SARS-CoV-2 virus dynamics in recently infected people – data from a household transmission study

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Notes

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Abstract

We used daily real-time reverse-transcription polymerase chain reaction (rRT-PCR) results from 67 cases of SARS-CoV-2 infection in a household transmission study, conducted April 2020--May 2021, to examine the trajectory of cycle threshold (Ct) values, an inverse correlate of viral RNA concentration. Ct values varied across RT-PCR platforms and by participant age. Specimens collected from children and adolescents had higher Ct values and adults aged ≥50 years showed lower Ct values than adults aged 18-49 years. Ct values were lower on days when participants reported experiencing symptoms, with the lowest Ct value occurring 2-6 days after symptom onset.

Keywords: SARS-CoV-2, cycle threshold values, age, viral dynamics, RT-PCR

Introduction

Cycle threshold (Ct) values, generated from real-time reverse-transcription polymerase chain reaction (RT-PCR) assays, represent the minimum number of amplification cycles needed to generate a signal for a specific target. Ct values are sometimes used as surrogate signals for SARS-CoV-2 viral loads[1], as they are inversely related to the amount of virus in the tested specimen. Widespread availability of RT-PCR has led to comparisons of Ct values at the patient and community levels to infer associations with illness severity and patient characteristics[2]. However, Ct values can vary by assay, specimen type and quality, and time during the infection course, especially complicating cross-sectional comparisons. Use of serial specimens collected from one individual over the course of infection on the same assay can partially mitigate these concerns, yet few investigations have used serial sampling to describe the natural history of SARS-CoV-2 infection[3-5] or included specimens from the general population with uncomplicated infection[6, 7].

We described SARS-CoV-2 RT-PCR Ct values in newly infected individuals who collected daily specimens as part of a prospective transmission study, and examined the impact of age and symptoms on Ct value trajectories.

Methods

We conducted a household transmission study of SARS-CoV-2 in Tennessee and Wisconsin[8, 9] between April 2020 and May 2021. Non-hospitalized individuals (index participants) who had tested positive for SARS-CoV-2 by a provider-ordered nucleic acid amplification test, and resided with at least one other individual, were recruited into the study and consented and enrolled in the study, along with their household contacts, within 6 days of the index participant's symptom onset. Study procedures included daily swabbing and symptom

diaries (Supplementary Table 1), for 14 consecutive days. All participants completed demographic surveys and self-reported pre-existing conditions (asthma, chronic liver disease, premature birth, cardiac conditions, diabetes, cancer, immunocompromising conditions, extreme obesity, kidney disease, or pregnancy) and, after COVID-19 vaccines became available, vaccination status (verified against health records and immunization information systems).

Anterior nasal swabs were self-/parent-collected by study participants. Swabs were either placed in viral transport media (Remel MicroTest M4RT®, Lenexa, KS USA) and refrigerated by participants for 7-10 days before transport, or placed in inactivating viral transport media (Primestore®, Longhorn Vaccines & Diagnostics LLC, Bethesda, MD) and stored at room temperature by participants for 1-3 days before transport to local laboratories for processing and freezing at -80°C prior to testing. All specimens were tested by RT-PCR using either the CDC 2019-Novel Coronavirus Real-Time RT-PCR Diagnostic Panel (EUA CDC-006-00019; with N1 and N2 gene targets and RNaseP control; CDC assay) or the ThermoFisher TaqPath™ COVID-19 ComboKit (with S and N gene and ORF1ab targets and MS2 spike control; ThermoFisher assay). Only Ct values from tests interpreted as positive (at least two SARS-CoV-2 target Ct values <40) were analyzed. As an additional quality control measure for this analysis, we excluded Ct values from any test where the control (RNaseP or MS2) result was interpreted as negative or where the RNaseP control target had a Ct value >35 (though this exclusion did not change results).

Viral culture was conducted on positive specimens from a subset of participants tested using the CDC assay. Wells were seeded with Vero E6-TMPRSS2 cells, to which 100µl of participant specimen was added. Wells were monitored daily for culture positivity for five days after inoculation. If >20% of cells were detached in wells exhibiting viral cytopathic effect, the

specimen was interpreted as culture positive. Additional detail of culture methods and results are described elsewhere[10].

To examine trajectories of Ct values over the course of infection, we selected data from household contacts who met the following criteria: individuals' first study specimen was negative, they must have tested positive on ≥3 different days, and all specimens must have been tested using the same assay (Supplemental Figure 1). Days of lowest Ct values were defined per target (N1, N2, N, S, or ORF1ab). After exploring multiple model specifications (Supplementary methods), we described Ct values over time using generalized additive models examining the effect of age (representing age categorically, in groups 0-11, 12-17, or ≥50 years compared to 18-49 years), controlling for the target of each assay (which also differed by assay type), with a random effect spline for repeated measurements and a smoothing thin plate spline for time since first positive test. We also explored the effects of symptoms on each day of infection, controlling for age; symptoms were considered binary (symptom present/absent) for both the primary results (on impact of any symptom) and post-hoc analysis of individual symptoms (Supplemental Table 1).

Results

A total of 577 household contacts from 302 households were enrolled in the parent study April 2020-May 2021. Sixty-seven contacts from 50 households met our criteria for "incident cases" (52.2% male; 82.1% non-Hispanic White; 19.4% with at least one underlying condition; 92.5% symptomatic; 26.8% aged 0-11, 16.4% aged 12-17, 40.3% aged 18-49, 16.4% aged ≥50; 10.4% having received one dose of an mRNA COVID-19 vaccine before enrollment; Table 1 and Supplemental Figure 1). Associations between other demographics and Ct values are presented

in Supplemental Table 2. A total of 544 specimens from incident cases were tested, including 1384 Ct values against SARS-CoV-2 targets.

The median observed number of positive days among incident cases was 10 (interquartile range [IQR]: 8, 12 days), although 58% of participants' last specimen collected were still positive for SARS-CoV-2 and participants were tested for a median of 10 days following first positivity. The median observed duration of symptoms was 10 (IQR: 7, 13) days. The median time from symptom onset among incident cases to their first positive test was 0 (IQR: -1, 3) days, with symptom onset preceding first positivity in 48% of symptomatic cases (Supplemental Figure 2). The median time from symptom onset to lowest Ct value was 4 (IQR: 2, 6) days, indicating that symptom onset preceded lowest Ct value. The median time from first testing positive to lowest Ct values was 3 (IQR: 2, 4) days. Among symptomatic incident cases, the median time from symptom onset to lowest Ct value was 4 (IQR: 2, 6) days. Among 93 specimens (from 13 incident cases, all culture positive at least once) that underwent attempted culture, Ct values were lower in culture-positive specimens (median N1 Ct value, 26.9 [IQR: 25.0, 30.0]; median N2 Ct value, 28.3 [IQR: 26.0, 30.7] from 63 specimens) than in culture-negative specimens (median N1 Ct value, 35.6 [IQR: 34.1, 38.5]; median N2 Ct value, 38.0 [IQR: 34.9, 39.0] from 30 specimens; Wilcox test $p < 0.001$ for both targets).

Supplemental Table 3 reports Ct values by target and age, with sample sizes of participants and tests. On average, children aged 0-11 years had Ct values that were 3.5 units higher than adults aged 18-49 years (95% confidence interval [CI]: 2.8, 4.1; $p < 0.001$). Adolescents aged 12-17 years also had higher average Ct values (absolute difference: 2.7; [CI: 1.9, 3.4]; $p < 0.001$) and older adults, aged ≥50 years, had significantly lower Ct values (absolute

difference: -1.7; [CI: -2.4, -1.0]; $p < 0.001$) compared with adults aged 18-49 years (Figure 1). As expected, Ct values differed between assays (higher in the CDC assay).

Reporting symptoms on a given day was associated with lower Ct values, controlling for both target and age (absolute difference: -0.84 [CI: -1.0, -0.7], $p < 0.001$). In post-hoc tests, Ct values were significantly lower on days incident cases reported fatigue, fever, aches, chills, diarrhea, cough, chest tightness/pain, shortness of breath, wheezing, nasal congestion, runny nose, sore throat, or headache (Supplemental Table 1). No significant difference in Ct values were noted on days the incident cases experienced abdominal pain, vomiting, or loss/change of taste/smell.

Discussion

Using data from incident cases from an intensive, prospective household study, this report contributes data on Ct values early in the infection period, which are difficult to capture using other designs. Compared to adults aged 18-49 years, we observed that Ct values were higher among children and adolescents (0-11 and 12-17 years; reflective of lower RNA levels), and lower among older adults (≥50 years) in this largely wild-type-predominant period. These results are consistent with previous findings of variable Ct values by RT-PCR assay and time course of infection[11].

Other studies have reported differences in Ct values across individuals who were persistently asymptomatic[7, 12]. This analysis further contributes that daily symptom status (and not just overall symptom presentation) is associated with daily Ct values, with lower Ct values on days when participants experienced symptoms. While Ct values cannot be used to directly infer infectiousness, changes in Ct value within an individual may represent a signal of

viral proliferation or eventual clearance. We specifically observed that individuals may have higher viral RNA concentrations while symptomatic.

While other studies have reported significant differences in Ct values as a function of age, the direction and interpretation of these results has differed. Cross-sectional and retrospective studies examining Ct values among children have observed lower Ct values in children under the age of 5 compared to adults over age 18[13] and compared to older children between age 5 and 14[14]. In this analysis of specimens collected daily since the first positive test result, Ct values among children and adolescents were higher than values among adults aged 18-49 years. The discrepancies between these findings and prior reports merit further investigation, and may have been driven by differences in the severity of illness, the time during infection, circulating variants, the particular age categories used, or the prospective versus cross-sectional study design.

Our observations of a median of 4 days from first positivity to peak viral RNA concentration are similar to prior reports[3, 7]. In this analysis, we observed that first positivity generally coincided with symptom onset, but that dates of symptom onset preceded dates of lowest Ct values by 2-6 days. One of the earliest reports on SARS-CoV-2 viral dynamics[5] observed that viral RNA concentrations were highest on the day of symptom onset, and fell thereafter (although no samples were collected prior to symptoms). A more recent model-based analysis of incident infections[7] found a median of 0.6 days from peak viral load to symptom onset among mildly symptomatic persons. Some of these differences may have emerged from the substantial heterogeneity we observed between timings of symptom onset and first positivity, which suggests that the natural history of infections can be variable. Our findings from this cohort with a broader range of ages and including only cases where date of first positivity is

known suggest relatively longer periods of rising viral RNA concentration following symptom onset. This supports the importance of following mitigation and infection control measures as symptoms develop and while ill to prevent onwards transmission.

Describing dynamics of Ct values based on frequent, systematic sampling of individuals over time ameliorates multiple concerns with the use of this data; however, these findings must still be interpreted with caution. Our selection of incident cases may have biased the sample towards those exhibiting delayed replication. Sample size and study period are also limitations, especially in our ability to assess the impact of vaccination status or dissociate vaccination or other demographics from age. Our incident cases, who were majority White, non-Hispanic, may not generalize to other populations. Ct values cannot be precisely converted to a quantitative representation of viral load, or used to directly infer differences in infectiousness. However, despite these limitations, clinical interpretation of Ct values (or their trajectories) may be "tempting" (*IDSA and AMP joint statement on the use of SARS-CoV-2 PCR cycle threshold (Ct) values for clinical decision-making,* page 3) [15]. The present data are directly relevant to these interpretations. Specifically, specimens that were collected within 4 days of symptom onset may represent periods when Ct values are still declining.

These findings contribute to our understanding of RT-PCR Ct values during relatively mild, uncomplicated SARS-CoV-2 infections over a broad range of ages, in a community setting, and among individuals with a known date of first shedding. While these data were collected prior to Delta and Omicron circulation, and prior to widespread vaccination, they may provide context for interpreting trajectories in Ct values in similar populations during later SARS-CoV-2 outbreaks.

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Inserts

Table 1. Characteristics of 67 incident cases of SARS-CoV-2 infection participating in a prospective household transmission study – Tennessee and Wisconsin, April 2020-May 2021

	Overall	Age 0-11	Age 12-17	Age 18-49	Age 50+	p value
n	67	18	11	27	11	
Partially vaccinated† (n, %)	7 (10.4)	0 (0.0)	1 (9.1)	4 (14.8)	2 (18.2)	0.337
Male (n, %)	35 (52.2)	9 (50.0)	5 (45.5)	16 (59.3)	5 (45.5)	0.807
Race-ethnicity (n, %)						0.754
Hispanic	10 (14.9)	3 (16.7)	2 (18.2)	3 (11.1)	2 (18.2)	
Non-white, non-Hispanic	2 (3.0)	0 (0.0)	0 (0.0)	2 (7.4)	0 (0.0)	
Non-Hispanic White	55 (82.1)	15 (83.3)	9 (81.8)	22 (81.5)	9 (81.8)	
Any underlying condition (n, %)	13 (19.4)	2 (11.1)	1 (9.1)	6 (22.2)	4 (36.4)	0.296
Number of household members, median [IQR]	6.0 [4.0, 6.0]	6.0 [6.0, 6.0]	6.0 [5.0, 7.0]	4.0 [3.8, 4.0]	2.0 [2.0, 3.0]	0.040
Days from first positive specimen to lowest Ct value, median [IQR]	3.0 [2.0, 4.0]	2.0 [1.0, 3.0]	3.0 [2.0, 3.0]	4.0 [3.0, 5.0]	3.0 [2.0, 3.3]	<0.001
Still positive at end of follow-up, n (%)	39 (58.2)	7 (38.9)	5 (45.5)	19 (70.4)	8 (72.3)	0.110
Duration of positivity in days*, median [IQR]	10.0 [8.0, 12.0]	8.0 [7.3, 9.8]	10.0 [5.5, 11.0]	10.0 [9.0, 12.0]	10.0 [8.0, 13.0]	-

Symptomatic (n, %)	62 (92.5)	16 (88.9)	10 (90.9)	26 (96.3)	10 (90.9)	0.805
Symptom duration in days*‡, median [IQR]	10.0 [7.0, 13.0]	6.5 [4.5, 10.3]	9.0 [4.0, 13.0]	12.0 [9.3, 13.8]	11.5 [10.3, 13.0]	-
Days from symptom onset to first positive specimen‡, median [IQR]	0.0 [-1.0, 3.0]	0.0 [-2.0, 2.0]	0.0 [-1.0, 3.0]	1.0 [-1.0, 3.0]	0.0 [0.0, 4.0]	0.315
Days from symptom onset to lowest Ct‡, median [IQR]	4.0 [2.0, 6.0]	3.0 [0.0, 4.0]	3.0 [0.3, 6.0]	4.0 [3.0, 6.0]	3.5 [3.0, 7.0]	0.008

*Comparison not performed due to censored data.

†Vaccination status defined at the time of study enrollment. Partial vaccination indicates having received one dose of a two-dose mRNA COVID-19 vaccine series. All other study participants had no vaccination documented.

§CDC assay indicates the CDC 2019-Novel Coronavirus Real-Time RT-PCR Diagnostic Panel. Remaining participants were tested with the ThermoFisher TaqPathTM COVID-19 ComboKit.

‡The number of days of symptoms, days from symptom onset to first positive test, and symptom onset to peak Ct are calculated only among symptomatic incident cases. The time from symptom onset to first positive specimen and from symptom onset to lowest Ct value are calculated per target for the CDC assay N1 and N2 targets, and ThermoFisher assay N, S, and ORF1ab targets before taking the median of all time differences.

Figure Legend

Figure 1. Ct value curves over time since each participant first tested positive against each target, within age groups. Dots represent mean observed values within age groups, and vertical bars show bootstrapped 95% confidence intervals. Smooth lines represent predicted values from the Generalized Additive Model of Ct values over time, accounting for age and repeated measurements. Panel A shows results from participants age 0-11 (square) compared to the reference group, age 18-49 (circle); Panels B and C repeat this comparison with age 12-17 (triangle) or 50+ (diamond). Each plot from left to right represents a SARS-CoV-2 target from one of the two included testing platforms (CDC 2019-Novel Coronavirus Real-Time RT-PCR Diagnostic Panel or the ThermoFisher TaqPathTM COVID-19 ComboKit).

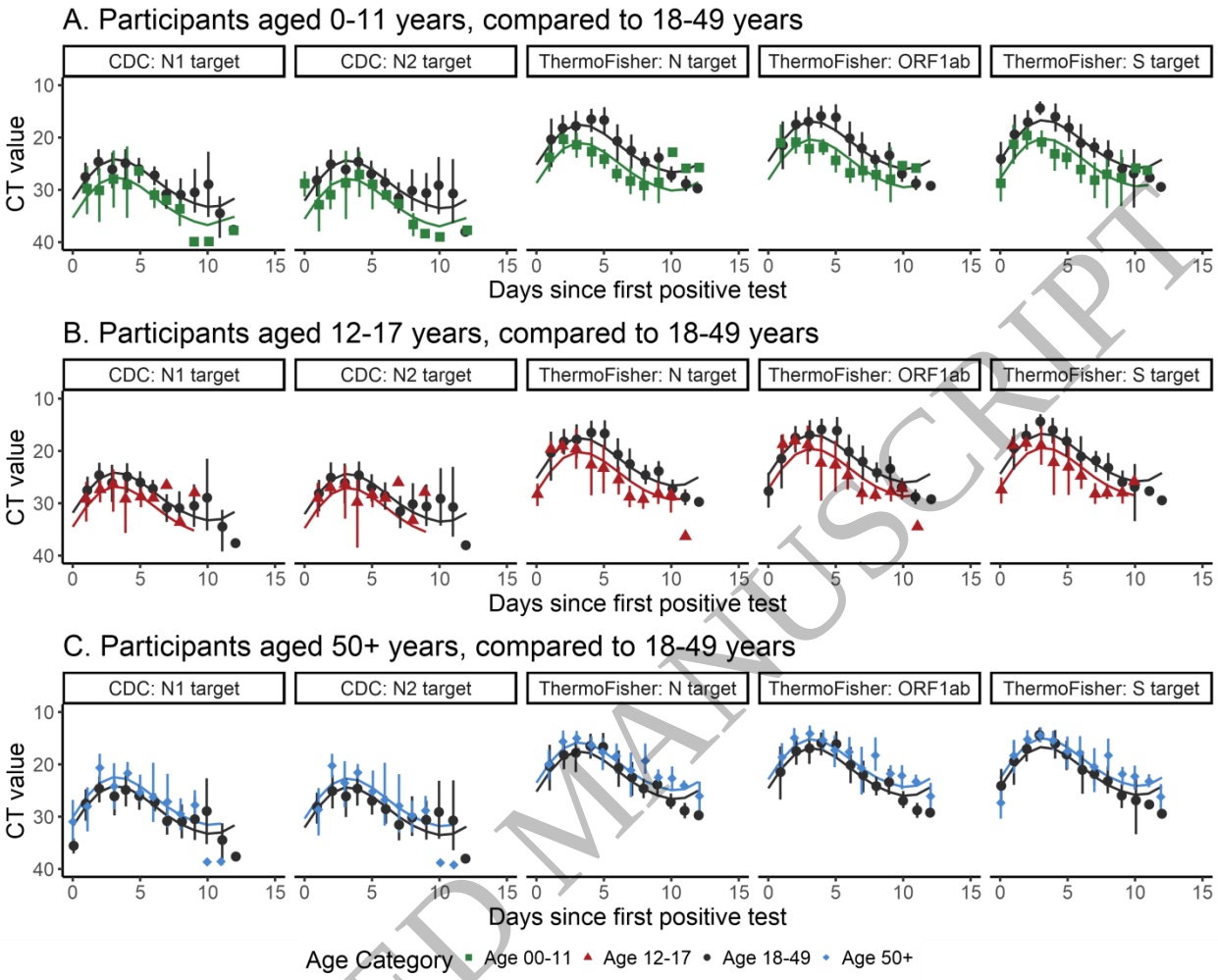

Figure 1
206x165 mm (.86 x DPI)