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Association of SARS-CoV-2 viral load with abnormal laboratory characteristics and clinical outcomes in hospitalised COVID-19 patients

Lilan Zheng[1†], Liping Qiu[1†], Luxi Wu[1†], Jianwei Wang[1], Haihua Xie[1], Junjun Wang[1], Yi Huang[2,3,4,5] and Fawen Chen[1]

[1]Department of Clinical Laboratory, Fujian Provincial Hospital South Branch, Fuzhou, Fujian, China; [2]Shengli Clinical Medical College, Fujian Medical University, Fuzhou, Fujian, China; [3]Department of Clinical Laboratory, Fujian Provincial Hospital, Fuzhou, Fujian, China; [4]Central Laboratory, Fujian Provincial Hospital, Fuzhou, Fujian, China and [5]Center for Experimental Research in Clinical Medicine, Fujian Provincial Hospital, Fuzhou, Fujian, China

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Corresponding authors:
Fawen Chen and Yi Huang;
Emails: chenfawenfj@126.com;
hyi8070@126.com

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

Abstract

We conducted a retrospective, analytical cross-sectional and single-centre study that included 190 hospitalised COVID-19 patients in the Fujian Provincial Hospital South Branch between December 2022 and January 2023 to analyse the correlation of viral loads of throat swabs with clinical progression and outcomes. To normalise 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 the Ct value of RNase P gene, termed ΔCt. Most patients were discharged (84.2%), and only 10 (5.6%) individuals who had a lower ΔCt value died. The initial ΔCt value 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 a lower ΔCt value at admission. Our study suggested that the ΔCt value may be used as a predictor of disease progression and outcomes in hospitalised COVID-19 patients.

Introduction

The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has last more than 3 years since it broke out in late 2019 [1–3]. 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 from the previous variants, Omicron, which possess an unprecedented number of mutations, rapidly spread around the world and became the dominant circulating variant due to its high transmissibility [7–9]. Although the Omicron variant is more transmissible, data and many studies have shown that unlike the previous variants, the Omicron variant is less virulent and causes 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 more likely 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 the viral load by a linear range of a SARS-CoV-2 RT-qPCR assay with a standard curve, which is accurate and gives a specific value [23, 24]. But each experiment adding a standard curve is time-consuming and incurs a high cost for mass screening of SARS-CoV-2. The second method is threshold cycle (Ct) values of reverse transcriptase polymerase chain reaction (RT-PCR), which can indicate the initial quantity of the template, and a lower Ct value denotes higher viral loads [25, 26]. Undoubtedly, the second method is more convenient and economical. However, both methods do not consider the potential variation in the process of sample collection and experiments.

To eliminate the sample collection and experimental errors, researchers include an internal control gene, RNase P gene, to obtain a normalised value, termed delta Ct(ΔCt), which is calculated by subtracting the Ct value of the target gene from the Ct value of the internal gene [27–30]. Additionally, further study has indicated that the ΔCt value could better reflect the exact viral loads [30]. But studies on the ΔCt value as an indicator of the viral load to explore the association of viral load with clinical progression of COVID-19 are scarce [28]. Therefore, we considered the clinical significance of the ΔCt value as a viral load indicator to monitor the disease severity and regression in hospitalised COVID-19 patients infected with the Omicron variant. In this study, we investigated the epidemiology and clinical and laboratory characteristics

of hospitalised COVID-19 patients from December 2022 to January 2023 in our hospital, using the △Ct value to analyse the correlation of viral loads with disease progression and outcomes in hospitalised COVID-19 patients.

Methods

Study design

We enrolled 190 hospitalised patients at the 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 1 December 2022 to 1 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 guidelines [31, 32]. Because the median Ct value of RNase P for the oropharyngeal swab (OPS) specimens in a previous study and our study was 23.9 and 27.9, respectively, and the common group of the Ct value was usually five intervals, which participants were stratified as <25, 25–30, and >30, therefore, in our study, patients were also stratified into three groups according to the △Ct value of initial collection: △Ct <0, 0–5, and >5 [28, 33, 34]. The △Ct value was calculated by subtracting the Ct value of the RNase P gene from that of the target gene as follows: $\triangle Ct = Ct_{N1}\text{-}Ct_{RNase\ P}$.

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 analysed in this study were within 3 days from the first collection of throat swab. As the early hospitalisation regulation required patients' RT-PCR results of SARS-CoV-2 during hospitalisation and required taking RT-PCR tests every 3 days, some patients could have a series of RT-PCR results.

Ethics declaration

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

Statistical analyses

Study data were analysed by Social Sciences (SPSS) version 20.0, and graphs were drawn by GraphPad Prism 8.0.2. Categorical variables were described as frequency and percentages with 95% confidence intervals (CIs), and continuous variables were displayed as 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 using the two-sample t test when analysing two groups, and when comparing multiple groups, it was performed by using one-way ANOVA with Welch's correction and post hoc multiple comparisons with LSD's test or Dunnett's T3, if equal variance was not assumed. The Pearson χ^2 test and Fisher's exact test were used to compare categorical variables. The relationship between variables was analysed by Pearson correlation test or the Spearman rank-based test in ordinal variables or when normal distribution was 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 shown in Table 1, a total of 190 hospitalised patients diagnosed with COVID-19 by RT-PCR were enrolled in this study. The median age of these participants was 74 years, with a majority of patients older than 65 years, and only one patient was younger than 18 years (7 years old). 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 hospitalisation time was almost 16 days, with the maximum of 142 days. The majority of the participants were discharged, and only a few patients transferred and died.

All the participants were further stratified into three groups according to the initial △Ct: lower than zero, between zero and five, and higher than five, and these groups accounted for 27.9%, 33.7%, and 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 △Ct values of initial sampling (Pearson $\chi 2$ test, $p<0.01$). Additionally, the date of initial sampling was between the 1st day and the 22nd day after onset, and most of the sample focused on the 5th to 15th day (Figure 1a).

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

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 △Ct values of initial sampling are significantly associated with lower lymphocyte counts (Spearman's r = 0.216, $p = 0.009$) and platelet counts (Spearman's r = 0.282, $p = 0.001$). However, there was no relationship of white blood cell and red blood cell counts with viral loads. Regarding cardiac biomarkers, higher levels of troponin I (TnI), creatine kinase (CK), and lactic dehydrogenase (LDH) were significantly associated with lower △Ct values 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), although there was no significant association between viral loads and interleukin-6 (IL-6). Besides, there was also significant relationships of aspartate transaminase (AST) and blood urea nitrogen (BUN) levels with viral loads.

Next, we stratified participants into three groups according to the initial △Ct values,<0, 0–5, and >5, and compared the difference in abnormal laboratory characteristics among these groups. As shown in Figure 2b, individuals with a △Ct value

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

Variable	All patients (n = 190)	△Ct of initial collection, n(%)			*p* value
		<0	0–5	>5	
△Ct,median(range)	3.70 (−7.40,15.0)	53(27.9)	64(33.7)	73(38.4)	
Age, years, median (IQR)	74(65,81)	72(65–82)	70(64,80)	72(65,82)	0.825
Age group, n(%)					0.643
<18 years	1(0.5)	1(1.9)	0(0)	0(0)	
18–45 years	10(5.3)	1(1.9)	4(6.2)	5(6.8)	
46–64 years	35(18.4)	10(18.9)	12(18.8)	13(17.8)	
≥65 years	144(75.8)	41(77.4)	48(75)	55(75.3)	
Gender, n(%)					0.359
Female	69(36.3)	15(28.3)	25(39.1)	29(39.7)	
Male	121(63.7)	38(71.7)	39(60.9)	44(60.3)	
Comorbidities, n (%)	156(82.1)				
Diabetes	76(40)	25(47.2)	24(37.5)	27(37.0)	0.454
Hypertension	107(56.3)	26(49.1)	38(59.4)	43(58.9)	0.454
Chronic lung	19(10.1)	6(11.3)	6(9.4)	7(9.7)	0.935
Cardiovascular disease	73(38.4)	22(41.5)	23(35.9)	28(38.4)	0.827
Renal disease	15(7.9)	6(11.3)	5(7.8)	4(5.5)	0.486
Cause of hospitalisation, n(%)					0.411
COVID-19	92(48.9)	26(49.1)	35(54.7)	31(43.7)	
Others	96(51.1)	27(50.9)	29(45.3)	40(56.3)	
Severity, n(%)					0.356
Non-severe	76(65)	16(57.1)	28(62.2)	32(72.7)	
Severe	41(35)	12(42.9)	17(37.8)	12(27.3)	
COVID-19 duration, days, median (range)	18.14(7,50)	19.43(14,35)	19.08(10,50)	16.88(7,29)	0.709
Length of hospitalisation, days, median(range)	15.71(3,142)	15.04(5,42)	17.62(3,49)	13.96(4,142)	0.383
Outcomes					**0.040**
Discharged	149(84.2)	35(71.4)	55(91.7)	59(86.8)	
Transfer	18(10.2)	8(16.3)	3(5.0)	7(10.3)	
Death	10(5.6)	6(12.2)	2(3.3)	2(2.9)	

Note: *p* values were calculated by Pearson $\chi 2$ test or Fisher's exact test or one-way ANOVA test according to the variable type. Statistically significant values are in bold.
Abbreviations: IQR, interquartile range.

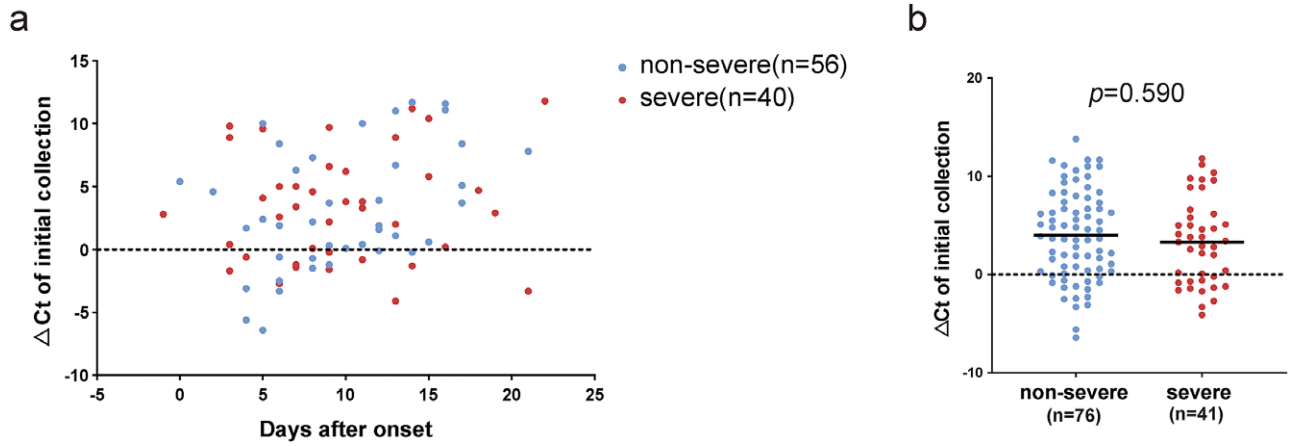

Figure 1. SARS-CoV-2 viral loads of hospitalized patients on admission. (a) Levels of SARS-CoV-2 viral loads at the time of initial collection across the days since the onset of symptoms and disease severity. The onset of symptoms was day 0. Each dot represents one patient, blue dot means non-severe patient, and red dot means severe patient. $n = 96$, and of these, 56 patients were non-severe and 40 patients were severe. (b) Comparison of SARS-CoV-2 viral loads between two groups: non-severe and severe. $n = 117$, and of these, 76 patients were non-severe and 41 patients were severe. Black line represents medians. *P* value was calculated by two-samples t test.

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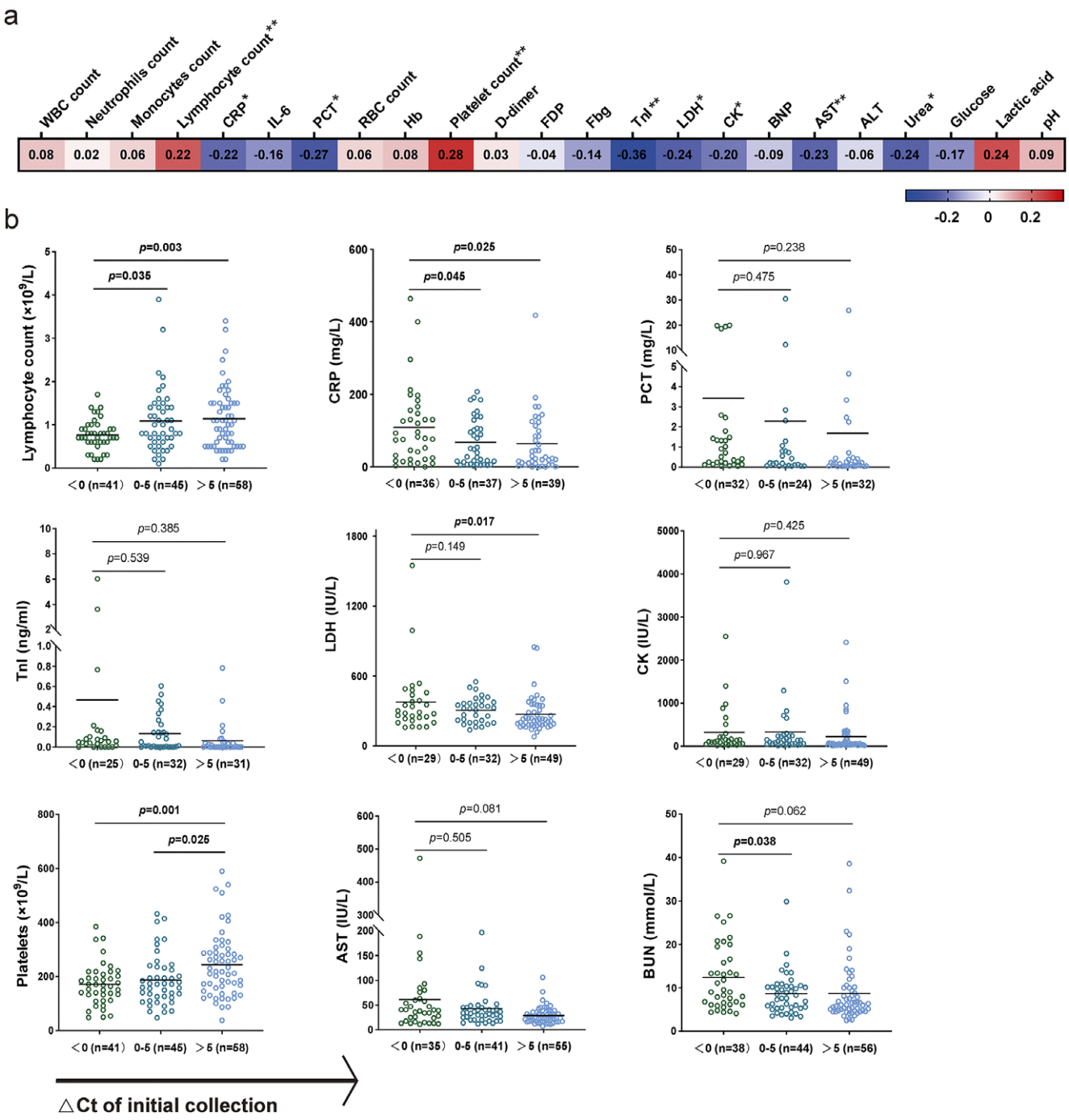

Figure 2. SARS-CoV-2 viral loads are associated with laboratory characteristics. (a) Heat map of Spearman's correlation value of initial sampling Ct with laboratory characteristics. **P* 0.05 ***P* 0.01. (b) The difference of laboratory characteristics among groups stratified by Ct of initial collection as three groups: Ct 0, 0-5, 5, analyzed by one-way ANOVA following post hoc multiple comparisons with LSD's test or Dunnett's T3 when equal variance not assumed. *P* values in bold are statistically significant. Black line represents medians. WBC, white blood cell, RBC, red blood cell, IL-6, interleukin-6, Hb, hemoglobin, CRP, C-reactive protein, PCT, procalcitonin, Fbg, fibrinogen, FDP, fibrinogen degradation products, TnI, troponin I, LDH, lactic dehydrogenase, CK, creatine kinase, BNP, brain natriuretic peptide, ALT, alanine aminotransferase AST, aspartate transaminase, BUN, blood urea nitrogen.

lower than zero were more likely to have abnormal laboratory results, such as lower lymphocyte and platelet counts. Besides, the levels of CRP, LDH, and BUN were also significantly different among patients with various △Ct values at admission. Nevertheless, there was no significant difference in △Ct values with other laboratory characteristics which was associate with △Ct value significantly when △Ct was analysed as continuous variable by Spearman's test above.

SARS-CoV-2 viral load is associated with the outcomes of hospitalised patients

As shown in Table 1, of all the participants, 149 patients discharged, 18 patients transfered and 10 patients died, which accounted for 84.2%, 10.2% and 5.6%, respectively. As analysed by the Pearson $\chi 2$ test, there was a significant correlation between the outcomes and the △Ct values at admission (p = 0.04).

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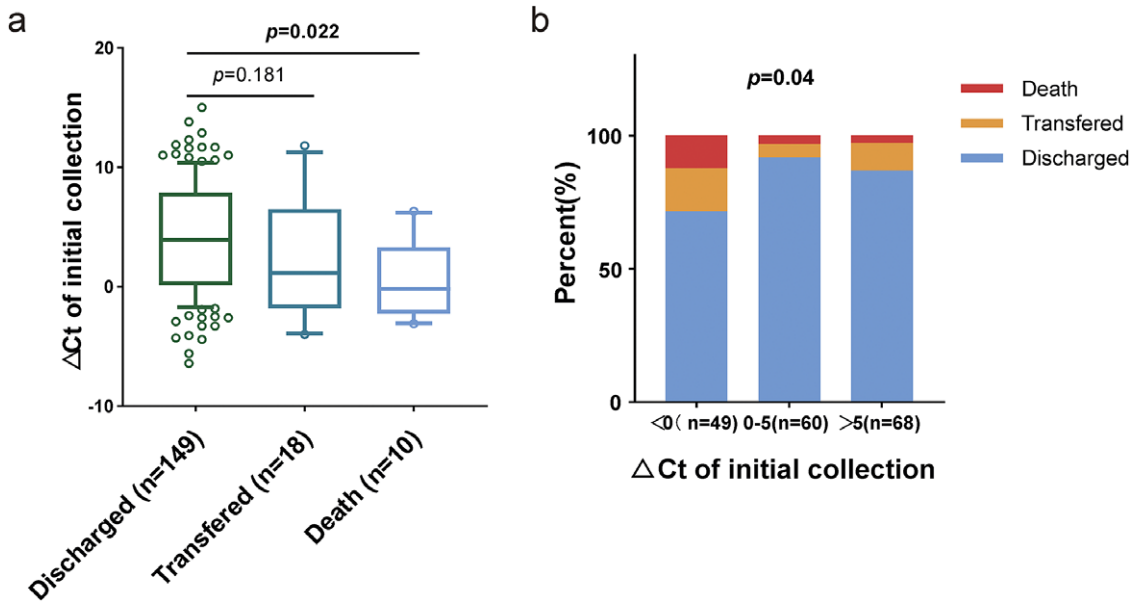

Figure 3. Correlation between viral loads and outcomes. (a) Participants who transferred or died had lower Ct of initial sampling compared to those who discharged. The center line represents median, and the whiskers represent the 10–90 percentile. *P* value was calculated by LSD's test. (b) The proportion of patients who eventually discharged, transferred, and died among groups stratified by Ct at admission. *P* value was calculated by Pearson 2 test. *P* values in bold are statistically significant.

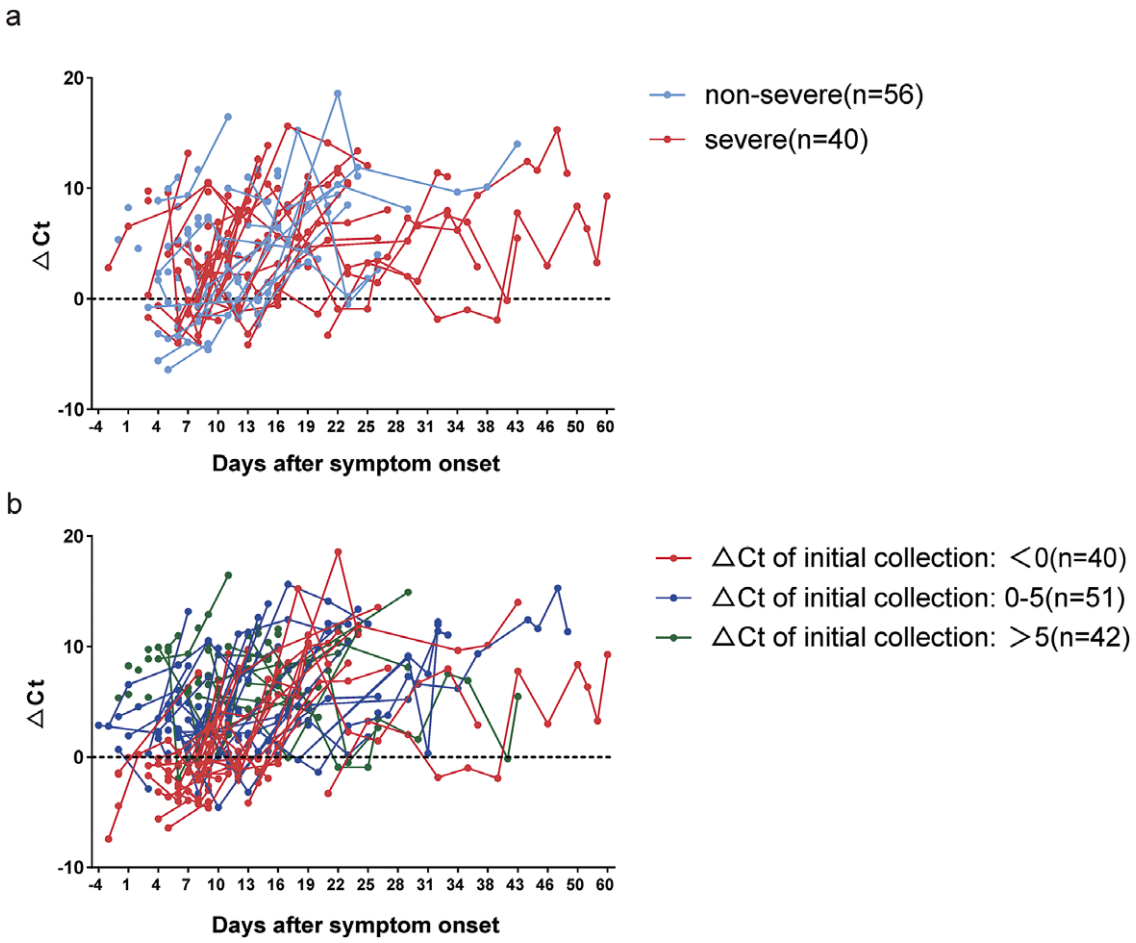

Figure 4. Viral load dynamics of SARS-CoV-2 across symptom onset. (a, b) Longitudinal viral load detection of hospitalized patients with days after symptom onset. Each line depicts a serial of sampling of Ct of one patient. (a) the blue dot means non-severe ($n = 56$), and red dot means severe ($n = 40$). (b) The green dot means Ct of initial sampling higher than 5 ($n = 42$), blue dot means Ct of initial sampling between 0 and 5 ($n = 51$), red dot means Ct of initial sampling lower than 0 ($n = 40$). Spearman's correlation test was used to analyze the relationship between Ct of initial collection and laboratory results

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Meanwhile, patients who eventually died had lower levels of initial sampling △Ct values than discharged patients, and the median △Ct values of patients who died were less than zero (Figure 3a). Additionally, as shown in Figure 3b and Table 1, we can find that compared to the patients whose △Ct values were between zero and five or greater than five (3.3% and 2.9%), patients with initial △Ct values 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 analysed. As depicted in Figure 4, the viral loads of most participants peaked at the second week after the symptom onset, irrespective of the disease severity or the level of △Ct values at the time of initial collection. Besides, the length of COVID-19 duration days was longer in severe patients than in non-severe patients. Moreover, compared to patients whose △Ct values were higher than five, patients with △Ct values lower than five had a longer duration time.

It was worth noting that there was a patient with initial △Ct values lower than zero and a duration time longer than 60 days. After in-depth analysis, we discovered that besides the lower initial △Ct values, this patient had severe symptoms and, importantly, the date of admission was at the 20th day after symptom onset, which indicated the importance of early treatment.

Discussion

In this study, we described the epidemiology and laboratory characteristics of 190 hospitalised COVID-19 patients infected with the Omicron variant and further analysed the association of viral loads with the abnormal laboratory profile and outcomes. It is the first study to apply △Ct values 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 hospitalised patients [22, 35]. Of all participants, 10 patients eventually died, and more than half of them had an initial △Ct value less than zero. Besides, the median of initial △Ct values in patients who finally died was lower than that of patients who were discharged. These results suggested that elderly patients with initial △Ct values less than zero at admission may need more attention. A previous study has reported that Omicron variant has lower mortality than the Delta variant (4.0% versus 8.3%), and in our study, mortality was 5.6%, which was also lower than that of the Delta variant [36]. Our results also indicated that the virulence of the Omicron variant 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 values are more likely to have abnormal laboratory results, such as lower platelet count and 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. The potential mechanism may be the hyperinflammatory response, which can be reflected in the elevated levels of CRP and PCT in COVID-19 patients in our study[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 values decreased. Therefore, the initial △Ct value could reflect the disease progression of COVID-19 in multi-organ injury.

However, in contrast to the previous studies [21, 28], the △Ct values 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 samples than throat swabs [22, 41]. On the other hand, researchers have reported that expired breath of confirmed cases contains high amounts of virus in the Omicron variant [42, 43], and the high titres in the upper respiratory tract are possible to weaken the difference in viral loads between non-severe and severe patients.

Notably, our results showed that the hospitalisation rate caused by Omicron only increased in elderly people, but not in children. Here, we excluded the neonatals who were delivered in our hospital. There was only one patient who was 7 years old, and the majority of hospitalised patients were older than 65 years. As for the reason of different hospitalization rate between children and elderly people, 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. As the elderly people may have lower vaccination rates than children and almost have more than one comorbidity, they are more susceptible to develop severe disease. On the other hand, as our hospitals are comprehensive hospital, paediatric patients with severe symptoms are likely go to other children's hospitals to get better therapy.

Nevertheless, there are also some limitations in our study. First, we can only gain the RT-PCR results after hospitalisation, while the data of viral loads in the early course of disease were dismissed. The initial △Ct values 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 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. Almost half of the participants lacked the final negative report, so it was difficult to analyse the exact viral dynamics in this epidemic. Moreover, the laboratory profile analysed in this study was within 3 days of initial △Ct values, and we did not further track the subsequent dynamic change of laboratory test results as the viral loads fluctuated. What's more, 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 hospitalised patients. Therefore, larger and multicentre studies are needed. In addition, the △Ct value in plasma would be more appropriate to monitor the disease progression and therapy response. Regrettably, we did not analyse the correlation of △Ct values with the management and treatment of hospitalised COVID-19 patients, which will be meaningful in the evaluation of treatment effectiveness.

In summary, we analysed the epidemiology and laboratory characteristics of hospitalised COVID-19 patients and further revealed the association of viral loads with abnormal laboratory results and outcomes. Our study suggested that △Ct values, which represent viral loads, could be used as a parameter for predicting the disease progression and outcomes of multi-organ injury outside of the respiratory system caused by SARS-CoV-2 infection in hospitalised patients.

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

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Author contribution. The study idea, approach, and methods were designed by Fawen Chen, Yi Huang, and Lilan Zheng. Liping Qiu and Luxi Wu contributed to data collection. 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.

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Competing interest. All authors declare no potential conflicts of interest.

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