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SARS-CoV-2 Identification in Saliva: Are We Interpreting the Results Correctly?

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Abstract

Background

In the present study, we obtained cycle threshold (Ct) values by qRT-PCR and compared them with clinical and laboratory data from saliva specimens of inpatients with COVID-19 and asymptomatic health workers (AHW).

Methods

Saliva specimens from inpatients with COVID-19 and AHW were studied by qRT-PCR using three sets of primers for the N (N1, N2, and N3) gene of SARS-CoV-2. The Ct values obtained were compared with the clinical and laboratory data.

Results

Data from 58 inpatients (37 critically ill patients and 21 patients with severe disease) and 105 AHW were analysed. In our system, the limit of viral detection corresponded to a Ct =46.5; therefore, our analysis focused on comparing the positivity rate obtained when using Ct <40 as the cut-off with that obtained using Ct <46 as the cut-off. The positivity rate was increased when the Ct cut-off of 46 was used as the criterion, yielding a sensitivity of 87.9% for patients and a sensitivity of 43% for AHW. The bivariate analysis revealed an association between Ct <40 for N2 and mechanical ventilation assistance among patients (p=0.013). In addition, the serological values of alanine transaminase (ALT), aspartate-transaminase (AST), lactate dehydrogenase (LDH), ferritin and creatine kinase–MB (CK-MB) showed significant correlations with the Ct values of N1 and N3.

Conclusion

Due to the intrinsic characteristics of the qRT-PCR process, obtaining amplification curves implies the presence of an active viral replication process, while Ct values may correlate with some clinical data. Our results support the claim that physicians should be informed of the Ct values obtained during the amplification of viral markers, as well as the Ct values that correspond to the limit of detection for viral RNA, which vary according to the characteristics of each system and amplification protocol used.

Background

Infection by SARS-CoV-2 causes the disease coronavirus disease 2019 (COVID-19), which has emerged as a global health problem and 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 who release the virus through bodily secretions, such as saliva and respiratory secretions or droplets, which are expelled when a person coughs, sneezes, talks or sings [1]. SARS-CoV and MERS-CoV RNA can be detected in saliva [2, 3], even before lung lesions appear [2], and saliva has a high

concordance rate of > 90% with nasopharyngeal samples in the detection of respiratory viruses [3]. Currently, molecular assays that use self-collected saliva samples are widely available [4].

SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) 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 lung [5]. An early study of serial saliva self-collected by patients infected with SARS-CoV-2 showed that 92% (11/12) of the saliva samples were positive for the virus. The patients' viral loads were monitored and generally showed a declining trend, leading the authors to conclude that saliva sampling could be a promising noninvasive method for diagnosis, monitoring, and control in patients with SARS-CoV-2 [7]. An Italian study performed with 25 patients with COVID-19 concluded that saliva is a reliable medium for detecting SARS-CoV-2 [8]. 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 [9].

The success of qRT-PCR relies on the amplification of tiny amounts of viral genetic material in a sample [10]. A variety of RNA target genes and protocols are now available [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. Most of the RT-PCR techniques show ≅100% specificity since the designed primers are specific to the genome sequence of SARS-CoV-2; however, false-negative results can occur [12] depending on the timing of sample collection in relation to illness onset and due to sampling errors [13]. These techniques have been performed with bronchoalveolar lavage fluid, fibrobronchoscope brush biopsy, sputum, nasal swab, nasopharyngeal swab, saliva, stool, blood and urine, yielding a wide range of positive results (from 0 to 93%) depending on the sample studied [7, 14, 15].

In quantitative PCR, a positive reaction is detected as the accumulation of fluorescent signal. The cycle threshold (Ct) is defined as the number of cycles required for the fluorescent signal to cross the threshold, i.e., to exceed the background level. Current RT-PCR protocols for SARS-CoV-2 detection suggest that samples with Ct values < 40 can be interpreted as positive for viral RNA. Some researchers 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 < 10^6 viral copies per ml (or copies per sample) may not yield an isolate [13, 16].

It has been argued that healthcare workers are at increased risk of exposure to SARS-CoV-2 and potentially influence in-hospital transmission [17, 18]. As of August 25 in Mexico, there have been 568,621 confirmed COVID-19 cases and 61,450 associated deaths, and 97,632 health workers have been infected with SARS-CoV-2 (42% nurses, 27% medical staff, and 31% other health workers). Most of the infected health workers (> 90%) have been treated as outpatients [19].

In the present study, we obtained Ct values and compared them with clinical and laboratory data from saliva samples of inpatients with COVID-19 and asymptomatic health workers (AHW).

Material And Methods

Participants

The 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 [11]. qRT-PCR confirmation was performed at one of the reference centres in Mexico City (Instituto Nacional de Ciencias Medicas y de la Nutrición “Salvador Zubiran”), with a Ct value less than 38 interpreted as a positive result. Patients’ clinical conditions were classified according to the Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment [20]. 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 according to [8, 21]. 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.

Nucleic acid extraction and qRT–PCR

Saliva specimens were frozen at -70 °C until processing. An aliquot of 500 µl saliva was collected, supplemented with 10 µl 2 M dithiothreitol (DTT) as mucolytic agent and shaken for 30 min [22]. Total RNA was extracted using TriPure® (Roche, Germany) 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, Madison, WI).

Real-time PCR was performed following WHO in-house protocols and the CDC 2019-nCoV Real-time RT-PCR Diagnostic Panel protocol [11], in which the oligonucleotide primers and probes for the detection of SARS-CoV-2 target regions of the virus nucleocapsid (N) gene (N1, N2 and N3). 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. Total mRNA expression was determined with a Light Cycler software, version 5.1 (Roche Diagnostic); bronchoalveolar lavage fluid from a patient was diluted to generate a standard curve of RNA concentration versus Cycle threshold (Ct). All samples were analysed in duplicate with a previously established positive control and saliva from healthy people, which had been stored at -70 °C prior to the COVID-19 pandemic and used as negative

controls. In cases where duplicates of a sample yielded Ct > 3, the sample was interpreted as inconclusive and analysed again. The lowest Ct value for a given sample was selected as the final value.

Statistical analysis

Most variables were expressed as the mean plus standard deviation (SD). To analyse the associations between each of the main clinical and laboratory variables or positivity level and Ct value, we performed bivariate analyses, calculating chi-square and Phi statistics to assess the strength of the relationship, as well as Student's t test and Mantel–Haenszel test, and we analysed correlations using Pearson's *r*. A p value < 0.05 was considered significant. Data analysis was performed with SSPS software version 15.0 (SPSS Institute, Chicago, IL) and Epi-Info6 v6.04 software.

Results

Table 1 summarizes the baseline clinical and laboratory characteristics of the 58 inpatients (69% male) with COVID-19, who comprised 37 critically ill patients and 21 patients with severe disease and had an average age of 52 ± 15 years. In addition, 105 (43% male) AHW (68% medical staff, 13% laboratory technicians/researchers, 11% administrative staff and 9% nurses) with a mean age of 32 ± 8 years were analysed in this study.

Table 1
Baseline clinical and laboratory characteristics of inpatients with COVID-19

	Severe disease (%)	Critically ill (%)
Clinical characteristic		
Female	33.3	27
Outcome/recovery	100	86.5
Assisted with mechanical ventilation	0	32.4
Dyspnoea	85.7	86.5
Arthalgia	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

	Severe disease (%)	Critically ill (%)
Peripherial blood profile		
Leukocytes (x10^9 cells/L)	8.3 ± 3.4	9.6 ± 4.3
Lymphocytes (%)	15.5 ± 9	8.2 ± 4.5
Hemoglobin (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
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)	57.1 ± 39.2	55.9 ± 43.3
Lactate dehydrogenase (LDH)	388.7 ± 182.4	456.7 ± 200.4
Inflammatory markers		
Oxygen saturation (SpO2)	88.1 ± 10.1	89.3 ± 6.6
Ferritin (µg/L)	859.1 ± 994.3	776.2 ± 711.5
C-reactive protein	15.1 ± 11.4	16.7 ± 9.2
D-dimer	1.5 ± 2.2	2.8 ± 8.0
Tropinin	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

During the standardization of the qRT-PCR, dilutions of the standard curve showed that the limit of viral detection in our system for each marker (N1, N2 and N3) corresponded to Ct = 46.5. Therefore, our analysis was focused on comparing the positivity rate achieved with our technique using Ct < 46 with that

achieved with the commonly used value of Ct < 40. Table 2 summarizes the percentage positivity and mean Ct values for critically ill patients, patients with severe disease and AHW. Most of the patient samples amplified for two and three markers; however, the samples of the AHW generally amplified a single marker (mainly N1), and 48 samples did not amplify any marker. In addition, 8 samples were associated with 2 or 3 amplified markers with high viral load (Ct < 22). Approximately 30% of all Ct values ranged between < 40 and < 46 (Fig. 1). The percentage positivity was considerably increased when a Ct of 46 was considered the cut-off value for a positive test in both the patient groups and the AHW. In addition, the main Ct value of the AHW was lower than that of patients. The bivariate analysis revealed an association between Ct < 40 for N2 and the need for assisted mechanical ventilation among patients ($p = 0.013$); furthermore, for the five blood biochemistry biomarkers alanine transaminase (ALT), aspartate-transaminase (AST), lactate dehydrogenase (LDH), ferritin and creatine kinase–MB (CK-MB), significant correlations were observed between their values and the Ct values of N1 and N3 (Fig. 2).

Table 2
General values on positivity and Ct values obtained for patients and asymptomatic health workers

	Number of positive samples					
	N1		**N2**		**N3**	
	Ct < 40	**Ct < 46**	**Ct < 40**	**Ct < 46**	**Ct < 40**	**Ct < 46**
Critically ill (n = 37)	10	29	14	16	13	14
Severe (n = 21)	6	14	6	7	5	5
Asymptomatic health workers (n = 105)	12	41	9	9	2	7
	Mean ± SD of Ct values from positive samples					
	N1		**N2**		**N3**	
	Ct < 40	**Ct < 46**	**Ct < 40**	**Ct < 46**	**Ct < 40**	**Ct < 46**
Critically (n = 37)	36.62 ± 3.52	40.39 ± 4.15	27.32 ± 12.40	29.28 ± 12.48	33.25 ± 4.81	33.97 ± 5.34
Severe (n = 21)	40.36 ± 0.13	41.87 ± 2.85	27.03 ± 8.35	29.80 ± 10.57	32.96 ± 10.90	30.05 ± 9.21
Asymptomatic health workers (n = 105)	19.04 ± 7.62	36.24 ± 11.99*	15.85 ± 1.82	15.85 ± 1.82	16.59 ± 1.02	36.27 ± 13.50
Test Positivity						
	With 1 marker (% accumulated)		**With 1 and 2 markers (% accumulated)**		**With 3 markers (% accumulated)**	
	Ct < 40	**Ct < 46**	**Ct < 40**	**Ct < 46**	**Ct < 40**	**Ct < 46**
Critically (n = 37)	6 (16)	17 (46)	14 (38)	23 (62)	19 (51)	33 (89)**
Severe (n = 21)	7 (33)	12 (57)	11 (52)	16 (76)	12 (57)	18 (86)**
Asymptomatic health workers (n = 105)	6 (6)	34 (32)**	13 (12)	44 (42)**	14 (13)	45 (43)**
*p < 0.05, Student's t test						
**p < 0.01, Mantel–Haenszel test						

Discussion

It has been emphasized that fast and accurate diagnostic tests are essential for controlling the ongoing COVID-19 pandemic. Some evaluations have shown that saliva specimens and nasopharyngeal swabs

have similar sensitivity in detecting SARS-CoV-2 [7–9]. In the present study, unexpectedly, a high number of samples with Ct values on the border of Ct < 40 and low sensitivity of our results for patients (53.4%) were observed. According to the CDC 2019-nCoV Real-time RT-PCR Diagnostic Panel protocol [11], we carefully reviewed our internal and external controls, which revealed the following: i) The known positive control consistently yielded a Ct value in the range of 30 to 33, and the negative controls did not yield fluorescence growth curves in any of the batches assayed. ii) Ct values were highly consistent between duplicates, and the external no-template control never yielded fluorescence growth curves. iii) The RP primers and probe set, as extraction RNA controls, yielded fluorescence growth curves; however, on many occasions, they crossed the Ct = 40 threshold, particularly in those patients from whom scarce saliva was obtained. For the mechanically ventilated patients, saliva specimens were obtained using a disposable sterile plastic like-Pasteur pipette, which yielded a low amount of saliva (approximately 500 µl). iv) All assays were performed with the same batch of primers, probes and reagents. The CDC 2019-nCoV Real-time RT-PCR Diagnostic Panel Results Interpretation Guide states that if only one of the two targets (N1 and N2) is positive, the test result is inconclusive, and the sample should be reanalysed, with new nucleic acid re-extraction and RT-PCR steps. In addition, the guide states that negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment decisions or other patient management decisions. In addition, false-negative results may occur if an inadequate number of organisms are present in the specimen [11].

Ct values may vary due to a variety of factors; however, the algorithm used to calculate them assumes that all amplification efficiencies are equal to 2 or at least equal among all reactions, and possible variation in amplification efficiency among sequences or samples [23] or among instruments [11] are not considered. Interestingly, in a retrospective study of nasopharyngeal-oropharyngeal specimens from symptomatic individuals, the proportion of specimens containing a viral load close to the assay limit of detection (LoD) was small; therefore, there was a low risk of false negatives when testing symptomatic patients by qRT-PCR [24].

The qRT-PCR has high sensitivity and is therefore helpful for the initial diagnosis of COVID-19 [13]. However, according to Tom and Mina [25], reporting the result as a binary measure, i.e., positive or negative, can confuse physicians by eliminating information useful for clinical decision making. 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 [26]. At later time points in such cases, Ct values are often very high, representing the presence of low copy numbers of viral RNA (as low as fewer than 100), but the results are reported to the clinicians simply as positive. This situation leaves the clinician with little choice but to interpret the results similarly to the clearly positive results of samples from patients with a high viral load, in which RNA copies typically reach 100 million or more [25]. Therefore, it is advisable that clinicians be informed of the Ct values obtained during the amplification of the viral markers as well as the Ct values corresponding to the LoD of viral RNA, which vary according to the characteristics of each system and amplification protocol used. As Binnicker [27] suggests, Ct value criteria must be established by each healthcare institution. In addition, the incorporation of viral load value would be useful to detail the number of copies of viral RNA in a sample.

A common concern is the presence of asymptomatic SARS-CoV-2-infected individuals at work (return-to-work) or hospital settings. A study regarding viral dynamics in 31 asymptomatic COVID-19 patients showed that 22 of them presented symptoms after their hospital admission and that their Ct values (Ct = 39) before hospital admission were significantly higher than those of symptomatic patients (Ct = 34.5). These findings showed that although the asymptomatic patients with COVID-19 exhibited a lower viral load, they undergo a certain period of viral shedding, allowing the possibility of transmission during their asymptomatic period [28]. In addition, some authors have stated that a positive qRT-PCR result may be due to the presence of active, replicating virus or residual viral nucleic acid, i.e., noninfectious virus; a small proportion of recovered patients may yield positive virus detection results after discharge, and this positivity does not necessarily mean that the patient is transmissive [29, 30]. In the present study, we performed reverse transcription using oligo(dT) primers, which is the method most widely used for the conversion of mRNA into cDNA. During reverse transcription, an oligo(dT) primer is first annealed to the poly(A) sequences present at the 3' end of nearly every mRNA by T:A base-pairing. Subsequently, the reverse transcriptase extends from the annealed oligo(dT) primer along the mRNA template, resulting in the copying of the mRNA sequence into the cDNA sequence [31]. Thus, during reverse transcription, we captured all viral mRNA templates, implying that a possible active viral transcription process is underway and that the potential infective SARS-CoV-2 virus may be shedding. Furthermore, differences within the fluorescence growth curves for the three markers (N1, N2, N3) of the same gene can be explained by the reverse transcription by oligo(dT) primers: A high frequency of truncated cDNAs through internal poly(A) priming has been demonstrated [31]; therefore, during qPCR, different amounts of cDNA template can modify the yield of the fluorescence growth curves. Although most of the AHW in the present study who were found positive by qRT-PCR showed high Ct values with 1 or 2 markers, suggesting a low viral load, the number of positive results increased almost 3 times when we changed the Ct cut-off from 40 to 46. Nevertheless, 8 AHW exhibited 2 or 3 amplified markers with high viral load (Ct < 20), implying a risk of transmission for their contacts. However, caution should be taken when interpreting the results, since wide flexibility in the Ct cut-off criteria could lead to an over-reporting of positive tests that would complicate potential clinical decisions.

Critically ill patients infected with SARS-CoV-2 have been reported to exhibit higher viral loads and more prolonged shedding in the lower respiratory tract than in the upper respiratory tract. Sampling from the lower respiratory tract may be required to assess the true viral clearance in such patients [32]. Therefore, considering the technical difficulties in obtaining a sufficient amount of saliva from patients who required assisted mechanical ventilation, it is not advisable to use saliva as test specimens in critically ill patients.

The Ct value is inversely related to the viral load, and every ~ 3.3 increase in Ct value reflects a 10-fold reduction in starting material [25]. Interestingly, a significant association between Ct < 40 for N2 and mechanical ventilation in patients ($p = 0.013$) was found, and significant correlations between the Ct values of N1 and N3 and the values of ALT, AST, LDH, CK-MB 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, in

which multiple copies must be expressed synchronously to assemble the SARS-CoV-2 virions that will be expelled from the infected cell. Nevertheless, our findings are in concordance with other reports. Azzi et al. [8] found an inverse correlation between the LDH values obtained in haematochemical analyses and Ct value; in addition, a retrospective cohort study of 678 inpatients showed that the risk of intubation was higher in patients with a high viral load (Ct < 25) than in those with a medium (Ct 25–30) or low viral load (Ct > 30) [33]. Furthermore, in a meta-analysis of 60 studies that reported laboratory findings, Nascimento et al. [21] found differences in patients with COVID-19 for ALT, AST and LDH. Another meta-analysis performed by Shi et al. [34] showed that elevated levels of CK-MB were associated with the severity of COVID-19 in patients. Furthermore, another study found that the serum levels of ferritin were markedly increased in patients with very severe COVID-19 compared with patients with severe COVID-19 [35]. 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 pro-inflammatory effects, contributing to the cytokine storm observed in patients with COVID-19 [36]. For this reason, it is important to continue carrying out virological 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.

Conclusions

Due to the intrinsic characteristics of the qRT-PCR process, obtaining amplification curves implies the presence of an active viral replication process, while Ct values may correlate with some clinical data. Our results support the claim that physicians should be informed of the Ct values obtained during the amplification of viral markers, as well as the Ct values that correspond to the limit of detection for viral RNA, which vary according to the characteristics of each system and amplification protocol used.

Abbreviations

COVID-19: disease coronavirus disease 2019; Ct: Cycle threshold; qRT–PCR: quantitative reverse transcription-polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome by coronavirus 2; AHW: asymptomatic health workers; WHO: World Health Organization; ACE2: angiotensin-converting enzyme 2, ALT: alanine transaminase; AST: aspartate-transaminase; LDH: lactate dehydrogenase; CK-MB: creatine kinase–MB, LoD: limit of detection; mRNA: messenger RNA.

Declarations

Ethical approval and informed consent

The study has been conducted in full accordance with ethical principles (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.

Consent for publication

Written consents for publication of clinical data were obtained from all participants or their relatives.

Availabilty of data and materials

All data supporting the conclusions of this article are included within the article.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

JPR-H, YR-S, AKR-G, MCh-G, MR-V, SA-E, collected the saliva samples; NRG-A, AI-A, SA-E, BZ-V, MR-V, performed the nucleic acid extraction and RT–qPCR; AO-D, LS-R performed the statistical analysis; JPR-H, LEE-P, AO-D, RH-C, GA-R, PM, MR-V, formulated the idea; HP-C, OS-M obtained the authorizations and funding, A-F, provided critical comments. All authors participated during the discussion and writing of the manuscript.

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Figures

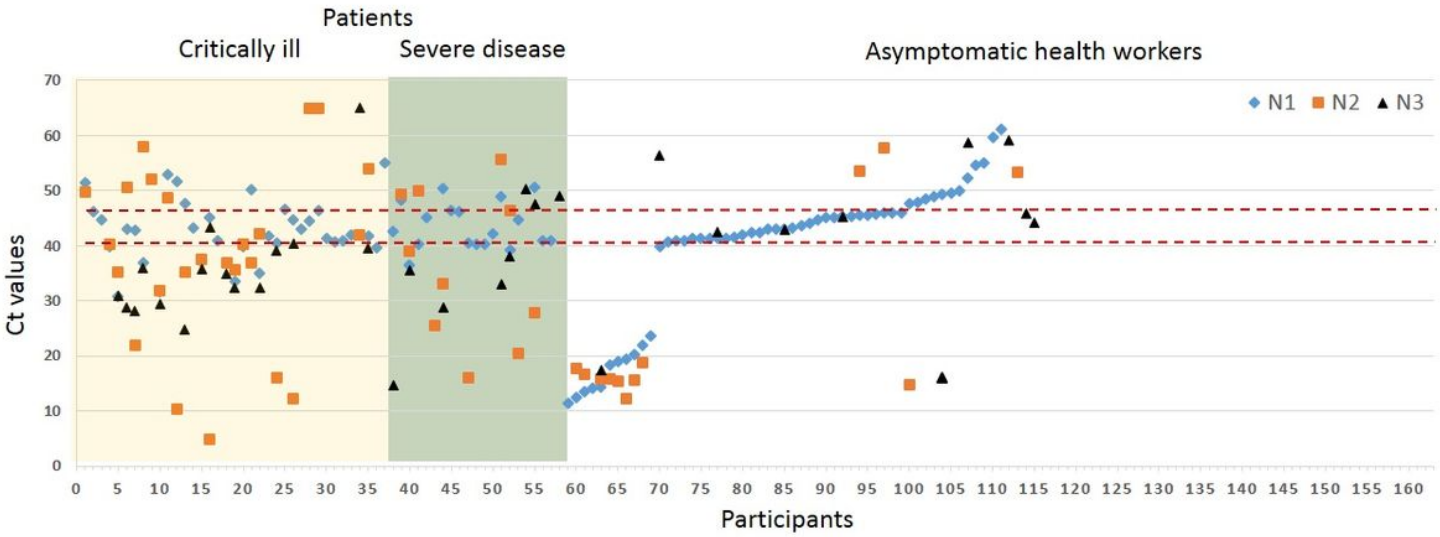

Figure 1

Ct values for N1, N2, N3 markers obtained by RT-qPCR of saliva specimens from 37 critically ill and 21 with severe disease inpatients with COVID-19 and 105 asymptomatic health workers. Discontinue lines mean Ct =040 and Ct =046, respectively.

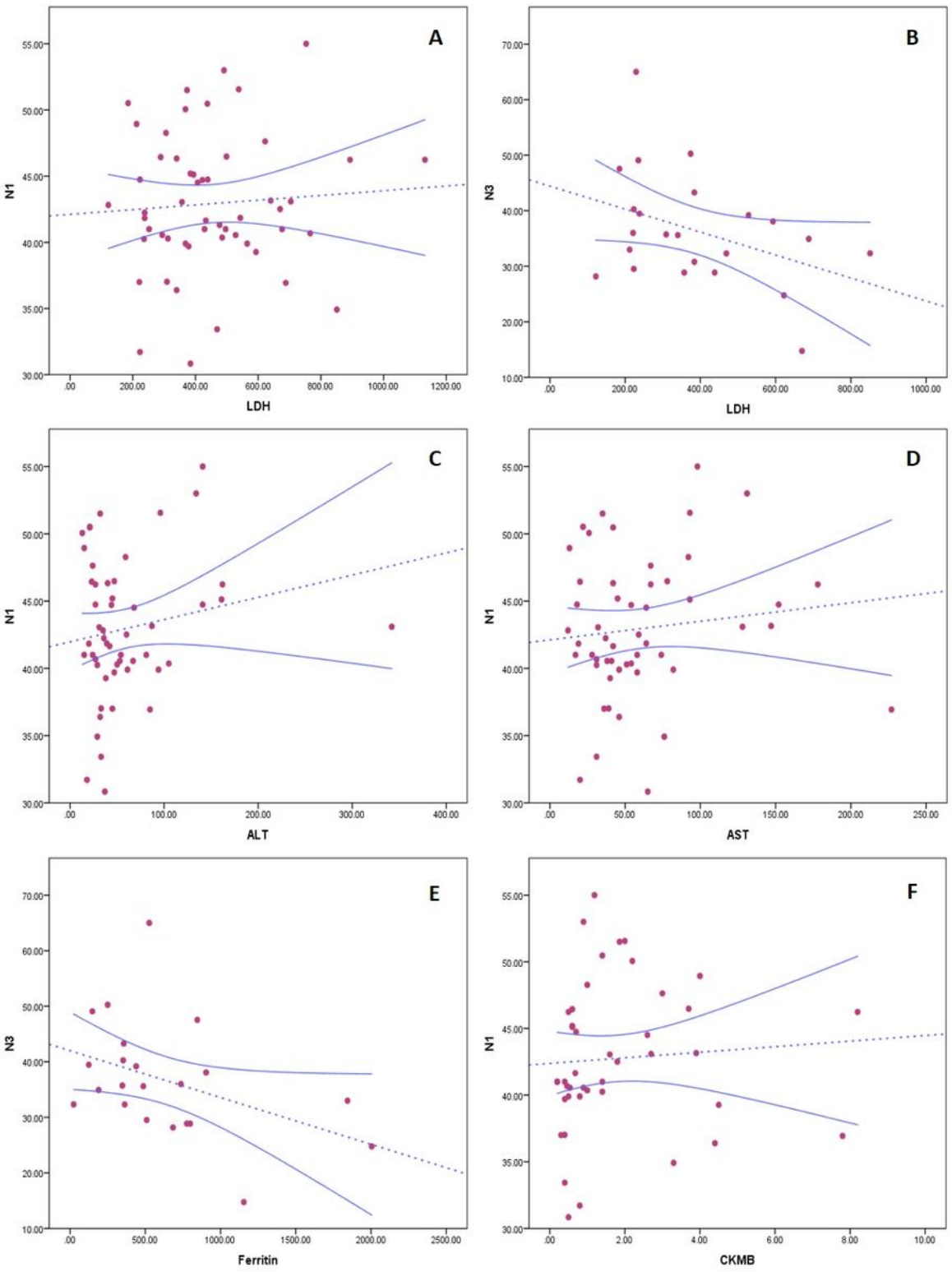

Figure 2

Scatter plot of LDH, ALT, AST, ferritin and CK-MB and the RT-qPCR cycle threshold for N1 (A, C and D) and N3 (B, E and F) from critically ill and severe disease inpatients with COVID-19. Discontinue blue lines represent the regression result and the solid blue lines is its 95% confidence intervals. The Pearson's r were: 0.252 and p=0.045 (A); -390, p=0.493 (B); 0.292 and p=0.200 (C); 0.253 and p=0.045 (D); -0.412 and p=0.049 (E); 0.300 and p=0.032 (F).