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Symptom progression and viral shedding dynamics in children and adolescents with asymptomatic and mild COVID-19 infections

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Abstract:

Objective: Limited research has examined the progression of disease symptoms and viral shedding dynamics using continuous daily cycle threshold (Ct) values in large pediatric populations infected with SARS-CoV-2.

Methods: This analysis included 7803 children and adolescents selected from a retrospective cohort of 174,371 individuals with COVID-19. Factors influencing symptom progression and viral shedding were evaluated across asymptomatic, asymptomatic-to-mild and mild symptom groups. Inverse Probability of Treatment Weighting (IPTW) was used to adjust for confounding and correct group imbalances.

Results: During follow-up, 86.2% of initially asymptomatic individuals progressed to mild disease. The median durations of hospital stay and viral shedding were both six days (interquartile range: 4-8 days). Older children (AOR: 1.13-1.53), females (AOR: 1.21), lower nadir N gene (AOR: 0.89) and vaccinated individuals (AOR by IPTW: 1.75-1.99) exhibited a higher likelihood of symptom progression. Older age (AHR: 1.13-1.44) and higher nadir N gene values (AHR: 1.15) were associated with an increased likelihood of viral shedding. Full vaccination demonstrated a modest association with viral shedding (AHR by IPTW: 1.08). Compared to the asymptomatic group, viral shedding was less likely in the asymptomatic-to-mild (AHR: 0.64) and mildly symptomatic (AHR: 0.60) groups.

Conclusion: In pediatric COVID-19 patients, older age, female sex, lower nadir N gene and vaccination are associated with an increased likelihood of progression from asymptomatic to mild symptoms. Older age and higher nadir N gene correlate with a greater probability of viral shedding. Vaccination status correlates with a higher probability of symptomatic

presentation, which may reflect a robust, rapid anamnestic immune response, and a weakly accelerated viral clearance.

Keywords: COVID-19; Viral Shedding; Asymptomatic infection; Mildly symptomatic infection

1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic broke out in Wuhan, China, in late 2019 and rapidly spread worldwide. By January 2025, over 777 million individuals have been infected with severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) globally, resulting in more than 7.1 million deaths[1]. Widespread vaccination campaigns and ongoing natural infections have contributed to the development of population-level immunity, which has helped to control further spread of the disease [2-4]. Although COVID-19 is no longer classified as a global public health emergency, it remains a significant public health concern worldwide [5].

Children and adolescents are defined as individuals under 18 years of age. Due to differences in immune system function compared to adults, they typically experience asymptomatic or mild SARS-CoV-2 infections [6, 7]. Understanding the progression of disease symptoms and viral shedding in pediatric COVID-19 cases is critical for effective disease prevention and control. As infection rates among children and adolescents are relatively low [8, 9], few studies have examined symptom progression and viral shedding at a large scale. In particular, research utilizing continuous daily data on symptoms and viral

changes from admission to discharge in pediatric patients remains limited. Although some studies have reported continuous monitoring data, most included fewer than 500 cases and used sampling intervals of three days or longer [8-10].

Shanghai, one of the largest cities in China, experienced a major outbreak of SARS-CoV-2 infections between April and May 2022, with a total of 600,000 confirmed cases. In response to this public health emergency and as a part of a proactive containment strategy, all residents were required to undergo daily nucleic acid testing. Individuals who tested positive were promptly transferred to Fangcang shelter hospitals for centralized isolation and treatment. The National Exhibition and Convention Center (NECC) Fangcang was the largest shelter hospital during the COVID-19 pandemic, primarily admitting patients with asymptomatic and mild cases. Between April 9 and May 23, 2022, NECC Fangcang admitted a total of 174,308 patients, including 7803 children and adolescents.

In this retrospective study, we utilized continuous daily cycle threshold (Ct) values and associated clinical factors from infection onset to hospital discharge in a large patient cohort. We used logistic regression, Cox regression, and mixed-effects models to assess the impact of various factors on symptom progression and viral shedding. We also applied inverse probability of treatment weighting (IPTW) to adjust for confounding and correct for group imbalances.

2. Materials and methods

2.1 Data source and study design

Data on individuals aged 18 years or younger with asymptomatic or mild symptoms were extracted from the NECC Fangcang cohort [11]. Eligibility criteria included a positive result

on the initial nucleic acid test conducted after admission. Patients with a Ct value above 35 on the day of admission were excluded, in accordance with national prevention and control guidelines[12], which specify that a Ct value below 35 indicates possible nucleic acid infection, whereas a Ct value ≥35 suggests that the patient is unlikely to be infectious. Patients without continuous nucleic acid testing after admission, as the absence of sequential test data precluded longitudinal analysis. In addition, patients who were transferred to other hospitals during hospitalization due to severe clinical conditions (e.g., persistent high fever) were excluded (Table S1), as their final viral shedding status could not be observed. Cases with missing values or outliers were also removed from the dataset. The final cohort comprised 7,803 pediatric patients. The study design flowchart is presented in Figure 1.

For each pediatric case, collected variables included age, gender, symptomatic status, admission date, discharge date, infection date, vaccination status, and location of first positive screening. Continuous RT-PCR assays were conducted on pharyngeal swab specimens, with daily recording of Ct values for both the ORF1ab and N genes recorded after admission. Hospital stay length was calculated as the difference between the discharge and admission dates.

Ct values fluctuated for some patients during their hospital stay, with values exceeding 35 on one day and dropping below 35 on others. Some maintained Ct values above 35 for two consecutive days before decreasing below 35 on the third day, while others consistently remained below 35 throughout the observation period. In our analysis, we did not make strict distinctions and combined all these data for a comprehensive assessment.

2.2 Case definitions

2.2.1 Classification of asymptomatic, asymptomatic-to-mild, and mild groups

According to the *Diagnosis and Treatment Guideline for COVID-19 (Ninth Edition)* [12] issued by the National Health Commission of China, infections were categorized into three groups based on clinical presentation at admission and symptom progression until discharge: asymptomatic, asymptomatic-to-mild, and mildly symptomatic. Asymptomatic infection was characterized as the patient not exhibiting any of the following symptoms: fever (>37.5°C), chills, myalgia, fatigue, rhinorrhea, nasal congestion, hyposmia, hypogeusia, sore throat, dyspnea, cough, sputum production, hemoptysis, headache, dizziness, anorexia, nausea, vomiting, abdominal pain, or diarrhea. Mildly symptomatic infection was defined by the presence of mild manifestations of any above symptoms, with imaging examinations showing no signs of pneumonia and oxygen saturation levels remaining above 93%. Asymptomatic-to-mild infection refers to individuals who were initially asymptomatic at admission but subsequently developed mild symptoms during hospitalization.

2.2.2 Positive cases and predominant variants

Ct values below 35 for the ORF1ab or N genes was considered indicative of a positive SARS-CoV-2 result[12]. During the study period in Shanghai, most positive samples were identified as the variant of Omicron BA.2, with no other variants detected[13-15].

2.2.3 Viral shedding

According to the *Diagnosis and Treatment Guideline for COVID-19 (Ninth Edition)* [14], viral shedding was defined as a decline in viral load until Ct values exceeded 35, confirmed by two consecutive negative nucleic acid tests performed at intervals greater than 24 hours. A Ct value ≥35 generally corresponds to a viral RNA load of less than 1×10^4 copies/mL, at

which point viable virus is rarely isolated in cell culture and infectivity is essentially lost.

Viral shedding time (VST) was defined as the duration from the initial positive result of a pharyngeal swab measured by quantitative RT-PCR and the first two consecutive negative test results. The initial positive result refers to the patient's first positive nucleic acid test, which is generally conducted before admission to the NECC Fangcang shelter hospital.

2.2.4 Vaccination status

In accordance with Chinese epidemic prevention and control strategies [16] and Shanghai's relevant policy of free vaccination for all residents [17-19], it can be inferred that the estimated timeline for the last vaccine doses administered to children in Shanghai was as follows: the partial vaccinations was given between August and September 2021, the full vaccinations between September and November 2021, and the booster dose between February and March 2022. The intervals between the administration of three doses and the time of infection were approximately 6 to 7 months for the partial vaccination, 5 to 6 months for the full vaccination, and 1 to 2 months for booster dose.Partial vaccinations were mainly given from April to June 2021, full vaccinations from May and July 2021, and booster doses from November 2021 to January 2022. Nearly all vaccines administered in this study were inactivated vaccines (Sinovac or Sinopharm), rather than mRNA-based vaccines [20].

Based on these timelines, vaccination status was categorized into four groups: unvaccinated, partially vaccinated, fully vaccinated, and booster. Partially vaccinated individuals are those who have received at least one dose of a vaccine but have not completed the full series as prescribed by the vaccination protocol. Fully vaccinated individuals have completed all doses prescribed by the vaccination protocol, and individuals who have received

a booster shot are classified as having received a booster vaccination. In accordance with the immunization schedule, full vaccinations were usually administered four weeks after partial vaccinations, and booster doses were administered six months after the full vaccinations.

2.3 Operating Procedure for SARS-CoV-2 Testing

2.3.1 Sample collection

The collection of SARS-CoV-2 samples was performed using nasopharyngeal swabs. Specifically, the procedures were as follows: the sampler gently supported the subject's head with one hand while holding the swab with the other hand. The swab was inserted along the base of the lower nasal passage, moving the swab backward along the nostril slowly and carefully. Once the tip of the swab reached the posterior nasopharyngeal wall, it was gently rotated once. If a reflex cough occurred, the swab was left in place for a moment before being slowly withdrawn. The swab tip was then placed into a tube containing 2–3 mL of viral transport medium. The swab stick was discarded, and the tube was tightly capped[12].

2.3.2 Specimens storage

Specimens for viral isolation and nucleic acid detection were generally sent for testing immediately. If testing could not be performed promptly due to special circumstances, specimens could be stored at 4°C for up to 24 hours[12].

2.3.3 RT-PCR kit

RT-PCR detection was performed using the SARS-CoV-2 Detection Kit (Easy Diagnosis Biomedicine Co., Ltd., Wuhan, China) in combination with the MA6000 Real-Time PCR Detection System (Molarray Bioscience Co., Ltd., Suzhou, China). All testing procedures were conducted at Shanghai Labway Clinical Laboratory.

2.4 Statistical method

We applied univariable and multivariable ordinal logistic regression to identify factors influencing the progression from asymptomatic to mild infections, using symptom classification (asymptomatic, asymptomatic-to-mild, mildly symptomatic) as the dependent variable. We performed univariable and multivariable Cox regression analyses, treating viral shedding as the dependent variable and viral shedding time (VST) as the time-to-event variable, to estimate adjusted hazard ratios (aHRs) across symptom classification groups. For the dependent variable, individuals who achieved viral shedding within two weeks (14 days) were coded as 1 (event), while those who did not were coded as 0.

We employed a mixed-effects model to estimate trajectories of viral load, as measured by Ct values of the N and ORF1ab genes, and to assess their association with symptom classification. In this model, symptom classification was included as the main effect, with an interaction term between symptom classification and time, and age, sex, and vaccination status were incorporated as covariates. Using this model, we predicted and estimated the viral area under the curve (AUC) for each patient.

Considering the uneven distribution of the four vaccination status types, we conducted sensitivity analyses using the IPTW method to adjust for indication-related confounding. Vaccination status was treated as the dependent variable, while age, sex, minimum ORF1ab, minimum N gene, and symptom classification were included as independent variables in a multinomial logistic regression model to estimate propensity scores (weights) for each patient. Symmetric trimming of the weights was performed, and the adjusted weights were incorporated into the dataset for subsequent modelling and analysis.

Additionally, we used the *Chi-square* test to compare categorical variables of age (n), gender, vaccination status, and swab location among three symptom groups, and the *Kruskal-Wallis H* test to compare differences in age (years), nadir Ct values, length of stay, and viral shedding time.

All statistical analyses were performed using R software (version 4.3.1) and RStudio (version 2024.12.0). Logistic regression analyses were computed using the "MASS" package. Survival analysis was performed with the "survival" and "survminer" packages. Mixed-effects models were primarily implemented using the “lmerTest” and “nlme” packages. $P < 0.05$ indicates statistical significance.

3. Results

3.1 Characteristics of asymptomatic, asymptomatic-to-mild, and mildly symptomatic groups

The study included 7803 children and adolescents admitted to NECC Fangcang. The median age of these individuals was 12 years (interquartile range(IQR): 8-16 years) (Table 1). Among them, 569 (7.3%) were under 4 years old, 2209 (28.3%) were aged 5 to 9 years, 2214 (28.4%) were aged 10 to 14 years, and 2811 (36.0%) were aged 15 to 18 years. The proportion of female and male participants was 41.9% and 58.1%, respectively. The median duration from symptom onset to cessation of viral shedding was 6 days (IQR: 4-8 days), which corresponded to the median length of hospital stay. A total of 5105 (65.5%) children and adolescents had received vaccination, including partial, full or booster doses.

In the entire cohort, 81.8% of cases were initially diagnosed with asymptomatic infections, and 18.2% presented with mild symptoms at admission. However, among those

initially diagnosed as asymptomatic, 82.8% (5285/6382) subsequently progressed to mildly symptomatic infections at follow-up, while only 17.2% (1097/6382) remained asymptomatic until their discharge (Table 1). As a result, the final proportion of asymptomatic infections was 14.1% (1097/7803).

3.2 Influencing factors for progression from asymptomatic to mildly symptomatic infections

We analyzed factors influencing progression from asymptomatic to mildly symptomatic infections using univariate and multivariate ordinal logistic regression. Both analyses yielded consistent results, showing that older children, female sex, and vaccinated individuals were more likely to progress from asymptomatic to mild disease. As shown in Figure 2, compared to the reference group of children aged 0-4 years, those aged 5-9 years exhibited a higher, though not statistically significant, with an odds ratio (OR) of 1.13 (95% CI 0.94-1.38, $P > 0.05$). This association became more pronounced with increasing age, reaching statistical significance in the 10-14 age group (OR=1.34; 95% CI, 1.10-1.63; $P < 0.05$), and further strengthening in the 15-18 years group (OR=1.53; 95% CI, 1.26-1.84; $P < 0.05$).

The analysis also revealed that female children and adolescents were more likely to transition from asymptomatic to mildly symptomatic infections compared to males (OR=1.21; 95% CI, 1.11-1.34; $P < 0.05$). A lower nadir N gene value, indicating a higher viral load, was associated with an increased risk of progressing from asymptomatic to mildly symptomatic infections. (OR=0.89; 95% CI, 0.85-0.93; $P < 0.05$).

Compared to unvaccinated individuals, those who received partial, full, or booster vaccinations exhibited increased odds of developing mild symptoms, with ORs of 1.84 (95%

CI 1.47-2.31) ($P < 0.01$), 2.20 (95% CI 1.97-2.47) ($P < 0.01$), and 1.91 (95% CI 1.34-2.73), respectively. The trend is further supported by the results of absolute risks. As shown in Table S2, unvaccinated individuals have a significantly higher probability of remaining asymptomatic (21.3%, 95% CI: 21.0–21.5%) than fully vaccinated individuals (12.0%, 95% CI: 11.9–12.1%). In contrast, fully vaccinated individuals have a higher risk of progressing to mild symptoms (19.4%, 95% CI: 19.3–19.6%) than unvaccinated individuals (10.8%, 95% CI: 10.7–10.9%). These findings suggest that unvaccinated individuals are more likely to remain asymptomatic, while vaccinated individuals are more likely to transition from asymptomatic to mildly symptomatic.

Furthermore, we conducted stratified analyses by age groups (0–4, 5–9, 10–14, and 15–18 years), as shown in Table S3. Across all four age groups, the effects of gender and nadir Ct values on symptom progression were consistent with the overall analysis. For vaccination status, full vaccination had no statistically significant effect in the 0–4 years group, while booster vaccination showed a significant but unstable effect due to wide confidence intervals. In the 5–18-year groups, associations between vaccination status and symptom progression were generally consistent with the overall findings.

3.3 Influencing factors associated with viral shedding

We performed univariate Cox regression to assess the relationship between each influencing factor and VST, and multivariate Cox regression analyses to evaluate their combined effect on VST. Results from both univariate (Figure 3a) and multivariate (Figure 3b) analyses were largely consistent, indicating that older children were more likely to experience viral shedding. Compared to the reference group aged 0-4 years, the hazard ratios (HR) for the

6-10, 10-14, and 15-18 age group gradually increased to 1.13 (95% CI: 1.03-1.24) ($P < 0.05$), 1.17 (1.05-1.29) ($P < 0.05$), and 1.44 (1.31-1.59) ($P < 0.05$), respectively. Moreover, Figure 3c shows that older children had shorter viral shedding durations than those aged ⩽5 years ($P < 0.05$). Gender analysis revealed no statistically significant differences in viral shedding between males and females ($P > 0.05$) (Figure 3b). Although the statistical difference in Figure 3d was significant, the actual shedding durations between groups were nearly identical. Additionally, higher nadir N gene values were associated with an increased likelihood of viral shedding (HR: 1.15, 95% CI: 1.13-1.18) ($P < 0.05$) (Figure 3b). Significant variations in nadir Ct values for both N and ORF1ab genes were observed across symptomatic groups (Figure 3f).

Figures 3a and 3b demonstrate that full vaccination is significantly associated with viral shedding, with HRs of 1.14 ($P < 0.05$) in univariate analysis and 1.02 ($P > 0.05$) in multivariate analysis, respectively. Partial and booster vaccination did not show statistically significant effects. This trend is also reflected in the calculated absolute risk estimates (Table S4). At a median survival time of 6 days, the probability of viral shedding among fully vaccinated patients was 60.1% (95% CI: 59.4% – 60.8%), which is higher than that observed in the unvaccinated (55.0%, 95% CI: 54.0% – 55.9%), partially vaccinated (58.5%, 95% CI: 56.0% – 61.0%), and booster groups (48.9%, 95% CI: 45.0% – 52.9%).

Stratified analyses by age groups (0–4, 5–9, 10–14, and 15–18 years) were conducted further to investigate the effect of vaccination on viral shedding. As shown in Table S5, partial vaccination did not result in significant differences in viral shedding across age groups. Booster vaccination demonstrated a significant effect in the 0–4 years group; however, this

result was highly unstable due to a wide confidence interval (1.00–10.05). Full vaccination was significant associations with viral shedding in the 0–4 and 15–18 years groups, with HRs of 1.57 (95% CI: 1.06–2.33) and 1.08 (95% CI: 1.06–1.18), respectively.

To clarify differences in viral shedding across vaccination types, Kaplan-Meier analysis were conducted to assess both overall and pairwise comparisons, applying the Bonferroni correction for multiple testing. Figure 4b demonstrates that viral shedding dynamics differed significantly among vaccination groups over time ($P < 0.001$). Specifically, the fully vaccinated group showed the most rapid decline in viral load compared to unvaccinated individuals, while the partially vaccinated and booster groups did not differ significantly. These findings align with the results presented in Figure 3.

Compared to the asymptomatic group, both the asymptomatic-to-mild and mildly symptomatic groups exhibited a lower likelihood of viral shedding, with HRs of 0.64 (95% CI: 0.60-0.69) ($P < 0.05$) and 0.60 (95% CI: 0.55-0.65) ($P < 0.05$), respectively. Furthermore, the duration of viral shedding was significantly longer in both asymptomatic-to-mild and mildly symptomatic groups relative to the asymptomatic group ($P < 0.05$) (Figure 3e). A Kaplan-Meier analysis was performed to further characterize viral shedding dynamics across groups. As illustrated in Figure 4a, viral shedding began on day two post-infection in all groups, with approximately 98% of individuals exhibiting viral shedding by day 15. The asymptomatic group exhibited the most rapid decline in viral shedding, followed by the asymptomatic-to-mild (HR: 0.60, 95% CI: 0.57-0.64, $P < 0.01$) and mildly symptomatic groups (HR: 0.53, 95% CI: 0.49-0.58, $P < 0.01$).

3.4 Dynamic variations in viral loads across different symptom groups

We employed a mixed-effects model to estimate the relationship between viral load trajectories and symptom groups. As shown in Table S6, for the main effect of symptom groups, the N gene viral load in the asymptomatic-to-mild group was, on average, 1.67 units lower than in the asymptomatic group (95% CI: -2.00 to -1.33, $P < 0.01$) , indicating a higher baseline viral load. The mildly symptomatic group exhibited the highest baseline N gene viral load, averaging 2.59 units lower (95% CI: -3.00 to -2.17, $P < 0.01$). The ORF1ab gene showed a similar trend, although with a slightly smaller effect size.

Regarding the interaction effects, the TIME×asymptomatic-to-mild group showed an increased slope of 0.12 units per day (95% CI: 0.05 – 0.19), indicating a slower viral clearance rate compared to the asymptomatic group. The TIME×mildly symptomatic group exhibited the highest slope increase of 0.20 units per day (95% CI: 0.12 – 0.28), reflecting the slowest viral clearance. The ORF1ab gene displayed a similar pattern, with a slightly slower viral clearance rate than the N gene.

We used a mixed-effects model to predict viral AUC for each patient and compared viral AUC differences among symptom groups using the Wilcoxon test with Bonferroni correction. Results presented in Figure S2 and Table S7 indicate that, for both the N gene and ORF1ab gene, the asymptomatic-to-mild (N gene: 142.28, ORF1ab gene: 146.76) and mild groups (N: 154.99, O: 157.17) exhibited significantly higher total viral exposure compared to the asymptomatic group (N: 79.04, O: 79.28) ($P < 0.05$), with the mild group showing greater viral exposure than the asymptomatic-to-mild group.

3.5 Sensitivity analyses to adjust for confounding factors related to vaccination

Due to the uneven distribution of vaccination status categories (Table 1: unvaccinated

34.6%, partially vaccinated 5%, fully vaccinated 58.4%, booster 2.0%), we employed the IPTW method for sensitivity analyses to address confounding by indication related to vaccination. As shown in Table 2, IPTW-adjusted results for symptom progression lign with findings from univariate and multivariate analyses, indicating that vaccinated individuals are more likely to transition from asymptomatic to mild symptoms. The highest OR was observed for the fully vaccinated group at 1.99 (95% CI: 1.77–2.23, $P < 0.05$), followed by the booster and partially vaccinated groups with ORs of 1.78 (95% CI: 1.57–2.02, $P < 0.05$) and 1.75 (95% CI: 1.56–1.96, $P < 0.05$), respectively. Furthermore, age-stratified analysis adjusted by IPTW (Table S8) showed that vaccination effects across age groups were largely consistent with those observed without IPTW adjustment (Table S3), demonstrating a positive association between vaccination and symptom progression in all age groups.

IPTW-adjusted results for viral shedding differ from univariate and multivariate analyses. As shown in Table 2, IPTW-adjusted analysis revealed a modest association between full vaccination and viral shedding (HR: 1.08; 95% CI: 1.02–1.15, $P < 0.05$). Partial vaccination was associated with a slight, but not statistically significant, increase in viral shedding (HR: 1.04; 95% CI: 0.93–1.17; $P > 0.05$). Booster vaccination shows an inverse association with viral shedding (HR: 0.81; 95% CI: 0.67–0.97; $P < 0.05$), which may be attributable to the limited number of booster recipients, as boosters were mainly administered to children aged 3 years or older. Further age-stratified analysis (Table S8) shows a significant positive association between full vaccination and viral shedding in the 0–4 and 15–18 year age groups, with HRs of 1.62 (95% CI: 1.20–2.19, $P < 0.05$) and 1.05 (95% CI: 1.01–1.13, $P < 0.05$), respectively. Although a weak positive association was observed in the 5–9-year age group, it

was not statistically significant.

4. Discussion

To our knowledge, few studies have comprehensively examined the progression of disease symptoms and viral shedding using continuous daily data in large pediatric populations infected with COVID-19. Although some studies have reported continuous monitoring data, most involved sample sizes of fewer than 500 cases and employed sampling intervals of three days or longer [8-10]. This study examined factors affecting disease symptom progression and viral shedding among asymptomatic, asymptomatic-to-mild, and mild symptom groups. Our findings demonstrate that older age, female sex, and vaccination are associated with an increased likelihood of progressing from asymptomatic to mild symptoms. Older age and higher nadir N gene correlate with a higher likelihood of viral shedding. Fully vaccinated individuals show a weak positive association with viral shedding. Furthermore, both the asymptomatic-to-mild and the mildly symptomatic groups had a lower likelihood of achieving viral shedding than the asymptomatic group.

The results highlight that age significantly influences disease progression in pediatric populations. Individuals under 18 years of age are more likely to progress from asymptomatic to mild conditions and tend to achieve viral shedding more efficiently as age increases, which aligns with previous findings in children[21]. Symptom characteristics vary across age groups, primarily due to differences in the human immune system. Specifically, as children grow older, both the quantity and functional capacity of immune cells, including T cells and B cells, increase, thereby enhancing the overall immune response[22-24]. Therefore, individuals with more robust immune systems are more likely to develop mild symptoms and progress from

asymptomatic to mild illness.

The results indicate that female children are more likely than male children to progress from asymptomatic status to mild symptoms (OR=1.21, $P<0.05$) (Figure 2b). Although this finding does not reach statistical significance, there is a trend suggesting that female children may experience faster viral shedding (HR=1.02, $P=0.06$) (Figure 3b). Previous studies have suggested that female sex hormones may modulate disease symptoms and severity[25, 26]. Although hormonal effects in children may be less pronounced than in adults, they may still represent a potential factor influencing variations in symptom progression and viral shedding.

We found that vaccinated children and adolescents were more likely to progress from asymptomatic infection to mild disease. The adjusted OR by IPTW were 1.75 for partial, 1.99 for full, and 1.78 for booster vaccination. This observation aligns with our previous study[27], which observed a similar effect in adults. Several studies suggest that mRNA-based vaccines enhance the immune response by stimulating the immune system to produce specific antibodies and T-cell responses against SARS-CoV-2, thereby strengthening host defenses and reducing the incidence of asymptomatic infections [28-33]. We hypothesize that vaccination does not delay symptom onset but instead accelerates it in pediatric patients. Most patients infected with the Omicron variant are asymptomatic or exhibit subtle clinical manifestations. Although patients infected with the Omicron variant present symptoms distinct from those of earlier COVID-19 strains and lack typical radiological and laboratory features, such as elevated neutrophil and white blood cell counts and increased C-reactive protein levels [34], studies indicate that breakthrough Omicron infections can robustly stimulate recall responses of pre-existing humoral and cellular immunity induced by

vaccination [35]. In vaccinated individuals, Omicron infections significantly activate spike-specific CD8+ T cells and follicular T helper cells, leading to a notable increase in spike-specific IgG+ B cells, plasmablasts, and certain memory B cells [36]. The rapid immunological recall hastens the emergence of symptoms [37, 38]. Especially during the Shanghai outbreak, the predominant strain was Omicron BA.2. Similar to Omicron BA.1, it possesses stronger immune escape capabilities than the previous Delta strain[39, 40]. In the context of high immune escape, vaccine-activated memory immune responses may manifest more rapidly as mild symptoms.

Our study demonstrates an association between vaccination and viral clearance, with vaccinated individuals more likely to achieve viral shedding. These findings align with previous research indicating that vaccination reduces viral RNA load in children [39] and that complete inactivated vaccine regimens effectively facilitate viral shedding in pediatric populations [41, 42]. However, the association observed in our current dataset is relatively weak; the hazard ratio for full vaccination is only 1.08, while booster vaccination appears to have a protective effect. This outcome may be attributable to incomplete vaccination schedules in partially vaccinated individuals, resulting in suboptimal vaccine-induced protection [43]. The limited impact observed for booster doses is likely due to the smaller sample size compared to the partially and fully vaccinated groups, as current immunization guidelines do not recommend booster administration for children aged $\leqslant$3 years [44]. These factors may have contributed to the discrepancies in the data analysis. Additional evidence supporting these findings is demonstrated by the data collection process, which showed that among 157 pediatric patients transferred to other medical facilities due to severe conditions

(Table S1), primarily persistent high fever, chest tightness, and dyspnea, 74.4% (116/157) were unvaccinated. This finding highlights the critical role of vaccination in mitigating the risk of severe disease in this population.

Our findings indicate that in pediatric COVID-19 cases, the N gene demonstrates a more pronounced effect than the ORF1ab gene on both progression from asymptomatic to mild symptoms and viral shedding dynamics. These findings are consistent with previous reports indicating that the N gene is more sensitive. Multiple studies have shown that, across various nucleic acid detection kits, the limit of detection (LoD) for the N gene is generally lower than that for the ORF1ab gene. For instance, in the PerkinElmer assay, the LoD for the N gene is 11.61 copies/mL, compared to 34.66 copies/mL for the ORF1ab gene[45]. Similar findings have been reported in evaluations of several other diagnostic kits [46]. During SARS-CoV-2 replication, large quantities of subgenomic RNAs containing the N gene and E gene (envelope protein gene) are produced, whereas the ORF1ab gene is transcribed exclusively from the full-length viral genomic RNA[47]. The replication and expression levels of subgenomic RNAs, particularly the N gene, which encodes the highly expressed nucleocapsid protein, greatly exceed those of the full-length genomic RNA. As a result, diagnostic assays targeting the N gene generally exhibit higher sensitivity, allowing for earlier and more reliable detection of viral infection.

This study has several limitations that may affect the interpretation and generalizability of the results. 1) Retrospective study design. As a retrospective study, this research may be subject to selection and information biases. Imbalances in the number of individuals across vaccination categories could not be fully corrected by age stratification and IPTW adjustment.

Symptom data were based on self-reports from patients or their parents, which may be subject to reporting bias. Additionally, some patients were excluded due to incomplete data during the inclusion and exclusion process, which may have introduced additional bias. Moreover, the absence of chest CT images and additional laboratory tests limited the comprehensive evaluation of patients' clinical status. 2) Ct values as a proxy for infectivity. Ct is an indirect marker of viral load and does not directly measure viral viability or true transmissibility. 3) Vaccine heterogeneity. We only have data on the overall estimated time since the last dose, do not have details regarding vaccine types, number of doses, time since last dose, and combined vaccination schedules, which may limit interpretation of vaccine-related associations. 4) Reinfection patients. Reinfection could lead to an overestimation of the duration of viral positivity and an underestimation of the viral shedding rate. Although the incidence of reinfection within the Fangcang shelter hospital is low [47], it may still modestly affect the robustness of our analysis. 5) Confounder factors. Some potential unmeasured confounders, including comorbidities, household transmission and genomic sequencing data, were not included in the analysis. Additionally, we did not collect information on patients' personal mediation use, which may also impact viral clearance. Literature reports that early use of drugs like pidotimod can prevent disease progression and promote more rapid virological recovery[48]. The use of deflazacort has also been shown to significantly reduce the risk of hospitalization due to metabolic disorders[49]. 6) Context and generalizability. The data for this study were obtained exclusively from the NECC Fangcang hospital in Shanghai, with the study population consisting soley of asymptomatic and mild cases, and no severe infections. All participants received only inactivated vaccines, which have different efficacy from mRNA

vaccines. The SARS-CoV-2 strain studied in this article was the Omicron BA.2 variant; differences in transmissibility, immune evasion, and cellular tropism between Delta, Omicron BA.1, BA.5 and XBB. These factors may reduce applicability of our findings to broader contexts.

5. Conclusions

In pediatric COVID-19 patients, older age, female sex, lower nadir N gene, and vaccination status are linked to a higher risk of progressing from asymptomatic to mildly symptomatic infections. Older age and higher nadir N gene levels are associated with a higher likelihood of viral shedding. Vaccination status correlates with a higher probability of symptomatic presentation, which may reflect a robust, rapid anamnestic immune response and a weakly accelerated viral clearance. The N gene exhibits a more pronounced effect than ORF1ab gene on both symptom progression and viral shedding dynamics. These results may inform the development of optimized prevention and management strategies for COVID-19 and other respiratory infections in pediatric populations.

Abbreviations

COVID-19	Coronavirus disease 2019
SARS-CoV-2	Severe acute respiratory syndrome-associated coronavirus 2
NECC	National exhibition and convention center
Ct	Cycle threshold
IPTW	Inverse probability of treatment weighting
VST	Viral shedding time
AUC	Area under the curve
IQR	Interquartile range
CI	confident interval
OR	Odds ratio

COR	crude odds ratio
AOR	adjusted odds ratio
HR	Hazard ratios
CHR	crude hazard ratio
AHR	adjusted hazard ratio

Supplementary Information

The online version contains supplementary material available at https://doi.org/...

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Author's contributions

LS and WZK conceived, designed and supervised the study. PY and XJ contributed to the initial manuscript draft. PY and YHZ analysed the data and prepared the figures. WK, YSL, YG, RJL, WW, TPW, WJZ, and YHZ provided critical revision to the manuscript. All authors have read and approved the final manuscript.

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Data availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Clinical Research and Ethics Committee (CREC) at Tangdu Hospital (Approval No. 202205-01). The requirement for informed consent was waived by the CREC of

Tangdu Hospital in accordance with Article 32 of the Chinese regulations titled “Ethical review measures for life sciences and medical research involving human subjects” [50].

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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684 **Figures**

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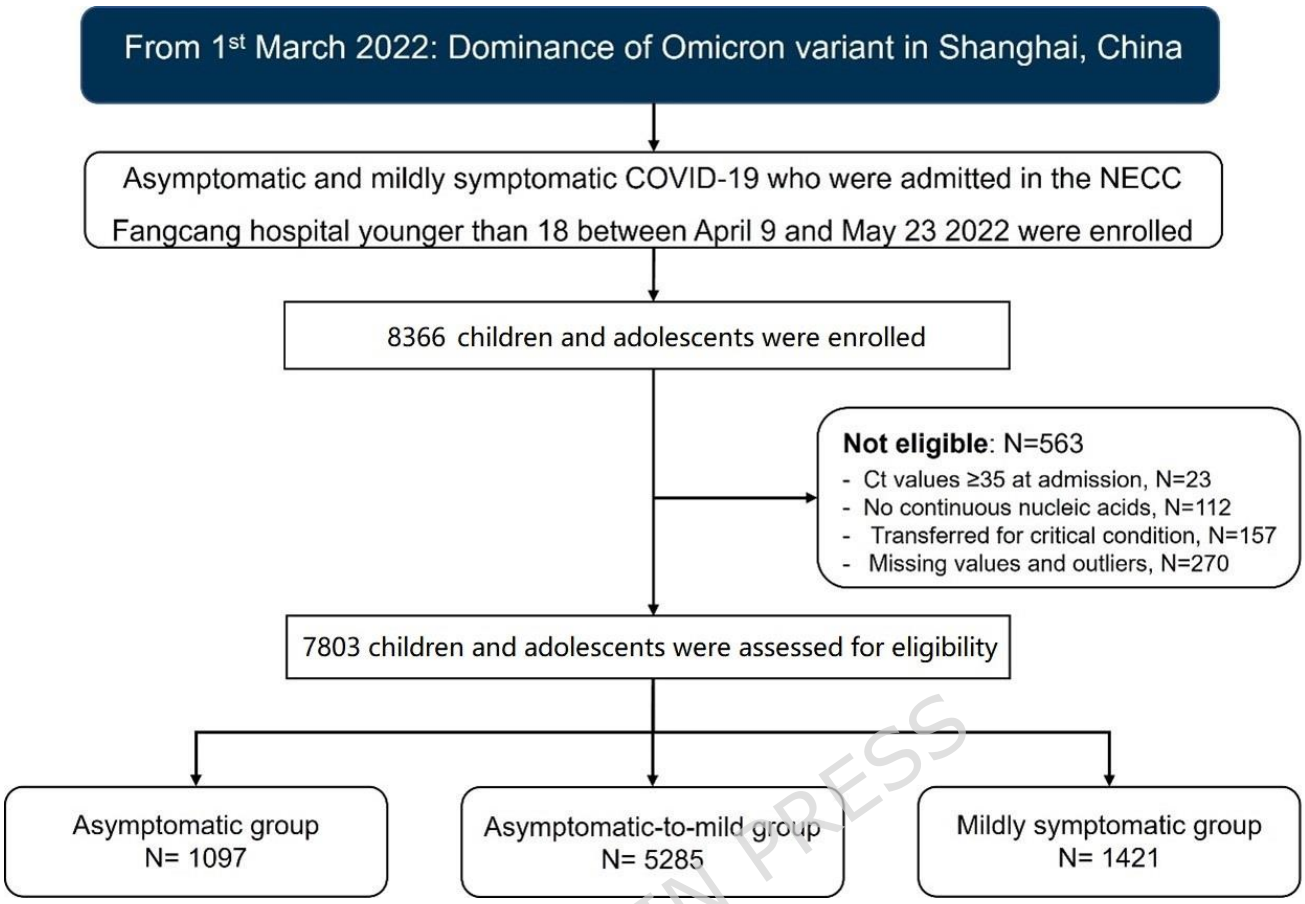

Figure 1. Flow chart of study design. Ct, cycle threshold; NECC, National Exhibition and Convention Center

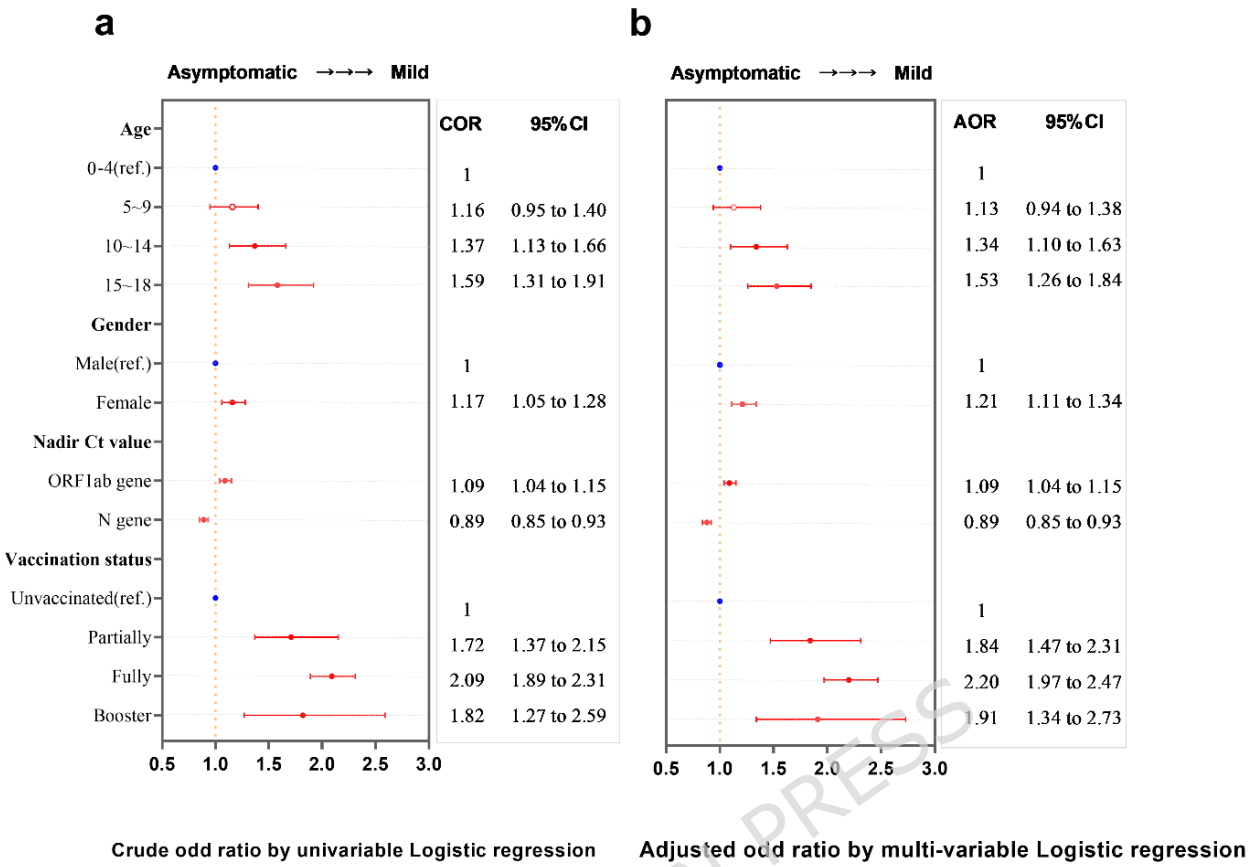

Figure 2. Influencing factors associated with progression from asymptomatic to mildly symptomatic infections. Figures a and b present the results of univariate and multivariate analyses, respectively, assessing factors influencing this progression. Solid blue dots denote the baseline reference categories for each group. Red-filled circles indicate statistical significance ($P < 0.05$), while unfilled circles denote non-significant. COR, crude odds ratio; AOR, adjusted odds ratio;CI, confident interval; ref., reference; Ct, cycle threshold

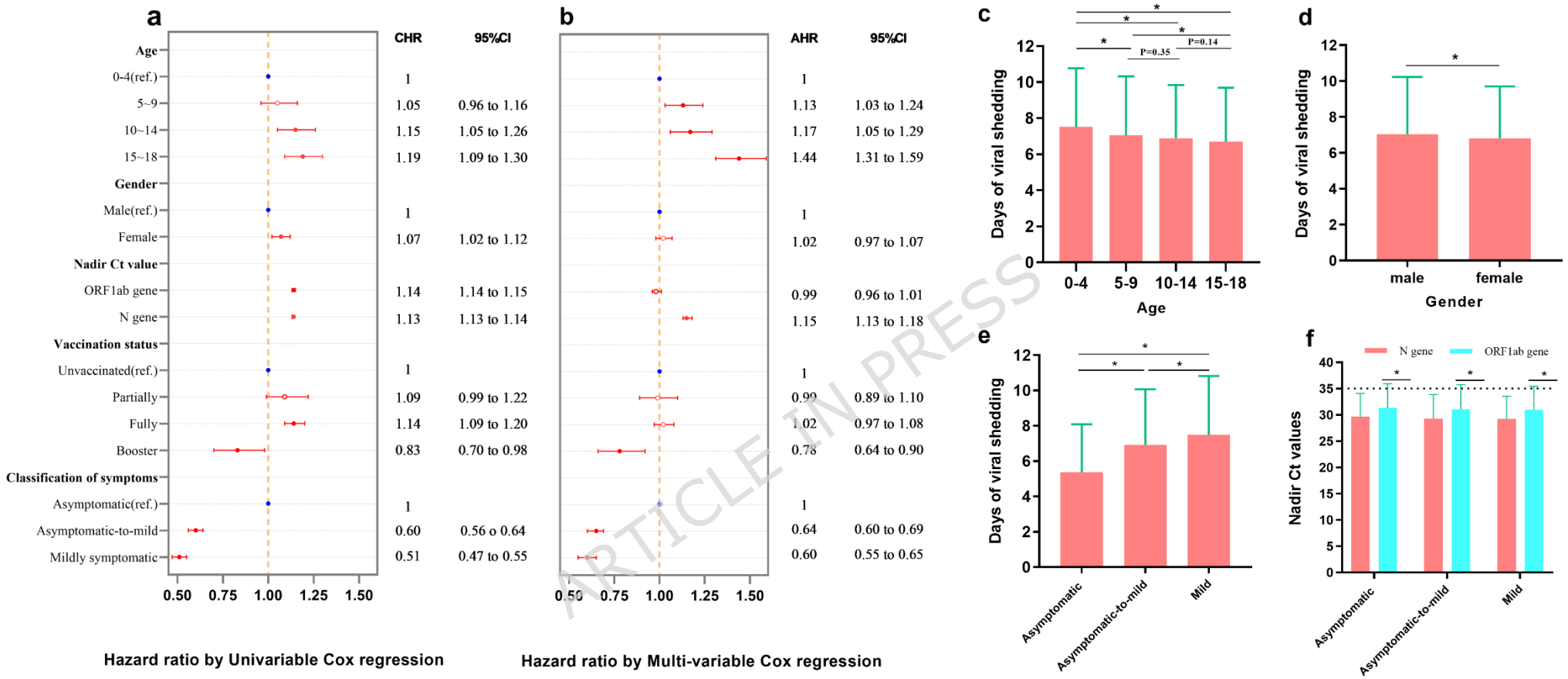

Figure 3. Association between viral shedding and influencing factors. Panels a and b display the univariate and multivariate analysis, respectively, evaluating the relationship between each factor and viral shedding. Solid blue dots represent baseline reference categories for each group. Red-filled circles indicate statistical significance ($P < 0.05$), while unfilled circle denote non-significant results. Panels c, d and e depict the days of viral shedding stratified by age, gender, and symptom, respectively. Panel f illustrates the differences of nadir Ct values among symptomatic groups. CHR, crude hazard ratio; AHR, adjusted hazard ratio; CI, confident interval; ref, reference; Ct, cycle threshold.

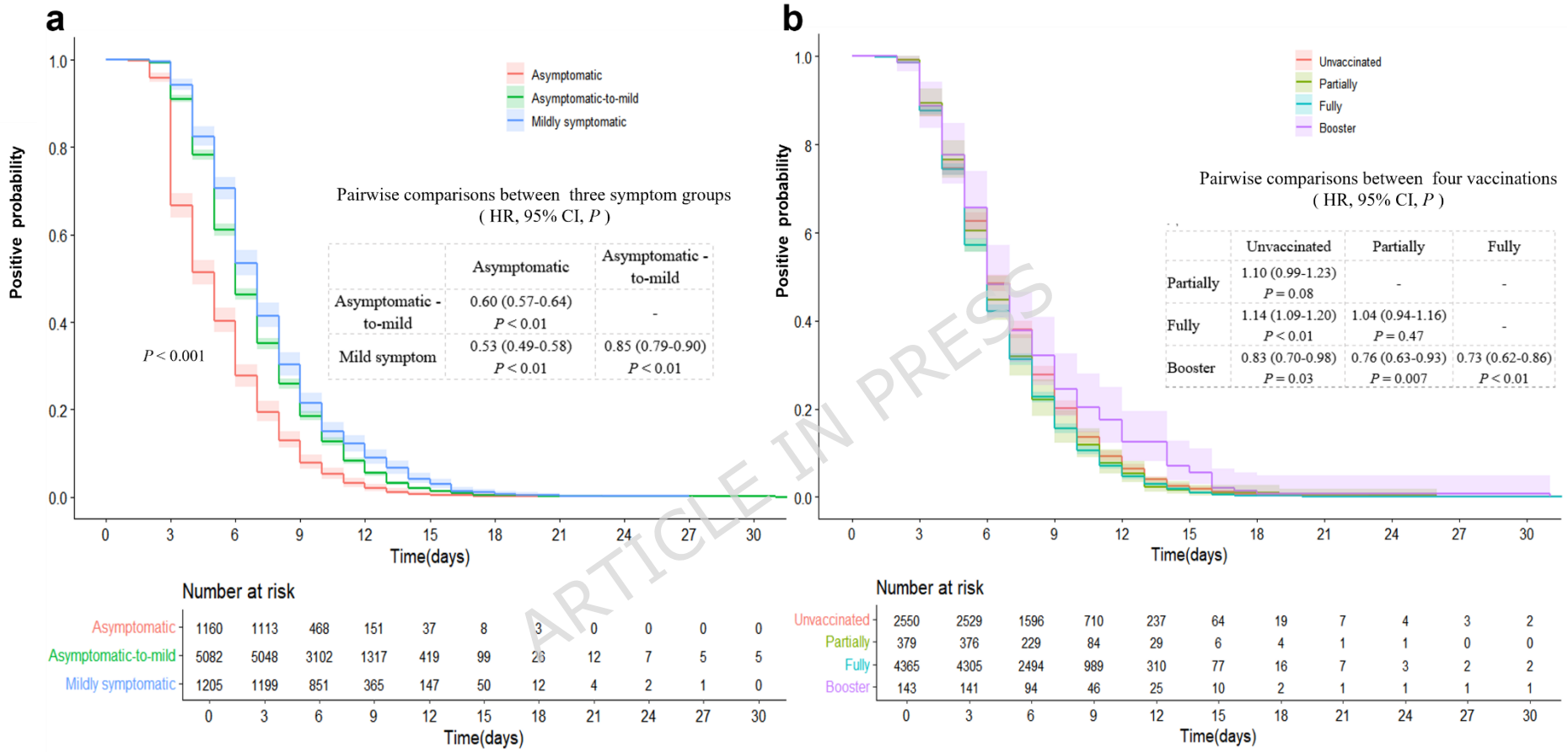

Figure 4. Survival curves of viral shedding duration stratified by symptoms classification (a) and vaccinations type (b). In panel a, from day 2 to 15 post-infection, 98% of individuals in the asymptomatic, asymptomatic-to-mild, and mildly symptomatic groups showed viral shedding. Significant differences in viral shedding trends were observed both overall and in pairwise comparisons among the three groups ($P <$ 0.01), with the asymptomatic group exhibited the fastest viral shedding, followed by the asymptomatic-to-mild and mildly symptomatic groups. Panel b shows significant differences in viral shedding dynamics over time across vaccination types. Compared to the unvaccinated group,

partially (P=0.08) and fully(P<0.01) group showed the fastest decline in viral shedding.

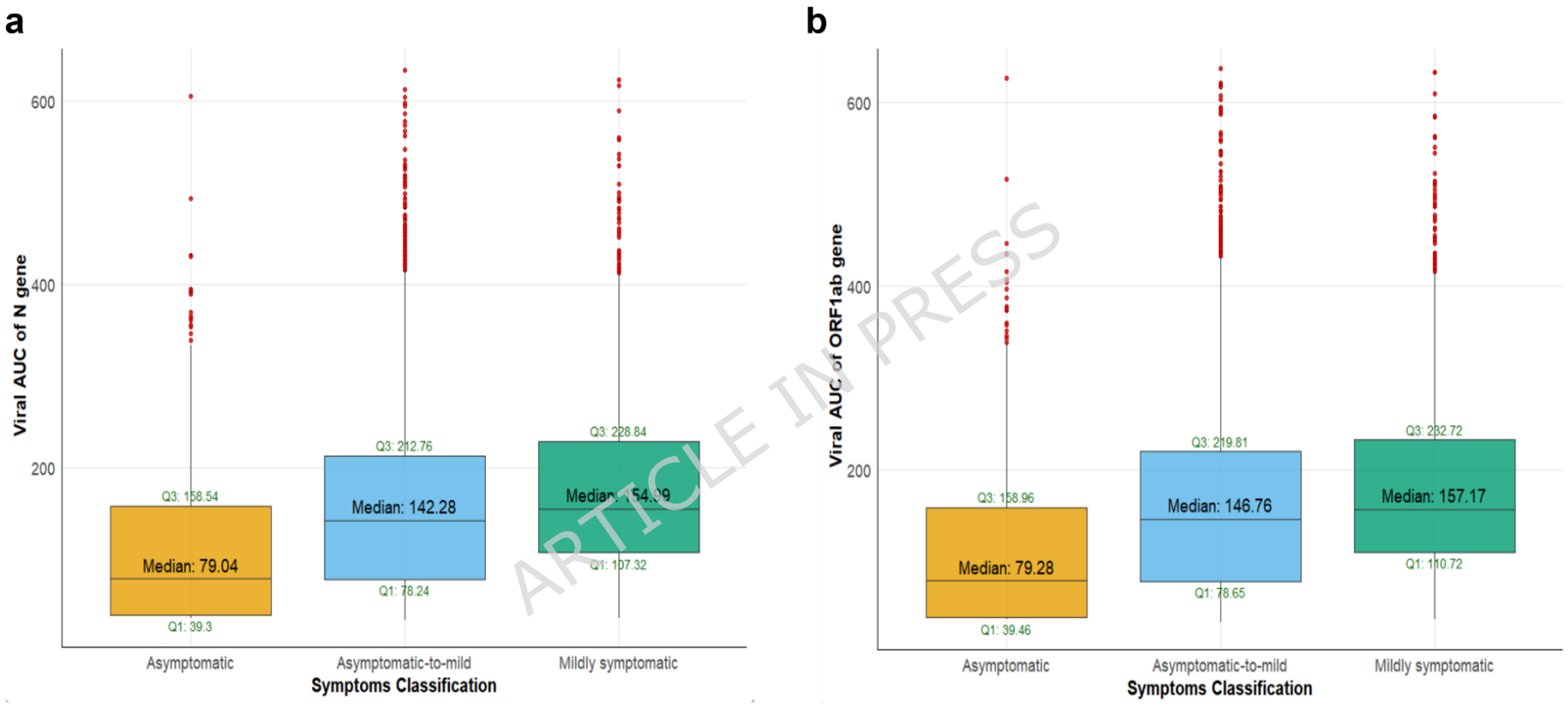

Figure S1. Differences in viral AUC values of patients among three symptom groups. For both the N gene and ORF1ab gene, the asymptomatic-to-mild and mild groups exhibited significantly higher total viral exposure compared to the asymptomatic group ($P < 0.05$), with the mild group showing greater exposure than the asymptomatic-to-mild group.

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Table 1 Characteristics of asymptomatic, asymptomatic-to-mild, and mildly symptomatic groups

Characteristics	Total	Classification of symptoms			*P* value
		Asymptomatic	Asymptomatic-to-mild	Mildly symptomatic	
N	7803	1097	5285	1421	
Age, years, Median (IQR)[a]	12 (8-16)	11 (7-16)	12 (8-16)	13 (9-17)	<0.001[b]
0-4, n (%)	569 (7.3)	93 (8.5)	405 (7.7)	71 (5.0)	<0.001[c]
5-9, n (%)	2209 (28.3)	337 (30.7)	1533(29.0)	339 (23.9)	
10-14, n (%)	2214 (28.4)	328 (29.9)	1456 (27.5)	430 (30.3)	
15-18, n (%)	2811 (36.0)	339 (30.9)	1891 (35.8)	581 (40.9)	
Gender, n (%)					<0.001[c]
Female	3271 (41.9)	444 (40.5)	2170 (41.1)	657 (46.2)	
Male	4532 (58.1)	653(59.5)	3115 (58.9)	764 (53.8)	
Nadir Ct values, Median (IQR)[a]					
N gene	29.5 (26.2-32.6)	29.6 (26.4-33.1)	29.5 (26.1-32.6)	29.3 (26.3-32.4)	0.421[b]
ORF1ab gene	31.2 (27.8-34.6)	31.3 (28.0-35.0)	31.2 (27.7-34.6)	31.0 (27.9-34.5)	0.326[b]
Length of stay, days, Median (IQR)[a]	6 (4-8)	5 (4-7)	6 (4-9)	7 (5-8)	<0.001[b]
Viral shedding time, days, Median (IQR)[a]	6 (4-8)	6 (3-8)	6 (4-8)	6(5-8)	<0.001[b]
Vaccination status, n (%)					<0.001[c]
Unvaccinated	2698 (34.6)	488 (44.4)	1944 (36.8)	266 (18.7)	
Partially	392 (5.0)	51(4.6)	266 (5.0)	75 (5.3)	
Fully	4556 (58.4)	536 (48.8)	2974 (56.3)	1046 (73.6)	
Booster	147 (2.0)	23 (2.1)	100 (1.9)	32 (2.4)	
The location of the swab for diagnosis, n (%)					<0.001[c]
Community screening	4071 (52.2)	552 (50.3)	2645(50.0)	874(61.5)	
Companies /schools screening	282 (3.6)	47(4.3)	180 (3.4)	55 (3.9)	
Active screening at nucleic acid sampling points	1325 (17.0)	156(14.2)	885 (16.7)	284 (20.0)	
Fever clinic screening	2125 (27.2)	342 (31.2)	1575 (29.9)	208 (14.6)	

[a] IQR, interquartile range,25-75%

[b] *P-value* calculated by the *Kruskal-Wallis H* test

[C] *P-value* calculated by the *Chi-squared* test

Table 2 Sensitivity analysis of symptom progression and viral shedding using IPTW method

Vaccination status	Univariate analysis		Multivariate analysis		IPTW method	
Ordinal logistic regression for symptom progression						
	OR	95%CI	OR	95%CI	OR	95%CI
Unvaccinated	1		1		1	
Partially	1.72	1.37-2.15	1.84	1.47-2.31	1.75	1.56-1.96
Fully	2.09	1.89-2.31	2.20	1.97-2.47	1.99	1.77-2.23
Booster	1.82	1.27-2.59	1.91	1.34-2.73	1.78	1.57-2.02
Cox regression for viral shedding						
	HR	95%CI	HR	95%CI	HR	95%CI
Unvaccinated	1		1		1	
Partially	1.09	0.99-1.22	0.99	0.89-1.10	1.04	0.93-1.17
Fully	1.14	1.09-1.20	1.02	0.97-1.08	1.08	1.02-1.15
Booster	0.83	0.70-0.98	0.78	0.64-0.90	0.81	0.67-0.97

Tables S1-S8

Table S1 Characteristics of the 157 cases excluded from the study due to the critical condition and transferred to other institutions

Table S2 Absolute risk by variable categories using ordinal logistic regression

Table S3 Influencing factors for symptom progression stratified by age group

Table S4 Absolute risk by variable categories using Cox regression with reference time at median survival day 6

Table S5 Influencing factors for viral shedding stratified by age group

Table S6 Mixed-effects models for N gene and ORFlab gene trajectories by symptom classification

Table S7 Differences in viral AUC values of patients across symptom classification

Table S8 Sensitivity analysis of symptom progression and viral shedding using IPTW method stratified by age groups

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