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LETTER TO THE EDITOR

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Comparing studies of SARS-CoV-2 viral loads requires caution

To the editor,

Since the emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), research groups around the world are unraveling key factors of the associated disease, coronavirus disease 2019 (COVID-19). In light of this, many studies have sought to elucidate predictors for COVID-19 severity to guide clinical management and prognosis of the disease.[1] With this in mind, a growing body of evidence suggests that severe cases of COVID-19 are linked with pronounced cytokine storm, high levels of C-reactive protein, D-dimer, immunoglobulin G, total antibodies, lymphopenia, lymphocyte dysfunction and activation, monocyte and granulocyte abnormalities.[1–4] Moreover, many studies have evaluated the association between viral load and COVID-19 severity with controversial findings.[3,5–12] In summary, a cumulative body of data obtained during the COVID-19 pandemic course has demonstrated high, little, or no statistical correlation between viral load and severity in COVID-19 patients. Taken together, these results published until now demonstrate that this is a question that remains unclear and undefined.

Throughout the COVID-19 pandemic, many studies have suggested that the high viral load was associated with a higher risk of severe disease in COVID-19 patients.[5,8] In one of the first reports assessing the relationship between viral load and COVID-19 severity, Liu et al.[5] analyzed the C_t values in patients classified with mild and severe disease using 76 respiratory specimens. After quantitative reverse transcription-polymerase chain reaction (RT-qPCR) analysis, the results demonstrated that the viral load in nasopharyngeal specimens of COVID-19 severe cases was around 60 times higher than mild cases, and this positive correlation was maintained during the first 12 days of infection.[5] In a similar study, SARS-CoV-2 RNA viral shedding was evaluated in 3497 samples (serum, respiratory, stool, and urine) from 96 consecutively admitted COVID-19 patients in a hospital in Zhejiang province, China.[8] Viral load in respiratory specimens, with exception of stool and serum, of individuals with severe disease was higher than in individuals with mild disease.[8] In severe ill patients, male gender and old age was associated with longer viral shedding.[8] Similarly, these findings also corroborate with outcomes reported by other research teams across the world.[10,13–15]

In contrast, several reports have pointed that the high viral load was not associated with a higher risk of severe disease in COVID-19 patients.[9,16,17] For instance, a multicenter cross-sectional retrospective study was conducted by Abdulrahman et al.[9] using data obtained from Bahrain's National COVID-19 Task force's centralized database to explore whether a correlation exists between viral load and COVID-19 severity. A multivariable logistic regression was applied to assess for a correlation using data from a total of 1057 admitted COVID-19 cases. In summary, the results showed that the C_t values obtained from RT-qPCR showed no statistical significance for an association with the requirement for oxygenation on admission among COVID-19 patients.[9] In the midst of the COVID-19 pandemic, what factors have led to this controversial association between viral load and COVID-19 severity? A probable answer is the use of RT-qPCR C_t values instead of true quantitative determinations.[18,19]

At present, most studies just considered the C_t value for analysis viral load among COVID-19 patients, instead of the number of RNA copies/ml. In fact, C_t values are correlated with the amount of viral RNA in a sample.[20] However, C_t values cannot be directly compared across RT-qPCR assays and, therefore, they must be interpreted with caution.[20] Notably, the exclusive use of C_t value to assess viral load can represent a bias during the statistical analysis since many technical issues that might impact and alter the C_t value during RT-qPCR reactions—including differences in protocols, threshold values, viral target, primers, enzymes, and research kits, calibration of RT-qPCR machine, type of biological samples, and period of sample collection,[19] which means that the C_t value not represent the best parameter to assess viral load in COVID-19 patients. With this in mind, I suggest that further studies should consider a combination of C_t values and RNA copies/ml for viral load analysis among COVID-19 patients. This new perspective, combined with the evaluation of host-related factors (e.g., age, sex, comorbidities, etc.)[19,21] will be critical to understand the real impact of SARS-CoV-2 viral load on COVID-19 disease severity.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Severino Jefferson Ribeiro da Silva conceived the work, wrote the original manuscript, and reviewed the final version of the submitted manuscript.

DATA AVAILABILITY STATEMENT

The author declares that there are no data available.

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REFERENCES

1. Yang L, Liu S, Liu J, et al. COVID-19: immunopathogenesis and immunotherapeutics. *Signal Transduct Target Ther*. 2020;5(1):128.
2. Cao X. COVID-19: immunopathology and its implications for therapy. *Nat Rev Immunol*. 2020;20(5):269-270.
3. Tan L, Kang X, Ji X, et al. Validation of predictors of disease severity and outcomes in COVID-19 patients: a descriptive and retrospective study. *Med (N Y)*. 2020;1(1):128-138.e3.
4. Zheng Z, Peng F, Xu B, et al. Risk factors of critical & mortal COVID-19 cases: a systematic literature review and meta-analysis. *J Infect*. 2020;81(2):e16-e25.
5. Liu Y, Yan LM, Wan L, et al. Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect Dis*. 2020;20(6):656-657.
6. Shi F, Wu T, Zhu X, et al. Association of viral load with serum biomakers among COVID-19 cases. *Virology*. 2020;546:122-126.
7. Yu F, Yan L, Wang N, et al. Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients. *Clin Infect Dis*. 2020;71:793-798.
8. Zheng S, Fan J, Yu F, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. *BMJ*. 2020;369:m1443.
9. Abdulrahman A, Mallah SI, Alqahtani M. COVID-19 viral load not associated with disease severity: findings from a retrospective cohort study. *BMC Infect Dis*. 2021;21(1):688.
10. KnudtzeN FC, Jensen TG, Lindvig SO, et al. SARS-CoV-2 viral load as a predictor for disease severity in outpatients and hospitalised patients with COVID-19: a prospective cohort study. *PLoS One*. 2021;16(10):e0258421.
11. Elzein S, Chehab O, Kanj A, et al. SARS-CoV-2 infection: initial viral load (iVL) predicts severity of illness/outcome, and declining trend of iVL in hospitalized patients corresponds with slowing of the pandemic. *PLoS One*. 2021;16(9):e0255981.
12. Magalhães JJF, Mendes RPG, Silva CTA, Silva SJR, Guarines KM, Pena L. Epidemiological and clinical characteristics of the first 557 successive patients with COVID-19 in Pernambuco state, Northeast Brazil. *Travel Med Infect Dis*. 2020;38:101884.
13. Boyapati A, Wipperman MF, Ehmann PJ, et al. Baseline severe acute respiratory syndrome viral load is associated with coronavirus disease 2019 severity and clinical outcomes: post hoc analyses of a phase 2/3 trial. *J Infect Dis*. 2021;224(11):1830-1838.
14. Pérez-García F, Martin-Vicente M, Rojas-García RL, et al. High SARS-CoV-2 viral load and low CCL5 expression levels in the upper respiratory tract are associated with COVID-19 severity. *J Infect Dis*. 2021:jiab604.
15. Fajnzylber J, Regan J, Coxen K, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. *Nat Commun*. 2020;11(1):5493.
16. Yilmaz A, Marklund E, Andersson M, et al. Upper respiratory tract levels of severe acute respiratory syndrome coronavirus 2 RNA and duration of viral RNA shedding do not differ between patients with mild and severe/critical coronavirus disease 2019. *J Infect Dis*. 2021;223(1):15-18.
17. Rodriguez C, de Prost N, Fourati S, et al. Viral genomic, metagenomic and human transcriptomic characterization and prediction of the clinical forms of COVID-19. *PLoS Pathog*. 2021;17(3):e1009416.
18. Silva LN, Oliveira S, Magalhães LB, et al. Clinical and laboratory diagnosis of SARS-CoV-2, the virus causing COVID-19. *ACS Infect Dis*. 2020;6:1273-1282.
19. da Silva SJR, de Lima SC, da Silva SC, Kohl A, Pena L. Viral load in COVID-19 patients: implications for prognosis and vaccine efficacy in the context of emerging SARS-CoV-2 variants. *Front Med*. 2021.8:1–15.
20. Shah VP, Farah WH, Hill JC, et al. Association between SARS-CoV-2 cycle threshold values and clinical outcomes in patients with COVID-19: a systematic review and meta-analysis. *Open Forum Infect Dis*. 2021;8(9):ofab453.
21. Barek MA, Aziz MA, Islam MS. Impact of age, sex, comorbidities and clinical symptoms on the severity of COVID-19 cases: a meta-analysis with 55 studies and 10014 cases. *Heliyon*. 2020;6(12):e05684.