EasyChair Preprint

№ 11678

Asymptomatic Carriers Are Associated with Shorter Negative Conversion Time in Children with Omicron Infections

Yang Xiang, Yang Zhou, Sicen Liu, Jinghua Wang, Fang Li, Siyuan Wang, Man Rao, Chuanchuan Yang, Buzhou Tang and Hongzhou Lu

January 4, 2024

Asymptomatic carriers are associated with shorter negative conversion time in children with Omicron infections

Yang Xiang[†,2], Yang Zhou[†,1], Sicen Liu[†,3], Jinghua Wang[4,1], Fang Li[5], Siyuan Wang[1], Man Rao[1],
Chuanchuan Yang[*,1], Buzhou Tang[*,3,2], Hongzhou Lu[*,1]

[1]Shenzhen Third People's Hospital, Shenzhen, Guangdong, China
[2]Peng Cheng Laboratory, China
[3]Harbin Institute of Technology at Shenzhen, Shenzhen, Guangdong, China
[4]Tianjin Medical University General Hospital, Tianjin, China
[5]University of Texas Health Science Center at Houston, Houston, Texas, U.S.
luhongzhou@fudan.edu.cn
†These authors share the first authorship.
*These authors share the corresponding authorship.

Abstract. Objective: Among the Omicron carriers, asymptomatic ones should be paid close attention to due to their silence in clinical symptoms and uncertainty in secondary transmission. The clinical characteristics associated with viral shedding of asymptomatic patients need to be carefully investigated especially for children. Methods: We revisited the clinical data from 471 pediatric patients who have been infected with the SARS-CoV-2 Omicron variant in 2022. The cases were divided into symptomatic and asymptomatic groups according to clinical manifestations. Descriptive analysis and survival analysis were applied for the comparison between the groups.Results: A total number of 333 patients were selected out of the original 471 children according to certain eligibility criteria, which resulted in 192 (57.7%) symptomatic and 141 (42.3%) asymptomatic cases. According to the univariate analysis, we discovered that the asymptomatic carriers had significantly shorter negative conversion time (NCT) (10±8 days) compared with the symptomatic ones (14±7 days) ($p<0.001$). Conclusion: The NCT of asymptomatic patients, is shorter than the symptomatic ones, signifying that the asymptomatic patients may be equipped with shorter periods of self- or centralized isolation. These results could provide important implications for future policy-making or anti-virus treatment to lower the overall transmission risk to society.

.

Keywords: Omicron; Asymptomatic; Negative Conversion; Survival Analysis; Children

1 Introduction

Starting from the end of 2021, Omicron has become one of the predominated variants of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the

infections have exploded worldwide [1]. A study from Shanghai, China shows that in the last year's largest Omicron wave in Shanghai (April-June, 2022), 94.3% of cases were asymptomatic [2]. Researchers found that the incidence rate of Omicron was 6 to 8 times that of the Delta variant in children younger than 5 years old [3]. Accelerating the negative conversion of SARS-CoV-2 for patients is beneficial in reducing the risk of secondary viral transmission [4]. However, systematic research on asymptomatic pediatric patients infected with Omicron remains lacking, and the corresponding information regarding the clinical manifestations and outcomes is still limited. Clinicians usually evaluate the virus infectivity for rapid antigen testing by RT-PCR cycle threshold (Ct) of nucleocapsid protein (N gene) and open reading frame lab (ORF1ab) values [5]. The corresponding Ct values and negative conversion time (NCT) of N genes and ORF1ab are significant indicators in determining the discharge criteria during hospitalization or centralized quarantine [6,7]. Therefore, we carefully considered the aforementioned immunologic factors during our analyses.

In this study, we carried out a retrospective study to analyze the clinical characteristics of a pediatric cohort aged between 1 and 12 years. Attention was especially paid to the asymptomatic cases who were diagnosed with SARS-CoV-2, and Omicron but without any relevant clinical symptoms before hospitalization. Univariate and multivariate analyses were conducted to investigate the clinical outcomes among different groups to benefit the understanding of NCT and reduce the transmission risk of asymptomatic pediatric patients. We experimentally proved the existence of varying NCTs between different groups, hoping that our findings could provide useful implications for the precise prevention and control of the disease.

2 Method

2.1 Cohort selection

This retrospective cohort study was conducted on 471 children aged between 1 and 12 years infected with SARS-CoV-2 Omicron (BA.2) who have been admitted to Shenzhen Third People's Hospital, China, between February 14, 2022, and April 14, 2022, for centralized isolation and clinical treatment. Patients diagnosed with SARS-CoV-2 were based on the positive RT-PCR SARS-CoV-2 testing results from the oropharyngeal and nasal swabs test. A total number of 333 patients were selected out of the original 471 children according to certain eligibility criteria, which resulted in 192 (57.7%) symptomatic and 141 (42.3%) asymptomatic cases.This study was approved by the Institutional Review Board (IRB) of the Shenzhen Third People's Hospital, China. All participants have been informed of the potential benefits, risks, and alternatives associated with this research.

2.2 Group and variable definitions

The clinical characteristics and laboratory test results were collected from the record of each patient. The clinical characteristics include demographic information (sex, age), vaccination status (including doses), and clinical manifestations (including cough, fever, sore throat, runny nose, nasal congestion, weak, bellyache, and vomiting). The laboratory test results include white blood cell count (WBC), lymphocytes (LYMPH), platelets (PLT), prothrombin time (PT), albumin (ALB), Natriuretic Peptide Tests (NT-proBNP), IgM antibody (IgM) and IgG antibody values (IgG), IL6, Ct values for ORF1ab (ORF1ab_Ct) and N gene (N_Ct), etc. Empty values were imputed using the CART algorithm to ensure consistency in distribution [8]. The symptomatic group was identified according to the chief complaint upon admission to the hospital. Cases with a record of any aforementioned clinical manifestations are considered symptomatic. The discharge criteria are defined as the Ct values of both N genes and ORF1ab are higher than 35. The NCT is subsequently identified as the latter time for the satisfaction of the two Ct values. Thus, the negative conversion period is defined as the duration between admission and NCT1. The age was stratified into two groups, i.e. $1 \leq age \leq 6$ and $6 < age \leq 12$.

2.3 Statistical Analyses

In the univariate analysis, the continuous variables which satisfy normal distribution (according to the Shapiro-Wilk normality test) were expressed as the mean and standard deviation (SD) and others as the median and interquartile range (IQR). Categorical variables were described as numbers (percentages). Comparisons between groups (p-values) were estimated by univariate analyses, i.e. Chi-square test for categorical variables, and t-test and Wilcoxon Rank sum test for continuous variables. The risks of asymptomatic or symptomatic manifestations in regard to NCT were estimated using a Kaplan-Meier method that can be used with time-varying covariates. Propensity score matching (1:1 matching according to the clinical variables with the “nearest” matching strategy) and multivariate Cox proportional hazards regression (Hazard ratio [HR] and 95% confidence interval [CI]) were further leveraged to estimate the risk of negative conversion comparing the asymptomatic with the symptomatic group. All analyses were conducted using RStudio with R version 4.1.2. Two-sided p-values less than 0.05 were considered statistically significant.

[1] We use NCT to denote the negative conversion period for simplicity below.

3 Method

3.1 Cohort characteristics

Among the 333 cases, 192 belong to the symptomatic group, and 141 belong to the asymptomatic group.

Table 1. Table captions should be placed above the tables.

Features	Asymptomatic	Symptomatic	p-value	Symptomatic	p-value
		Before PSM		After 1:1 PSM	
Cases, n (%)	141 (42.3)	192 (57.7)		141	
Sex, n (%)			*0.037**		0.339
male	72 (51.1)	120 (62.5)		80 (56.7)	
female	69 (48.9)	72 (37.5)		61 (43.3)	
Age group, n (%)			*0.027**		0.074
≤6	65 (46.1)	112 (58.3)		80 (56.7)	
>6	76 (53.9)	80 (41.7)		61 (43.3)	
Vaccination, n (%)			0.134		0.302
unvaccinated	78 (55.3)	127 (66.1)		90 (63.8)	
vaccinated (1 dose)	34 (24.1)	35 (18.2)		25 (17.7)	
boosted (2 doses)	29 (20.6)	30 (15.6)		26 (18.4)	
vaccinated (1 dose + 2 doses)	63 (44.7)	65 (33.9)		51 (36.2)	
SNI, n (%)			0.63		0.812
No	66 (46.8)	95 (49.5)		71 (50.4)	
Yes	75 (53.2)	97 (50.5)		70 (49.6)	
Laboratory results					
WBC (× 10^9/L), median (IQR)	6.6 (5.2,8.5)	5.5 (4.6,7.6)	*0.001***	5.6 (4.7,7.9)	*0.029**
LYMPH (× 10^9/L), median (IQR)	2.7 (1.9,3.8)	2.4 (1.4,3.4)	*0.02**	2.6 (1.8,3.6)	0.321
PLT (× 10^9/L), median (IQR)	286 (229,342)	256 (212.8,306)	*0.015**	270 (221,323)	0.21
AST(g/L), median (IQR)	35.2 (28.9,41.9)	37.5 (31.2,45.4)	*0.002***	36.3 (29.7,43)	0.25

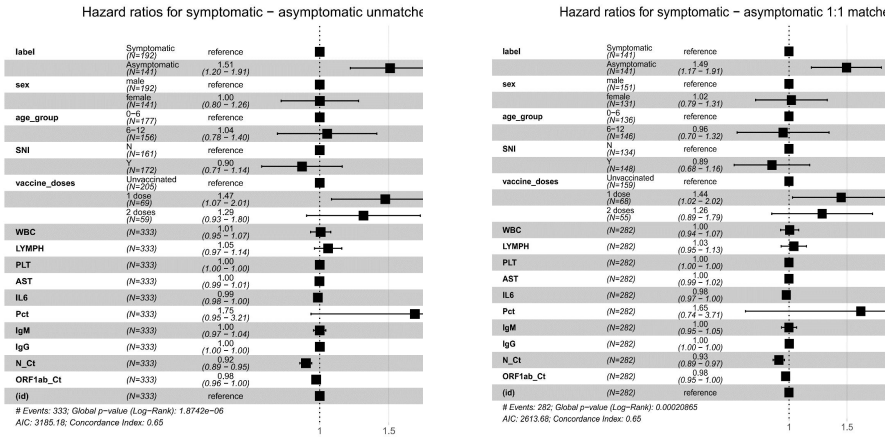
ALB (g/L), mean (SD)	44.9 (43.1,47.8)	45.1 (43.4,46.9)	0.7	45.3 (43.4,46.9)	0.856
PT(second), median (IQR)	13.5 (0.7)	13.7 (0.8)	0.132	13.6 (0.7)	0.414
NT.proBNP （pg/m), median (IQR)	34.8 (19.3,68.1)	36.9 (18.5,76.6)	0.621	34.8 (16.9,72)	0.794
IgM (g/L), median (IQR)	1.1 (0.3,3.1)	0.5 (0.2,1.5)	*0.001***	0.6 (0.2,2)	*0.044**
IgG (g/L), median (IQR)	44.4 (0.6,108.3)	7.2 (0.3,55.5)	< *0.001****	16.7 (0.4,68.3)	*0.012**
IL6 (pg/ml), median (IQR)	6.9 (2.6,11)	7.4 (4.1,12.6)	0.073	7.4 (5,12.6)	0.103
N_Ct, median (IQR)	36.6 (35.1,37.8)	35.8 (33.4,37.3)	*0.002***	36.4 (34.5,37.6)	0.279
ORF1ab_Ct, median (IQR)	36.7 (31.4,38.6)	35.3 (31.1,37.9)	0.069	35.7 (31.2,38.1)	0.2
NCT of N gene (day), median (IQR)	7 (1,15)	13 (9,17)	< *0.001****	13 (9,17)	< *0.001****
NCT of ORF1ab (day), median (IQR)	4 (0,12)	11 (7,14)	< *0.001****	11 (8,14)	< *0.001****
NCT, median (IQR)	10 (3,16)	14 (11,18)	< *0.001****	14 (11,18)	< *0.001****

Significance levels: ***’ 0.001 ‘**’ 0.01 ‘*’ 0.05

The characteristics of these patients are listed in Table 1. For the unmatched cohort, this descriptive analysis demonstrates that male (p=0.037) and younger aged patients (≤ 6) (p=0.027) are more likely to be symptomatic. There are significant differences in several laboratory results including WBC (p=0.001), LYMPH (p=0.02), PLT (p=0.015), AST (p=0.002), IgM (p=0.001), IgG (p<0.001), and N_Ct (p=0.002) between the two groups. Specifically, the NCTs of both N genes and ORF1ab in the asymptomatic group are shorter than those in the symptomatic group (p<0.001), resulting in the overall NCT of the asymptomatic group being significantly shorter (10 [3, 16] days versus 14 days [11, 18], p<0.001). After PSM, the remaining significant laboratory variables are WBC (p=0.029), IgM (p=0.044), and IgG (p=0.012). And the NCTs still show significant differences (p<0.001).

3.2 Hazard ratios of negative conversion

After adjusting to the considered variables, the results of Cox proportional hazard models showed the hazard ratio of each factor considering possible confounders. From Figure 3, we notice that the “asymptomatic” condition is significantly associated with the negative status (1.51 [1.20-1.91], p<0.001 and 1.49 [1.17-1.91], p=0.001). In addition, vaccinated one dose (p=0.017 and p=0.036), a higher level of IgG (p=0.049 and p=0.036), and a lower value of the N gene Ct (p<0.001) show increasing hazards with negative conversion, both before and after PSM.



a) The cox result before PSM. b) The cox result after PSM.

Figure 1. The forest plot shows hazard ratios generated by the Cox model.

4 Discussion

The percentage of asymptomatic infections was 42.3% among pediatric patients in our study, which was higher than that of the general population reported in [9] (32.40% [25.30-39.51%]). In the pooled percentage of asymptomatic infections was 43.75% in the Omicron-positive individuals when the median age is less than 20 years, which was similar to the result in our study. The univariate analysis revealed that the WBC and lymphocytes of the asymptomatic infections are generally higher than those of the symptomatic infections (with minor exceptions). During the same period, the children infected with the Omicron variant with reduced lymphocyte proportion demonstrated a longer time of viral nucleic acid negative conversion in Shanghai [10], which was similar to those discovered in our study. This could be explained by the “cytokine storm” of symptomatic infections. The higher Th1 cytokines including IL-2, IL-8, IL-2, IL-8, IL-12p70, IFN-γ, and TNF-α, as well as Th2 cytokines including IL-10 and IL-13 lead to the depletion of WBC and lymphocytes [11].

We next compared the differences in SARS-CoV2-specific IgM and IgG antibody values and positive rates between symptomatic and asymptomatic infections (Table 1). The IgM value of the asymptomatic group was higher than that of the symptomatic group (1.1 [0.3, 3.1] vs. 0.6 [0.2, 0.2], p=0.044, after PSM). More significantly, the IgG value of asymptomatic infected children was higher than that of the symptomatic

infected children (44.4 [0.6, 108.3] vs. 16.7 [0.4, 68.3], p=0.012, after PSM). These results may indicate that the virus in asymptomatic infected children can stimulate the immune system to produce higher levels of SARS-CoV2-specific IgM and longer-lasting IgG antibodies. Our findings suggested that asymptomatic infected children may produce higher levels of SARS-CoV2-specific IgM and IgG to avoid body damage, thus affecting the clinical manifestations and clinical type of children.

. In our study, we found that vaccination (including one dose and two doses) raises the possibility of asymptomatic manifestation (46.3 % asymptomatic without vaccination versus 55.3% with vaccination), which means vaccination can effectively decrease the risk of appearing symptoms. Results also verified that vaccination was associated with a faster negative conversion (1.37, [1.04-1.80], p=0.027). Children show some loss of cross-neutralization against all variants of SARS-CoV-2, with the most pronounced loss against Omicron, while vaccination can effectively increase high titers cross-neutralization against Alpha, Beta, Gamma, Delta, and Omicron[11].

The transmission risk of children Omicron infections may be different from that of adults. This study found that the NCT of the asymptomatic infected children averages 10 days (3, 16), which is analogous to the NCT (averages 10 days) of the asymptomatic children with the SARS-CoV-2 infection reported in [12]. But the NCT of symptomatic infected children (average 14 days) is longer than the NCT of infected Omicron adults reported in (6-9 days) [13]. The time of nasopharyngeal / pharyngeal swab viral RNA turning negative was reported as ranging from 6 to 22 days (mean 12 days) in previous research [14]. Compared with the symptomatic infected children, the NCT of asymptomatic infected children was shorter. It indicates that the risk of transmission of asymptomatic infections is lower than that of the symptomatic infections, which is similar to the result of adults in a previous study [15].

Multivariate Cox proportional hazards regression demonstrated that the NCT of asymptomatic infection was inversely correlated with the Ct thresholds of N gene ($p<0.001$) and ORF1ab (mildly significant with p-value of 0.066 after PSM) in our research. It showed that even previous studies have summarized that having higher Ct values links to lower amount of viral RNA, which means cases with higher Ct values tend to being asymptomatic [15,16],

This study has several limitations. Firstly, the study found that vaccination is more prone to asymptomatic infection with clinical features, suggesting that vaccination has a protective effect, but we did not further analyze more stratified situations.Secondly, there is a proportion of empty values in the data.In addition, symptomatic and asymptomatic children also have the risk of Long COVID disease. There is an urgent to build a follow-up survey of those children to investigate the risk of long COVID.

5 Conclusion

We conducted a systematic analysis of children infected with Omicron and found that the NCT of asymptomatic patients, is shorter than the symptomatic ones, signifying that the asymptomatic patients may be equipped with shorter periods of

self- or centralized isolation, in front of determining the measures to prevent community transmission. It is also confirmed that vaccination assists in the acceleration of negative conversion. These results could provide important implications for future policy-making, e.g. informing targeted isolation periods, to lower the overall transmission risk to society.

6 Author Contributions

YX, YZ and SL had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis

Concept and design: YX, YZ, BT, HL

Acquisition, analysis, or interpretation of data: SL, SW, MR, CY

Visualization: YX, SL

Statistical analysis: YX, SL

Drafting of the manuscript: YX, YZ, SL

Critical revision of the manuscript for important intellectual content: JW, FL, CY, BT, HL

Supervision: CY, BT, HL

References

1. Karim SSA, Karim QA. Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic. The lancet 2021; 398:2126–2128.
2. Huang L. Adjusted control rate closely associated with the epidemiologic evolution of the recent COVID-19 wave in Shanghai, with 94.3% of all new cases being asymptomatic on first diagnosis. J Infect 2022; 85:e89–e91.
3. Wang L, Berger NA, Kaelber DC, Davis PB, Volkow ND, Xu R. Incidence rates and clinical outcomes of SARS-CoV-2 infection with the omicron and delta variants in children younger than 5 years in the US. JAMA Pediatr 2022; 176:811–813.
4. Zhou L-K, Zhou Z, Jiang X-M, et al. Absorbed plant MIR2911 in honeysuckle decoction inhibits SARS-CoV-2 replication and accelerates the negative conversion of infected patients. Cell Discov 2020; 6:54.
5. Pickering S, Batra R, Merrick B, et al. Comparative performance of SARS-CoV-2 lateral flow antigen tests and association with detection of infectious virus in clinical specimens: a single-centre laboratory evaluation study. Lancet Microbe 2021; 2:e461–e471.
6. Chan JF-W, Yip CC-Y, To KK-W, et al. Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens. J Clin Microbiol 2020; 58:e00310-20.
7. To KK-W, Tsang OT-Y, Leung W-S, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020; 20:565–574.

8. Doove LL, Van Buuren S, Dusseldorp E. Recursive partitioning for missing data imputation in the presence of interaction effects. Comput Stat Data Anal 2014; 72:92–104.
9. Shang W, Kang L, Cao G, et al. Percentage of asymptomatic infections among SARS-CoV-2 omicron variant-positive individuals: a systematic review and meta-analysis. Vaccines 2022; 10:1049.
10. Yin R, Lu Q, Jiao JL, et al. Characteristics and related factors of viral nucleic acid negative conversion in children infected with Omicron variant strain of SARS-CoV-2. Zhonghua Er Ke Za Zhi 2022:1307–1311.
11. 11. Tang J, Novak T, Hecker J, et al. Cross-reactive immunity against the SARS-CoV-2 Omicron variant is low in pediatric patients with prior COVID-19 or MIS-C. Nat Commun 2022; 13:2979.
12. Yin Rong, Lu Quan, Jiao Jiali, et al. Characteristics and related factors of viral nucleic acid negative conversion in children infected with Omicron variant strain of SARS-CoV-2. Chin J Pediatr 2022; 12:1307–1311.
13. Yin Y, Lin J, Yuan S, et al. The relationship between early isolation and the duration of viral shedding of mild and asymptomatic infection with SARS-CoV-2 Omicron BA. 2 variant. J Infect 2022; 85:e184–e186.
14. Jiehao C, Jin X, Daojiong L, et al. A case series of children with 2019 novel coronavirus infection: clinical and epidemiological features. Clin Infect Dis 2020; 71:1547–1551.
15. Kimball A, Hatfield KM, Arons M, et al. Asymptomatic and presymptomatic SARS-CoV-2 infections in residents of a long-term care skilled nursing facility — King County, Washington, March 2020. Morb Mortal Wkly Rep 2020; 69:377.
16. Hall SM, Landaverde L, Gill CJ, et al. Comparison of anterior nares CT values in asymptomatic and symptomatic individuals diagnosed with SARS-CoV-2 in a university screening program. PLoS One 2022; 17:e0270694.

Conflict of Interest Disclosures

All authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Acknowledgment

This work is funded by the National Natural Science Foundation of China (Grant Number: 62106115) [YX], the Shenzhen Science and Technology Plan Project (No. JCYJ20210324132012035) [YZ].

Contact Information

Hongzhou Lu: luhongzhou@fudan.edu.cn
Shenzhen Third People's Hospital, the second affiliated hospital of Southern University of Science and Technology, National Clinical Research Center for Infectious Diseases. No. 29, Bulan Road, Longgang District, Shenzhen 518112, P. R. China.