or ORF8* genes. In addition, most of the RT-PCR protocols have shown 100% of specificity, since the designed primers are specific to the genome sequence of SARS-CoV-2; however, false-negative results can occur depending on the timing of sample collection in relation to illness onset and due to sampling errors.[12–14] These techniques have been performed with bronchoalveolar lavage fluid, fibrobronchoscope brush biopsy, sputum, nasal swab, nasopharyngeal swab, saliva, stool, blood and urine, yielding up to 93% positive results depending on the sample studied.[15–17]

In quantitative (q) PCR, a positive reaction is detected as the accumulation of fluorescent signal. Cycle threshold (C_t) is defined as the number of cycles required for the fluorescent signal to cross the threshold, i.e. to exceed the background level. Current qRT-PCR protocols for SARS-CoV-2 detection suggest that samples with C_t values less than 40 can be interpreted as positive for viral RNA. Some authors have argued that a "positive" PCR result during virological load assessment in hospitalized patients reflects only the detection of viral RNA and not necessarily the presence of viable virus. Furthermore, the success of virus isolation by culture has been found to depend on viral load; samples containing less than 10^6 viral copies per ml (or copies per sample) may not yield an isolate.[13,18]

Healthcare workers are at increased risk of exposure to SARS-CoV-2 and potentially influence in-hospital transmission.[19,20] As of February 22, 2021 in Mexico, there have been 2,247,852 confirmed COVID-19 cases, 181,809 associated deaths and 226,581 health workers have been infected with SARS-CoV-2 (40% nurses, 26% medical staff, and 34% other health workers) available at Secretaria de Salud, Mexico (https://www.gob.mx/cms/uploads/attachment/file/607909/COVID-19_Personal_de_Salud_2021.01.18.pdf).[21] In addition, an alternative to support the diagnosis of SARS-CoV-2 without the use of commercial kits, is the performance of in-house assays.[11]

We obtained C_t values and compared them with clinical and laboratory data from saliva of inpatients with COVID-19 and asymptomatic health workers (AHW) for the present study.

2 | MATERIALS AND METHODS

2.1 | Participants

Participants in this study were hospitalized patients with COVID-19 symptoms who were diagnosed with COVID-19 based on clinical respiratory symptoms and radiological images and confirmed to be infected with SARS-CoV-2 by qRT-PCR using nasopharyngeal specimens following Charité, Germany/WHO protocols by WHO.[11] qRT-PCR confirmation was performed at one of the reference centres in Mexico City (Instituto Nacional de Ciencias Médicas y de la Nutrición "Salvador Zubiran"), with a C_t value less than 38 interpreted as a positive result. Patients' clinical conditions were classified according to the Chinese Clinical Guide for COVID-19 Pneumonia Diagnosis and Treatment available at (https://www.acc.org/latest-in-cardiology/articles/2020/03/17/11/22/chinese-clinical-guidance-for-covid-19-pneumonia-diagnosis-and-treatment).[22] During admission, clinical and laboratory data were obtained, such as age, sex, comorbidities (hypertension, diabetes, obesity, and previous lung or mediastinal diseases), drugs, inflammatory indexes or tissue damage biomarkers and peripheral blood and biochemistry profiles.[9,23] In addition, AHW (medical staff, nurses, researchers and administrative personnel) were invited to participate in the study. Approximately 2 ml saliva was obtained from each participant by self-collection; participants spat into sterile 15 ml conical tubes during their hospital admission or in clean, no-COVID-19 areas; for patients in severe/critically ill condition, saliva specimens were obtained using a disposable sterile plastic transfer pipette.

The present study was approved by the Research and Ethics Committees of the "Dr. Manuel Gea Gonzalez" General Hospital with reference number 12-26-2020, and written consent was obtained from all participants or their relatives.

2.2 | Nucleic acid extraction and quantitative reverse transcription polymerase chain reaction

Saliva specimens were frozen at −70°C until processing. An aliquot of 500\ µl saliva was collected, supplemented with 10 µl of 2 M dithiothreitol (DTT) as mucolytic agent and shaken for 30 min.[24] Total RNA was extracted using TriPure according to the manufacturer's instructions and eluted in 20 µl Tris-EDTA buffer. Reverse transcription was performed with oligo(dT) primers using 5 U GoScript Reverse Transcriptase (Promega).

Real-time PCR was performed following WHO in-house protocols and the CDC 2019-nCoV Real-time RT-PCR Diagnostic Panel protocol by Centers for Disease Control and Prevention (https://www.fda.gov/media/134919/download)[10] with oligonucleotide primers and probes for the detection of SARS-CoV-2 target regions of the virus nucleocapsid (*N*) gene (*N*1, *N*2, and *N*3). According to the CDC protocol, the panel specifically detects SARS-CoV-2 (two primers/probe sets). An additional primer/probe set detects the human RNase P gene (*RP*) as an amplification control. Viral RNA was determined with a Light Cycler software, version 5.1 (Roche Diagnostic); standard curves were performed by using serial 10-fold dilutions, starting with 400 ng of DNA from a plasmid that contains full SARS-CoV-2 N-gene (see below) and 100 ng of total RNA from bronchoalveolar lavage fluid of a critical patient and fluorescence data were plotted versus C_t. All saliva samples were analyzed in duplicate with a previously established positive control and saliva from healthy people, which had been stored at −70°C before the COVID-19 pandemic and used as negative controls. In cases where duplicates of a sample yielded $C_t > 3$, the sample was interpreted as inconclusive and analysed again. The lowest C_t value for a given sample was selected as the final value.

2.3 | Cloning of the N SARS-CoV-2 sequence

The SARS-CoV-2 N gene was cloned in the plasmid pProEX HTb (Invitrogen). Briefly, total RNA was extracted from samples of nasopharyngeal swab carried out using the Quick-RNA-Viral Kit (Zymo Research), according to the manufacturer's instruction. The N sequence from SARS-CoV-2 was amplified by reverse transcription PCR, using the primer pair: 5′-CGCTCTAGAATGTCTGATAATGGA-3′ (forward), 5′-TCAACTCAGGCCTAAGGTACCGAA-3′ (reverse). The PCR product and pProEX HTb plasmid were digested with XbaI and KpnI and then ligated together. Finally, the plasmid from the resultant colonies was purified using EndoFree Plasmid Purification Kit (Qiagen) and construction was verified by automated DNA sequencing.

TABLE 1 Baseline clinical and laboratory characteristics of inpatients with COVID-19[a]

	Severe disease (%)	Critically ill (%)
Clinical characteristic		
Female	33.3 (*n* = 7)	29.7 (*n* = 11)
Fever (>38.3°C)	76.2	91.9
Outcome/recovery	100	86.5
Assisted with mechanical ventilation	0	32.4
Dyspnoea	85.7	86.5
Arthralgia	75	89.2
Chills	55	33.3
Headache	52.4	70.3
Rhinorrhea	51	44.4
Chest pain	19	26.5
Abdominal pain	10.5	0
Vomit	0	5.5
Cyanosis	0	0
Anosmia	0	0
Ageusia	0	0
Comorbilities		
Diabetes mellitus	33.3	27
Hypertension	28.5	18.9
Obesity	23.8	18.9
Smoking	23.8	16.2
Chronic renal insufficiency	4.7	0
Chronic obstructive pulmonary emphysema	9.5	2.7
Asthma	0	2.7
Immunosuppression	0	0
HIV/AIDS	0	0
Heart disease	0	0
Peripherial blood profile		
Leukocytes (x10^9 cells/L)	8.3 ± 3.4	9.6 ± 4.3
Lymphocytes (%)	15.5 ± 9	8.2 ± 4.5
Haemoglobin (g/dl)	14.7 ± 2.6	14.1 ± 3.4
Platelets (x10^9 cells/L)	285.9 ± 126.1	253.4 ± 123.9
Basophils (%)	0.5 ± 0.7	0.4 ± 0.6
Eosinophils (%)	0.7 ± 1.0	0.5 ± 0.9
Blood biochemistry		
Glucose (mg/dl)	135.4 ± 67.7	158.5 ± 98.1
Creatinine (mg/dl)	1.7 ± 3.8	1.5 ± 2.4
Albumin (g/L)	31.7 ± 0.6	32.1 ± 0.4

(Continues)

TABLE 1 (Continued)

	Severe disease (%)	Critically ill (%)
Total bilirubin (mg/dl)	0.8 ± 0.7	0.9 ± 0.7
Indirect bilirubin (mg/dl)	0.4 ± 0.2	0.6 ± 0.3
Direct bilirubin (mg/dl)	0.3 ± 0.5	0.3 ± 0.4
Alanine transaminase (ALT)	53.8 ± 44.6	53.1 ± 56.6
Aspartate-transaminase (AST)	77.28 ± 52.74	64.64 ± 51.19
Lactate dehydrogenase (LDH)	467.68 ± 273.48	515.15 ± 267.72
Inflammatory markers		
Oxygen saturation (SpO_2)	88.1 ± 10.1	89.3 ± 6.6
Ferritin (μg/L)	1242.07 ± 994.3	1011.73 ± 1015.0
C-reactive protein	15.1 ± 11.4	16.7 ± 9.2
D-Dimer	1.5 ± 2.2	2.8 ± 8.0
Troponin	0.3 ± 1.3	0.2 ± 1.3
Myoglobin	145.4 ± 341.8	106.2 ± 174.7
Creatine kinase–MB (CK-MB)	7.23 ± 21.4	1.4 ± 1.5

Abbreviation: COVID-19, coronavirus disease 2019.

[a]The values were recorded in the first 48 h of hospital admission.

2.4 | Statistical analysis

Most variables were expressed as the mean ± *SD*. To analyse the associations between each of the main clinical and laboratory variables or positivity level and C_t value, we performed bivariate analyses, calculating chi-square and Phi statistics to assess the strength of the relationship, as well as one-way ANOVA and Bonferroni's post hoc test was used for multiple comparisons and we analyzed correlations using Pearson's r. A p value <.05 was considered significant. Data analysis was performed with SSPS software version 15.0 (SPSS Institute) and Epi-Info6 v6.04 software.

3 | RESULTS

Table 1 summarizes the baseline clinical and laboratory characteristics of the 58 inpatients (69% male) with COVID-19, who comprised 21 patients with severe disease and 37 critically ill patients and had an average age of 52 ± 15 years. In addition, 105 (43% male) AHW (68% medical staff, 12% laboratory technicians/researchers, 11% administrative staff and 9% nurses) with a mean age of 32 ± 8 years were analyzed in this study. The baseline clinical and laboratory values of patients were recorded in the first 48 h after hospital admission; since the objective of the present study was a cross-sectional analysis, no follow-up was considered in the changes of the clinical or laboratory values for all participants. Overall, no differences were found between the clinical and laboratory data from severe disease and critically ill patients; however, in a couple of markers (oxygen saturation and ferritin), particularly from those patients who died (group of critically ill, $n = 5$), some differences were high; that is, oxygen saturation (SpO_2) values were lower among the patients who died (81.8 ± 12.4%) compared to the global values of the patients with severe disease, and critically ill; for ferritin, patients who died exhibited very high values (>2500 μg/L).

During beginning of present study, obtaining saliva for those patients with assisted mechanical ventilation was complicated due to their condition and limited saliva was obtained (~500 μl), however, specimens were sufficient for qRT-PCR assays; therefore, obtaining saliva samples from patients was focused on those in whom the sample could be taken during their hospital admission.

Profile of both standard curves (cloned plasmid containing full SARS-CoV-2 N-gene and RNA from bronchoalveolar lavage fluid of a critical patient) were very similar (image not shown), strengthening the certainty in our qRT-PCR results. The positivity rate using saliva specimens was 87.9% for patients and 8% for AHW. Table 2 summarizes the mean C_t values obtained for patients and asymptomatic health workers. Most of patient samples exhibited high C_t values, in contrast, those AHW who were positive, showed low C_t values.

Interestingly, the bivariate analysis revealed an association between $C_t < 38$ values for *N*2 and mechanical ventilation assistance among patients ($p = .013$).

For three blood biochemistry biomarkers (Figure 1), aspartate-transaminase (AST), lactate dehydrogenase (LDH), and ferritin, significant correlations were observed with C_t values of *N*1 (for AST and LDH) and *N*3 (ferritin); no significant correlation was found for blood biochemistry biomarkers in positive AHW.

4 | DISCUSSION

More than a year after the emergence of SARS-CoV-in China, there are still multiple gaps in knowledge which are debated. In the present study we detected SARS-CoV-2 in 87.9% of inpatients and 8% AHW; similarly, Wyllie et al.,[25] reported a positivity of 81% for SARS-CoV-2 in inpatients using the protocol from the Centers for Disease Control and Prevention with saliva samples, and only 2.6% in AHW. In a study performed in China, comparing 37 asymptomatic individuals with 37 symptomatic individuals, C_t values were similar among them (mean $C_t = 32.8$ vs. ~31.7 for *ORF1ab* marker and mean $C_t = 32.6$ vs. 33.5 for *N* marker).[26] During a cohort in South Koreans, two out of three presymptomatic patients showed $C_t < 20$ and asymptomatic subjects had C_t values ranging from 24 to 40 for *RdRp* gene.[27] Another comparative cohort with symptomatic and asymptomatic South Korean subjects, showed that there was no significant difference in the first follow-up C_t value for the *E* (32.2 vs. 33.0), *RdRp* (33.1 vs. 33.1), and *N* (32.4 vs. 32.7) genes between the two groups, however, serial changes in C_t values for the three genes with rebound, even with measurements of $C_t < 35$, were observed for both groups.[28] During a cohort of 202 Colombian workers, C_t values in

TABLE 2 General positivity values and C_t values obtained for patients and asymptomatic health workers

	Mean; IQR[a] C_t values from positive samples		
	N1	N2	N3
Critically ($n = 37$)	37.68; 37.15–38.20	30.73; 25.62–38.5	37.74; 37.32–38.17
Severe ($n = 21$)	37.94; 37.53–38.30	30.01; 25.64–35.66	37.71; 37.41–38.06
AHWb ($n = 8$)	17.06; 13.24–19.67	15.98; 15.45–17.42	18.24; 16.03–20.84
	Statistically differences among groups[c]		
	N1	**N2**	**N3**
Comparison between groups	Critically versus AHW severe vs AHW Critically versus severe	Critically versus AHW severe versus AHW	Critically versus AHW severe versus AHW

Abbreviations: ANOVA, analysis of variance; C_t, cycle threshold.

[a]IQR: The interquartile range.

[b]AHW: Asymptomatic health workers.

[c]$p < .05$ by one-way ANOVA, Bonferroni's post hoc test.

asymptomatic patients did not differ significantly from the symptomatic ones, the mean C_t value in the asymptomatic group were 33.53 for *ORF1ab* gene and 33.61 for *N* gene, C_t value in the symptomatic group was 34.13 for *ORF1ab*.[29,30] In contrast, a study regarding viral dynamics in 31 asymptomatic COVID-19 patients showed that 22 presented symptoms after their hospital admission and that their C_t values ($C_t = 39$) previously were significantly higher than those of symptomatic patients ($C_t = 34.5$).[30] In the present study, we found that patients with COVID-19 exhibited higher C_t values than AHW, similarly to C_t values described by Kim et al.,[27] but in our study, positive AHW were not presymptomatic subjects since they never exhibited symptoms related to COVID-19.

On the other hand, in the present study, the bivariate analysis revealed an association between $C_t < 38$ values for *N*2 and mechanical ventilation assistance among patients ($p = .013$), *N*2 had low C_t values (inferring high viral load) with respect to *N*1 and *N*3. Interestingly, a retrospective cohort study of 678 inpatients attended in a New York Hospital, showed that the risk of intubation was higher in patients with a high viral load than in those with a medium or low viral load.[31]

C_t value are inversely related to the viral load, and every ~3.3 increase in C_t value reflects a 10-fold reduction in starting material.[32] Interestingly, a significant association between $C_t < 38$ for N2 and mechanical ventilation in patients ($p = .013$) was found, and significant correlations between C_t values of *N*1 and *N*3 and values of AST, LDH, and ferritin were identified. We do not have a clear virological explanation for this finding. Different expression levels between markers can arise due to the reverse transcription process used in the present protocol; however, we cannot rule out differential expression in certain regions of the structural genes of the virus, especially the nucleocapsid gene, where multiple copies must be expressed synchronically to assemble the SARS-CoV-2 virions that will be expelled from the infected cell. Our findings are in concordance with other reports; Azzi et al.,[9] found an inverse correlation between the LDH values obtained in haematochemical analyses and C_t value.

Furthermore, in a meta-analysis of 60 studies that reported laboratory findings, Borges do Nascimento et al.,[23] found differences in patients with COVID-19 for AST and LDH. Another study found that serum levels of ferritin were markedly increased in patients with very severe COVID-19 compared with patients with severe COVID-19.[31] Although, in present study patients with severe disease and critically ill showed similar ferritin values, patients who died (inside the critically ill group) exhibited very high values (>2500 µg/L). Ferritin is particularly relevant as it is a mediator of immune dysregulation; it has been proposed that under extreme hyperferritinaemia, ferritin exerts direct immune-suppressive and proinflammatory effects, contributing to the cytokine storm observed in patients with COVID-19.[33,34] For this reason, it is important to continue carrying out virological and immunological studies of this new coronavirus to clearly understand the molecular process of viral replication and find potential markers of disease progression as well as targets of therapeutic drugs that will allow the control of COVID-19.

It has been argued that knowledge of viral load is essential to formulate strategies for antiviral treatment, vaccination, and epidemiological control of COVID-19; however, C_t values alone are often used as viral load indicators, which may be a mistake;[35] it is important to consider the viral dynamics, because several reports on viral dynamics indicated that viral shedding peaked on or before symptom onset case after symptoms onset, viral loads decreased; in addition, the variability observed in the C_t value that discriminates between infective and noninfective viruses does not allow us to select a single C_t value, since this value depends on multiple technical factors (e.g the number and type of target genes). Therefore, a more precise approach to transmissibility would be to jointly evaluate the C_t value and the time of evolution (or the time since contact in asymptomatic people), clinical course, severity of the disease and immunosuppression.

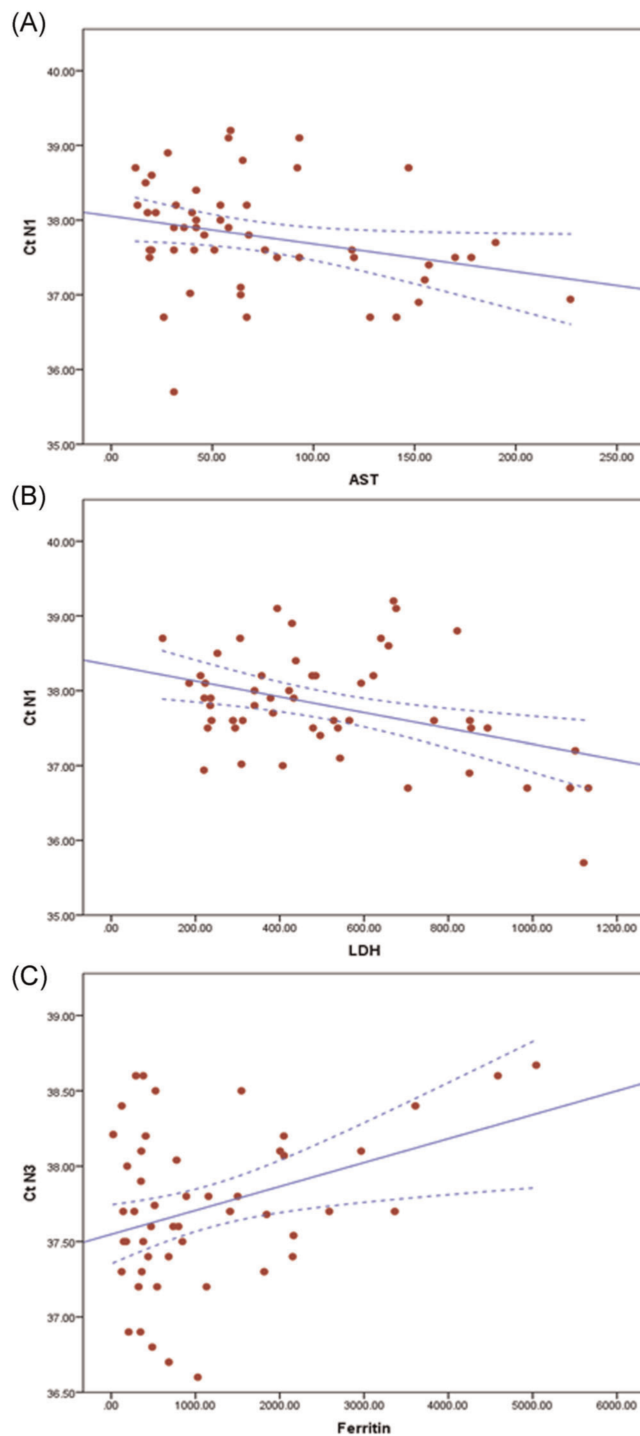

FIGURE 1 Scatter plot of AST, LDH, and ferritin and the RT-qPCR cycle threshold for (A, B) N1 and (C) N3 from critically ill and severe disease inpatients with COVID-19. Noncontinuous blue lines represent the regression result and the solid blue lines is its 95% confidence intervals. The Pearson's *r* were: (A) −0.276 and $p = .048$; (B) −0.40 and $p = .003$; (C) 0.357 and $p = .011$. AST, aspartate-transaminase; COVID-19, coronaviru disease; LDH, lactate dehydrogenase; RT-qPCR, reverse-transcription quantitative polymerase chain reaction

Finally, the qRT-PCR has high sensitivity and is therefore helpful for the initial diagnosis of COVID-19.[13] However, according to Tom and Mina,[32] reporting the result as a binary measure, i.e., positive or negative, can confuse physicians by eliminating information useful to make decisions. It has been reported that after complete resolution of symptoms, patients infected with SARS-CoV-2 continue to yield positive qRT-PCR results for many weeks.[36] Therefore, it is advisable that clinicians be informed of the C_t values obtained during amplification of viral markers as well as C_t values corresponding to the assay detection limit of viral RNA, which vary according to the characteristics of each system and amplification protocol used. As Binnicker[37] suggests, C_t value criteria must be established by each healthcare institution; in adittion, a careful interpretation of results with high C_t values needs to be undertaken in the context of the clinical situation and timing of testing relative to symptoms or exposure.[38]

Although the present study shows some limitations, such as cross-sectional study design with a small number of patients, grouped into groups of severe disease and critically ill, the information obtained is relevant because it shows that C_t values correlate with some relevant clinical data for hospitalized patients with COVID-19, supporting the use of saliva for internal tests for the detection of SARS-CoV-2, to obtain useful information to support clinical decisions.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

AUTHOR CONTRIBUTIONS

Juan Pablo Ramirez-Hinojosa, Yunuen Rodriguez-Sanchez, Angel Kaleb Romero-Gonzalez, Marisol Chavez-Gutierrez, Mirza Romero-Valdovinos, and Sara Arroyo-Escalante collected the saliva samples. Nelly Raquel Gonzalez-Arenas, Aurora Ibarra-Arce, Sara Arroyo-Escalante, Beatriz Zavaleta-Villa, and Mirza Romero-Valdovinos performed the nucleic acid extraction and RT–qPCR. Angelica Olivo-Diaz and Lourdes Suarez-Roa performed the statistical analysis. Juan Pablo Ramirez-Hinojosa, Luz Elena Espinosa de los Monteros-Perez, Angelica Olivo-Diaz, Rigoberto Hernandez-Castro, Guillermina Avila-Ramirez, Pablo Maravilla, and Mirza Romero-Valdovinos formulated the idea. Hector Prado-Callero and Octavio Sierra-Martinez obtained the authorizations and funding. Ana Flisser

provided critical comments. All authors participated during the discussion and writing of the manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are shown within the paper.

ETHICS STATEMENT

The study was conducted in full accordance with ethical principles (World Medical Association Declaration of Helsinki), and written informed consent was obtained from all participants or their relatives. The present study was approved by the Research and Ethics Committees of the "Dr. Manuel Gea Gonzalez" General Hospital with reference number 12-26-2020.

ORCID

Pablo Maravilla http://orcid.org/0000-0003-2534-9447

REFERENCES

1. World Health Organization. Transmission of SARS-CoV-2: implications for infection prevention precautions. 2020. Available at: https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions/. Accessed March 17, 2021.
2. Wang WK, Chen SY, Liu IJ, et al. Detection of SARS-associated coronavirus in throat wash and saliva in early diagnosis. *Emerg Infect Dis*. 2004;10(7):1213-1219.
3. To KK, Lu L, Yip CC, et al. Additional molecular testing of saliva specimens improves the detection of respiratory viruses [published online ahead of print June 7, 2017]. *Emerg Microbes Infect*. 6(6):e49. https://doi.org/10.1038/emi.2017.35
4. Rutgers The State University of New Jersey. New Rutgers saliva test for coronavirus gets FDA approval. 2020. https://www.rutgers.edu/news/new-rutgers-saliva-test-coronavirus-gets-fda-approval. Accessed March 17, 2021.
5. Tan HW, Xu YM, Lau ATY. Angiotensin-converting enzyme 2: The old door for new severe acute respiratory syndrome coronavirus 2 infection. *Rev Med Virol*. 2020;30(5):e2122.
6. Xu X, Chen P, Wang J, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci*. 2020;63(3):457-460.
7. Fakheran O, Dehghannejad M, Khademi A Saliva as diagnostic specimen for detection of SARS-CoV-2 in suspected patients: a scoping review. *Infect Dis Poverty*. 2020; 9(1):100.
8. Xu R, Cui B, Duan X, Zhang P, Zhou X, Yuan Q. Saliva: potential diagnostic value and transmission of 2019-nCoV. *Int J Oral Scien*. 2020;12(1):11.
9. Azzi L, Carcano G, Gianfagna F, et al. Saliva is a reliable tool to detect SARS-CoV-2. *J Infect*. 2020;81(1):e45-e50.
10. Centers for Disease Control and Prevention. CDC-201-novel coronavirus (2019-nCoV) real-time reverse transcriptase (RT-) PCR diagnostic panel. 2020. https://www.fda.gov/media/134919/download. Accessed March 17, 2021.
11. World Health Organization. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance. 2020. https://apps.who.int/iris/handle/10665/331329. Accessed March 17, 2021.
12. Arevalo-Rodriguez I, Buitrago-Garcia D, Simancas-Racines D, et al. False-negative results of initial RT-PCR assays for COVID-19: a systematic review. *PLoS One*. 2020;15(12):e0242958.
13. Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. *JAMA*. 2020;323(22):2249-2251.
14. Xing Y, Mo P, Xiao Y, Zhao O, Zhang Y, Wang F. Post-discharge surveillance and positive virus detection in two medical staff recovered from coronavirus disease 2019 (COVID-19), China, January to February 2020. *Euro Surveill*. 2020;25(10):2000191.
15. Carter LJ, Garner LV, Smoot JW, et al. Assay techniques and test development for COVID-19 diagnosis. *ACS Cent Sci*. 2020;6(5): 591-605.
16. To KK, Tsang OT, Yip CC, et al. Consistent detection of 2019 novel coronavirus in saliva. *Clin Infect Dis*. 2020;71(15):841-843.
17. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA*. 2020;323(18):1843-1844.
18. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature*. 2020;581(7809): 465-469.
19. Sikkema RS, Pas SD, Nieuwenhuijse DF, et al. COVID-19 in health-care workers in three hospitals in the south of the Netherlands: a cross-sectional study. *Lancet Infect Dis*. 2020;20(9):e215-e1280.
20. Siddiqui MK, Parcell B, Allstaff S, Palmer C, Chalmers JD, Bell S. Characteristics and outcomes of health and social care workers testing positive for SARS-CoV-2 in the Tayside region of Scotland. *Eur Respir J*. 2020;56(3):2002568.
21. Secretaria de Salud. Mexico, on COVID-19, Technical report. https://www.gob.mx/cms/uploads/attachment/file/607909/COVID-19_Personal_de_Salud_2021.01.18.pdf. Accesed March 2021.
22. American College of Cardiology, Chinese National Health Commission. Chinese clinical guidance for COVID-19 pneumonia diagnosis and treatment. 2020. https://www.acc.org/latest-in-cardiology/articles/2020/03/17/11/22/chinese-clinical-guidance-for-covid-19-pneumonia-diagnosis-and-treatment. Accessed March 17, 2021.
23. Borges do Nascimento IJ, Cacic N, Abdulazeem HM, et al. Novel coronavirus infection (COVID-19) in humans: a scoping review and meta-analysis. *J Clin Med*. 2020;9(4):941-955.
24. Paska C, Barta I, Drozdovszky O, Antus B. Improving gene-expression studies from sputum: a multistep optimization of RNA isolation and qPCR protocols. *Am J Respir Cell Mol Biol*. 2017;57(5): 626-628.
25. Wyllie AL, Fournier J, Casanovas-Massana A, et al. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. *N Engl J Med*. 2020;383(13):1283-1286.
26. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med*. 2020; 26(8):1200-1204.
27. Kim SE, Jeong HS, Yu Y, et al. Viral kinetics of SARS-CoV-2 in asymptomatic carriers and presymptomatic patients. *Int J Infect Dis*. 2020;95:441-443.
28. Uhm JS, Ahn JY, Hyun J, et al. Patterns of viral clearance in the natural course of asymptomatic COVID-19: Comparison with symptomatic non-severe COVID-19. *Int J Infect Dis*. 2020;99: 279-285.
29. Malagón-Rojas J, Parra E, Mercado M. Letter to the editor on "Asymptomatic infection by SARS 2 coronavirus: Invisible but invincible" by Nikolai et al. 2020. *Int J Infect Dis*. 101, 2020: 391-392.
30. Malagón-Rojas J, Gómez-Rendón C, Parra EL, et al. SARS-CoV-2 y RT-PCR en pacientes asintomáticos: resultados de una cohorte de trabajadores del Aeropuerto Internacional El Dorado de Bogotá, 2020. *Biomedica*. 2020;40(Suppl 2):166-172.
31. Magleby R, Westblade LF, Trzebucki A, et al. Impact of SARS-CoV-2 viral load on risk of intubation and mortality among hospitalized patients with coronavirus disease 2019. *Clin Infect Dis*. 2020;30: ciaa851.
32. Tom MR, Mina MJ. To Interpret the SARS-CoV-2 test, consider the cycle threshold value. *Clin Infect Dis*. 2020;71(16):2252-2254.
33. Vargas-Vargas M, Cortés-Rojo C. Ferritin levels and COVID-19. *Rev Panam Salud Publica*. 2020;44:e72.

34. Zhou B, She J, Wang Y, et al. Utility of ferritin, procalcitonin, and C-reactive protein in severe patients with 2019 novel coronavirus disease. https://doi.org/10.21203/rs.3.rs-18079/v1. Accessed March 17, 2021.
35. Miranda RL, Guterres A, de Azeredo Lima CH, Filho PN, Gadelha MR. Misinterpretation of viral load in COVID-19 clinical outcomes. *Virus Res*. 2021;296:198340.
36. Xiao AT, Tong YX, Zhang S. Profile of RT-PCR for SARS-CoV-2: a preliminary study from 56 COVID-19 patients. *Clin Infect Dis*. 2020; 71(16):2249-2251.
37. Binnicker MJ. Can the SARS-CoV-2 PCR cycle threshold value and time from symptom onset to testing predict infectivity? *Clin Infect Dis*. 2020;6:ciaa735.
38. Drew RJ, O'Donnell S, LeBlanc D, McMahon M, Natin D. The importance of cycle threshold values in interpreting molecular tests for SARS-CoV-2. *Diagn Microbiol Infect Dis*. 2020;98(3):115130.

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