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Can quantitative RT-PCR for SARS-coV-2 help in better management of patients and control of coronavirus disease 2019 pandemic

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ABSTRACT

The emergence of SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19), represents a public health emergency of unprecedented proportion. The global containment efforts have been focused on testing, tracing of contacts and treatment (isolation) of those found COVID-19 positive. Since the whole genome sequences of a number of strains of this novel RNA virus were available in the public domain by early January, a number of real-time polymerase chain reaction (RT-PCR) protocols were designed and used for diagnosis of this infection. Most RT-PCRs are designed for qualitative COVID-19 reporting (of SARS-CoV-2 detected or not detected), but have been used for semi-quantitative estimation of viral load based on cycle threshold value. Our manuscript discusses the utility of quantitative PCR testing for COVID-19 and its patient management benefits.

Introduction

The emergence of SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19), represents a public health emergency of unprecedented proportion. The global containment efforts have been focused on testing, tracing of contacts and treatment (isolation) of those found COVID-19 positive. Since the whole genome sequences of a number of strains of this novel RNA virus were available in the public domain by early January, a number of real-time polymerase chain reaction (RT-PCR) protocols were designed and used for diagnosis of this infection. The term RT-PCR is also used synonymously for reverse transcriptase PCR (rt-PCR) for RNA viruses which require additional step of converting RNA to complementary DNA to DNA as in case of SARS-CoV-2.

Consequent on the explosive nature of spread of infection in different parts of the world, different RT-PCR protocols were made available for clinical use without first ascertaining the sensitivity of detection. In order to make diagnostic kits easily available, the USA Food and Drug Administration permitted emergency use authorization of both RT-PCR and serology kits and regulatory authorities worldwide followed suit making unvalidated and not fully characterized kits available for clinical use.

RT-PCR emerged as the gold standard for the diagnosis of COVID-19 infection. Reports on viral dynamics indicated that viral shedding peaked on or before symptom onset and a substantial proportion of transmission probably occurred before first symptoms in the index case. After symptoms onset, viral loads decreased monotonically. Virus was detected for a medium of 20 days after symptom onset, but infectiousness declined significantly 8 days after symptom onset [1].

Since accurate testing for SARS-CoV-2 followed by appropriate preventive measures are paramount in health-care setting to prevent both nosocomial and community transmission, it is essential to characterize the sensitivity and specificity as well as predictive value of the results knowing well that the ‘window period’ after acquisition could produce false-negative results. Since RT-PCR tests are being used not only to diagnose infection but also to ‘rule out’ infection to conserve scarce personal protective equipment and preserve the workforce, it is essential to understand how the predictive value of the test varied with time from exposure and symptom onset to avoid being falsely reassured by negative results from tests done early in the course of infection.

After exposure to an infected patient, over the 4 days of infection before the typical time of symptom onset (day 5), the probability of a false-negative result in an infected person decreased from 100% on day
1%–67% on day 4. On the day of symptom onset, the median false-negative rate was 38%, this decreased to 20% on day 8 (3 days after symptom onset) then began to increase again from 21% on day 9–66% on day 21. Serial testing in symptomatic patients would almost certainly result in false-negative rates [2].

In a recent study by Wölfel et al. in 2020, seroconversion was detected by IgG and IgM immunofluorescence using cells that expressed the spike protein of SARS-CoV-2 and a virus neutralization assay using SARS-CoV-2 in 50% of patients by day 7 and in all patients by day 14. All patients showed detectable neutralizing antibodies, the titers of which did not suggest close correlation with clinical courses. Whereas the virus was readily isolated during the 1st week of symptoms from considerable fraction of samples, no isolates were obtained from samples after day 8 in spite of ongoing high viral load [3].

Simultaneously, performance of RT-PCR and virus isolation in cell culture in a study from Canada demonstrated that infectivity (as defined by growth in cell culture), is significantly reduced when RT-PCR cycle threshold (Ct) values were > 24. For every unit increase in Ct, the odds ratio for infectivity decreased by 32%. The high specificity of Ct and symptom onset to test suggested that Ct values > 24, along with duration of symptoms > 8 days may be used in combination to determine duration of infectivity in patients [4]. It is also noteworthy that the Ct value for detecting live virus may differ based on the context of testing: setting (hospital vs. community); depending on COVID-19 symptoms (asymptomatic vs. symptomatic); severity of infection and duration of symptoms as well as the quality of the testing.

Similar observation had been made earlier from China by Zou et al., who detected COVID-19 infection by RT-PCR for L and Orf 1b genes in an asymptomatic patient with Ct values of 30–32, when tested on days 7, 10 and 11 after contact. In their study, higher viral load (inversely related to Ct value) were detected soon after symptom onset, with higher viral loads detected in the nose than in the throat [5].

In another similar study by La Scola et al., they observed a strong correlation between Ct value and sample infectivity in a cell culture model. On the basis of their data, they deduced that with their system, patients with Ct values equal or above 34 did not excrete infectious viral particles. It was observed that SARS-CoV-2 was detected up to 20 days after onset of symptoms by PCR in infected patients but that the virus could not be isolated after day 8 in spite of ongoing high viral loads of approximately 10^3 RNA copies/mL of sample, using the RT-PCR system [6].

In view of different cut-off of Ct reported by Bullad and La Scola et al., Binnicker [7] in an invited editorial to caution that though data indicate that PCR positivity was not a reliable surrogate marker for determining the infectious status of COVID-19 patients, the fact that SARS-CoV-2 culture positivity declined with increasing PCR Ct values and SARS-Co-V was not isolated in culture from any sample that had a PCR Ct value > 34 (which was different from the Ct value of 24 reported from Canada, despite the same PCR gene target being used), the Ct value threshold correlating with SARS-Co-V culture positivity may vary significantly between tests. Therefore, the author suggests, Ct value criteria must be established by each healthcare institution. The common thread in all the studies was that no replicative virus was isolated after 8 days of appearance of symptoms. Thus, it is important to know that the viral load at end of PCR cycle (> 34) may not represent infectious virus. This is an inherent limitation of PCR technology and therefore determination of viral load especially in the late cycles may be subgenomic RNA and should be interpreted with caution and clinically correlated [4,7].

Another important point with respect to quantitative PCR is as cautioned by Han et al., that quantitative RT-PCR was entirely different from qualitative RT-PCR. Ct values itself could not be directly interpreted as viral load without a standard curve using reference material. Hence, there is some lack of consensus on using Ct values as indicator of lack of infectivity, but all authors have reported similar results that replication efficient virus is not found after 8 days of appearance of symptoms [8].

It was also demonstrated by Poon et al. in 2004, that quantitative real-time RT-PCR assay was more sensitive than conventional RT-PCR for the detection of SARS-CoV-2 in samples collected early in the course of the disease. At days 1–3, the quantitative RT-PCR assay was able to detect SARS-CoV-2 in one half of nasopharyngeal samples, by contrast only one-third of these samples were positive by conventional RT-PCR. At days 7–10, the detection rates of the quantitative assay became comparable to those for conventional RT-PCR assay. These results indicated that the real-time quantitative assay was better diagnostic method for early SARS diagnosis [9].

Multi-centric comparison of quantitative PCR based assays to detect different genes of SARS-CoV-2 has been carried out in seven laboratories and results indicate that most methods reliably detect the sample at 10^-3 dilution, which was equivalent to ca 5 RNA copies for CDC N1, N2, N3 and E reactions based on the absolute quantifications by One-Step RT-digital droplet (dd) PCR [10].

The need to determine viral load by quantitative assay was brought home by Pujadas et al. from New York who reported on 1145 SARS-CoV-2 positive hospitalised patients and were followed up for 66 days. Only 807 (70.5%) patients were alive at the end of the study. The viral load in those who survived had a mean viral load of log_{10} 5.19 virus copies/mL, while the viral load in those who expired was 6.44 viral copies/mL. There was a statistically significant survival probability between those with a high defined as > 5.557 log_{10} viral load and lower viral load. Thus, understanding who is at risk for worse outcomes early in their illness could help clinician patients with higher viral load and closely monitor them, while those with lower viral load could safely convalesce at home [11].

Coronaviruses are known to contain one linear RNA but also many subgenomic RNA and these subgenomic RNA are closely associated with the membrane and thus very stable. It is likely that what is being detected for a protracted time after replicative virus has ceased, are these subgenomic RNA and the sometime negative and sometimes positive RT-PCR results that are obtained later in the course of the disease are to a certain extent related to how samples were taken and treated. Not only are these sub-genomic RNA responsible for extended period of PCR positivity but also this may in part explain conflicting findings about reinfection as well as discrepancies amongst diagnostic PCRs detecting targets in different parts of the SARS-CoV-2 genome. The clinical reporting of positive cases with low viraemia is important as RNA from RT-PCR may continue to be detected in positive cases long after infection had resolved. This may be possible because inactivated RNA degrades slowly over time and it may still be detected months after infectiousness [12,13].

It is based on these evidence that most global authorities have shifted from two RT-PCR negatives to make patients eligible for discharge from being asymptomatic for 3 days after 10 days have passed after first appearance of symptoms. Hence, a change from test based to a symptom-based discharge policy has been implemented by all authorities [14-16].

Looking at the viral load dynamics of SARS-CoV-2, it has been projected that test sensitivity is secondary to frequency and turnaround time in COVID-19 surveillance since control of this pandemic is critically dependent on quickly identifying an infected person and isolating him to interrupt the spread of infection [17]. In this endeavour RT-PCR with its central laboratory positioning and slow turn round time has proved unequal to the task. If instead a rapid antigen test which may have lower sensitivity than RT-PCR but would give results in minutes would help identify symptomatic patients with higher viral load (and thus more infective) leading to immediate isolation. Symptomatic patients could be retested after a day or two if negative in the first test, as progression of the disease would have increased the viral load to detectable levels in the next couple of days.

Quantitative RT-PCR could be gainfully employed for risk stratification as well as for the detection of asymptomatic patients in whom the viral load could be lower but they too need to be isolated to stop the spread of infection.

Clinical sensitivity of PCR decreased with days post symptom onset.
with >90% clinical sensitivity during the first 5 days after symptom onset, 70%–71% from days 9–11, 50% and 30% at day 21. In contrast, serological sensitivity increased with days post symptom onset with >50% of patients seropositive by at least one antibody isotype after day 7, >80% after day 12, and 100% by day 21. Therefore, although serology has no role in rapid diagnosis followed by isolation of infected persons as antibodies appear only after 7 days of symptoms, at a time when the patient maybe at the end of his infectious stage. However, many reports have indicated that if used after 14 days of infection, serology may help play an important complementary role in completing the diagnostic evaluation of an infected person [18–20]. PCR and serology are complimentary modalities that require time-dependent interpretation. Superimposition of sensitivities over time indicates that serology can function as a reliable diagnostic aid indicating recent or prior infection.

The construction of a pseudo virus which expresses spike protein on the surface but does not contain the RNA of SARS-CoV-2, should make the possibility of performing neutralizing antibody tests in clinical diagnostic laboratories a real possibility [21] and the growing understanding of the role of T cells may help us is devising an evidence-based best strategy to diagnose, treat and contain COVID-19 infection and stop the pandemic [22,23].

In conclusion, quantitation of SARS-CoV-2 viral load by RT-PCR detection is helpful for patient management considering its merit and simultaneously limitation of detecting minute quantities of RNA and need for reporting COVID-19 tests with caution after clinical correlation, as it may not detect infectious virus especially in asymptomatic cases with low viraemia.

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Declaration of competing interest
There are no conflicts of interest.

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