

Dynamic Changes of ORF1ab and N Gene Ct Values in COVID-19 Omicron Inpatients of Different Age Groups — Beijing Municipality, China, November–December 2022

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ABSTRACT

Introduction: In November 2021, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron variant was identified as the variant of concern and has since spread globally, replacing other cocirculating variants. To better understand the dynamic changes in viral load over time and the natural history of the virus infection, we analyzed the expression of the open reading frames 1ab (ORF1ab) and nucleocapsid (N) genes in patients infected with Omicron.

Methods: We included patients initially admitted to the hospital for SARS-CoV-2 infection between November 5 and December 25, 2022. We collected daily oropharyngeal swabs for quantitative reverse transcriptase-polymerase chain reaction tests using commercial kits. We depicted the cycle threshold (Ct) values for amplification of ORF1ab and N genes from individual patients in age-specific groups in a time series.

Results: A total of 480 inpatients were included in the study, with a median age of 59 years (interquartile range, 42 to 78; range, 16 to 106). In the <45-year-old age group, the Ct values for ORF1ab and N gene amplification remained below 35 for 9.0 and 11.5 days, respectively. In the ≥80-year-old age group, the Ct values for ORF1ab and N genes stayed below 35 for 11.5 and 15.0 days, respectively, which was the longest among all age groups. The Ct values for N gene amplification took longer to rise above 35 than those for ORF1ab gene amplification.

Conclusion: The time to test negative varied among different age groups, with viral nucleic acid shedding taking longer in older age groups compared to younger age groups. As a result, the time to resolution of Omicron infection increased with increasing age.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 and has been prevalent for over three years (1–3). As of January 8, 2023, more than 659 million confirmed cases and over 6.6 million deaths have been reported globally (4). The continuous evolution of SARS-CoV-2 has produced multiple variants of concern, including Alpha, Beta, Gamma, Delta, and Omicron, which have caused several waves of coronavirus disease 2019 (COVID-19) during the pandemic (5).

The Omicron variant was first detected in November 2021 in South Africa and contained extensive mutations that enhanced its transmissibility, allowing it to spread globally within a month (6). As a result, Omicron quickly replaced the other cocirculating variants worldwide.

As indicated in the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 9), a sample with cycle threshold (Ct) values greater than 35 for both open reading frames 1ab (ORF1ab) and nucleocapsid (N) genes by two consecutive tests (with at least 24 hours in between) is the laboratory criterion for hospital discharge of the patient. Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) of nasopharyngeal swabs or oropharyngeal swabs is recommended for clinical diagnosis of SARS-CoV-2 infection (7). The Omicron variant has a shorter incubation period compared to that of previous variants. Currently, patients infected with the Omicron variant generally recover within 7 days. However, the dynamic changes of SARS-CoV-2 infection have not been systematically analyzed. In this study, we collected samples from 480 patients with SARS-CoV-2 and assessed the amplification of target SARS-CoV-2 genes to describe the natural history of SARS-CoV-2 Omicron variant infection through the

viral load over time in infected patients.

METHODS

Between November 5 and December 25, 2022, patients hospitalized in Beijing Ditan Hospital, the designated hospital for clinical treatment of COVID-19 in Beijing, with confirmed COVID-19 in the early stages of acute infection, whose Ct values of SARS-CoV-2 gradually decreased after admission, were enrolled in this study. All infections belonged to the Omicron BF.7 sub-variant, as established via next-generation sequencing. Oropharyngeal swabs were collected daily from the enrolled cases during hospitalization, and viral RNA was extracted from the swabs using a Nucleic Acid Extraction System (Xi'an Tianlong Science and Technology CO., Ltd., Xi'an, China). qRT-PCR was used to detect SARS-CoV-2 RNA from patient samples, with RNA expressed from the N and ORF1ab genes of SARS-CoV-2 being detected. Ct values based on amplification from detected RNA were used as indicators of the copy number of SARS-CoV-2 RNA in specimens; lower Ct values represented a higher viral load in the specimens. Additionally, general and clinical information of the patients was collected, including sex, age, and underlying medical comorbidities. Patients were divided into four groups according to their age, and a boxplot of Ct values over time was plotted.

RESULTS

A total of 480 cases were enrolled from November 5 to December 25, 2022, with a median age of 59 years (interquartile range, 42 to 78; range, 16 to 106). The

male-to-female sex ratio of all cases was 0.81. Patients were divided into four age groups: <45, 45–59, 60–79, and ≥80 years old (Table 1), containing 130, 112, 128, and 110 patients, respectively. The proportion of male and female patients in each age group was similar. In the <45-year-old age group, 93.8% of patients had no underlying medical comorbidities, whereas 88.2% of patients in the ≥80-year-old age group had one or more underlying medical comorbidities (Table 1). The risk of developing underlying medical comorbidities increased with increasing age.

The Ct values for amplification of ORF1ab and N RNA changed over time during hospitalization, initially decreasing and then increasing (Figure 1 and Supplementary Table S1, available in <https://weekly.chinaccdc.cn/>). This indicated that all cases were in the early stages of infection. Except for those in the <80-year-old age group, the Ct values for the ORF1ab and N genes were lowest at day 3, indicating the highest viral load at the inflection point. However, in the ≥80-year-old age group, the Ct values were lowest at day 2 (Figure 1 and Supplementary Table S1), which differed from the other age groups. As the viral load peak was reached, the Ct values gradually increased, indicating a gradual decrease in viral load.

The median time for the Ct value for ORF1ab RNA amplification to reach >35 in the <45-year-old group was 9 days (range: 4 to 26) (Figure 1), whereas this was 11.5 days for the Ct value for N RNA to reach >35 (range: 7 to 26). In the 45–59-year-old group, the Ct values for ORF1ab and N genes reached >35 after a median of 9 (range: 7 to 23) and 11 days (range: 8 to 23), respectively (Figure 1). In the 60–80-year-old group, these medians were 11 (range: 6 to 24) and 14 days (range: 7 to 28), respectively. In the ≥80-year-old group, the times for the Ct values for ORF1ab and N

TABLE 1. Baseline characteristics of the enrolled coronavirus disease 2019 patients by age group in Beijing (n=480).

Variable	Age group			
	<45 years (n=130)	45–59 years (n=112)	60–79 years (n=128)	≥80 years (n=110)
Sex, n (%)				
Male	53 (40.8)	52 (46.4)	59 (46.1)	51 (46.4)
Female	77 (59.2)	60 (53.6)	69 (53.9)	59 (53.6)
Underlying medical comorbidities*, n (%)				
No underlying disease	122 (93.8)	73 (65.2)	27 (21.1)	13 (11.8)
One condition	3 (2.3)	16 (14.3)	43 (33.6)	34 (30.9)
Two conditions	4 (3.1)	15 (13.4)	32 (25.0)	29 (26.4)
Three and more conditions	1 (0.8)	8 (7.1)	26 (20.3)	34 (30.9)

* The underlying medical comorbidities included coronary heart disease, high blood pressure, diabetes, thyroid disease, and so on.

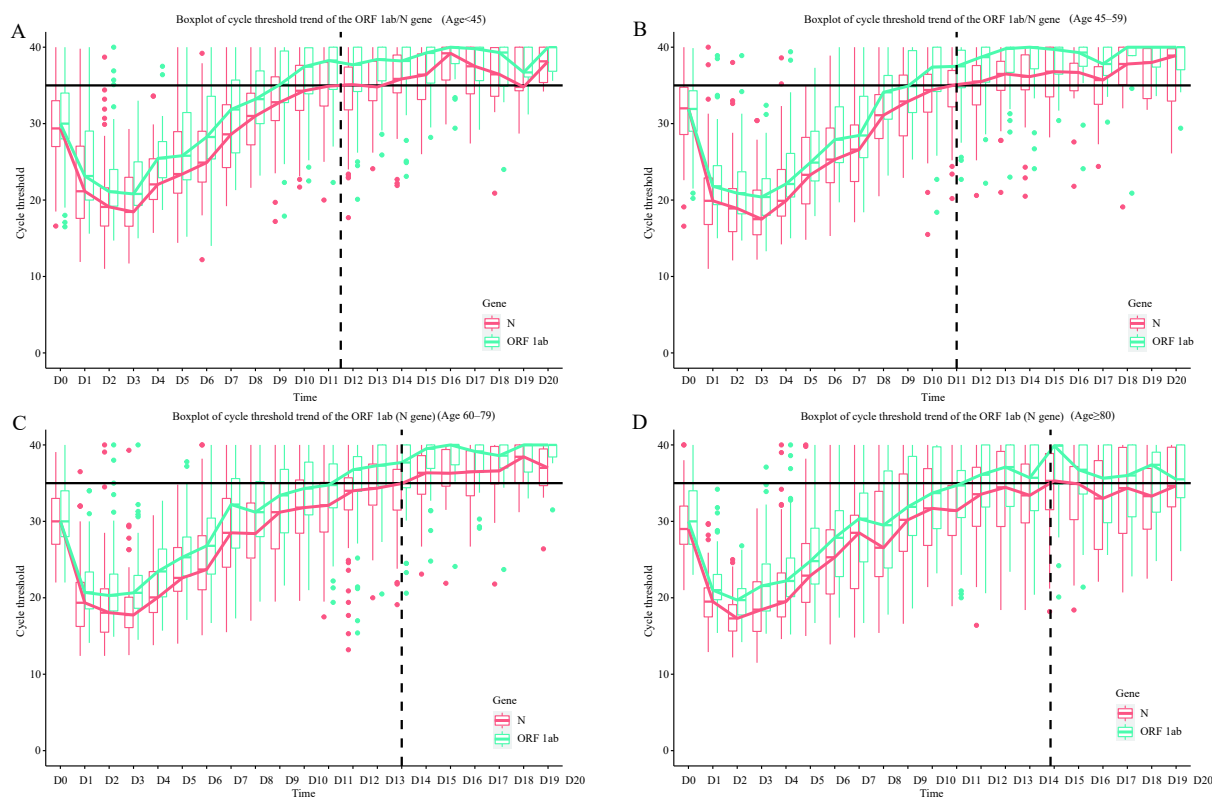


FIGURE 1. Dynamic trends in cycle threshold (Ct) for ORF1ab and N RNA amplification in patients with COVID-19 in different age groups. (A) <45-year-old group, (B) 45–59-year-old group, (C) 60–79-year-old group, and (D) ≥ 80 -year-old group.

Note: Blue boxes and lines represent the ORF1ab gene. The red boxes and lines represent the N gene. Day 0 represents the first positive test for nucleic acid, based on onset date of the disease.

Abbreviation: COVID-19=coronavirus disease 2019; ORF1ab=open reading frames 1ab; N=nucleocapsid.

amplification to reach >35 were 11.5 (range: 6 to 39) and 15 days (range: 6 to 39), respectively, which were the longest times in the study (Figure 1). These results indicated that the time required for a negative result ($Ct > 35$) based on the presence of N RNA in COVID-19 infections was longer than that for ORF1ab RNA. Furthermore, the time required for detection of the N and ORF1ab Ct values to reach >35 in people ≥ 60 years old was longer than that in people < 60 years old.

CONCLUSIONS

In this study, we analyzed the dynamic change of ORF1ab and N RNA in patients with COVID-19 over time. We found that the time to a negative result varied among different age groups, with viral nucleic acid shedding persisting for longer in the older age groups than in the younger groups. This may be due to underlying medical comorbidities in the older age groups. We also observed that it took longer for the N gene Ct value to rise to 35 than for the ORF 1ab gene,

indicating that the N gene is more sensitive than the ORF 1ab gene in diagnosis.

Early studies of the COVID-19 outbreak reported the viral load of SARS-CoV-2 in different types of clinical specimens from infected patients (8–10). These studies suggested that the infectious period of Omicron was shorter than the previous strain (8). However, the sample size was small and patients were not always continuously sampled for testing. In our study, we collected samples from hospitalized patients on a daily basis and graphically depicted the changing trend of ORF1ab and N RNA detection via their Ct values.

Our study has several limitations. We only collected oropharyngeal swab samples from patients, so we were unable to assess the trend of viral load in other sample types. Additionally, the assignment of people with very mild symptoms to makeshift hospitals instead of designated hospitals for treatment may have introduced selection bias. Furthermore, some patients did not have detailed clinical information, so we were unable to determine whether Ct values changed with

different severity of disease in these patients. Treatment of different cases may have impacted on the change of Ct values.

In conclusion, we examined the natural history and dynamic changes of ORF1ab and N RNA in patients with COVID-19 across different age groups. Patients in the older age group took longer to test negative compared to those in the younger age group, indicating that the time to resolution of Omicron infection increased with age.

Conflicts of interest: No conflicts of interest.

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SUPPLEMENTARY TABLE S1. Changes of Ct values for N and ORF1ab genes from Day 0 to 20 in each age group of COVID-19 patients in Beijing.

Time	Age group, <45 years			Age group, 45-59 years			Age group, 60-79 years			Age group, ≥80 years		
	N, Ct, M* (Q ₁ , Q ₃)	ORF1ab, Ct, M (Q ₁ , Q ₃)	N, Ct, M (Q ₁ , Q ₃)	ORF1ab, Ct, M (Q ₁ , Q ₃)	N, Ct, M (Q ₁ , Q ₃)	ORF1ab, Ct, M (Q ₁ , Q ₃)	N, Ct, M (Q ₁ , Q ₃)	ORF1ab, Ct, M (Q ₁ , Q ₃)	N, Ct, M (Q ₁ , Q ₃)	ORF1ab, Ct, M (Q ₁ , Q ₃)		
Day0	29.4 (27.0, 33.0)	30.0 (28.0, 34.0)	32.0 (28.6, 34.8)	31.9 (29.0, 34.3)	30.0 (27.0, 33.0)	30.0 (28.0, 34.0)	29.0 (27.0, 32.0)	30.0 (27.3, 34.0)				
Day1	21.2 (17.6, 27.1)	23.1 (20.0, 29.2)	19.9 (16.8, 22.9)	21.8 (19.4, 24.5)	19.4 (16.3, 22.0)	20.5 (18.5, 23.5)	19.5 (17.5, 21.3)	21.0 (19.7, 22.6)				
Day2	19.1 (16.6, 21.6)	21.1 (19.2, 24.1)	18.9 (15.9, 21.5)	20.9 (18.2, 23.7)	18.3 (15.5, 21.4)	20.3 (18.3, 23.1)	17.3 (15.7, 19.1)	19.7 (17.8, 21.2)				
Day3	18.5 (16.6, 23.0)	20.8 (19.3, 25.0)	17.5 (15.5, 21.3)	20.4 (18.0, 22.8)	17.8 (16.1, 20.1)	20.7 (18.6, 23.0)	18.5 (15.6, 22.1)	21.6 (18.3, 24.4)				
Day4	22.1 (20.1, 25.4)	25.5 (22.9, 27.7)	19.9 (17.9, 24.0)	22.1 (20.4, 26.1)	20.1 (18.1, 23.4)	23.5 (20.4, 26.5)	19.5 (17.5, 23.1)	22.2 (20.2, 25.2)				
Day5	23.4 (20.9, 29.0)	25.8 (22.7, 31.5)	23.3 (19.5, 28.2)	24.9 (22.5, 29.0)	22.6 (20.9, 26.6)	25.3 (22.8, 27.8)	22.9 (19.4, 27.1)	24.8 (21.8, 29.1)				
Day6	25.0 (22.5, 29.2)	28.3 (25.4, 33.6)	25.3 (22.3, 29.4)	28.0 (25.0, 32.4)	23.7 (21.1, 28.1)	27.0 (23.3, 30.6)	25.3 (20.5, 28.9)	27.9 (22.8, 32.3)				
Day7	28.6 (24.3, 32.5)	31.9 (26.4, 35.8)	26.6 (22.4, 29.8)	28.6 (25.3, 33.7)	28.5 (24.0, 33.0)	32.1 (26.5, 35.5)	28.5 (21.2, 30.8)	30.4 (24.8, 33.7)				
Day8	31.0 (27.2, 34.0)	33.3 (30.0, 37.0)	31.1 (28.1, 33.8)	34.4 (30.7, 37.1)	28.4 (25.2, 32.4)	31.2 (28.0, 35.2)	26.6 (22.8, 33.9)	29.5 (23.9, 36.6)				
Day9	32.9 (30.4, 36.3)	35.7 (32.8, 40.0)	32.9 (28.4, 36.4)	34.9 (31.5, 39.6)	31.2 (26.4, 35.0)	33.7 (29.1, 38.1)	30.5 (26.3, 36.9)	33.0 (28.6, 39.6)				
Day10	34.3 (31.7, 37.6)	37.5 (34.2, 40.0)	34.4 (31.5, 36.5)	37.5 (34.3, 40.0)	31.8 (28.5, 35.4)	34.3 (30.9, 37.8)	31.7 (26.9, 36.9)	33.7 (29.1, 39.8)				
Day11	35.0 (32.4, 38.1)	38.6 (34.8, 40.0)	35.1 (32.2, 37.0)	37.5 (34.7, 40.0)	32.1 (28.5, 34.8)	35.0 (32.2, 37.6)	31.4 (28.2, 34.7)	34.7 (30.5, 36.7)				
Day12	35.1 (31.8, 37.5)	38.3 (35.0, 40.0)	35.4 (32.4, 37.5)	39.0 (35.5, 40.0)	34.0 (31.5, 35.7)	36.8 (34.0, 38.7)	33.6 (29.4, 37.1)	36.7 (32.0, 40.0)				
Day13	35.0 (33.1, 38.6)	38.5 (36.0, 40.0)	36.5 (34.4, 38.2)	39.8 (37.1, 40.0)	34.4 (32.1, 37.5)	37.4 (35.1, 40.0)	34.5 (29.3, 39.2)	37.4 (32.0, 40.0)				
Day14	35.9 (34.0, 39.0)	38.4 (35.9, 40.0)	36.2 (34.4, 39.0)	40.0 (37.6, 40.0)	35.1 (31.5, 37.1)	37.8 (34.4, 40.0)	33.4 (29.8, 37.5)	35.9 (31.6, 40.0)				
Day15	36.3 (33.0, 39.1)	40.0 (36.0, 40.0)	37.0 (33.6, 40.0)	40.0 (36.3, 40.0)	36.5 (33.8, 38.6)	39.9 (37.0, 40.0)	35.3 (31.5, 38.9)	39.9 (34.9, 40.0)				
Day16	39.2 (35.7, 40.0)	40.0 (38.0, 40.0)	36.7 (34.3, 37.9)	39.6 (37.7, 40.0)	36.3 (34.6, 39.4)	40.0 (37.6, 40.0)	34.9 (30.2, 37.2)	36.8 (33.6, 40.0)				
Day17	37.5 (33.9, 40.0)	40.0 (36.2, 40.0)	35.7 (32.5, 37.5)	39.5 (36.7, 40.0)	36.5 (33.4, 39.3)	39.2 (36.4, 40.0)	33.0 (26.3, 38.0)	36.5 (29.8, 40.0)				
Day18	36.5 (33.6, 40.0)	39.3 (34.9, 40.0)	38.5 (35.2, 40.0)	40.0 (38.9, 40.0)	36.6 (34.9, 39.8)	39.2 (37.6, 40.0)	34.4 (28.4, 39.7)	37.2 (30.8, 40.0)				
Day19	34.8 (34.3, 40.0)	36.7 (36.1, 40.0)	38.0 (33.3, 39.8)	40.0 (37.4, 40.0)	38.5 (35.1, 40.0)	40.0 (36.5, 40.0)	33.3 (29.0, 36.9)	37.4 (29.8, 39.1)				
Day20	38.2 (35.4, 40.0)	40.0 (37.4, 40.0)	39.3 (33.9, 40.0)	40.0 (38.5, 40.0)	37.0 (34.7, 39.5)	40.0 (38.4, 40.0)	34.7 (31.9, 39.7)	36.7 (33.8, 40.0)				

* M indicates the median age in each age group. Q1 and Q3 represent the lower quartile and upper quartile, respectively. Abbreviation: Ct=cycle threshold; N=nucleocapsid; ORF1ab=open reading frames 1ab; COVID-19=coronavirus disease 2019.