Epidemiological Correlates of PCR Cycle Threshold Values in the Detection of SARS-CoV-2

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Summary: Ct values were lower among COVID-19 patients under 18 years of age and those reporting upper respiratory symptoms at the time of sample collection and were correlated with time since onset. These results indicate populations who may be most infectious.

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ABSTRACT

Background

Detection of SARS-CoV-2 infection has principally been performed through the use of real-time reverse-transcription PCR (rRT-PCR) testing. Results of such tests can be reported as cycle threshold (Ct) values, which may provide semi-quantitative or indirect measurements of viral load. Previous reports have examined temporal trends in Ct values over the course of a SARS-CoV-2 infection.

Methods

Using testing data collected during a prospective household transmission investigation of outpatient and mild COVID-19 cases, we examined the relationship between Ct values of the viral RNA N1 target and demographic, clinical, and epidemiological characteristics collected through participant interviews and daily symptom diaries.

Results

We found Ct values are lowest (corresponding to higher viral RNA concentration) soon after symptom onset and are significantly correlated with time elapsed since onset ($p<0.001$); within 7 days after symptom onset, the median Ct value was 26.5 compared with a median Ct value of 35.0 occurring 21 days after onset. Ct values were significantly lower among participants under 18 years of age ($p=0.01$) and those reporting upper respiratory symptoms at the time of sample collection ($p=0.001$) and were higher among participants reporting no symptoms ($p=0.05$).

Conclusions

These results emphasize the importance of early testing for SARS-CoV-2 among individuals with symptoms of respiratory illness and allows cases to be identified and isolated when their viral shedding may be highest.

Keywords: COVID-19, SARS-CoV-2, RT-PCR, Cycle Threshold, Epidemiology

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INTRODUCTION

Since its emergence in 2019, the SARS-CoV-2 virus has caused over 19 million cases of COVID-19 and over 700,000 deaths globally [1]. Diagnosis of COVID-19 relies primarily on the use of real-time reverse-transcription PCR (rRT-PCR) tests, including the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel [2]. Such rRT-PCR tests amplify and detect target viral genetic sequences and generate cycle threshold (Ct) values [3]. Qualitative rRT-PCR tests do not measure the viral load within a clinical specimen, but Ct values offer a semi-quantitative assessment of viral RNA concentration: lower Ct values correspond to higher viral RNA concentrations. While quantitative interpretation of Ct values is dependent on multiple factors including reaction conditions and amplification efficiency, a common theoretical value can provide a useful benchmark for interpreting Ct values: an increase of 3.3 units in Ct value corresponds to 10-fold less target RNA under optimum conditions[3]. As a result, Ct values can serve as an indirect indicator of relative viral load in diagnostic samples of persons tested for SARS-CoV-2. However, relatively few studies have found significant associations between epidemiological factors and SARS-CoV-2 Ct values [4-9]. Given the paucity of data examining associations of these factors with Ct value at the time of diagnosis, we sought to identify relationships between Ct values and time since onset, demographic factors, and symptoms among laboratory-confirmed COVID-19 cases identified in a multistate investigation of SARS-CoV-2 household transmission.

METHODS

Individuals included in this analysis were participants in a household transmission investigation in Utah and Wisconsin between March 23 and May 13, 2020 who tested positive for SARS-CoV-2 on an nasopharyngeal (NP) swab at enrollment or during follow-up, including index cases and household members [10-12]. Briefly, households identified by local health authorities as having a member with laboratory-confirmed COVID-19 were approached for participation. NP specimens were collected from household members on the day of enrollment and repeatedly during follow-up. All participants completed questionnaires that included questions on demographics, medical history, timelines for exposure, and symptom assessment. Symptom assessments were conducted by CDC investigators at the time of specimen collection and by participants daily using symptom

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diaries. Household visits were made to collect NP specimens from all participating household members on the day of enrollment, 14 days later, and on any day in between when a household member reported the onset of a new symptom. All participants were outpatients at the time of data and specimen collection.

NP specimens were collected by CDC clinicians using flexible minitip flocked swabs (Becton, Dickinson, and Company, Franklin-Lakes, New Jersey, USA). Specimens were transported in three milliliters of viral transport media. For participants who tested positive more than once during the investigation period, Ct values from only the first positive test were included. All specimens were tested at the Utah Public Health Laboratory or the Milwaukee Public Health Laboratory using the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel [2]. Briefly, this assay amplifies and detects two targets (N1 and N2) of the viral nucleocapsid gene with a limit of detection in the range of 1000-3,162 viral RNA copies per mL. The human housekeeping gene target RNAse P (RP) was also measured in each sample for use in normalization. Viral RNA extraction procedures varied by instrument (using either the QIAgen EZ1 Advanced XL or the bioMérieux EMAG) and followed manufacturer's instructions; RT-PCR was performed using the ABI 7500. Results were considered positive if signal was detected (Ct<40) for RP, N1, and N2 genes. Results were classified as "not detected" if RP was detected but no signal was observed (Ct≥40) from either N1 or N2. Results were classified as inconclusive if RP was detected (Ct<40) and either N1 or N2 was detected (but not both). Results were classified as invalid if no RP was detected in the sample. Any specimens for which results were inconclusive were re-tested; specimens which produced inconclusive results after re-testing were excluded from the analysis (n=17 specimens, 6 participants). Ct values for amplification of both viral targets (N1 and N2 probes) and the human housekeeping gene RNase P (for specimen quality assessment) were analyzed.

Symptoms at the time of NP swab collection were classified into categories. Respiratory symptoms included runny nose, nasal congestion, or sore throat (upper respiratory); cough, discomfort while breathing, shortness of breath, wheezing, or chest pain (lower respiratory). COVID-19-like symptoms were defined as fever, cough, or shortness of breath [13]. The Council for State and Territorial Epidemiologists case definition for COVID-19 (adopted by several public health organizations) included any of cough, shortness of breath, or

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discomfort while breathing, or, alternatively, two or more of the following: fever, myalgia, headache, chills, sore throat, loss of taste, and loss of smell [14]. The criteria used by CDC for surveillance and monitoring of seasonal influenza-like illness activity included fever and either cough or sore throat [15]. The World Health Organization (WHO) surveillance definition of acute respiratory infection included shortness of breath, cough, sore throat, nasal congestion, or runny nose [16]. Further details on symptom categorization can be found in Supplemental Table S1. Categories of examined comorbid conditions were diabetes, any immunosuppressive condition, and any other reported comorbidity. Because Ct values are semi-quantitative, differences in Ct values between groups were assessed nonparametrically using the Mann-Whitney U test. Correlations between Ct value and time since symptom onset were assessed nonparametrically using tie-corrected Spearman rank correlation coefficients. Statistical tests for which $p<0.05$ were reported as statistically significant.

CDC collaborated with state and local health departments in the public health response to COVID-19 cases. This activity involved identification, control, or prevention of disease in response to an immediate public health threat; it was determined not to be public health research. Therefore, this activity did not require IRB review.

RESULTS

Characteristics of the population evaluated in that investigation have been described previously [10-12]. A total of 93 household members (including index cases) who tested positive for SARS-CoV-2 by NP swab were included in this analysis. A majority of those with a positive NP swab were female (53%), white (78%), and non-Hispanic (87%). The median age of participants was 37 years (interquartile range, IQR: 21-53 years) and 13 participants were under 18 years of age. All participants reported symptoms before, after, and/or on the day the day their positive NP swab was collected. Among participants reporting symptoms on or before the day their positive NP swab was collected (n=90), a median of 8 days (IQR: 6-11 days) elapsed between the day of the participant's symptom onset and the day of collection.

Ct values ranged from 14.4 to 38.4 (median, 29.7) for the N1 probe, were similar to those obtained for the N2 probe and did not vary by RNAse P value (Supplemental Figure S1). For this analysis, we focused on

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values of the N1 probe. Among participants who developed symptoms on or before their positive NP was collected (n=90), Ct values were significantly and positively correlated with time elapsed since earliest symptom onset (p<0.001; Figure 1A, 1C). Among individuals who tested positive within 7 days of symptom onset, the median Ct value was 26.5 (IQR: 20.8-29.9). By comparison, among individuals who tested positive 21 days or more after symptom onset, the median Ct value was 35.0 (IQR: 33.5-35.7). Ct values were not significantly correlated with age as a continuous variable due to variability in Ct values among adults (Figure 1B). However, when dichotomized, Ct values were significantly lower among participants under 18 years of age (Mann-Whitney p=0.01, Figure 1C). Age was significantly correlated with time from onset to collection (Spearman p=0.002). Ct values were not significantly associated with sex, race, or ethnicity (Figure 1C) or comorbid conditions (Supplemental Figure S2).

Ct values varied by symptom or combination of symptoms present at the time of specimen collection. Median Ct values for participants reporting specific symptoms (whether alone or accompanied by other symptoms) ranged from 25.1 (among those reporting chills) to 33.0 (among those reporting abdominal pain; Figure 2A). Although adults reported cough more frequently than children (Fisher's p<0.01, Supplemental Figure S3A), Ct values among participants reporting different symptoms did not vary by age group (Supplemental Figure S3B). Participants meeting the criteria for acute respiratory infection used by the WHO [16] had significantly lower Ct values than participants who did not meet these criteria (median Ct values of 29.3 and 33.3, respectively; p= 0.008; Figure 2B). Similarly, Ct values were significantly lower among those reporting one or more respiratory symptoms compared to those with no respiratory symptoms (median Ct values of 29.3 and 33.4, respectively; p=0.012; Figure 2B). The significant difference noted among those reporting any respiratory symptoms was likely due to significantly lower Ct values among those reporting upper respiratory symptoms (p<0.001). Additionally, median Ct values were lower among participants falling into several symptom categories and syndromes compared to participants who did not fall into these categories, but these were not statistically significant differences (Figure 2B). Ct values were higher among participants reporting no symptoms at the time of collection than among those reporting any symptoms (median Ct values: 33.3 and 29.3, respectively;

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p=0.0496); the p-value for this relationship approaches, but falls under, the *a priori* alpha threshold of 0.05, driven by wide confidence intervals among participants reporting any symptoms (95% CI: 16.4-37.5). Among participants reporting no symptoms at the time of collection (n=13), all reported symptoms either prior to collection (n=6), after collection (n=7), or both (n=4). Although these patients did not report symptoms at the time of NP collection, they represent a mixed group with some being post-symptomatic and some being pre-symptomatic. Among these participants, Ct values were lower among pre-symptomatic participants (median: 23.1) than among participants who were post-symptomatic (median: 35.9; p=0.036, Figure 3). Of these 13, three were children under 18 years of age; one was pre-symptomatic and two reported symptoms both before and after symptom collection. For symptoms and symptom combinations significantly associated with Ct value, medians, 95% confidence intervals, and associated p-values are presented in Table 1.

DISCUSSION

In this analysis, we examined associations between SARS-CoV-2 Ct values and epidemiological characteristics of participants with confirmed COVID-19. Among participants who were symptomatic on or before the day of their positive NP, we found that Ct values were significantly correlated with time since symptom onset. While Ct values are not direct quantitative measures of viral load, these results suggest that viral RNA levels in the nasopharynx (and, by extension, possible infectiousness) are highest soon after symptom onset. Additionally, we identified several clinical presentations significantly associated with lower Ct values, including upper respiratory symptoms and the WHO definition of acute respiratory infection [16]. Of note, we found that participants without symptoms at the time of collection exhibited higher Ct values than those with symptoms. However, this relationship was influenced by high Ct values among post-symptomatic participants. These observations illustrate the importance of rapid quarantine and testing of individuals with high-risk exposures (such as close contacts of known cases) who may be infectious while pre-symptomatic or asymptomatic. Our results also support recent findings that children are likely to contribute to transmission of SARS-CoV-2 [17, 18].

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This analysis is subject to several limitations. Participants were categorized according to their symptoms at the time of testing, which may not capture the full trajectory of a participant's symptoms over time [11]. Simultaneously, the household-based nature of this investigation captured a population of outpatient and mild COVID-19 cases and therefore was not powered to detect correlations between Ct values and more severe COVID-19 symptoms or presentations (which occur more rarely in outpatient/mild cases). As a result, this household transmission investigation may have captured some individuals who would not have sought care otherwise (e.g., those with mild symptoms) but may still contribute to household transmission [12]. We excluded six participants for whom only inconclusive results were obtained. Finally, though some subgroups exhibited significantly higher Ct values, all participants had positive NP swabs, and associations between high Ct values and symptoms or syndromes should be interpreted cautiously. Despite these limitations, this approach provides unique insight using prospective specimen collection among household members and the testing of individuals who were not seeking care. This has the potential to provide a more complete description of infection beyond healthcare settings.

Our investigation adds to a growing body of literature examining characteristics of SARS-CoV-2 and clinical/epidemiological factors. While viral load is only indirectly assessed by Ct values, both may present information useful in understanding clinical and epidemiological correlates of possible infectiousness. Previous studies have reported differing evidence regarding the timing of peak viral load in individuals with COVID-19. Some report no correlation or association between viral load (or, separately, Ct value) and time since onset of symptoms [4, 5], while others observed signals of greatest viral RNA amounts closest to the onset of symptoms [6-8, 19] or in the first week following symptom onset [20-22]. There is some evidence that the timing of peak viral load may be dependent on the anatomical source of the specimen tested (e.g., peaking earlier in throat swabs than in sputum specimens [23]) or extent of disease (e.g., peaking later in patients showing signs of lung infection [23] or with more severe disease [24]). Fewer studies have examined possible relationships between clinical presentation and Ct value. While one analysis found higher loads in patients with severe disease [24], multiple studies in which these associations were examined found no significant relationship between viral load

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(or, separately, Ct value) and patient demographics [4, 8], comorbidities [4], symptom status [5, 7, 9], disease severity [8], or medical intervention [6]. Our findings – that Ct values are significantly lower among individuals with respiratory symptoms – represent a novel addition and enhanced granularity to this growing body of evidence. Our results bolster the importance of early access to SARS-CoV-2 diagnostic testing for individuals who develop symptoms of respiratory illness or who have high-risk exposures (even while pre-symptomatic) and reinforce existing guidance for testing [25]. By quickly identifying such individuals early (when Ct values are lowest and infectiousness may be high) and implementing isolation protocols, public health practitioners might more effectively interrupt transmission of SARS-CoV-2.

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Competing Interests Statement

All authors have completed ICMJE conflict of interest disclosure statements have no competing interests to declare.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

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FIGURE LEGENDS

Figure 1: Correlates of Cycle Threshold Values from Participants in COVID-19 Household Transmission Investigations – Utah and Wisconsin 2020

Cycle threshold (Ct) values for SARS-CoV-2 rRT-PCR target probes N1 and N2 are plotted against the time elapsed between symptom onset and NP specimen collection in panel 1A. Ct values for the N1 probe are plotted against participant age in panel 1B. Distributions of N1 probe Ct values among participants categorized by time between symptom onset and NP collection, age, sex, race, and ethnicity are displayed in panel 1C. Age was significantly correlated with time between symptom onset and NP collection. "Other" race includes American Indian/Alaska Native, Native Hawaiian/Other Pacific Islander, and Multiracial, which were grouped due to low sample sizes. Boxes illustrate interquartile ranges, whiskers illustrated 95% distributions, and medians are highlighted in white.

Figure 2: Participants' Cycle Threshold Values from Nasopharyngeal Swabs, by Reported Symptoms and Syndromes in COVID-19 Household Transmission Investigations – Utah and Wisconsin 2020

Distributions of N1 probe Ct values among participants exhibiting specific symptoms or combinations of symptoms are displayed in panels 2A-2B. Boxes illustrate interquartile ranges, whiskers illustrated 95% distributions, and medians are highlighted in white. Each column of paired data (colored and grey) depicts the set of participants that reported the symptom(s) labeled on the x-axis (colored distribution) compared with the set of participants that did not report the same symptom(s) (grey distribution). Mann-Whitney p-values for each comparison are displayed beneath data pairs. Panel 2A illustrates distributions of Ct values from participants categorized by individual symptoms. Panel 2B illustrates distributions of Ct values from participants meeting the definition of various clinical categories or syndromes.

Figure 3: SARS-CoV-2 N1 Probe rRT-PCR Cycle Threshold Values among Participants Reporting No Symptoms at the Time of Positive Nasopharyngeal Swab Collection in COVID-19 Household Transmission Investigations – Utah and Wisconsin 2020

Points illustrate individual cycle threshold (Ct) values among participants reporting no symptoms on the day of positive NP specimen collection. "No Symptoms at Collection" refers to all such participants. "Pre-Symptomatic" refers to participants who reported no symptoms at or before the day of collection but reported symptoms later in the follow-up period. (Participants whose positive NP was collected on the final day of follow-up were excluded from this group.) "Post-Symptomatic" refers to participants who reported symptoms prior to collection but none on or after the day of collection. "Mid-Symptomatic" refers to participants who reported symptoms prior to and following the day of collection but not on the day of collection

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Table 1. Summary of Statistically Significant Associations between Ct Value and Clinical Presentation

	Ct Values of Participants Meeting Symptom/Syndrome Criteria Median (95% CI)	Ct Values of Participants Not Meeting Symptom/Syndrome Criteria Median (95% CI)	p-value (Mann-Whitney U)
Runny Nose	27.4 (16.3-33.5)	31.3 (20.3-37.7)	p<0.001
Abdominal Pain*	33.0 (27.1-36.3)	29.3 (16.4-37.5)	p= 0.042
Acute Respiratory Infection†	29.3 (16.2-37.6)	33.3 (22.7-37)	p= 0.008
Respiratory Symptoms†	29.3 (16.3-37.6)	33.4 (22.7-37)	p= 0.012
Upper Respiratory Symptoms†	29.3 (16.5-35.9)	32.3 (20.5-37.6)	p= 0.001
No Symptoms at Collection‡	33.3 (22.6-37.2)	29.3 (16.4-37.5)	p= 0.0496

Abbreviations: Ct – cycle threshold; CI – confidence interval.
*Ct values were lower in participants reporting no abdominal pain compared to participants who reported abdominal pain.
†Details on criteria for different clinical syndromes can be found in Supplementary Table S1.
‡ For the category of "No Symptoms at Collection", participants meeting the symptom/syndrome criteria includes participants who did not report symptoms at the time of collection; participants not meeting symptom/syndrome criteria includes participants who reported any symptoms at the time of collection.

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Figure 1

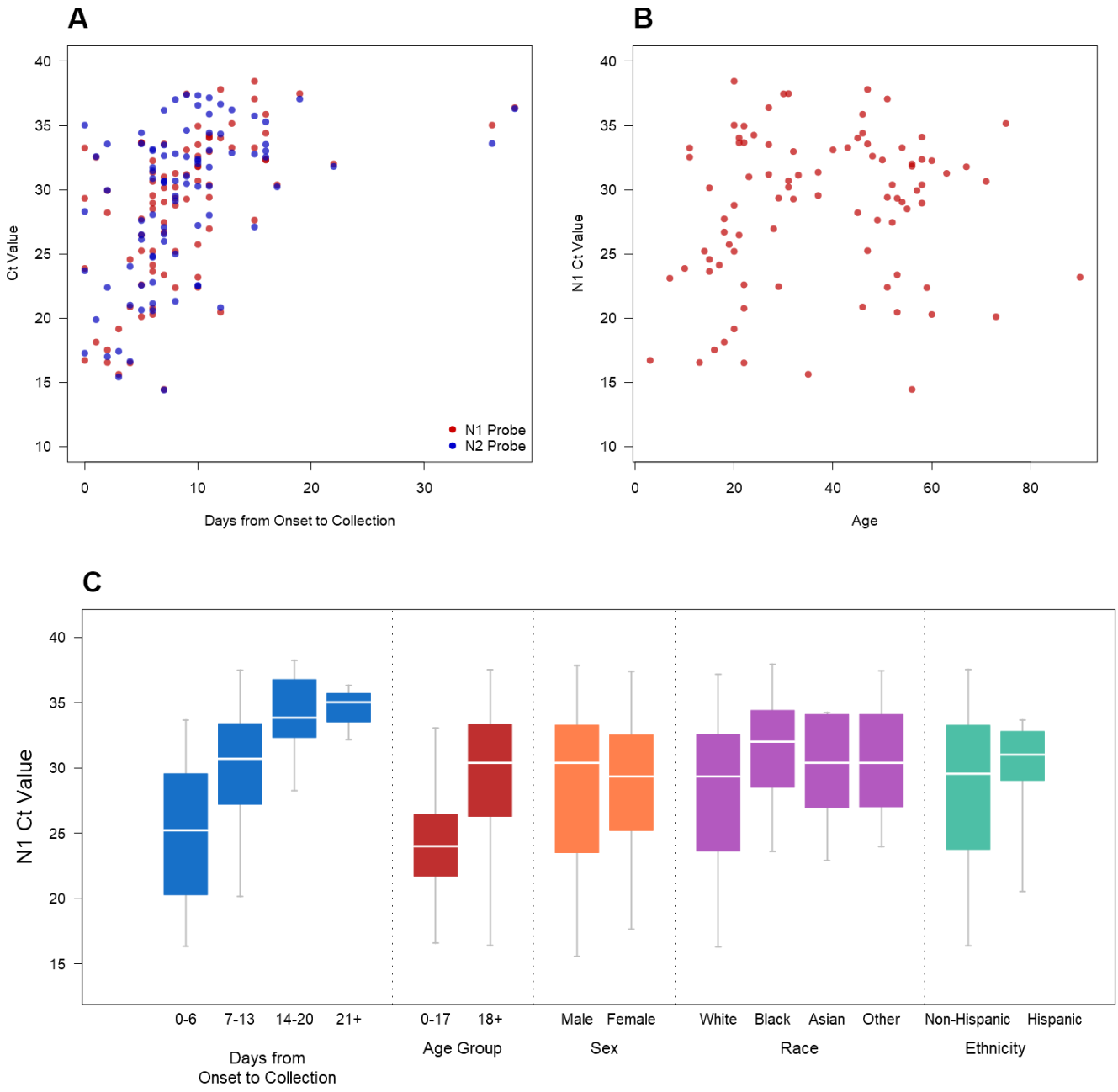

A
Ct Value
Days from Onset to Collection
N1 Probe
N2 Probe
B
N1 Ct Value
Age
C
N1 Ct Value
0-6
7-13
14-20
21+
Days from Onset to Collection
0-17
18+
Age Group
Male
Female
Sex
White
Black
Asian
Other
Race
Non-Hispanic
Hispanic
Ethnicity

Figure 2

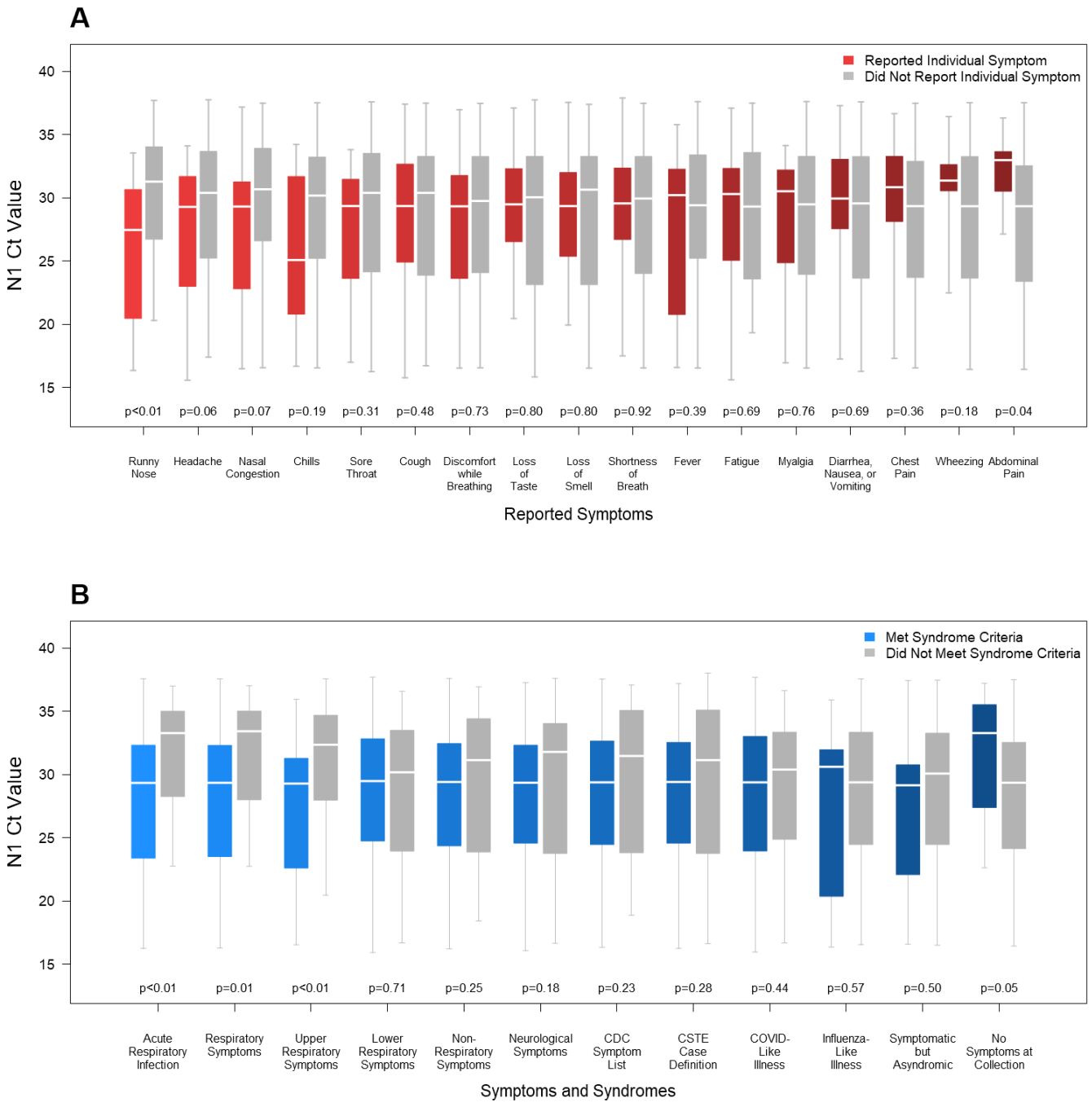
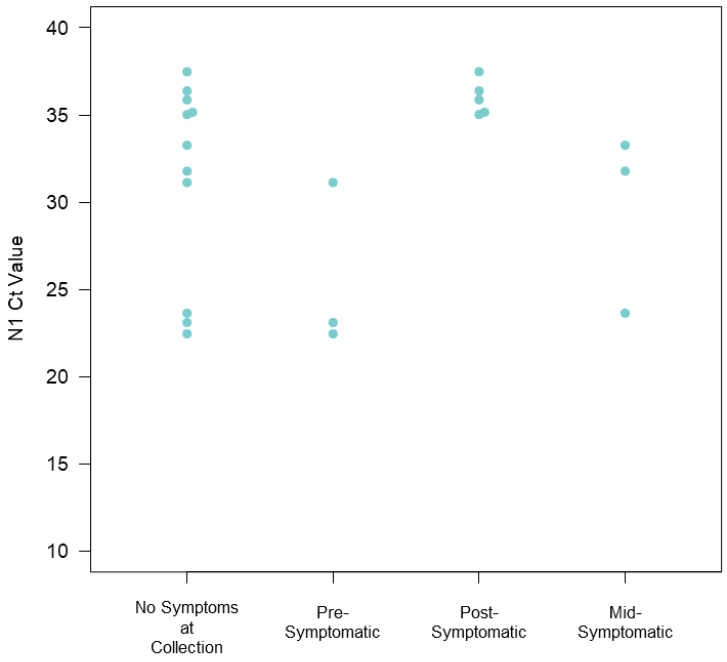

A
Reported Individual Symptom
Did Not Report Individual Symptom
N1 Ct Value
40
35
30
25
20
15
p<0.01
p=0.06
p=0.07
p=0.19
p=0.31
p=0.48
p=0.73
p=0.80
p=0.80
p=0.92
p=0.39
p=0.69
p=0.76
p=0.69
p=0.36
p=0.18
p=0.04
Runny Nose
Headache
Nasal Congestion
Chills
Sore Throat
Cough
Discomfort while Breathing
Loss of Taste
Loss of Smell
Shortness of Breath
Fever
Fatigue
Myalgia
Diarrhea, Nausea, or Vomiting
Chest Pain
Wheezing
Abdominal Pain
Reported Symptoms
B
Met Syndrome Criteria
Did Not Meet Syndrome Criteria
N1 Ct Value
40
35
30
25
20
15
p<0.01
p=0.01
p<0.01
p=0.71
p=0.25
p=0.18
p=0.23
p=0.28
p=0.44
p=0.57
p=0.50
p=0.05
Acute Respiratory Infection
Respiratory Symptoms
Upper Respiratory Symptoms
Lower Respiratory Symptoms
Non-Respiratory Symptoms
Neurological Symptoms
CDC Symptom List
CSTE Case Definition
COVID-Like Illness
Influenza-Like Illness
Symptomatic but Asyndromic
No Symptoms at Collection
Symptoms and Syndromes

Figure 3


N1 Ct Value
40
35
30
25
20
15
10
No Symptoms at Collection
Pre-Symptomatic
Post-Symptomatic
Mid-Symptomatic