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Short Communication

Clinical Utility of Ct Value in Covid-19 Infection

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The clinical spectrum of Covid-19 infection caused by SARS CoV 2 is very heterogenous varying from completely asymptomatic infection to severe life threatening infection requiring admission and treatment in ICU and still ending fatally.

Concentration of SARS CoV 2 RNA measured in respiratory specimen declines after onset of symptoms. In laboratories where both viral cultures onto Vero cell lines and RT PCR was simultaneously carried out on respiratory samples from suspected Covid-19 patients, while replicative viruses were isolated in Vero cells only upto the first eight days from onset of symptoms, RT PCR continued to be positive for upto 5 to 12 weeks. Recovery of replication competent virus between 10 and 20 days after symptom onset has only been documented in immunocompromised patients with severe Covid-19 disease [1].

Since Real Time PCR has emerged as the Gold Standard for diagnosis of Covid-19 infection after converting the RNA of SARS CoV2 into cDNA, it is interesting to note that Real Time PCR is also known as qPCR where q standards for quantitative! Ct (cycle threshold values) represents the cycle of PCR when the fluorescence crosses the threshold of detection and has an inverse relationship with starting load of DNA (in this case cDNA [complementary] from Corona virus). More the quantity of starting cDNA, the smaller would be the Ct value, conversely when starting cDNA is sparse, it would take many cycles for the fluorescence to cross the threshold and hence Ct value would be high. Kit manufacturers have suggested any value below a Ct of 40 to be reported as positive by RT PCR for SARS CoV 2! [2].

La Scola., *et al*. [3] from Italy assessed the correlation of SARS CoV 2 isolation in cell culture with RT PCR Ct values and reported culture positivity declined with increasing Ct values and replication competent SARS CoV 2 was not isolated in culture from any sample that had a Ct value for E gene of > 34. Bullard., *et al*. [4] from Canada found that when E gene Ct was more than 24, it was not possible to isolate the virus in cell culture. Singanayagam., *et al*. [5] from UK found that chances of isolating SARS CoV 2 was less than 8 % if the Ct value was over 35. In view of the different Ct values reported Binnicker [6] in an editorial cautioned that although real-time PCR Ct values can be used to estimate the relative concentration of target nucleic acid in clinical samples, Ct values are not interchangeable between assays. The PCR Ct value can be impacted by the assay's gene target(s) and by factors affecting the efficiency of the PCR reaction, including the nucleic acid extraction system and PCR amplification chemistry. Han., *et al*. [7] also cautioned that quantitative RTPCR was entirely different from qualitative RTPCR. Ct values itself could not be directly interpreted as viral load without a standard curve using reference material.

Belabed [8] reported that Tissue inflammation and organ dysfunction in fatal Covid-19 did not map to the tissue and cellular distribution of SARS-CoV-2, demonstrating tissue-specific tolerance. They concluded that death in Covid-19 was primarily a consequence of immune-mediated, rather than pathogen mediated, organ inflammation and injury.

However, Pujadas., *et al*. [9] studied 1145 hospitalized SARS CoV 2 positive patients in New York and followed them over 66 days af-

ter admission. The overall mean log10 viral load for the group was 5.56 viral copies/mL, and the median log10 viral load was 6.16 viral copies/mL. By the end of the study period, 807 were alive (70.5%; mean log10 viral load 5.19 +/- 2.99 viral copies/mL) and 338 had died (29.5%; mean log10 viral load was 6.44 +/- 2.66 viral copies/mL). A Cox proportional hazards model was performed to evaluate the association between viral load and mortality, adjusting for multiple baseline clinical and demographic characteristics including age, sex, race, asthma, atrial fibrillation, coronary artery disease, chronic kidney disease, chronic obstructive pulmonary disease, heart failure, hypertension, and stroke that yielded a statistically significant independent association between viral load and mortality (HR 1.069, CI 1.026 - 1.11; p = 0.0014). Furthermore, a univariate survival analysis revealed a statistically significant survival probability between those with a high (defined as greater than the log10 viral load mean of 5.557) and low viral load, with a mean follow up time of 12.8 days, and a maximum follow up of 66 days.

Westbalde., *et al*. [10] revealed that admission viral load independently predicted mortality in hospitalized patients. They felt that providing viral load information to clinicians may guide the care of hospitalized patients with Covid- 19. Liu., *et al*. [11] found viral load in severe cases 60 times more than in mild cases and felt that calculating Delta Ct value (Ct of reference control - Ct of patient sample) would be useful for patient management and resource allocation.

Coronaviruses are known to contain one linear RNA but also many sub-genomic RNA and these sub-genomic 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 sub-genomic RNA and the sometime negative and sometimes positive RTPCR results that are obtained later in the course of the disease are to a certain extend related to how samples were taken and treated.

Quantitative RTPCR 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.

It is of paramount importance to define when a treated patient can be considered as no longer contagious. Correlation between successful isolation of virus in cell culture and Ct value of quantitative RT-PCR targeting E gene suggests that patients with Ct above 33 - 34 using our RT-PCR system are not contagious and thus can be discharged from hospital care or strict confinement for non-hospitalized patients.

It is based on these evidence that most global authorities have shifted from two RTPCR 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 symptombased discharge policy has been implemented by all authorities [1].

In view of the mounting evidence that Ct values would help identify persons at greatest risk of in hospital mortality, would help identify patients most likely to progress to severe infection as well inform about their infectiveness and in light of the fact that Real Time PCR is inherently a quantitative PCR, we would recommend that Ct values should be shared with the clinicians.

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