Temporal, Spatial, and Epidemiologic Relationships of SARS-CoV-2 Gene Cycle Thresholds: A Pragmatic Ambi-directional Observation

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Abstract

Prospective serial sampling of 70 patients revealed clinically relevant cycle thresholds (Ct) occurred 9, 26, and 36 days after symptom onset. Race, gender, or corticosteroids did not appear to influence RNA-positivity. Retrospective analysis of 180 patients revealed that initial Ct did not correlate with requirement for admission or intensive care.

Key words: RNA Positivity, SARS-CoV-2, Corticosteroids, Cycle threshold

Background:

The optimal timing of public health decisions, including when to discontinue isolation precautions (IP) for patients hospitalized with SARS-CoV-2 infection remains unclear. Federal, state, and professional guidelines are not consistently harmonized. In many locations, the availability of testing remains limited, and repeated testing after symptom resolution for patient disposition further stresses scarce resources.

Despite several reports of viral RNA kinetics [1-6], U.S. data, especially those correlating viral kinetics to infection control practice, are scarce. Also unclear is whether dexamethasone, now widely used, prolongs RNA-positivity. Against this backdrop, we sought to evaluate temporal and spatial associations between demographic factors, disease severity, corticosteroid use and SARS-CoV-2 real-time PCR cycle threshold (RT-PCR Ct) to inform the application of IP.

Methods:

From 03/14/2020 to 06/01/2020, nasopharyngeal (NP) swabs from SARS-CoV-2 infected hospitalized patients were prospectively collected weekly from admission. Samples were collected in a standardized protocol using flocked or polyester swabs applied to each middle turbinate and rotated for 5-10 seconds, placed in 3 ml viral transport medium, and immediately analyzed using an emergency-use authorized laboratory-developed-EUA assay or commercial platforms. Each platform targeted the RNA-dependent RNA polymerase, N, ORF1ab, S, and/or PanSARS-E genes. The commercial platforms were: the cobas® 6800 System, (Roche Diagnostics, North America); the BD SARS-CoV-2 Reagents for BD MAX™ (Becton Dickinson and Company, Franklin Lakes, NJ); and the Simplexa® COVID-19 Direct kit (DiaSorin Molecular, Minneapolis, MN). For the laboratory-developed assay, positive and negative controls are included with each batch/run.

Patients were stratified by corticosteroid exposure. Only patients who did not receive any steroids or other immunosuppressive were included in the negative group, and only patients who received steroids but no other immunosuppressive (i.e. IL-6 inhibitors, cancer chemotherapy) were included in the positive group. (5 of the 70 patients in the prospective group received tocilizumab.

These were excluded from both groups.) To be included in the steroid group, patients had to receive at least one full day of therapy, defined as >/=4 mg of methylprednisolone or >/=7.5 mg prednisone or prednisolone per 24 hrs.

Separately, a regional registry of SARS-CoV-2 positive ambulatory and hospitalized patients was retrospectively queried. The registry had been created on 03/21/20 to capture detailed clinical data from every patient with a positive SARS-CoV-2 RT-PCR in a five-hospital healthcare system in upstate NY. It currently contains data from 399 RT-PCR positive patients.

Symptom onset (SO) was determined from the history and defined as how many days the patient felt ill before presenting for care and having a positive test. For the retrospective sample, author JH manually reviewed every record.

ANOVA, Mann-Whitney U, and the 2-sample percent defective tests were performed in R or Minitab.

Results:

Prospective sample

235 NP samples from 70 hospitalized patients were prospectively evaluated. The demographic, co-morbidities, outcomes, and isolation status are presented in Table 1. 80 % (n=188) of all the samples were analyzed on the lab-developed platform. The coefficient of variation (CV) of the Ct values of the positive controls were as follows: 2.81% (SD=0.78) for plasmid (n=41), 2.45 % (SD=0.57) for the RNA (n=31), and 1.75% (SD=0.49) for RNase P (n=112). 10% of the samples were run on the Roche Cobas 6800. Roche did not reply to an inquiry about the precision of that platform for SARS-COV-2, but literature review reveals CVs between 10% and 20% for HIV, HBV, and HCV [S1-3]. The remaining 10 % were analyzed on the DiaSorin Simplexa which reported "an almost perfect agreement" among the Simplexa and the reference samples with standard deviation of the mean Ct of 0.06 – 0.83 and a K = 0.938 SE 0.021; 95% CI = 0.896-0.980) [S4].

Mean time to negative NP swab RT-PCR was 28.5 days from symptom onset (SO) and 24.1 days from initial test. Duration of positivity ranged from 7 to 78 days from SO. Baseline RT-PCR Ct were significantly lower compared to those on week three (Supplemental Figure 1) with 3.3%, 8.9%, 31.3% and 50.0% tested negative at weeks 1, 2, 3, and 4 from SO respectively. The result of linear regression (Ct = 19.08 + (0.58 x days of symptoms)) is graphically presented in Supplemental Figure 2. The Ct increased by 0.58 per day, and Ct trajectories did not differ by race, gender, or comorbidity.

10 of 19 patients who received more than one dose of corticosteroid remained positive beyond 14 days from SO as compared to 12 of 17 patients who did not receive any corticosteroids (p=0.32). As of 06/14/2020, 11 of 70 (16%) patients had IP discontinued during admission (mean time to discontinuation 15.8 days; range 12-40), and none were linked to new transmissions during the subsequent 30-day follow-up period. Twenty two percent of patients reverted to at least one SARS-CoV-2 positive NP RT-PCR after previously testing negative.

Retrospective sample

From the registry, Ct data were available from the first 180 patients. These patients were cared for at one of the five hospitals in the healthcare system between 03-13-2020 and 04-30-2020. Mean, median, SD, and IQR of SO to time of initial test was 5.9, 4.0, 7.1 and 2.0-7.5 days, respectively. Mean Ct of the initial diagnostic RT-PCR did not differ between patients that were released after evaluation in the emergency department (23.0; IQR: 17.93-27.32) and those who were admitted (22.6; IQR 18.5-28.39) (p = 0.99). Similarly, mean initial Ct between ward (23.1; IQR 19.20-28.30) and intensive care unit patients (20.2; IQR 18.25-28.34) were not significantly different (p =0.42).

Discussion:

Regarding the prospectively studied cohort, exposure to corticosteroids did not appear to prolong RNA positivity; however, our sample size might be too small to detect a difference. Nonetheless, to our knowledge it is the first study of a diverse cohort U.S. patients to include steroid exposure in analysis of Ct kinetics for IP application [7]. It was previously reported that the RT-

PCR Ct breakpoint above which virus was uncultivable varies from > 24 to ≥ 34, and perhaps higher for severely immunocompromised patients [1, 2, 8]. In our study, linear regression derived from a diverse cohort of 70 patients with various disease severities revealed that a Ct of 24, 34, and negative (>40) occurred 9, 26, and 36 days after SO (Supplemental Figure 2). This timing is consistent with those from a cohort of nine patients with relatively mild disease that included viral culture [3], study that included cultures, but did not provide number of patients [2] and, a smaller study evaluating viral kinetics in saliva [4]. Together, these data offer reassurance that discontinuation of enhanced isolation/ PPE after day 10 is probably safe for immunocompetent patients who are improving and afebrile. Waiting for a completely negative Ct is probably unnecessary and contributes to avoidable consumption of personal protective equipment.

For the retrospectively analyzed cohort, initial Ct could not be used to differentiate those needing admission from those released after emergency department evaluation. Nor could initial Ct values be used to distinguish between those patients needing intensive care versus ward level care.

Our study has several limitations. First, the cultivation of the virus was not available due to safety concerns. Second, prospective data come from a single center. Third, a single assay could not be used for the entire duration of the observation period due to the initial limitation of test systems or reagents. As mentioned, most of the tests were performed on the laboratory-developed-EUA assay, and the variation was negligible as indicated by the CVs. The volume of sample required per assay, along with the ongoing shortage of kits and reagents, precludes re-testing samples on all platforms simultaneously. Nonetheless, it has recently been shown that commercially available and laboratory-developed platforms are quite comparable [9-11, S1-4].

Despite these limitations, our findings are notable for several reasons. We provide actionable RT-PCR Ct values, other than negative, that can complement infection control decisions based on illness duration and symptom resolution. Furthermore, reports from non-university-based locations are underrepresented in research, and studies are carried out in large academic institutions. However, the majority of health care in the U.S. is provided at non-university-based facilities. Our data help

close this gap. 20% of the prospectively evaluated patients reverted to RT-PCR positivity after symptom improvement and defervescence for > 72 hours. Table 1.In contrast, a previously reported study that found only 3% reverted to positivity [12]. Finally, although race and gender are known to affect SARS-CoV-2-related morbidity and mortality, they did not appear to influence RNA-positivity and therefore provide reassurance that infection control measures do not require adjustment for race or gender.

In conclusion, regression analysis of 235 patient samples from 70 patients was consistent with and supported by culture-derived data from other, albeit smaller or more homogenous studies [1-3]. RT-PCR Ct obtained at day nine can inform IP decisions. 43% of patients re-tested 4 weeks after SO had detectable RNA. Retrospective analysis of a 180-patient registry revealed that duration of RNA-positivity did not appear to be affected by race or gender. Initial Ct did not correlate with requirement for admission or intensive care. These data provide a foundation for hypothesis generation and more in-depth and well controlled follow up studies.

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Table 1:

Demographics and Clinical Characteristics of patients	
Variables	**All Patients (N=70)**
Age	Range=25-94 Mean=63 Median=65
Gender, n (%)	
Male	44 (63%)
Female	26 (37%)
Race, n (%)	
Black/AA	25 (36%)
Non-Hispanic, White	25 (36%)
Hispanic	16 (23%)
Other	4 (5%)
Comorbidities, n (%)	
Diabetes	34 (49%)
HTN/Heart disease	46 (66%)
Obesity	24 (34%)
Immunocomp/suppressed	6 (9%)
CKD/ESRD	11 (16%)
No major comorbidities	11 (16%)
Inpatient Unit, n (%)	
ICU	24 (34%)
Ward	45 (64%)
Unknown unit	1 (2%)
Isolation status	
Removed while inpatient	8 (11%)
Removed at discharge/death	52 (74%)
Remains on isolation	8 (13%)
Unknown	2 (2%)
Status at end of study, n (%)	
Remains inpatient	15 (23%)
Discharged	45 (67%)
Deceased	8 (12%)
Unknown	2 (3%)
Positive-Negative-Positive Result trend, n (%)	
Yes	14 (20%)
No	56 (82%)

Table 1 continued Positive/Negative by week			
Total Patients Tested: 70 (Day 0)	**Number of patients tested that week**	**Number positive (+)**	**Number negative (-)**
Week 1 (Days 6-8)	30	29	1
Week 2 (Days 13-15)	45	41	4
Week 3 (Days 20-27)	32	22	10
Week 4 (>28 days)	14	6	7