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MAJOR ARTICLE

Predicting Infectious Severe Acute Respiratory Syndrome Coronavirus 2 From Diagnostic Samples

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(See the Editorial Commentary by Binnicker on pages 2667–8.)

Background. Reverse-transcription polymerase chain reaction (RT-PCR) has become the primary method to diagnose viral diseases, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). RT-PCR detects RNA, not infectious virus; thus, its ability to determine duration of infectivity of patients is limited. Infectivity is a critical determinant in informing public health guidelines/interventions. Our goal was to determine the relationship between E gene SARS-CoV-2 RT-PCR cycle threshold (Ct) values from respiratory samples, symptom onset to test (STT), and infectivity in cell culture.

Methods. In this retrospective cross-sectional study, we took SARS-CoV-2 RT-PCR–confirmed positive samples and determined their ability to infect Vero cell lines.

Results. Ninety RT-PCR SARS-CoV-2–positive samples were incubated on Vero cells. Twenty-six samples (28.9%) demonstrated viral growth. Median tissue culture infectious dose/mL was 1780 (interquartile range, 282–8511). There was no growth in samples with a Ct > 24 or STT > 8 days. Multivariate logistic regression using positive viral culture as a binary predictor variable, STT, and Ct demonstrated an odds ratio (OR) for positive viral culture of 0.64 (95% confidence interval [CI], .49–.84; < .001) for every 1-unit increase in Ct. Area under the receiver operating characteristic curve for Ct vs positive culture was OR, 0.91 (95% CI, .85–.97; < .001), with 97% specificity obtained at a Ct of > 24.

Conclusions. SARS-CoV-2 Vero cell infectivity was only observed for RT-PCR Ct < 24 and STT < 8 days. Infectivity of patients with Ct > 24 and duration of symptoms > 8 days may be low. This information can inform public health policy and guide clinical, infection control, and occupational health decisions. Further studies of larger size are needed.

Keywords. SARS-CoV-2; COVID-19; RT-PCR; infectivity; public health.

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), represents a public health emergency of historic proportion. The global containment efforts have had broad societal and economic impacts. Policy decisions to relax public health measures will require a better understanding of duration of infectivity. This information will also impact infection control practices and occupational health.

To date, the diagnosis of COVID-19 has relied on the detection of SARS-CoV-2 through molecular detection. While this method is both rapid and highly sensitive, there are important limitations. Several studies describe the persistence of SARS-CoV-2 RNA within different body sites [1, 2]. It is known from other viruses that viral RNA can persist beyond infectivity [3, 4]. As a result, demonstration of in vitro infectiousness on cell lines is a more informative surrogate of viral transmission. The ability of viral culture to inform infectivity is an important aspect of diagnostics, but its use is hampered by its difficult and labor-intensive nature. This is further complicated by the need for Biosafety Level 3 facilities in the case of SARS-CoV-2. In a recent cohort study of 9 patients, no virus could be recovered beyond 7 days after symptom onset [1]. This important study is limited by the small number of patients examined and the fact that all 9 cases are linked; therefore, the data may represent a unique viral subpopulation. Here we add to the existing body of literature by presenting viral culture results on a larger cross-sectional group of patients, compared to polymerase chain reaction (PCR) data and time of symptom onset.

MATERIALS AND METHODS

SARS-CoV-2 Reverse-Transcription PCR Cycle Threshold Values and Symptom Onset to Test

All samples in this study were obtained to support routine care and surveillance of the public health response in the province of Manitoba, Canada. All suspected COVID-19 cases had

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SARS-CoV-2 reverse-transcription PCR (RT-PCR) performed on nasopharyngeal (NP) or endotracheal (ETT) samples at Cadham Provincial Laboratory (CPL), the public health laboratory.

The NP swabs and ETT specimens in viral transport media were stored at 4°C for 24–72 hours until they were tested for the presence of SARS-CoV-2 RNA using real-time RT-PCR targeting a 122-nt portion of the Sarbecovirus envelope gene (E gene) [5]. Fifty-five microliters of RNA was extracted from 200 µL of a respiratory specimen using the Ambion AM1836 RNA kit (Thermo Fisher) paired with the Kingfisher Flex instrument (Thermo Fisher). The 20 µL reactions, comprised of TaqMan Fast Virus One-step master mix and 5 µL of RNA, were cycled for 5 minutes at 50°C, 20 seconds at 95°C followed by 40 cycles of 5 seconds at 95°C and 30 seconds at 58°C on a Bio-Rad CFX96 thermal cycler. RT-PCR results were analyzed with the CFX manager software (version 3.1).

Through public health and epidemiology/surveillance and laboratory records, date of symptom onset was determined. Time from symptom onset to RT-PCR, or symptoms to test (STT), was calculated based on laboratory records. For all positive samples, the cycle threshold (Ct) was obtained. The study was performed in accordance with protocol HS23906 (H2020:211), approved by the University of Manitoba Research Ethics Board.

Median Tissue Culture Infectious Dose Assay

Samples were stored at −80°C for between 2 and 4 weeks before being processed for culture. Viral titers of patient samples were determined through median tissue culture infectious dose ($TCID_{50}$) assays inside a Biosafety Level 4 laboratory. In brief, Vero cells (ATCC: CCL-81), maintained in modified Eagle's medium (MEM) supplemented with 5% fetal bovine serum (FBS), 1% penicillin/streptomycin, 0.5 µg/mL amphotericin B, and 1% L-glutamine, were seeded into 96-well plates (Thermo Scientific, 167008) at 70% confluency. Using dilution blocks, patient samples were serially diluted 10-fold from 10^{-1} to 10^{-8} in MEM supplemented with 2% FBS, 1% penicillin/streptomycin, 0.5 µg/mL amphotericin B, and 1% L-glutamine. Dilutions were placed onto the Vero cells in triplicate and incubated at 37°C with 5% carbon dioxide for 96 hours. Following incubation of 4 days, cytopathic effect was evaluated under a microscope and recorded. $TCID_{50}$ and $TCID_{50}$/mL were calculated using the Reed and Muench method previously described [6]

Statistical Methods

Data are presented as mean ± standard deviation for normally distributed data and as median with interquartile range (IQR) for nonnormally distributed data. values are reported as 2-tailed. All statistical analysis was performed with Stata version 14.2 (StataCorp, College Station, Texas). Between-group comparisons were performed using a Student *t* test or Mann-Whitney test. Normality was assessed using the Kolmogorov-Smirnov test, and logistic regression was performed with robust standard errors.

RESULTS

A total of 90 samples were analyzed. Median age of the patients sampled was 45 (IQR, 30–59) years. Almost half (49%) of our samples were from males. SARS-CoV-2 was successfully cultivated from 26 (28.9%) of the samples. The samples included in this study included those positive for SARS-CoV-2 by RT-PCR from day of symptom onset (day 0) up to 21 days after symptom onset. Within this range of samples, positive cultures were only observed up to day 8 after symptom onset (Figure 1). Median Ct count of all samples was 23 (IQR, 17–32). The median $TCID_{50}$/mL was 1780 (IQR, 282–8511). Positive culture samples had a significantly lower Ct compared with culture-negative samples (17 [IQR, 16–18] vs 27 [IQR, 22–33]; < .001; Figure 2). Symptom to test time was also significantly lower in culture-positive vs culture-negative samples (3 [IQR, 2–4] days vs 7 [IQR, 4–11] days; < .001; Figure 2).

Multivariate logistic regression using positive culture as a predictor variable (binary result) and STT, age, and sex as independent variables showed Ct as being significant (odds ratio [OR], 0.64 [95% confidence interval {CI}, .49–.84]; < .001). This implies that for every 1-unit increase in Ct, the odds of a positive culture decreased by 32%. Increasing symptom to test time was also significantly associated with a negative culture (OR, 0.63 [95% CI, .42–.94]; = .025). For every 1-day increase in STT, the odds ratio of being culture positive was decreased by 37%. Receiver operating characteristic curves constructed using Ct vs positive culture showed an area of 0.91 (95% CI, .85–.97; < .001) with 97% specificity obtained at a Ct of > 24. Similarly, STT vs positive culture showed an area of 0.81 (95% CI .73–.90; < .001), with 96% specificity at > 8 days. The probability of successfully cultivating SARS-CoV-2 on Vero cell culture compared to STT is demonstrated in Figure 3. The probability of

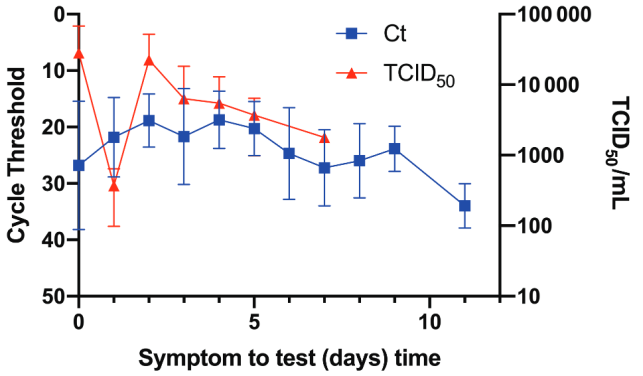

Figure 1. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral dynamics as expressed by E gene reverse-transcription polymerase chain reaction cycle threshold (Ct) value and cell culture median tissue culture infectious dose ($TCID_{50}$)/mL, over time (days).

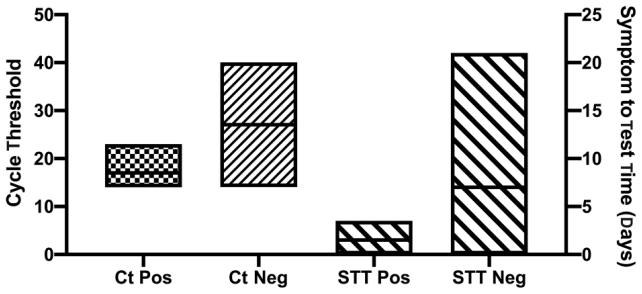

Figure 2. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) E gene reverse-transcription polymerase chain reaction cycle threshold (Ct) values and symptom to test time (STT) in samples that were culture positive or negative. Positive SARS-CoV-2 culture samples had a significantly lower Ct compared with culture-negative samples (17 [interquartile range {IQR}, 16–18] vs 27 [IQR, 22–33]; $P < .001$). STT was also significantly lower in culture-positive vs culture-negative samples (3 [IQR, 2–4] days vs 7 [IQR, 4–11] days; $P < .001$).

obtaining a positive viral culture peaked on day 3 and decreased from that point.

DISCUSSION

PCR and other nucleic amplification (NA) strategies have surpassed viral culture as the gold standard viral diagnostic, because of their wider application, higher sensitivity, rapid performance, and ability for field deployment. A major drawback to PCR and other diagnostic approaches (including other NA, serology, and antigen detection) is that they all fail to determine virus infectivity; PCR sensitivity is excellent but specificity for detecting replicative virus is poor [7]. Our study utilized a cross-sectional approach to correlate COVID-19 symptom onset to specimen collection with SARS-CoV-2 E gene RT-PCR and virus viability as determined by cell culture.

These results demonstrate that infectivity (as defined by growth in cell culture) is significantly reduced when RT-PCR Ct values are > 24. For every 1-unit increase in Ct, the odds ratio for infectivity decreased by 32%. The high specificity of Ct and STT suggests that Ct values > 24, along with duration of symptoms > 8 days, may be used in combination to determine duration of infectivity in patients. Positive cell culture results in our study were most likely between days 1 and 5. This finding is consistent with existing literature [1, 2].

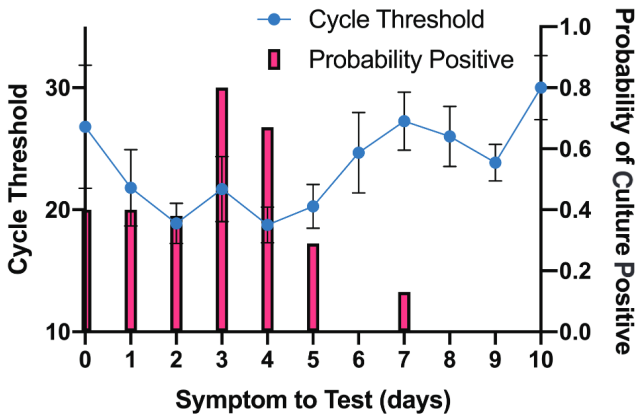

Figure 3. Comparison of symptom onset to test (days) to the probability of successful cultivation on Vero cells (Probability Positive) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) E gene reverse-transcription polymerase chain reaction cycle threshold (Ct) value.

This study is the first to report a large enough data set that demonstrates a link between in vitro viral growth, Ct value, and STT. These results have implications for clinical care, infection prevention and control, and public health. These data can be used to efficiently target case finding efforts by better defining the period of maximal transmission risk. This will be of particular importance in the maintenance phase of the response, where case finding efforts to rapidly interrupt chains of transmission will be essential. Isolation of COVID-19 cases in the community is typically recommended for at least 10 days after symptom onset. Our data supports this approach. Jurisdictions across Canada and the United States are recommending a variety of strategies to discontinue isolation of hospitalized COVID-19 cases [8–13]. Clinical criteria including 14 days from symptom onset or 72 hours symptom free (whichever is longer) are being used in some, while other jurisdictions are using 2 negative NP RT-PCR results 48 hours apart after 14 days of symptoms. Our data support the former approach since RT-PCR positivity persists significantly beyond infectivity; the alternative approach may lead to unnecessary isolation, and use of personal protective equipment and testing resources. The qualitative reporting of results of SARS-CoV-2 RT-PCR as positive or negative is sufficient for diagnosis but may be supplemented by Ct, a semiquantitative value, as well as time of symptom onset to guide infection control, public health, and occupational health decisions.

Our study has important limitations. First, our study utilized a single SARS-CoV-2 gene target (E gene). Though other gene targets may offer greater specificity, the SARS-CoV-2 E gene is more consistently used in both laboratory-developed tests and commercial assays. The testing criteria in Manitoba had sufficient pretest probability to make the likelihood of a false-positive remote. In addition, the first 71 of 90 samples were confirmed using the described protocol with the Centers for Disease Control and Prevention N1 gene target [14]. Second target confirmation was discontinued at that time based on being satisfied with testing criteria and assay sensitivity to accurately identify true COVID-19 cases. Reagent supply also played a role. Second, the recall bias of symptom onset is possible, but this likely would have been equally distributed between those who were culture positive and negative. Third, the infectivity of certain individual cases and the accuracy of our culture assay may have unique variations. Though some individuals in our cross-sectional study would be considered immunocompromised, patients with these conditions could have prolonged shedding of infective SARS-CoV-2 and may not be

fully represented here. Few children have been diagnosed with COVID-19 in our province (median age of positive PCR, 45 [IQR, 30–59] years). With other respiratory viruses, children may have prolonged shedding. Finally, our patient numbers remain small and larger studies are needed to establish Ct criteria that reliably correlate with loss of infectivity and that utilize additional SARS-CoV-2 gene targets.

In conclusion, the SARS-CoV-2/COVID-19 pandemic represents a dynamic situation where decisions and policy must be guided by evidence. Our study showed no positive viral cultures with a Ct > 24 or STT > 8 days. The odds of a positive culture were decreased by 32% for each unit increase in Ct. These data, if confirmed, may help guide isolation, contact tracing, and testing guidelines.

Notes

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Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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