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Case Report

Cycle threshold values versus reverse transcription-polymerase chain reaction positivity in COVID-19 de-isolation

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ABSTRACT

In the modern COVID-19 pandemic, reverse transcription-polymerase chain reaction (RT-PCR) positivity has a major role in the diagnosis of the disease. However, in deciding the patient's discharge or de-isolation, its role is still debatable. We are, hereby, describing three cases (an intern, a nursing officer and a caretaker of another patient) where only RT-PCR could not help much since it was persistently positive for >20 days of the illness course. Instead, the cycle threshold (Ct) values could have better correlated with the infectivity of COVID. We propose a rising trend (24 h apart) and absolute Ct value > 25, instead of RT-PCR negativity (which was taken as Ct value > 36 in our laboratory), to be used in deciding the infective potential of the patients, their discharge from the hospital and de-isolation of the patients. This will help in the timely discharge of patients from health-care institutions and home isolation, which, as a result, will lead to optimal utilisation of the limited hospital resources we have available in the line of the ongoing pandemic. Future studies are required to define the exact cut-off of Ct value for de-isolation purposes.

1. Introduction

COVID-19 is the ongoing pandemic that has the world in its grip. Initial containment and mitigation strategies were primarily oriented towards early detection and prolonged isolation in hospitals or homes, but as the number of cases progresses day by day, our hospital resources are being overburdened with patients leading to lack of availability of logistics. Furthermore, prolonged home isolation is also de-accelerating our economy and reducing the availability of our health-care workers (HCWs). Prolonged admissions mean lesser availability of beds for new patients; hence, our approach has to be modified in such a way that allows the patients to be discharged or shifted from COVID-19 hospitals as soon as possible after ensuring that the patient is not infective. As per various guidelines including the CDC, World Health Organization (WHO) and other nation-specific ones, this infectivity conversion is decided by reverse transcription-polymerase chain reaction (RT-PCR) positivity [1].

RT-PCR converts RNA into DNA (complementary DNA or cDNA) and amplifies specific DNA targets using PCR [2]. In this process, the cycle threshold (Ct) is the number of cycles required for the fluorescent signal to cross the threshold (exceed the background level). Ct values are inversely proportional to the amount of target nucleic acid in the sample (lower Ct value means a higher amount of target nucleic acid in the sample). Ct < 40 is considered positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [3]. Most centres use RT-PCR positivity to determine when the patient has lost infectivity and when to discharge the patients. However, RT-PCR may remain positive even for a month, and merely this detection of the virus does not correlate with infectivity or viability of virus [4]. Ct values better correlate with infectivity of COVID.

Hereby, we report three case scenarios where Ct value could have been used rather than RT-PCR positivity in deciding their discharges from the hospital, resulting in more workforce availability and better economic viability.

2. Case report

In a tertiary care hospital, designated with levels of care for all categories of COVID-19 patients, we hereby share relevant details of three cases (an intern, a nursing officer and a caretaker of another patient) (Table 1). 'SD Biosensor standard M nCoV real-time detection kit' was used for PCR, with the primer probe provided by the same company. This is according to the WHO interim guidance for laboratory testing of 2019-

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Table 1
Details of three COVID-19 positive cases.

Cases	Age/sex	Complaints	Disease severity (ICMR)	Date of COVID hospital admission	RT-PCR positive with cycle threshold values (nasopharyngeal samples with days of hospitalisation)
1	28/male	Fever, sore throat, myalgia (unknown source), resolved after 3 days	Uncomplicated illness	25 April 2020	D1-18.26 D6-24.25 D10–26.32 D14–25.85 D18–34.77 D22–31.32 D26–34.45
2	23/ female	Running nose (low-risk contact), resolved after 2 days	Uncomplicated illness	1 May 2020	D1-14.47 D6-29.19 D10–30.31 D14–34.98 D18–34.73 D22–36.61
3	38/ female	Asymptomatic (close and high-risk contact)	Uncomplicated illness	26 April 2020	D1-20.69 D8-30.55 D12–32.91 D18–34.28 D23-Negative

RT-PCR: Reverse transcription-polymerase chain reaction.

nCoV. It is based on TaqMan probe real-time fluorescent PCR technology. We used Biorad CFX 96 real-time thermocycler, and for RNA extraction, Thermo flex 96 extractor machine was used. The target genes were 'E-gene and RdRp' and housekeeping genes Rnase P. We used a Ct cut-off of 36 for both E gene and RdRp.

As seen, all the patients had a very mild disease or were asymptomatic, and also their blood laboratory workup was within the normal range, except for a minor rise in D-dimer in case 2, which resolved over time. All had an uncomplicated illness. As is easily observable, despite RT-PCR positivity, Ct values constantly show rising trends (decreasing viral nucleic acid). However, patients still remained hospitalised for 25–30 days since the RT-PCRs did not come out negative. A summary of the discharge criteria used by various organisations is presented (Table 2). Most institutions are still going for negative RT-PCR testing only to document the loss of infectivity; hence, our discharge criteria need urgent modifications to avoid major confusion.

3. Discussion

The SARS-CoV-2 virus is a highly infective one leading to mass fear, with many HCWs themselves getting infected resulting in a future possibility of mass exposure and complete hospital shutdowns.

It is already proved that RT-PCR detection does not correlate with infectivity, and accordingly, statements have been released by various countries. Then, what is the gold standard to decide the infective potential of SARS-CoV-2 so that necessary guidelines could be generated? Viral culture is the definitive answer. A small but important study from Germany found that no viral isolates could be obtained from nasopharyngeal samples after day 8 despite high viral loads [8]. This was confirmed by viral replicative RNA intermediates or subgenomic (sg) mRNAs, which are only present in actively infected cells and are not packaged into virions. Sputum sgRNA values declined over days 10–11, whereas throat swab sgRNA levels were undetectable after 5 days, implying loss of infectivity. This is in contrast to the persistent viral PCR positivity from these specimens. Viral culture and sgRNA determination are not possible in each hospital and not economically viable for general public use. Another study from France showed a significant relationship between Ct value and viral culture positivity rates. Samples with Ct value of 13–17 had positive culture growths. Culture positivity rates declined progressively with the rise in Ct values to reach 12% at 33 Ct value and no culture being obtained from samples with Ct > 34 [9]. This suggests higher Ct values could be used as a marker for loss of infectivity and de-isolation strategy. If we see our cases, Ct value > 25 correlates with >10 days of symptom onset, hence instead of two negative RT-PCR reports, 24 h apart, the test-based strategy should focus on the rising trends of Ct values and decide de-isolation/discharge strategy.

Our three cases had hospital stays averaging 1 month. The beds occupied by these patients are isolation beds, with strictest precautions and a single set of HCWs being dedicated to them because of patient care. Apart from HCWs, all these isolation practices require gigantic amounts of personal protective equipment, disinfectants, sanitizers and other hospital logistics. The mental impact on the patients themselves is immense, as they are being forced to stay in isolation rooms with no outside contact for months altogether. This is a stressful task that too with the additional stress of being COVID positive. The HCWs are under the mental stress of catching the disease when they work in this environment, even though the patient may be completely non-infective. All this hampers efficient patient care for non-COVID patients as well. Those who are in home isolation have compromised productivity, hence the economy is severely affected as a whole. As of now, everything is revolving around RT-PCR positivity, and wastage of many resources could be avoided by utilisation of Ct values along with RT-PCR reporting. All cases could have been discharged considering symptom-based de-isolation strategy after 10 days of symptom onset or diagnosis and 3 days of the asymptomatic period. This would have decreased the hospital burden, and also the hospital would have regained its two HCWs, including case 3 who had to take care of her admitted mother in the general ward [1]. All this was halted because a test-based strategy (at least two negative RT-PCR reports, 24 h apart) is also there in the guidelines for de-isolation.

In summary, Ct value is a semi-quantitative measure of viral loads and is undeniably better than mere RT-PCR positivity. We propose a rising trend (24 h apart) and absolute Ct value > 25, instead of RT-PCR negativity (Ct value > 36 in our laboratory), be used in deciding the infective potential and de-isolation of patients. This will help in the timely discharge of patients from health-care institutions and home isolation and as a result, the optimal utilisation of the limited hospital resources we have available in the line of the ongoing COVID-19 pandemic. Future studies are required to define the exact cut-off Ct values for de-isolation purposes.

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Table 2
Summary of de-isolation (or discharge) criteria used by various organisations.

Agency	Hospitalised, symptomatic	Home isolated, asymptomatic
India (ministry of health and family welfare) [5]	Severe: Discharge after clinical recovery and one negative RT-PCR Moderate: Discharge after the resolution of symptoms and no oxygen requirement without RT-PCR testing* Patient to be home isolated for 7 days*	Discharge after 10 days of symptom onset and no fever for 3 days* Patient to be home isolated for 7 days*
Italy (ministero della salute, consiglio superiore di sanità) [6]	COVID-19 patient can be considered cured after symptom resolution and two negative tests for SARS-CoV-2 at 24-h intervals. For patients with clinical recovery earlier than 7 days after onset, an interval of 7 days between the first and the final test is advised NB: Virus clearance is defined as viral RNA disappearance from bodily fluids of symptomatic and asymptomatic persons, accompanied by the appearance of specific IgG	Negative SARS-CoV-2 RNA test 14 days after the first test (end of the quarantine period)
CDC China [6]	Patients to be discharged to meet the following criteria Afebrile for >3 days Improved respiratory symptoms Pulmonary imaging showing obvious resolution of inflammation and nucleic acid tests negative for respiratory tract pathogen twice consecutively (sampling interval of ≥24 h) After discharge, patients are recommended to continue 14 days of isolation and health monitoring, wear a mask, live in a single room with good ventilation, reduce close contact with family members, eat separately, keep hands clean and avoid outdoor activities	
CDC USA [1]	For discontinuation of transmission-based precautions (not a prerequisite for discharge) Symptom-based strategy: At least 3 days (72 h) have passed since recovery-defined as resolution of fever without the use of fever-reducing medications and improvement in respiratory symptoms (e.g., cough and shortness of breath), and at least 10 days have passed since symptoms first appeared Test-based strategy: Resolution of fever without the use of fever-reducing medications and improvement in respiratory symptoms	Time-based strategy: 10 days have passed since the date of their first positive COVID-19 diagnostic test, assuming they have not subsequently developed symptoms since their positive test. Note, because symptoms cannot be used to gauge where these individuals are in the course of their illness, it is possible that the duration of viral shedding could be longer or shorter than 10 days after their first positive test Test-based strategy: Negative results of an FDA emergency use authorised COVID-19 molecular assay for detection of SARS-CoV-2 RNA from at least two consecutive respiratory specimens collected ≥24 h apart (total of two negative specimens). Note, because of the absence of symptoms, it is not possible to gauge where these individuals are in the course of their illness. There have been reports of prolonged detection of RNA without a direct correlation to viral culture Note that detecting viral RNA through PCR does not necessarily mean that the infectious virus is present. Consider consulting with local infectious disease experts when making decisions about discontinuing transmission-based precautions for patients who might remain infectious longer than 10 days (e.g., severely immunocompromised)
NCID Singapore [7]	Discharge patient with follow-up if indicated and daily wellness calls until day 14 after last possible exposure if the following conditions are met Afebrile ≥24 h two respiratory samples tested negative for SARS-CoV-2 by PCR in ≥24 h Day of illness from onset ≥6 days OR Alternative aetiology found (e.g., influenza and bacteraemia) OR Not a close contact of a COVID-19 case It does not require inpatient care for other reasons	

RT-PCR: Reverse transcription-polymerase chain reaction, NCID: National centre for infectious diseases, FDA: Food and Drug Administration, SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

Declaration of competing interest

There are no conflicts of interest.

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