Clinical utility of cycle threshold values in the context of COVID-19

Sonia N. Rao  
QIAGEN Inc

Davide Manissero  
QIAGEN Manchester Ltd

Victoria Steele  
Ashfield Healthcare Communications, UK

Josep Pareja (✉ josep.pareja@qiagen.com)  
STAT-Dx Life, S.L. (a QIAGEN Company)

Systematic Review

Keywords: COVID-19, Cycle threshold, PCR, SARS-CoV-2, Viral Load

DOI: https://doi.org/10.21203/rs.3.rs-41867/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

Background

The ability to predict likely prognosis and infectiousness for patients with COVID-19 would aid patient management decisions. Diagnosis is usually via real-time PCR and it is unclear whether the semi-quantitative capability of this method, determining viral load through cycle threshold (Ct) values, can be leveraged.

Objectives

We aim to review available knowledge on correlations between SARS-COV-2 Ct values and patient- or healthcare-related outcomes to determine whether Ct values provide useful clinical information.

Sources

A PubMed search was conducted on 1st June 2020 based on a search strategy of (Ct value OR viral load) AND SARS-CoV-2. Data was extracted from studies reporting on the presence or absence of an association between Ct values, or viral loads determined via Ct value, and clinical outcomes.

Content

Data from 18 studies were relevant for inclusion. One study reported on the correlation between Ct values and mortality and one study reported on the correlation between Ct values and progression to severe disease; both reported a significant association (p < 0.001 and p = 0.008, respectively). Fourteen studies reported on the correlation between Ct value or viral loads determined via Ct value and disease severity and an association was observed in 8 (57%) studies. Studies reporting on the correlation of viral load with biochemical and haematological markers showed an association with at least one marker, including increased lactate dehydrogenase (n = 4), decreased lymphocytes (n = 3) and increased high-sensitivity troponin I (n = 2). Two studies reporting on the correlation with infectivity showed that lower Ct values were associated with higher viral culture positivity.

Implications

Data suggest that lower Ct values may be associated with worse outcomes, and that Ct values may be useful in predicting the clinical course and prognosis of patients with COVID-19; however, further studies are warranted to confirm clinical value.

Introduction

Patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) display disparate disease severity, ranging from an absence of symptoms to requiring intensive care and fatal outcomes. Therefore, the ability to predict the likely prognosis and infectiousness of patients at diagnosis would greatly aid treatment and patient management decisions.

The standard molecular method for coronavirus disease 2019 (COVID-19) diagnosis is via real-time reverse transcription polymerase chain reaction (RT-PCR). Real-time RT-PCR cycle threshold (Ct) values represent the
number of amplification cycles required for the target gene to exceed a threshold level. Ct values are therefore inversely related to viral load and can provide an indirect method of quantifying the copy number of viral RNA in the sample; however, the use of Ct values as a proxy of viral load is influenced by the assay itself (correlation would stand in the linear dynamic range of the specific RT-PCR assay used) and factors within the sample matrix that can affect amplification efficiency.  

It has previously been suggested that the viral load of SARS-CoV-2 may be an important factor in determining both disease severity and likelihood of transmission.  

Although there are many differences between the current SARS-CoV-2 pandemic compared to the SARS-CoV epidemic of 2002, evidence from SARS-CoV indicated that higher viral load was associated with increased need for intensive care and overall worse prognosis.  

In a clinical setting, the results of SARS-CoV-2 RT-PCR diagnostic tests are usually reported qualitatively as a binary positive or negative result using a specified cut-off, either based on Ct or integrated by an automatic algorithm interpreting different parameters of the potential amplification; Ct values themselves are not normally reported. It is currently unclear as to whether SARS-CoV-2 Ct values could be leveraged to guide patient management decisions.

In this review, we assessed the available global literature to determine whether there is evidence that SARS-CoV-2 Ct values correlate with clinical outcomes and therefore, whether they could provide valuable information to clinicians for more tailored decision-making.

**Methods**

This review was undertaken according to the principles outlined in the Cochrane handbook. A comprehensive search of PubMed was conducted on 1st June 2020 based on the following search strategy: (Ct value OR viral load) AND SARS-CoV-2. Titles and abstracts were screened for relevance by two independent reviewers, and a third reviewer resolved conflicts. All studies that were conducted in humans diagnosed with COVID-19 and reported on the presence or absence of an association between real-time RT-PCR Ct values, or viral load specifically determined via real-time PCR Ct value, and clinical or healthcare-associated outcomes were eligible for inclusion. Studies that reported only on the time course of SARS-CoV-2 viral load or that only compared viral load in different sample types or using different methodologies were not included. Pre-review articles, animal studies and reviews were excluded, but additional publications were identified by manual citation searching of appropriate reviews. The full-texts of relevant studies were assessed for inclusion by two independent reviewers and key data from all included studies were captured using a data extraction form. All extracted data were verified by an independent reviewer.

**Results**

*Included studies*

PubMed searches identified 162 unique records for screening and one study was identified through manual citation searches. The PRISMA flowchart of included studies is shown in Figure 1. Data from 18 studies were relevant for inclusion and are summarised in Tables 1 and 2.
Twelve studies (63%) were conducted in China or Hong Kong, and three studies were performed in Europe. Nearly all studies reported data for specimens from the respiratory tract, although two studies analysed saliva, one used stool and serum samples in addition to respiratory samples, and one used both throat and anal swabs. The real-time PCR targets varied between studies and included ORF1ab (including RdRp), N, E genes and the 5’ untranslated genome region. Six studies analysed SARS-CoV-2 Ct values at multiple time points for each patient, and seven studies determined Ct values at hospital admission or diagnosis. Fourteen studies reported on the direct correlation of outcomes with Ct values. Three studies reported on the correlation of outcomes with viral load, determined using standard curves of Ct values versus RNA copy number, and one study correlated outcomes with the inverse of Ct values, taken as a proxy for viral load.

**Mortality**

Only one study reported on the correlation between SARS-CoV-2 Ct values and mortality (Table 1). In 308 hospitalised adult patients in China, average Ct values multiple time points during the disease course were lower in patients who died compared with those who had recovered or who were still hospitalised at the end of the study (recovered: median 37.43 [IQR 34.94–38.67]; still hospitalised: median 36.97 [IQR 34.33–38.70]; deceased: median 34.79 [IQR 24.46–37.65]; p < 0.001).

**Disease progression**

One study reported that SARS-CoV-2 Ct values at hospital admission negatively correlated with the probability of progression to severe disease in 62 patients who presented with mild–moderate disease (Table 1). Lower Ct values were observed in specimens from patients who became severely ill during hospitalisation than those who did not (24 vs. 29; p = 0.008).

**Disease severity**

Eleven studies (with numbers of PCR-positive patients ranging from 10 to 308) reported on the correlation between Ct value and disease severity. Lower Ct values from respiratory samples were associated with more severe disease in 7 (64%) of these (Table 2). Three studies (with numbers of PCR-positive patients ranging from 23 to 114) reported on the correlation between viral load determined via Ct values and disease severity and one of these (which included 96 patients) reported that higher viral loads were significantly associated with more severe disease (Table 2).

Of the 15 studies reporting on the correlation between Ct value or viral load determined via Ct value and disease severity, 11 were performed in hospitalised patients and three included non-hospitalised patients. Of the eleven studies performed in hospitalised patients only, eight (73%) reported an association between Ct value and disease severity, of which six showed statistical significance. None of three studies that included non-hospitalised patients reported that patients with severe disease had higher viral loads compared with those with mild disease.

**Biochemical and haematological markers**
All five studies (with numbers of PCR-positive patients ranging from 12 to 308) reporting on the correlation of Ct value with biochemical and haematological markers showed a correlation with at least one marker (Table 2).11,13,14,18,22 Lower Ct values were significantly associated with: higher lactate dehydrogenase levels (n = 4);11,13,18,22 lower lymphocyte counts and/or percentages (n = 3);11,13,14 lower T-cell counts (n = 3);11,13,18 lower serum albumin levels (n = 2);11,14 increased levels of creatinine kinase myocardial band (n = 2);11,18 and increased levels of high-sensitivity troponin 1 (n = 2).11,13 Two studies showed that lower Ct values were associated with higher neutrophil counts and/or percentages,11,14 whereas one study showed a negative correlation.18 One study in 12 patients showed that C-reactive protein levels negatively correlated with Ct value (r = −0.584; p = 0.03),14 whereas another in 25 patients showed no significant association (p = 0.07).22 Associations were also reported between Ct values and angiotensin II,14 IL-2R,13 basophil and eosinophil counts, and levels of myoglobin, N-terminal pro-brain natriuretic peptide, inorganic phosphorus and calcium.11

**Infectivity**

Two studies reported on the correlation between Ct value and infectivity and showed that lower Ct values were associated with higher probability of a positive viral culture (Table 2).24,27 In one study of 155 patients, multivariate logistic regression analyses using time from symptom onset to test, age and gender as independent variables showed a significant effect of Ct value on the culture positivity of samples (OR 0.64 [95% confidence interval 0.49–0.84], p < 0.001) suggesting that for every one unit increase in Ct, the odds of positive culture decreased by 32%.27 The results demonstrated that infectivity (defined as growth in cell culture) was significantly reduced when RT-PCR Ct values were greater than 24 (p < 0.001).

**Discussion And Conclusion**

The majority of the 18 studies identified in this review reported an association between SARS-CoV-2 Ct values or viral load determined via Ct values and clinical outcomes. Higher Ct values generally correlate with lower viral loads, although Ct value and log viral load may not be directly proportional due to the linear dynamic range of the assay and potential presence of inhibitory factors within clinical samples.28 Fifteen (79%) of the studies included in this review investigated the direct association of Ct values with clinical outcomes, rather than viral load itself, but it was assumed by authors that Ct values are an appropriate surrogate for viral load. Clinical knowledge of COVID-19 is constantly evolving, with studies being published at a high rate; however, there is currently only limited data relating to the correlation of viral loads with patient prognoses, such as mortality or disease progression. Only one study reported on the association between mortality and SARS-CoV-2 Ct value and showed that lower Ct values correlated with increased risk of death,11 which is consistent with data for previous epidemic-causing coronaviruses.9,29 Given the wide range in disease course for COVID-19, the ability to predict which patients are at particularly high risk of deterioration and negative outcomes would be of particular value in the clinical setting; it would therefore be useful to continue to assess the value of SARS-CoV-2 Ct as further data become available.

Eleven studies reported on the correlation of Ct values with symptom severity at presentation and seven of these indicated that lower Ct values were associated with more severe disease. This is consistent with some
previous studies of Ct values in other respiratory infections,\textsuperscript{29-31} although other studies do not show correlation.\textsuperscript{32} Whilst correlation between Ct value and disease severity was observed for 73\% of studies in hospitalised patients, correlation between Ct or viral load determined via Ct value and disease severity was observed in none of the studies that included patients with COVID-19 who were not hospitalised. Studies in hospitalised patients are unlikely to include asymptomatic patients or those with very mild symptoms, but are likely to be more controlled, making correlations with Ct more probable. The role that symptoms play in viral shedding remains to be determined; in a large study of 5,830 patients with COVID-19, which was pre-review at the time that this review was conducted, viral load determined via Ct values in nasal swabs of asymptomatic and symptomatic patients was not statistically different (median $4.7 \log_{10}$ copies/ml vs. $5.0 \log_{10}$ copies/ml; $p = 0.51$).\textsuperscript{33}

Ct values were found to correlate with a number of clinical markers. Lower Ct values were associated with elevated LDH levels in all four studies in which it was assessed, which is consistent with reports that elevated LDH can act as an indicator of poor prognosis in patients with COVID-19.\textsuperscript{34,35} Increased LDH reflects tissue destruction and in interstitial pulmonary fibrosis is seen as an important prognostic marker for lung injury.\textsuperscript{36} Lower Ct values were associated with lower lymphocyte levels in all three studies in which it was assessed, which is consistent with reports that lymphopaenia could act as a predictor of higher disease severity in patients with COVID-19.\textsuperscript{35,37} Similarly, correlation of Ct values was also seen with high-sensitivity troponin I, which has been suggested as a marker of COVID-19 disease progression and mortality.\textsuperscript{35}

Both studies that investigated the correlation between SARS-CoV-2 Ct values and infectivity showed that samples with higher Ct values had lower culture positivity. It has been shown that following resolution of COVID-19 symptoms, people can have prolonged positive SARS-CoV-2 real-time PCR results for several weeks\textsuperscript{38} and at late time points, Ct values are often very high representing low copies of viral RNA.\textsuperscript{20} Therefore, as suggested previously,\textsuperscript{4} considering Ct values in conjunction with the clinical context of patients may help in patient management decisions such as the need for isolation, use of PPE and testing resources.

To the best of our knowledge, this is the first report to systematically assess the globally available literature data relating to the predictive value of SARS-CoV-2 Ct values; however, it is associated with a number of limitations. The majority of the studies included in this review contained a relatively small number of patients; only four studies included more than 100 patients with COVID-19.\textsuperscript{11,15,18,24} The viral load of SARS-CoV-2 is known to vary during the course of infection.\textsuperscript{10,11,13,19,20,39-42} The time from onset of symptoms to sampling varied between studies, and in most of the included studies, varied between patients. Time from onset of symptoms was included as an independent variable in only one of the analyses presented\textsuperscript{27} and therefore this may be a confounding factor in many of the studies reported. The type of sample used varied between studies. Sample type is known to affect the Ct values and detected viral load,\textsuperscript{20,40} and therefore this may have affected results. The variability within and between the included studies is not consistent with previously reported considerations regarding variability around factors involved (sample type, workflow, assay) in robust viral load measurement using RT-PCR.\textsuperscript{3} Reproducible experimental layouts to assess viral load from patient samples are key to establish any correlation to patient outcome.
Reporting of qualitative SARS-CoV-2 test results as positive or negative is sufficient for diagnosis, but the totality of currently available data indicate that the reporting of Ct values may offer benefit to clinicians in making clinical and patient-management decisions for patients with COVID-19, as well as guide infection control, public health and occupational health decisions. However, additional data and prospective studies are required to support this.

**Declarations**

**Acknowledgements**

**Funding**

This study was funded by Qiagen Manchester Ltd

**Authorship**

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published. All authors contributed to data analysis, data interpretation and writing of this report.

**Medical writing, editorial and other assistance**

The authors would like to acknowledge Tom Hudson, PhD and Sarah Rossall, PhD of Ashfield Healthcare Communications, part of UDG Healthcare plc, for assistance with literature screening that was funded by Qiagen Manchester Ltd.

**Disclosures**

SR is an employee of QIAGEN Inc, DM is an employee of QIAGEN Manchester Ltd, VS is an employee of Ashfield Healthcare communications, part of UDG Healthcare plc, which received funding from Qiagen Manchester Ltd to conduct the study, and JP is an employee of STAT-Dx Life, a QIAGEN company.

**Compliance with ethics guidelines**

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

**References**

1. Poletti P, Tirani M, Cereda D, et al. Probability of symptoms and critical disease after SARS-CoV-2 infection. *arxiv*. 2020;Preprint.

2. Tang Y-W, Schmitz JE, Persing DH, Stratton CW. Laboratory Diagnosis of COVID-19: Current Issues and Challenges. 2020;58(6):e00512-00520.
3. Bustin SA, Mueller R. Real-time reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis. *Clinical science (London, England : 1979).* 2005;109(4):365-379.

4. Tom MR, Mina MJ. To Interpret the SARS-CoV-2 Test, Consider the Cycle Threshold Value. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2020.

5. Joynt GM, Wu WK. Understanding COVID-19: what does viral RNA load really mean? *The Lancet. Infectious diseases.* 2020;20(6):635-636.

6. Geddes L. Puzzle over viral load. *New scientist (1971).* 2020;245(3276):8.

7. Cheng VC, Hung IF, Tang BS, et al. Viral replication in the nasopharynx is associated with diarrhea in patients with severe acute respiratory syndrome. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2004;38(4):467-475.

8. Ng EK, Hui DS, Chan KC, et al. Quantitative analysis and prognostic implication of SARS coronavirus RNA in the plasma and serum of patients with severe acute respiratory syndrome. *Clinical chemistry.* 2003;49(12):1976-1980.

9. Chu CM, Poon LL, Cheng VC, et al. Initial viral load and the outcomes of SARS. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne.* 2004;171(11):1349-1352.

10. He XL, E. H. Y.; Wu, P.; Deng, X.; Wang, J.; Hao, X.; Lau, Y. C.; Wong, J. Y.; Guan, Y.; Tan, X.; Mo, X.; Chen, Y.; Liao, B.; Chen, W.; Hu, F.; Zhang, Q.; Zhong, M.; Wu, Y.; Zhao, L.; Zhang, F.; Cowling, B. J.; Li, F.; Leung, G. M. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nature medicine.* 2020;26(5):672-675.

11. Huang JTR, R. X.; Lv, Z. H.; Feng, L. N.; Ran, C. Y.; Tong, Y. Q.; Li, D.; Su, H. W.; Zhu, C. L.; Qiu, S. L.; Yang, J.; Xiao, M. Y.; Liu, M. J.; Yang, Y. T.; Liu, S. M.; Li, Y. Chronological Changes of Viral Shedding in Adult Inpatients with COVID-19 in Wuhan, China. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2020.

12. Liu Y, Yan LM, Wan L, et al. Viral dynamics in mild and severe cases of COVID-19. *The Lancet. Infectious diseases.* 2020;20(6):656-657.

13. Liu YL, W.; Wan, L.; Xiang, T.; Zhang, W. Correlation Between Relative Nasopharyngeal Virus RNA Load and Lymphocyte Count Disease Severity in Patients with COVID-19. *Viral immunology.* 2020.

14. Liu YY, Y.; Zhang, C.; Huang, F.; Wang, F.; Yuan, J.; Wang, Z.; Li, J.; Li, J.; Feng, C.; Zhang, C.; Wang, L.; Peng, L.; Chen, L.; Qin, Y.; Zhao, D.; Tan, S.; Yin, L.; Xu, J.; Zhou, C.; Jiang, C.; Liu, L. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Science China. Life sciences.* 2020;63(3):364-374.

15. Shi FW, T.; Zhu, X.; Ge, Y.; Zeng, X.; Chi, Y.; Du, X.; Zhu, L.; Zhu, F.; Zhu, B.; Cui, L.; Wu, B. Association of viral load with serum biomakers among COVID-19 cases. *Virology.* 2020;546:122-126.

16. Xia XYW, J.; Liu, H. L.; Xia, H.; Jia, B.; Huang, W. X. Epidemiological and initial clinical characteristics of patients with family aggregation of COVID-19. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* 2020;127:104360.

17. Yu XS, S.; Shi, Y.; Wang, H.; Zhao, R.; Sheng, J. SARS-CoV-2 viral load in sputum correlates with risk of COVID-19 progression. *Critical care (London, England).* 2020;24(1):170.
18. Yuan CZ, H.; Yang, Y.; Cai, X.; Xiang, F.; Wu, H.; Yao, C.; Xiang, Y.; Xiao, H. Viral loads in throat and anal swabs in children infected with SARS-CoV-2. *Emerging microbes & infections*. 2020:1-17.

19. Zheng SF, J.; Yu, F.; Feng, B.; Lou, B.; Zou, Q.; Xie, G.; Lin, S.; Wang, R.; Yang, X.; Chen, W.; Wang, Q.; Zhang, D.; Liu, Y.; Gong, R.; Ma, Z.; Lu, S.; Xiao, Y.; Gu, Y.; Zhang, J.; Yao, H.; Xu, K.; Lu, X.; Wei, G.; Zhou, J.; Fang, Q.; Cai, H.; Qiu, Y.; Sheng, J.; Chen, Y.; Liang, T. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. *BMJ (Clinical research ed.)*. 2020;369:m1443.

20. Zou LR, F.; Huang, M.; Liang, L.; Huang, H.; Hong, Z.; Yu, J.; Kang, M.; Song, Y.; Xia, J.; Guo, Q.; Song, T.; He, J.; Yen, H. L.; Peiris, M.; Wu, J. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *The New England journal of medicine*. 2020;382(12):1177-1179.

21. To KKT, O. T.; Leung, W. S.; Tam, A. R.; Wu, T. C.; Lung, D. C.; Yip, C. C.; Cai, J. P.; Chan, J. M.; Chik, T. S.; Lau, D. P.; Choi, C. Y.; Chen, L. L.; Chan, W. M.; Chan, K. H.; Ip, J. D.; Ng, A. C.; Poon, R. W.; Luo, C. T.; Cheng, V. C.; Chan, J. F.; Hung, I. F.; Chen, Z.; Chen, H.; Yuen, K. Y. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *The Lancet. Infectious diseases*. 2020;20(5):565-574.

22. Azzi LC, G.; Gianfagna, F.; Grossi, P.; Gasperina, D. D.; Genoni, A.; Fasano, M.; Sessa, F.; Tettamanti, L.; Carinci, F.; Maurino, V.; Rossi, A.; Tagliabue, A.; Baj, A. Saliva is a reliable tool to detect SARS-CoV-2. *The Journal of infection*. 2020.

23. Schwierzeck VK, J. C.; Kuhn, J.; Mellmann, A.; Correa-Martinez, C. L.; Omran, H.; Konrad, M.; Kaiser, T.; Kampmeier, S. First reported nosocomial outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a pediatric dialysis unit. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2020.

24. La Scola BLB, M.; Andreani, J.; Hoang, V. T.; Grimaldier, C.; Colson, P.; Gautret, P.; Raoult, D. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology*. 2020;39(6):1059-1061.

25. Arons MMH, K. M.; Reddy, S. C.; Kimball, A.; James, A.; Jacobs, J. R.; Taylor, J.; Spicer, K.; Bardossy, A. C.; Oakley, L. P.; Tanwar, S.; Dyal, J. W.; Harney, J.; Chisty, Z.; Bell, J. M.; Methner, M.; Paul, P.; Carlson, C. M.; McLaughlin, H. P.; Thornburg, N.; Tong, S.; Tamin, A.; Tao, Y.; Uehara, A.; Harcourt, J.; Clark, S.; Brostrom-Smith, C.; Page, L. C.; Kay, M.; Lewis, J.; Montgomery, P.; Stone, N. D.; Clark, T. A.; Honein, M. A.; Duchin, J. S.; Jemigan, J. A. Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility. *The New England journal of medicine*. 2020;382(22):2081-2090.

26. Kimball AH, K. M.; Arons, M.; James, A.; Taylor, J.; Spicer, K.; Bardossy, A. C.; Oakley, L. P.; Tanwar, S.; Chisty, Z.; Bell, J. M.; Methner, M.; Harney, J.; Jacobs, J. R.; Carlson, C. M.; McLaughlin, H. P.; Stone, N.; Clark, S.; Brostrom-Smith, C.; Page, L. C.; Kay, M.; Lewis, J.; Russell, D.; Hiatt, B.; Gant, J.; Duchin, J. S.; Clark, T. A.; Honein, M. A.; Reddy, S. C.; Jemigan, J. A. Asymptomatic and Presymptomatic SARS-CoV-2 Infections in Residents of a Long-Term Care Skilled Nursing Facility - King County, Washington, March 2020. *MMWR. Morbidity and mortality weekly report*. 2020;69(13):377-381.

27. Bullard JD, K.; Funk, D.; Strong, J. E.; Alexander, D.; Garnett, L.; Boodman, C.; Bello, A.; Hedley, A.; Schiffman, Z.; Doan, K.; Bastien, N.; Li, Y.; Van Caeseele, P. G.; Poliquin, G. Predicting infectious SARS-CoV-2 from
diagnostic samples. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America.* 2020.

28. Aquino-Jarquin G. The raw Ct values from RT-PCR detection are not viral load quantitation units. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America.* 2020.

29. Feikin DR, Alraddadi B, Qutub M, et al. Association of Higher MERS-CoV Virus Load with Severe Disease and Death, Saudi Arabia, 2014. *Emerging infectious diseases.* 2015;21(11):2029-2035.

30. Lee N, Chan PK, Hui DS, et al. Viral loads and duration of viral shedding in adult patients hospitalized with influenza. *The Journal of infectious diseases.* 2009;200(4):492-500.

31. Wishaupt JO, Ploeg TV, Smeets LC, Groot R, Versteegh FG, Hartwig NG. Pitfalls in interpretation of CT-values of RT-PCR in children with acute respiratory tract infections. *Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology.* 2017;90:1-6.

32. Feikin DR, Fu W, Park DE, et al. Is Higher Viral Load in the Upper Respiratory Tract Associated With Severe Pneumonia? Findings From the PERCH Study. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America.* 2017;64(suppl_3):S337-s346.

33. Cereda D, Tirani M, Rovida F, et al. The early phase of the COVID-19 outbreak in Lombardy, Italy. *arXiv.* 2020;Preprint.

34. Yan L, Zhang H-T, Goncalves J, et al. An interpretable mortality prediction model for COVID-19 patients. *Nature Machine Intelligence.* 2020;2(5):283-288.

35. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A. The role of biomarkers in diagnosis of COVID-19 - A systematic review. *Life sciences.* 2020;254:117788.

36. Kishaba T, Tamaki H, Shimaoka Y, Fukuyama H, Yamashiro S. Staging of Acute Exacerbation in Patients with Idiopathic Pulmonary Fibrosis. *Lung.* 2014;192(1):141-149.

37. Tan L, Wang Q, Zhang D, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduction and Targeted Therapy.* 2020;5(1):33.

38. Xiao AT, Tong YX, Zhang S. Profile of RT-PCR for SARS-CoV-2: a preliminary study from 56 COVID-19 patients. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America.* 2020.

39. Huang YC, S.; Yang, Z.; Guan, W.; Liu, D.; Lin, Z.; Zhang, Y.; Xu, Z.; Liu, X.; Li, Y. SARS-CoV-2 Viral Load in Clinical Samples of Critically Ill Patients. *American journal of respiratory and critical care medicine.* 2020.

40. Pan YZ, D.; Yang, P.; Poon, L. L. M.; Wang, Q. Viral load of SARS-CoV-2 in clinical samples. *The Lancet. Infectious diseases.* 2020;20(4):411-412.

41. Yu FY, L.; Wang, N.; Yang, S.; Wang, L.; Tang, Y.; Gao, G.; Wang, S.; Ma, C.; Xie, R.; Wang, F.; Tan, C.; Zhu, L.; Guo, Y.; Zhang, F. Quantitative Detection and Viral Load Analysis of SARS-CoV-2 in Infected Patients. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America.* 2020.

42. Wolfel RC, V. M.; Guggemos, W.; Seilmaier, M.; Zange, S.; Muller, M. A.; Niemeyer, D.; Jones, T. C.; Vollmar, P.; Rothe, C.; Hoelscher, M.; Bleicker, T.; Brunink, S.; Schneider, J.; Ehmann, R.; Zwigrlmaier, K.; Drosten, C.; Wendtner, C. Virological assessment of hospitalized patients with COVID-2019. *Nature.* 2020.
Tables

Table 1 Summary of reported data relating to SARS-CoV-2 real-time PCR Ct value correlation with mortality, disease progression and severity
| Outcome          | Study               | Country | Number of PCR+ patients | Patients Sample type | Sample type | RT-PCR target | Timepoint of assessment | Association | P value | Outcome measure                                                                 |
|------------------|---------------------|---------|-------------------------|---------------------|-------------|---------------|------------------------|--------------|---------|----------------------------------------------------------------------------------|
| Mortality        | Huang *et al.*11    | China   | 308                     | Hospitalised adult patients | Nasal and pharyngeal swab | ORF1ab gene | Multiple time points after admission | Yes          | < 0.001  | Average Ct values were lower in patients who died during the study than those who did not (discharged from hospital: median 37.43 [IQR 34.94–38.67]; still hospitalised: median 36.97 [IQR 34.33–38.70]; deceased: median 34.79 [IQR 25.46–37.65]) |
| Disease progression | Yu *et al.*17   | China   | 92                      | Hospitalised patients | Sputum from the lower respiratory tract | N and Orf1b genes | Hospital admission | Yes          | 0.008    | Lower Ct values were observed in specimens from patients who became severe during hospitalisation than those did not (Ct values 24 vs 29). |
| Severity of disease | Arons *et al.*26 | USA     | 57                      | Patients in a long term skilled nursing facility | Nasopharyngeal and oropharyngeal swabs | N1 and N2 genes | 10 days after first patient tested positive | No           | NR       | Ct values negatively correlated with the probability of progression to severe disease in patients representing mild-moderate disease at admission. |
| Study Reference | Country | N | Study Population | Sample Type | Gene(s) Tested | Sample Collection | Result | Ct Value Difference or Correlation |
|-----------------|---------|---|------------------|-------------|---------------|------------------|--------|-----------------------------------|
| He et al.10      | China   | 94 | Hospitalised patients | Throat swab | N gene        | Symptom onset to Day 32 | No NR | There was no obvious difference in Ct values by disease severity |
| Huang et al.11   | China   | 308 | Hospitalised patients | Nasal and pharyngeal swab | ORF1ab gene | Multiple time points after admission | Yes NR | The Ct values of critical patients were much lower than general patients and severe patients in the early stages of hospitalisation. The overall Ct values of general patients were higher than severe patients |
| Kimball et al.27 | USA     | 23 | Patients in a long term skilled nursing facility | Nasopharyngeal and oropharyngeal swabs | N1 and N2 genes | 12 days after an HCP within the facility tested positive | No 0.3 | Mean Ct values in the four symptom status groups did not show any difference (typical symptoms: 18.6–29.2; atypical symptoms only: 24.3–26.3; presymptomatic: 15.3–37.9; asymptomatic: 21.9–31.0) |
| Liu et al.14     | China   | 12 | Hospitalised patients | Respiratory samples, including throat swabs | ORF1ab and N genes | Hospital admission | Yes 0.01 | The Ct value was positively linked to lung disease severity measured by Murray score ($r = -0.765$) |

- Mean Ct values in the four symptom status groups did not show any difference (typical symptoms: 18.6–29.2; atypical symptoms only: 24.3–26.3; presymptomatic: 15.3–37.9; asymptomatic: 21.9–31.0)
| Study          | Country | Sample Size | Test Site | Test Type | Time of Collection | Result | Notes |
|---------------|---------|-------------|-----------|-----------|--------------------|--------|-------|
| Liu et al.13  | China   | 76          | Hospitalised patients | Nasopharyngeal swab | Hospital admission | 0.017  | Patients with severe disease had significantly lower Ct values than mild-moderate cases at admission (25 vs. 28) |
| Liu et al.12  | China   | 76          | Hospitalised patients | Nasopharyngeal swab | At diagnosis | < 0.00001 | ΔCt values (Ct of sample minus Ct of reference sample) of severe cases were significantly lower than those of mild cases at the time of admission (−1.42 ± 3.62 vs 4.44 ± 3.99); the mean viral load of severe cases was around 60 times higher than that of mild cases |
| Schwierzeck et al.24 | Germany | 12          | Paediatric dialysis patients | Nasopharyngeal swab | >5 days after contact with index case | 0.007  | Ct values of the symptomatic cases (22.55 [range 16.03–23.50]; n = 6) were lower compared with asymptomatic cases (29.94 [range 21.89–37.49]; n = 6), indicating an approximately 200-fold higher viral load |
| Shi et al.15  | China   | 114         | Hospitalised patients | Pharyngeal swab | Hospital admission | NR     | The mean viral load was lower in cases with pneumonia (5.15 log_{10} |
followed by non-pneumonia cases (5.22 log\textsubscript{10} copies/ml), and highest in severe pneumonia cases (5.58 log\textsubscript{10} copies/ml), but the differences was not significant < 0.05.

Among female cases, the mean viral load in patients with severe pneumonia was higher and significantly differed from non-pneumonia patients and pneumonia patients < 0.05.

Within the CRP(+) / SAA(+) group, mean viral load was higher in patients with severe pneumonia than those without pneumonia (4.80 log\textsubscript{10} copies/ml vs 5.50 log\textsubscript{10} copies/ml) < 0.05.

To et al.\textsuperscript{21} Hong Kong 23 Hospitalised patients Early morning saliva from the posterior pharynx RdRP gene 0 to 29 days after symptom onset No* 0.56

The median initial viral loads in severe cases were higher than those in mild cases (log 10 copies/ml 6.17 [IQR 4.18–7.13] vs 5.11 [IQR
The median peak viral loads in severe cases were higher than those in mild cases \((6.91 \log_{10} \text{copies/ml} [\text{IQR} 4.27–7.40] \text{ vs } 5.29 \log_{10} \text{copies/ml} [\text{IQR } 3.91–7.56])\), although the difference was not significant. 

| Author et al. | Country | Total | Population | Sample Type | Gene(s) | Sample Collection Time after Admission | Hospital admission | p-value | Findings |
|--------------|---------|-------|------------|-------------|---------|----------------------------------------|-------------------|--------|----------|
| Xia et al. | China | 10 | Hospitalised patients | Nasopharyngeal swab | ORF1ab and N genes | Hospital admission | Yes | NR | The Ct values for severe cases \((n = 3)\) were lower than those of other patients \((n = 7)\) |
| Yu et al. | China | 92 | Hospitalised patients | Sputum from the lower respiratory tract | N and Orf1b genes | Hospital admission | Yes | 0.017 | Severe patients had lower Ct values than mild-moderate cases at admission \((25 \text{ vs. } 28)\) |
| Zheng et al. | China | 96 | Hospitalised patients | Respiratory ORF1ab | Multiple time points after admission | Hospital admission | Yes* | 0.03 | Patients with severe disease had significantly higher viral loads than patients with mild disease |
|             |         |      |            | Stool ORF1ab | No* | 0.83 | Viral loads in stool samples showed no significant difference between patients with mild disease and patients with severe disease |
|             |         |      |            | Serum ORF1ab | No* | 0.09 | Viral loads in serum samples showed no |
significant difference between patients with mild disease and patients with severe disease.

Zou et al.\textsuperscript{20} China 18 Nasal and throat swabs Orf1b 0 to 21 days after symptom onset No NR The viral load that was detected in the asymptomatic patient (n = 1) was similar to that in the symptomatic patients.

*Presence or absence of correlation of outcome with viral load determined via use of a Ct standard curve, rather than directly with Ct values.

APACHE, Acute Physiology and Chronic Health Evaluation; Ct, cycle threshold; HCP, healthcare professional; IQR, inter-quartile range; RT-PCR, real-time polymerase chain reaction.

Table 2 Summary of reported data relating to SARS-CoV-2 real-time PCR Ct value correlation with clinical biomarkers and infectivity.
| Outcome measure                  | Study         | Country | Number of PCR+ patients | Patients | Sample type                      | RT-PCR target               | Timepoint of assessment | Association | P value | Outcome measure                                                                 |
|---------------------------------|---------------|---------|-------------------------|----------|---------------------------------|----------------------------|-------------------------|-------------|---------|--------------------------------------------------------------------------------|
| **Clinical markers**            | Azzi *et al.*22 | Italy   | 25                      | Hospitalised patients with severe disease | Saliva | 5’ untranslated region | After hospital admission | Yes         | 0.04    | There was an inverse correlation between LDH levels and Ct values               |
|                                 |               |         |                         | Hospitalised patients | Nasal and pharyngeal swab | ORF1ab gene             | Multiple time points after admission | Yes         | < 0.0001 | Cases with Ct<median had higher neutrophil percentages than cases with Ct>median (62.4 [54.8–74.2] vs. 61.8 [52.6–70.5]) |
|                                 | Huang *et al.*11 | China   | 308                     | Hospitalised patients | Nasal and pharyngeal swab | ORF1ab gene             | Multiple time points after admission | Yes         | 0.0007  | Cases with Ct<median had lower lymphocyte percentages than cases with Ct>median (24.1 [16.1–30.2] vs. 25.7 [17.6–32.4]) |
|                                 |               |         |                         | Hospitalised patients | Nasal and pharyngeal swab | ORF1ab gene             | Multiple time points after admission | Yes         | 0.0002  | Cases with Ct<median had lower basophil percentages than cases with Ct>median (0.40 [0.20–0.70] vs. 0.60 [0.30–0.80]) |
|                                 |               |         |                         | Hospitalised patients | Nasal and pharyngeal swab | ORF1ab gene             | Multiple time points after admission | Yes         | 0.0001  | Cases with Ct<median had lower eosinophil percentages than cases with Ct>median (1.60 [0.50–2.80] vs. 2.00 [0.98–3.20]) |
|                                 |               |         |                         | Hospitalised patients | Nasal and pharyngeal swab | ORF1ab gene             | Multiple time points after admission | Yes         | < 0.0001 | Cases with Ct<median had lower T cell counts than cases with Ct>median (783 cell/µl [466–1126] vs. 916 cells/µl [692–1132]) |
|                                 |               |         |                         | Hospitalised patients | Nasal and pharyngeal swab | ORF1ab gene             | Multiple time points after admission | Yes         | < 0.0001 | CK-MB levels were                                                                 |
| p-value | Description |
|---------|-------------|
| < 0.0001 | Myoglobin levels were increased in cases with Ct<median compared with cases with Ct>median (32.8 µg/L [25.0–68.2] vs. 26.4 µg/L [21.1–38.6]) |
| < 0.0001 | Ultrasensitive troponin-1 levels were increased in cases with Ct<median compared with cases with Ct>median (0.01 µg/L [0.01–0.03] vs. 0.01 µg/L [0.01–0.01]) |
| < 0.0005 | N-terminal pro-brain natriuretic peptide levels were increased in cases with Ct<median compared with cases with Ct>median (173.4 ng/L [43.3–646.1] vs. 91.4 ng/L [28.0–278.0]) |
| < 0.0001 | Cases with Ct<median had lower serum albumin compared with cases with Ct>median (36.3 g/L [32.9–39.2] vs. 37.7 g/L [34.8–40.0]) |
| < 0.0001 | Cases with Ct<median had lower inorganic phosphorus levels compared with cases with Ct>median (1.15 mmol/L [0.99–1.30] vs. 1.22 mmol/L [1.07–1.35]) |
| Study | Country | Number of Patients | Sample Type | Test | Admission | p Value | Findings |
|-------|---------|--------------------|-------------|------|-----------|---------|----------|
| Liu et al. | China | 12 | Hospitalised patients, Respiratory samples, including throat swabs | ORF1ab and N genes | Yes | 0.035 | Angiotensin II level in plasma samples was markedly elevated and negatively correlated with Ct value (r = −0.669) |
| Liu et al. | China | 76 | Hospitalised patients, Nasopharyngeal swab | | Yes | < 0.001 | The ΔCt value (Ct of sample minus Ct of reference sample) was positively correlated with lymphocyte counts (r = 0.548) |

Calcium levels compared with cases with Ct > median (2.47 [2.27–2.67] vs. 2.40 mmol/L [2.25–2.59]) < 0.0001

LDH levels were increased in cases with Ct < median compared with cases with Ct > median (220.0 mmol/L [187.0–287.5] vs. 204.0 mmol/L [174.0–240.0])

Albumin levels correlated with Ct value (r = 0.717) 0.01

The percentage of lymphocytes correlated with Ct value (r = 0.717) 0.01

The percentage of neutrophils negatively correlated with Ct value (r = −0.529) 0.05

CRP levels negatively correlated with Ct value (r = −0.584) 0.03

The ΔCt value was positively correlated with CD4+ T lymphocyte counts (r = 0.478) < 0.001

The ΔCt value was positively correlated with CD8+ T lymphocyte counts (r = 0.525) < 0.001
| Study            | Country | Patients | Sample Type      | Target Gene | Days Post-Onset | Ct Value | Comparison                                      |
|------------------|---------|----------|------------------|-------------|----------------|----------|------------------------------------------------|
| Yuan et al.      | China   | 217      | Throat swab      | ORF1ab gene | 0 to 24 days   | Yes      | Viral loads (assumed to be inversely related to Ct value) were positively correlated with myocardial zymogram, CK-MB, LDH and IL-10, while negatively correlated with neutrophils, CD8+ T cells and white blood cells. |
| Bullard et al.   | Canada  | 90       | Nasopharyngeal or endotracheal samples | E gene | 0 to 21 days | Yes       | Positive culture samples had a significantly lower Ct values compared with culture negative samples (17 [16–18] vs 27 [22–33], respectively). |

The ΔCt value was negatively correlated with interleukin-2R levels \( (r = -0.323) \). The ΔCt value was negatively correlated with LDH levels \( (r = -0.339) \). The ΔCt value was negatively correlated with high-sensitivity troponin T levels \( (r = -0.537) \).
| Study | Country | Samples | Sample Type | Target Gene | Report Culture | Positive | NR | Notes |
|-------|---------|---------|-------------|-------------|----------------|----------|----|-------|
| La Scola et al.²⁵ | France | 155 | Nasopharyngeal swab or sputum | E gene | Not reported | Yes | NR | An association between Ct value and culture positivity rate was observed: samples with Ct values of 13–17 all led to positive culture; culture positivity rate then decreased progressively according to Ct values to reach 12% at Ct = 33; no culture was obtained from samples with Ct > 34 |

CK-MB, creatinine kinase myocardial band; Ct, cycle threshold; IQR, inter-quartile range; LDH, lactate dehydrogenase; RT-PCR, real-time polymerase chain reaction

**Figures**
Figure 1

PRISMA flow diagram