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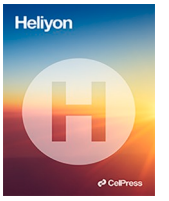

Research article

Epidemiological and immunological characteristics of middle-aged and elderly people in housing estates after Omicron BA.5 wave in Jinan, China

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ABSTRACT

A great number of COVID-19 patients was caused by Omicron BA.5 subvariant between December 2022 and January 2023 after the end of the zero-COVID-19 policy in China. In this study, we clarified the epidemiological and immunological characteristics of 457 enrolled middle-aged and elderly population in two housing estates after Omicron BA.5 wave. A total of 89.9 % (411/457) individuals have suffered Omicron BA.5 infection, among which 78.1 % (321/411) were symptomatic. The elderly patients were more likely to show fatigue and had longer symptomatic period than that of middle-aged patients post Omicron BA.5 infection. Omicron XBB and BA.2.86 subvariants extensively escaped the immunity elicited by Omicron BA.5 infection. The level of neutralizing antibody was mostly affected by vaccination doses rather than underlying disease status in these participants. It is very important to strengthen the epidemiological investigation and immune resistance assessment among elderly population for control of emerging SARS-CoV-2 variants.

1. Introduction

In December 2022, the zero-COVID-19 policy was end in China [1]. As reported by China CDC, a sharp increase of COVID-19 cases occurred between December 2022 and January 2023 [2]. However, a great many infected individuals may not be confirmed by nucleic acid detection or self-antigen test, potentially resulting in an imprecise representation of the actual infection data. An online survey has estimated that, with the end of the zero-COVID-19 policy, over 80 % populations in China have infected severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron BA.5 (BA.5.2 and BF.7) [3].

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Indeed, vaccination is widely accepted as one of the most effective measures in controlling the COVID-19 pandemic, could effectively reduce the death, severe cases, symptomatic cases, and infections across the world [4]. Additionally, vaccination elicits effective immune responses to neutralize SARS-CoV-2 [5]. However, the continued evolution of SARS-CoV-2 leads to increased immune resistance elicited by vaccination and infection [6–8], especially for the emerging Omicron subvariants, such as Omicron XBB.1.5, XBB.1.16, EG.5.1, and BA.2.86 subvariants [9–12].

Previous studies have found that the antibody level is correlated with protection against SARS-CoV-2 infection [13–15]. However, documented studies have identified that old age is a risk factor for poor antibody responses post vaccination or infection [16–19], and is associated with increased severe cases and deaths [20,21]. Several previous studies have identified the epidemiological features in children and measured the immune responses in general population post Omicron BA.5 infection [22–24], while the study focused on elderly people in communities is limited [25]. Therefore, immune resistance assessment in elderly population in communities is needed for further development of vaccination strategies and control of emerging SARS-CoV-2 variants. In this study, we conducted a cross-sectional study to identify the epidemiological and immunological characteristics of elderly population in housing estates in February 2023 after Omicron BA.5 wave in Jinan, China.

2. Materials and methods

2.1. Study participants

Middle-aged and elderly participants were randomly enrolled in two housing estates approximately 2 months after Omicron BA.5 wave in Jinan, China, with the assistance of Licheng Center for Disease Control and Prevention. Serum samples and epidemiological data were collected from each participant. And the SARS-CoV-2 Omicron BA.5 infection of subjects was mostly confirmed by self-antigen test and the real-time surveillance data of circulating SARS-CoV-2 variants (Fig. S1), while the rest was diagnosed by the quantitative reverse transcription polymerase chain reaction (qRT-PCR) method at the time of seeking medical treatment. The detailed information about sex, age, vaccination doses, underlying diseases and symptoms are shown in Table S1. All participants enrolled for further analysis were immunized with inactivated vaccines (CoronaVac or BBIBP-CorV), while the few people who vaccinated with other technical routes have been excluded in advance. Written informed consents were obtained from all participants. All collections were conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the Academy of Military Medical Science (IRB number: AF/SC-08/02.245) and the Shandong Center for Diseases Control and Prevention (IRB number: 2021-61).

2.2. Variant SARS-CoV-2 spike plasmid pseudovirus production

SARS-CoV-2 pseudoviruses were generated by co-transfecting HEK-293T cells (ATCC) with human immunodeficiency virus backbones expressing firefly luciferase (pNL4-3-R-E-luciferase) and pcDNA3.1 vector encoding either Omicron BA.5, XBB.1.5, XBB.1.16, EG.5.1 or BA.2.86 spike (S) proteins plasmid as previously described [26]. The medium was refreshed at 24 h, and the supernatants were harvested at 48 h post-transfection and clarified by centrifugation at 400*g* for 10 min before aliquoting and storing at −80 °C until use.

2.3. Pseudovirus neutralization test

The pseudovirus neutralization test (pVNT) was conducted as previously described [27,28]. Briefly, pseudoviruses were firstly titrated on HEK-293T-ACE2 cells (Vazyme) before conducting the neutralization assays to normalize the viral input between assays. Heat-inactivated sera were serially diluted starting from 1:30 with a dilution factor of three. Then, 50 μL of diluted pseudovirus was added and incubated with diluted serum for 1 h at 37 °C. After that, 2×10^4 HEK-293T-ACE2 cells per well were added and incubated at 37 °C, 5 % CO_2 for 48 h. Subsequently, luciferase activity was quantified using the Luciferase Assay System (Vazyme) using the GloMax Luminometer (Promega). Neutralization ID_{50} values for serum were calculated by a four-parameter nonlinear regression inhibitor curve in GraphPad Prism 8.0.2 (version 8.0.2, La Jolla, California, USA). A sample with ID_{50} values no more than 30 (the detectable limit) was considered negative for neutralizing antibodies and was assigned a value of 10 for geometric mean titer (GMT) calculations.

2.4. Statistical analysis

The Mann-Whitney test was used for comparisons between unpaired two groups, Friedman test and Kruskal-Wallis test with the false discovery rate method was used for comparisons among the paired and unpaired multi-groups, respectively. All statistical analyses were performed using GraphPad Prism (version 8.0.2, La Jolla, California, USA), and all statistical tests were 2-sided with a significance level of 0.05.

3. Results

During 7th February to 22nd February of 2023, we collected serum samples and questionnaire data from 457 participants in two housing estates of Licheng district (Jinan, China), approximately 2 months after the termination of zero-COVID-19 policy (Table S1).

These participants were categorized into two groups by the age: 43–59 years (middle-aged group, n = 101) and 60–83 years (elderly group, n = 356) (Fig. 1A). Of these participants, 409 (89.5 %) subjects previously vaccinated two or more doses of inactivated COVID-19 vaccines. The fully vaccination rates among the cohort were 95.0 % (96/101) in middle-aged group and 87.9 % (313/356) in elderly group (Fig. 1B). Meanwhile, 59.4 % (60/101) and 75.0 % (267/356) participants had underlying diseases in middle-aged group and elderly group, respectively (Fig. 1C). Notably, participants in elderly group had significant higher risk to get coronary heart disease than those in middle-aged group (Odds ratios [OR] = 3.393, 95 % confidence interval [CI] 1.635–7.153, $p = 0.0009$) (Fig. 1G).

We next compared the epidemiological characteristics of the two groups. 93.1 % (94/101) and 89.0 % (317/356) participants got Omicron BA.5 infection in middle-aged group and elderly group, respectively (Fig. 1D). Detailly, 14.9 % (15/101) and 78.2 % (79/101) participants were asymptomatic and symptomatic in middle-aged group, respectively. Similarly, 21.1 % (75/356) and 68.0 % (242/356) participants were asymptomatic and symptomatic in elderly group, respectively. Of these 321 symptomatic subjects, cough (62.0 %), fever (54.2 %), fatigue (43.6 %), sore throat (41.4 %), muscle aches (24.9 %), taste or smell loss (18.4 %), expectoration (12.8 %), headache (4.7 %), and nausea and vomiting (4.7 %) were the common clinical symptoms (Table S1). Of the 15 symptoms assessed, fatigue was significantly more prevalent in elderly group than the middle-aged group (OR = 1.816, 95 % CI 1.068–3.042, $p = 0.0272$) (Fig. 1H). Further comparison showed that the values of peak temperature after fever were significant higher in middle-aged group than that of elderly group, while the days of symptoms duration were shorter in middle-aged group than that in elderly group (Fig. 1E and F).

Then we measured the neutralizing antibody (NAb) titers of serum samples against Omicron BA.5, XBB.1.5, XBB.1.16, EG.5.1 and BA.2.86 subvariants by pseudovirus neutralization test (pVNT) (Fig. S2). Firstly, we observed that Omicron BA.5 infected participants had significantly higher NAb titers against all measured SARS-CoV-2 variants compared to that of non-infected participants. For these non-infected participants, only 11 (23.9 %) of them had detectable NAbs against Omicron BA.5, while the other subjects lost the ability to neutralize Omicron XBB.1.5, XBB.1.16, EG.5.1 and BA.2.86 subvariants (Fig. 2A). For the infected participants, the geometric mean titer (GMT) of NAb against Omicron BA.5 was 776, while the GMT of NAb were significant reduced by 18.0–37.0 times against Omicron XBB.1.5, XBB.1.16, EG.5.1 and BA.2.86 subvariants than that against Omicron BA.5 (Fig. 2A). The lowest GMT of NAb was observed against BA.2.86, which was significant reduced than that against XBB.1.5, XBB.1.16, and EG.5.1.

Further analysis showed that significant higher NAb titers were observed in participants with boosted vaccination against Omicron BA.5 than that in participants who were non-vaccinated or previously vaccinated with 1–2 doses (Fig. 2B). Additionally, the NAb titers were not affected by factors of age and whether they had underlying diseases (Fig. 3A and B, and Supplementary Fig. S3). Asymptomatic participants had significant higher NAb titers against EG.5.1 than that in symptomatic participants (Fig. 3C). Males only had significant higher NAb titers against EG.5.1 than that in females (Fig. 3D).

4. Discussion

In this study, a cross-sectional investigation was conducted to clarify the epidemiological and immunological characteristics of middle-aged and elderly population in communities after Omicron BA.5 wave in Jinan, China. Approximately 90 % participants suffered SARS-CoV-2 infection in housing estates after Omicron BA.5 wave. Cough (62.0 %), fever (54.2 %), fatigue (43.6 %), and sore throat (41.4 %) were the most common clinical symptoms, in which fatigue was significantly more prevalent in elderly group than in middle-aged group. The values of peak temperature after fever were significant higher in middle-aged group than that in elderly group, while the days of symptoms duration were shorter in middle-aged group than that in elderly group. In addition, the neutralizing titers elicited by Omicron BA.5 infection are significant higher against Omicron BA.5 than that against Omicron XBB and BA.2.86. The people who completed booster vaccination had significant higher NAb titers against Omicron BA.5 than others.

A previous study has estimated that 82.4 % of individuals in China being infected as of February 7, 2023 [3]. However, 89.9 % population in housing estates were diagnosed infection by self-antigen test or qRT-PCR method in this study, suggesting that the actual number of infected people in China after Omicron BA.5 wave is likely to exceed previous expectations. Documented studies have shown that fever is the most obvious symptoms after Omicron BA.5 infection, then followed by sore throat and cough [22,29]. Slightly different, the percentage of cough in middle-aged and elderly people is higher than that of fever in this study.

Previous studies have shown that the neutralizing antibodies against Omicron XBB.1.5, XBB.1.16, EG.5.1, and BA.2.86 elicited by BA.5 infection were relatively lower than that against BA.5 among adolescents and general population [10,30–33]. Consistent with the previous studies, robust neutralizing antibodies are generated against Omicron BA.5, while XBB and BA.2.86 subvariants showed immune resistance post Omicron BA.5 infection, revealing that the population in housing estates are at high risk of suffering SARS-CoV-2 reinfection.

Documentied studies have found that vaccination type and doses are closely associated with subsequent immune response [34,35]. In this study, we also find that vaccinated doses is the primary factor that is closely related to NAb titers in middle-aged and elderly population. Unexpectedly, the participants with underlying diseases have comparable NAb titers with those without underlying diseases, which is different from the documented studies [36,37]. We speculate that Omicron BA.5 breakthrough infection may eliminate the influence of underlying disease status on NAb titers.

5. Conclusion

Compared with middle-aged population, the elderly are more likely to show fatigue and have longer lasting time for clinical symptoms post Omicron BA.5 infection. Importantly, the antibody responses induced by Omicron BA.5 infection may not provide enough protection for population in communities against the emerging Omicron XBB and BA.2.86 variants. In addition, the

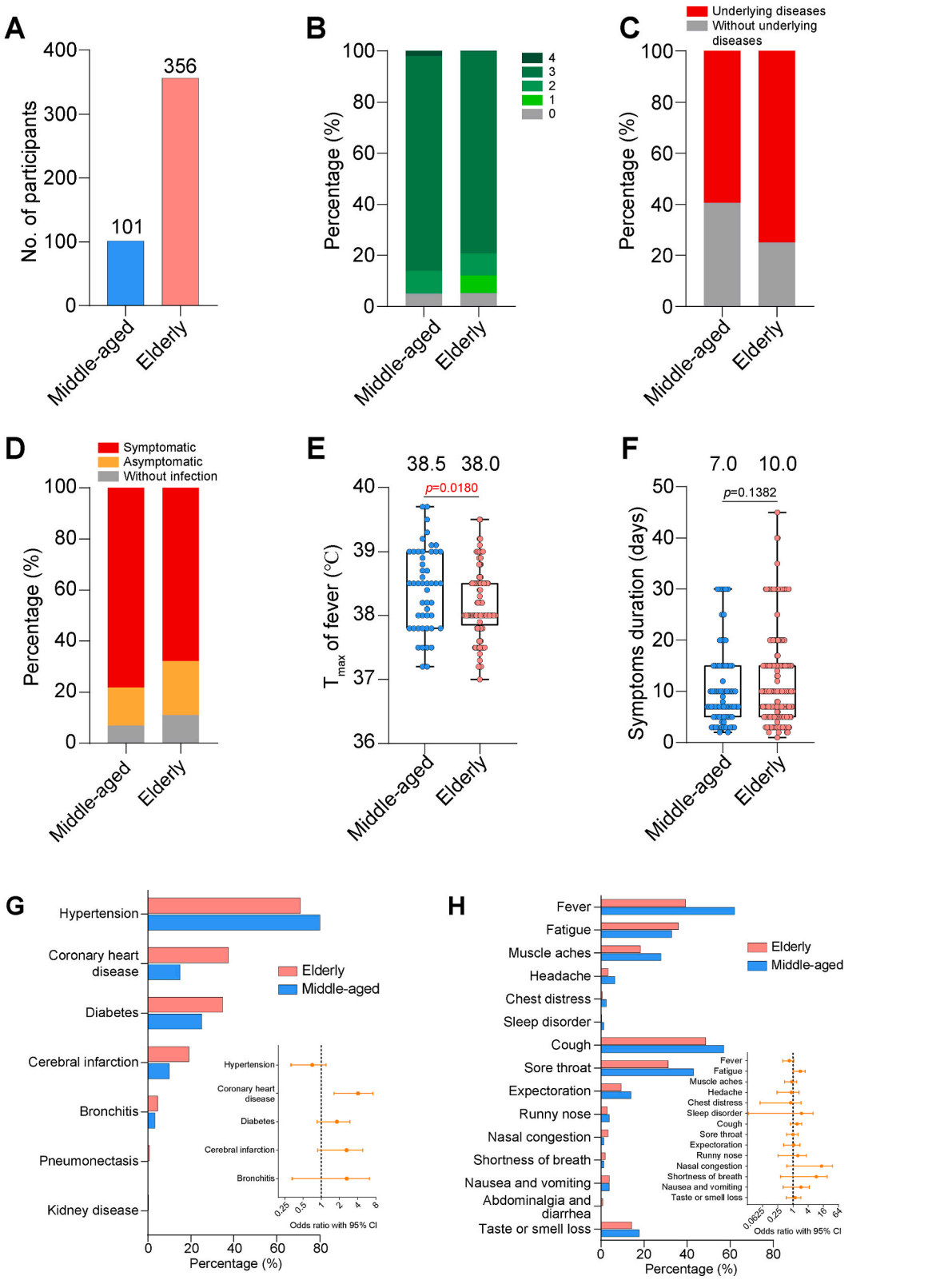

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Fig. 1. Epidemiological and immunological characteristics of middle-aged and elderly people in housing estates **after Omicron BA.5 wave.** (**A**) The number of participants enrolled in middle-aged group and elderly group. (**B**) The percentage of participants in two age groups who vaccinated with inactivated COVID-19 vaccines. (**C**) The percentages of participants in two age groups with or without underlying diseases. (**D**) The percentages of participants in two age groups without infection or with asymptomatic, symptomatic SARS-CoV-2 infection. (**E**) Fever peaks in symptomatic patients of two age groups. (**F**) Symptoms duration days in symptomatic patients of two age groups. (**G**) Prevalence of underlying diseases reported in two age group. Odds ratios (OR) with 95 % confidence interval (CI) is shown. (**H**) Prevalence of symptoms reported by symptomatic patients in two age groups. OR with 95 % CI is shown.

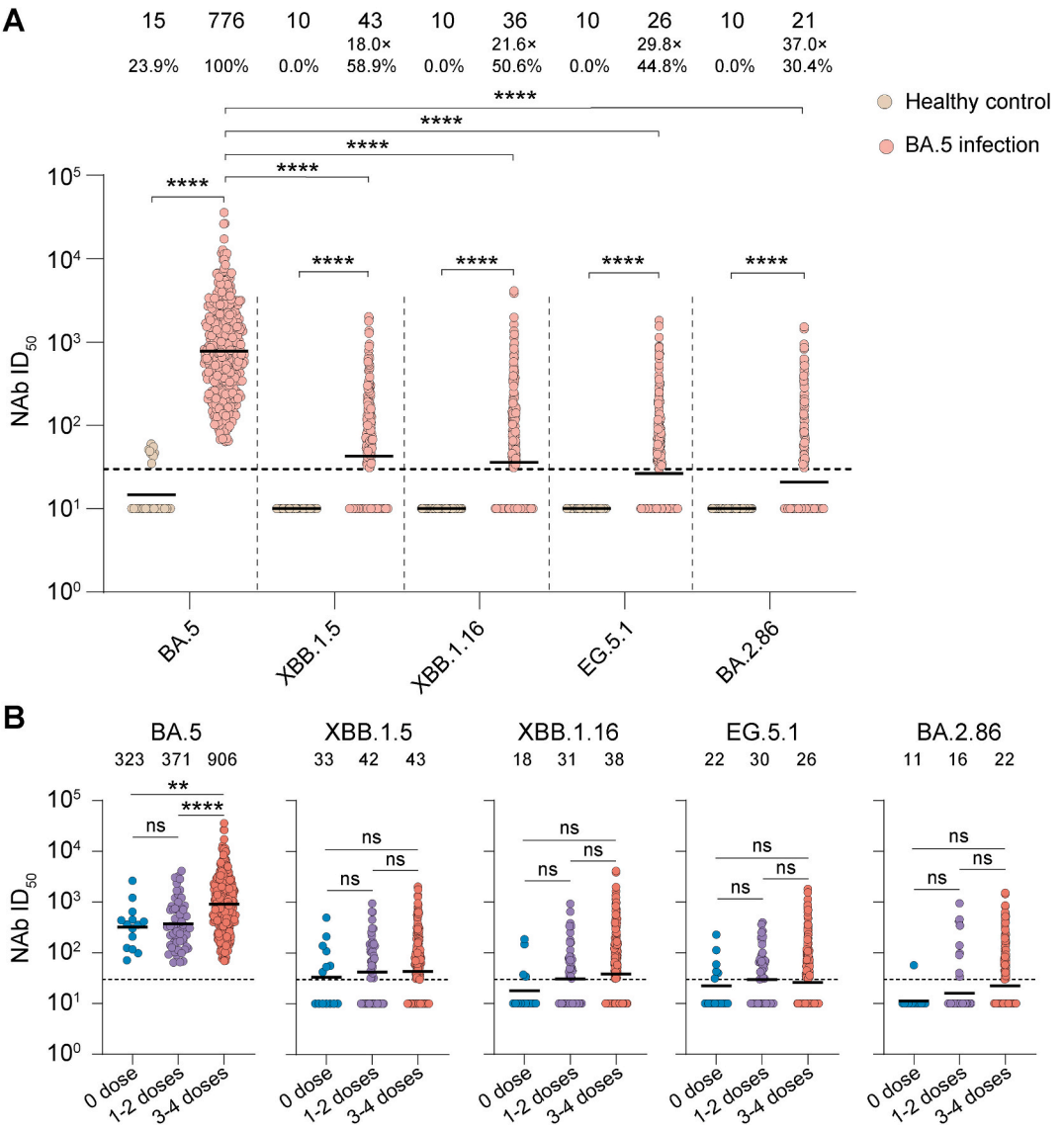

Fig. 2. Neutralizing antibody titers against different Omicron subvariants of participants in communities after Omicron BA.5 wave. (**A**) Neutralizing antibody titers against Omicron BA.5, XBB.1.5, XBB.1.16, EG.5.1, and BA.2.86 subvariants of health control (n = 46) and participants with Omicron BA.5 infection (n = 411). (**B**) Comparison of neutralizing antibody titers against Omicron BA.5, XBB.1.5, XBB.1.16, EG.5.1, and BA.2.86 subvariants by factors of vaccination doses in participants with BA.5 infection (n = 411). Geometric mean titers (GMT) of 50 % inhibitory dilution (ID50) are shown at the top of panels A and B, along with the ratio of the GMT against BA.5 by each omicron subvariants of BA.5 infection participants and the percentage of individuals with ID_{50} values above the limit of detection in panel A. The horizontal dotted lines represent the lower limit of detection of the assay (ID50 GMT 30) in panels. Mann-Whitney test and Friedman test with the false discovery rate method were used for comparison in panel A. Kruskal-Wallis test with the false discovery rate method was used for comparison in panel B. p values < 0.05 was considered statistically significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. ns indicates p values > 0.05.

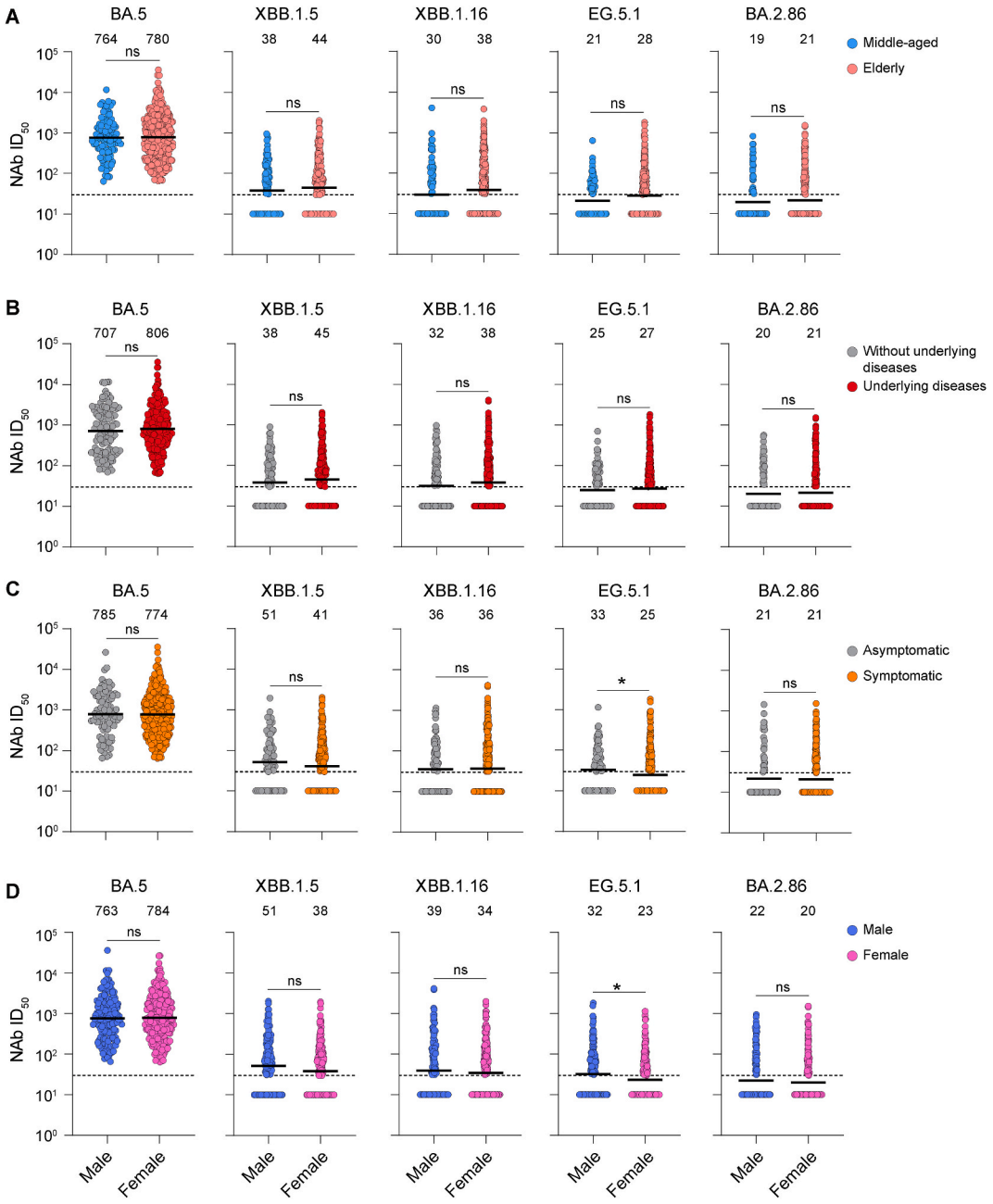

Fig. 3. Comparison of neutralizing antibody titers against different Omicron variants by different factors. Comparison of neutralizing antibody titers against different Omicron variants by different age (**A**), with or without underlying diseases (**B**), asymptomatic or symptomatic (**C**), and different sex (**D**). Geometric mean titers (GMT) of 50 % inhibitory dilution (ID_{50}) are shown at the top of panels A–D. The horizontal dotted lines represent the limit of detection of the assay (ID_{50} GMT 30). Mann-Whitney test was used for comparison in all panels. p values < 0.05 was considered statistically significant. *$p < 0.05$. ns indicates p values > 0.05.

participants completed booster vaccination had higher NAb titers than others. Therefore, immune resistance assessment of novel SARS-CoV-2 variants and development of SARS-CoV-2 updated vaccines are important for prevention and control of COVID-19 among elderly population in communities.

6. Data availability statement

The data associated with our study are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Xin-Jing Zhao: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Min Li:** Writing – review & editing, Investigation. **Sheng Zhang:** Writing – review & editing, Methodology, Formal analysis. **Ke Li:** Writing – review & editing, Investigation. **Wang-Qian Wei:** Writing – review & editing, Methodology. **Jin-Jin Chen:** Writing – review & editing, Methodology. **Qiang Xu:** Writing – review & editing, Formal analysis. **Chen-Long Lv:** Writing – review & editing, Formal analysis. **Ti Liu:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Guo-Lin Wang:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Li-Qun Fang:** Writing – review & editing, Supervision, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38382.

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