Association of SARS-CoV-2 Viral Load with Abnormal Laboratory Characteristics and Clinical Outcomes in Hospitalized COVID-19 Patients

Lilan Zheng†, Liping Qiu†, Luxi Wu†, Jianwei Wang†, Haihua Xie†, Junjun Wang†, Yi Huang**, Fawen Chen†

†Department of Clinical Laboratory, Fujian Provincial Hospital South Branch, Fuzhou, Fujian, China.

‡Department of Clinical Laboratory, Fujian Provincial Hospital, Fuzhou, Fujian, China.

††Lilan Zheng, Liping Qiu and Luxi Wu contributed equally to this work.

*Corresponding Author: Fawen Chen, M.D., Department of Clinical Laboratory, Fujian Provincial Hospital South Branch, Fuzhou, Fujian, China. Email: chenfawenfj@126.com.

Yi Huang, M.D., PH.D., Department of Clinical Laboratory, Fujian Provincial Hospital, Fuzhou, Fujian, China. Email: hyi8070@126.com.

This is an Open Access article, distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives licence (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is unaltered and is properly cited. The written permission of Cambridge University Press must be obtained for commercial re-use or in order to create a derivative work.
Summary

We conducted a retrospective, analytical cross-sectional and single-center study that included 190 hospitalized COVID-19 patients in Fujian Provincial Hospital South Branch between December 2022 and January 2023 to analyze the correlation of viral loads of throat swabs with clinical progression and outcomes. To normalize the Ct value as quantification of viral loads, we used RNase P gene as internal control gene, and subtracted the Ct value of SARS-CoV-2 N gene from Ct value of RNase P gene, termed △Ct. Most patients were discharged (84.2), only 10 (5.6%) individuals who had a lower level of △Ct died. The initial △Ct of participants was also significantly correlated with some abnormal laboratory characteristics. And the duration time of SARS-CoV-2 was longer in patients with severe symptoms and lower △Ct at admission. Our study suggested that △Ct may aid as a predictor of disease progression and outcomes in hospitalized COVID-19 patients.
Introduction

The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been last more than three years since it broke out in late 2019[1-3]. The numerous variants have emerged in the world, such as alpha (B.1.1.7), beta (B.1.351), gamma (P.1), delta (B.1.617.2), and Omicron (B.1.1.529)[4-6]. Different to the previous variants, Omicron, possessing an unprecedented number of mutations, rapidly spread around the world and became the dominant circulating variant for its high transmissibility[7-9]. Though Omicron variant was more transmissible, many data and studies have shown that unlike the previous variants, Omicron variant was less virulent and cause less severe illness and mortality[10-14]. However, it was worthy to note that Omicron cases in paediatric and elderly patients had a higher admission frequency[15-18]. Moreover, elderly populations with comorbidities such as diabetes, hypertension, cardiovascular disease and chronic lung disease were danger to develop severe symptoms[17, 19].

A number of studies have revealed the association of SARS-CoV-2 viral loads with disease severity and outcomes in COVID-19 patients[20, 21]. Notably, viral loads, especially in the plasma, are associated with laboratory abnormalities and mortality[22]. There are two common methods to measure viral loads. One is to calculate viral load by a linear range of a SARS-CoV-2 RT-qPCR assay with a standard curve, which is accurate and give a specific value[23, 24]. But each experiment adding a standard curve is time-consuming and high cost for mass screening of SARS-CoV-2. Another method is threshold cycle (Ct) values of reverse transcriptase polymerase chain reaction (RT-PCR) which can indicate the initial quantity of template, and the lower Ct means higher viral loads[25, 26]. Undoubtedly, the second method is more convenient and economical.
However, both of these methods do not consider the potential variation in the process of sample collection and experiments.

To eliminate the mistakes caused by the sample collection and experiments, researchers add an internal control gene, RNase P gene to obtain a normalized value, termed delta Ct (\( \Delta \)Ct) which calculated by subtracting Ct value of target gene from the Ct value of internal gene[27-30]. Additionally, further study have indicated that \( \Delta \)Ct could better reflect the exact viral loads[30]. But the application of \( \Delta \)Ct as indicator in viral load associated studies are scarce [28]. Therefore, we considered the clinical significance of \( \Delta \)Ct as viral loads indicator to monitor the disease severity and regression in hospitalized COVID-19 patients infected with Omicron. In this study, we investigated the epidemiology, clinical and laboratory characteristics of hospitalized COVID-19 patients from December 2022 to January 2023 in our hospital, using \( \Delta \)Ct to analyze the correlation of viral loads with disease progression and outcomes in hospitalized COVID-19 patients.

**Methods**

**Study design**

We enrolled 190 hospitalized patients at Fujian Provincial Hospital South Branch with COVID-19 confirmed by RT-PCR tests (SARS-CoV-2 Nucleic Acid Detection Kit, XY-202210146, DAAN GENE) from 1st December 2022 to 1st February 2023, excluding pregnant women and newborns. Patients were classified into two groups: non-severe and severe (including severe and critical) according to the severity of COVID-19 defined by the WHO guideline[31, 32]. Because the median Ct of RNase P for the oropharyngeal swab (OPS) specimens in previous study and our study was 23.9 and 27.9, respectively, and the common group of Ct value was usually five interval, which participants were stratified as <25, 25-30, >30, so in our study patients were also stratified into
three groups according to △Ct of initial collection: △Ct < 0, 0-5, >5 [28, 33, 34]. △Ct was calculated by subtracting the RNase P gene Ct from the target gene Ct: △Ct = Ct_{N1} - Ct_{RNase P}.

Epidemiological, clinical, and laboratory characteristics, outcomes data and RT-PCR results (the Ct number of N gene of SARS-CoV-2 viral RNA and RNase P gene region) were obtained from hospital electronic medical records. The laboratory characteristics analyzed in this study were within three days after the first collection of throat swab. As the early hospitalization regulation required patients’ RT-PCR results of SARS-CoV-2 when hospitalization and take RT-PCR test every three days, so some patients could have a series of RT-PCR results.

Ethics declaration

This study was approved by Ethics Review Committee of Fujian Provincial Hospital. Informed written consent was not obtain as it is a retrospective study that had not any risk to the patients. No patients were directly involved in the study process or asked questions in the study.

Statistical analyses

Study data were analyzed by the Social Sciences (SPSS) version 20.0 and graphs were draw by GraphPad Prism 8.0.2. Categorical variables were described by frequency and percentages with 95% confidence intervals (CIs) and continuous variables were displayed by the median and interquartile range (IQR) with 95% CIs. The homogeneity of data was performed by Levene’s test. The equality of means of continuous variables were compared by two-samples t test when analyzed between two groups, and when compared multiple groups, performed by One-Way ANOVA with Welch’s correction following post hoc multiple comparisons with LSD’s test or Dunnett’s T3 if equal variance not assumed. The Pearson χ² test and Fisher’s exact test were used to compared categorical variables. The relationship between variables was analyzed by Pearson’s
correlation test or Spearman rank-based test in ordinal variable or when normal distribution not assumed. All statistical tests were two-tailed, and $p<0.05$ was considered statistically significant.

Results

Characteristics and SARS-CoV-2 viral loads of participants.

As Table 1 shown, a total of 190 hospitalized patients diagnosed with COVID-19 by RT-PCR were enrolled in this study. Of these participants, the median age was 74 years with a majority of patients older than 65 years, and patients younger than 18 years were only one patient with age of 7 years. The majority of the participants had comorbidities with hypertension (56.3%) being the most frequent, following by diabetes (40%) and cardiovascular disease (38.4%). In patients with definite negative result of RT-PCR, the median days of SARS-CoV-2 duration time was 18 days with the longest time of 50 days. The median of hospitalization time was almost 16 days with the maximum of 142 days, and majority of the participants were discharged, only a few patients transferred and died.

All the participants were further stratified into three groups according to the initial $\Delta$Ct: lower than zero, between zero to five, higher than five, and these groups accounted for 27.9%, 33.7%, 38.4%, respectively. There was no statistically significant difference in age and gender among these three groups. From Table1, we can find that there was significant association between the outcomes and $\Delta$Ct of initial sampling (Pearson $\chi^2$ test, $p<0.01$). Additionally, the date of initial sampling was across from the -1st day to the 22nd day after onset, and most of the sample focused on the 5th to 15th day (figure 1a).

Furthermore, to analyze the relationship of viral loads with disease severity, we compared the $\Delta$Ct between non-severe and severe groups. As figure 1a shown, the date of hospital admission
and the level of $\Delta$Ct were evenly distributed between non-severe and severe groups. Meanwhile, there was no significant difference in $\Delta$Ct value between non-severe and severe groups ($p=0.590$, figure 1b). On the other hand, analyzed as categorical variable, there was also no significant association between $\Delta$Ct and disease severity ($p=0.356$, Table1).

The association of SARS-CoV-2 viral loads with abnormal laboratory characteristics

Moreover, the viral loads of SARS-CoV-2 were significantly correlated with several abnormal laboratory characteristics (figure 2a). As for haematology investigations, lower $\Delta$Ct of initial sampling is significantly associated with lower lymphocyte count (Spearman’s $r=0.216$, $p=0.009$) and platelet count (Spearman’s $r=0.282$, $p=0.001$). However, there was no relationship of white blood cell count and red blood cell count with viral loads. Regarding cardiac biomarkers, the higher level of troponin I (TnI), creatine kinase (CK), and lactic dehydrogenase (LDH) was significantly associated with lower $\Delta$Ct at the time of initial collection. In addition, significant associations with viral loads were also observed in some inflammation makers, such as C-reactive protein (CRP) and procalcitonin (PCT), though there was no association between viral loads and interleukin-6 (IL-6). Besides, there was also significant relationships in levels of aspartate transaminase (AST) and blood urea nitrogen (BUN) with viral loads.

Next, we stratified participants into three groups according to the initial $\Delta$Ct: $<0$, 0-5, $>5$, and compared the difference of abnormal laboratory characteristics among these groups. As figure 2b shown, individuals with $\Delta$Ct lower than zero more likely have abnormal laboratory results, such as lower lymphocyte count and platelets. Besides, the levels of CRP, LDH, and BUN were also significantly different among patients with various $\Delta$Ct at admission. Nevertheless, there was no significant difference of $\Delta$Ct with other laboratory characteristics that existed significant
association when $\Delta Ct$ was analyzed as continuous variable by Spearman’s test.

**SARS-CoV-2 viral loads is associated with the outcomes of hospitalized patients.**

As table 1 shows, of all the participants, 18 patients survived and 10 patients died, accounted for 10.2% and 5.6%, respectively. Analyzed by Pearson $\chi^2$ test, there was significant correlation between the outcomes and the $\Delta Ct$ at admission ($p=0.04$). Meanwhile, patients who were eventually died had lower levels of initial sampling $\Delta Ct$ than discharged patients, and the median $\Delta Ct$ of patients who were died was less than zero (figure 3a). Additionally, from figure 3b and table 1, we can find that compared to the patients whose $\Delta Ct$ was between zero and five or greater than five (3.3% and 2.9%), patients with initial $\Delta Ct$ less than zero had a higher mortality (12.2%).

On the other hand, as for the dynamics of SARS-CoV-2, sequential RT-PCR results of some patients were analyzed. As figure 4 depicted, the viral loads of most participants peaked at the second week after symptom onset, irrespective of the disease severity or the level of $\Delta Ct$ at the time of initial collection. Besides, the length of COVID-19 duration days was longer in severe patients than non-severe patients. And compared to patients whose $\Delta Ct$ was higher than five, patients with $\Delta Ct$ lower than five had a longer duration time.

It was worth to note that there was a patient whose initial $\Delta Ct$ was lower than zero having a duration time longer than sixty days. After in-depth analysis, we discovered that otherwise the lower initial $\Delta Ct$, this patient had a severe symptom, and importantly, the date of admission was at the 20th days after symptom onset, which indicated the importance of early treatment.

**Discussion**

In this study, we described the epidemiology and laboratory characteristics of 190 hospitalized COVID-19 patients infected with Omicron variant, and further analyzed the association of viral
loads with the abnormal laboratory profile and outcomes. It is the first study that apply △Ct to examine the relationship of SARS-CoV-2 viral loads with disease progression and outcomes.

Consistent with studies of other variants, our results also suggested that the viral loads in Omicron infection had a clear relationship with the outcomes of hospitalized patients [22, 35]. Of all participants, there are 10 patients eventually died, and more than half of them had an initial △Ct less than zero. Besides, the median of initial △Ct in patients who finally died was lower than patients who were discharged. These results suggested that elderly patients with initial △Ct less than zero at admission may need more attention. A previous study has reported that Omicron variant has lower mortality than Delta variant (4.0% versus 8.3%) and in our study, the mortality was 5.6%, which was also lower than Delta variant [36]. Our results also indicated that the virulence of Omicron variant was decreased.

Moreover, we also revealed that the higher viral loads are significantly linked with abnormality of laboratory tests. In our study, patients with lower △Ct are more likely to have abnormal laboratory results, such as lower platelet count, higher levels of CRP, AST, and BUN. It is clear that except causing lung injury, SARS-CoV-2 infection could also result in multi-organ dysfunction, and the potential mechanism may be the hyperinflammatory response, which can be reflected in the elevated levels of CRP and PCT of COVID-19 patients in our study, as well [37-39]. Also, the decreased platelet count may result from the hyperinflammatory state induced platelet destruction, named immune thrombocytopenia (ITP)[40]. Besides, the levels of AST, BUN and TnI, which were respectively liver, kidney and heart disease related biomarkers, increased as the △Ct decreased. Therefore, the initial △Ct could reflect the disease progression of COVID-19 in multi-organ injury.

However, in contrast to the previous studies[21, 28], the levels of △Ct between non-severe
and severe patients were not significantly different in our study. One of the reasons may be the sample types. As the report indicated, virus detected in plasma is more likely to be harmful and clinically meaningful than the virus existing in the respiratory tract, therefore, the relationship between viral loads and disease severity may be more significant in plasma sample than throat swab [22, 41]. On the other hand, researchers have reported that expired breath of confirmed cases contains high amounts of virus in Omicron variant [42, 43], and the high titres in the upper respiratory tract are possible to weaken the difference of viral loads between non-severe and severe patients.

Notably, our results showed that the hospitalization rate caused by Omicron only increased in elderly people, but not in children. Here, we excluded the neonatal who delivered in our hospital. There was only one patient who was seven years, and the majority of hospitalized patients was older than 65 years. On the one hand, we think it may profit from the general vaccination in children, which protect them from severe symptoms, and can be treated well at home. While the elderly people may have lower vaccination rates than children and almost have more than one comorbidity, which make them more susceptible to develop severe disease. On the other hand, as our hospital are comprehensive hospital, so pediatric patients with severe symptoms are likely go to to other Children's hospitals to get better therapy.

Nevertheless, there are some limitations in our study. First, we can only gain the RT-PCR results after hospitalization, while the data of viral loads in early course of disease were dismissed. The initial ΔCt at different stages after symptom onset may interfere with the analysis, although most collections were at the second week of onset. Besides, the RT-PCR report prior to December of 2022 had only qualitative results without specific Ct values, which contributed to a few patients
not being included. Additionally, only a few patients had a series of RT-PCR results and almost half of participants were lack of the final negative report, so it was difficult to analyze the exact viral dynamics in this epidemic. Moreover, the laboratory profile analyzed in this study was within three days of initial \( \Delta Ct \), and we did not further track the subsequent dynamic change of laboratory test results as the viral loads fluctuated. Moreover, we had little information on transferred patients, and the number of death patients was limited, which may affect the analysis of relationships between viral loads and outcomes of hospitalized patients. Therefore, larger and multi-center studies are further needed. In addition, the \( \Delta Ct \) value in plasma would be more appropriate to monitor the disease progression and therapy response. Regrettably, we did not analyze the correlation of \( \Delta Ct \) with the management and treatment of hospitalized COVID-19 patients, which will meaningful in the evaluation of treatment effectiveness.

In summary, we analyzed the epidemiology and laboratory characteristics of hospitalized COVID-19 patients, and further revealed the association of viral loads with abnormal laboratory results and outcomes. Our study suggested that \( \Delta Ct \), which represent the viral loads, could become a parameter to predict the disease progression and outcomes of multi-organ injury outside respiratory system caused by SARS-CoV-2 infection in hospitalized patients.

**Funding**

There was no specific funding received for this study.

**Data Availability Statement**

The data that support the findings of this study are available from Fujian Provincial Hospital. Restrictions apply to the availability of these data, which were used under licence for this study.
Data are available from the authors with the permission of Fujian Provincial Hospital.

**Potential Conflicts of Interest**

All authors declare no potential conflicts of interest.

**Author contributions**

The study idea, approach, and methods were designed by Fawen Chen, Yi Huang and Lilan Zheng. Data collection were achieved by Liping Qiu and Luxi Wu. Lilan Zheng and Liping Qiu were responsible for the data analysis. Lilan Zheng, Liping Qiu and Luxi Wu wrote the manuscript. All authors reviewed the manuscript and approved the final version.

**Acknowledgements**

We would like to thank all the individuals who participated in the study and all the staff members and physicians at Fujian Provincial Hospital South Branch who assisted with implementing this project.
References
(9) Davies MA, et al. Outcomes of laboratory-confirmed SARS-CoV-2 infection during resurgence driven by Omicron lineages BA.4 and BA.5 compared with previous waves in the Western Cape Province, South Africa. Journal of Infectious Disease 2023.
(17) Fericean RM, et al. Outcomes of Elderly Patients Hospitalized with the SARS-CoV-2 Omicron B.1.1.529 Variant: A Systematic Review. International Journal of Environmental...
Research and Public Health 2023; 20(3).


Table 1. Demographics and clinical characteristics of COVID-19 hospitalized patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>All patients (n=190)</th>
<th>△Ct of initial collection, n(%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>△Ct, median(range)</td>
<td>3.70 (-7.40,15.0)</td>
<td>△Ct of initial collection, n(%)</td>
<td></td>
</tr>
<tr>
<td>Age, years, median(IQR)</td>
<td>74(65.81)</td>
<td>△Ct</td>
<td>0.825</td>
</tr>
<tr>
<td>Age group, n(%)</td>
<td></td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>&lt; 18 years</td>
<td>1(0.5)</td>
<td>△Ct</td>
<td>0.643</td>
</tr>
<tr>
<td>18-45 years</td>
<td>10(5.3)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>46-64 years</td>
<td>35(18.4)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>≥65 years</td>
<td>144(75.8)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Gender, n(%)</td>
<td></td>
<td>△Ct</td>
<td>0.359</td>
</tr>
<tr>
<td>Female</td>
<td>69(36.3)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>121(63.7)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Comorbidities, n(%)</td>
<td></td>
<td>△Ct</td>
<td>0.411</td>
</tr>
<tr>
<td>Diabetes</td>
<td>76(40.1)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>107(56.3)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Chronic Lung disease</td>
<td>19(10.1)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>73(38.4)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Renal disease</td>
<td>15(7.9)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Cause of hospitalization n(%)</td>
<td></td>
<td>△Ct</td>
<td>0.411</td>
</tr>
<tr>
<td>COVID-19</td>
<td>92(48.9)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>96(51.1)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Severity n(%)</td>
<td></td>
<td>△Ct</td>
<td>0.356</td>
</tr>
<tr>
<td>Non-severe</td>
<td>76(65)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>41(35)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>COVID-19 duration, days, median(range)</td>
<td>18.14(7,50)</td>
<td>△Ct</td>
<td>0.709</td>
</tr>
<tr>
<td>Length of hospitalization, days, median(range)</td>
<td>15.71(3,142)</td>
<td>△Ct</td>
<td>0.383</td>
</tr>
<tr>
<td>Outcomes</td>
<td></td>
<td>△Ct</td>
<td>0.040</td>
</tr>
<tr>
<td>Discharged</td>
<td>149(84.2)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Transfer</td>
<td>18(10.2)</td>
<td>△Ct</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>10(6.6)</td>
<td>△Ct</td>
<td></td>
</tr>
</tbody>
</table>

IQR: Inter quartile range. p values were calculated by Pearson χ² test or Fisher’s exact test or One-Way ANOVA test according to the variable type. Statistically significant values are in bold.