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Effect of second booster vaccination on clinical outcomes of Omicron-variant breakthrough infection: A propensity score matching cohort study

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ABSTRACT

Objective: To further explore the effect of vaccination regimen and frequency on clinical outcomes of breakthrough infections caused by the Omicron variant, as well as the durability of vaccine effectiveness.
Methods: A retrospective, propensity score matching, real-world cohort study was conducted. Vaccination frequency was categorized into regular vaccination, first booster, and second booster.
Results: A total of 7428 cases were included, with 3910 (53 %) being male. The median age was 39 years. BA.2 than BA.5/5.2 infection presented with more pulmonary symptoms and fewer influenza-like symptoms. Among the 3516 cases of BA.5/5.2 breakthrough infections, patients who received the second booster than the first booster or regular vaccination had higher first IgM and IgG titers and first cycle thredhold values for N gene on admission, a lower percentage of fever, lower peak body temperatures, and a higher percentage of asymptomatic cases. Patients who received the first booster vaccinated with homologous mRNA or heterologous inactivated plus mRNA vaccines than homologous inactivated vaccines had higher first IgM and IgG titers, a higher percentage of asymptomatic cases, and a lower percentage of fever. Moreover, significantly different first IgG titers were observed among patients receiving the second booster vaccinated with any of the three regimens. There was no statistical difference between booster regimens of homologous mRNA vaccines and heterologous inactivated plus mRNA vaccines. Patients in Month 7- than Month 0–6 after the first booster had lower first IgM and IgG titers and

Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; Ct, cycle thredhold; EMRs, electronic medical records; FiO_2, fraction of inspired oxygen; IQR, interquartile range; PaO_2, partial arterial oxygen pressure; PSM, propensity score matching; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; STROBE, STrengthening the Reporting of OBservational studies in Epidemiology; VOC, variant of concern; WHO, World Health Organization.

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first cycle thredhold values, a lower percentage of asymptomatic cases, and a higher percentage of fever; and a higher percentage of pneumonia after the second booster.
Conclusions: Repeated booster vaccinations every six months, with priority given to heterologous mRNA vaccine booster regimens in countries previously primarily using inactivated vaccines, may provide protection for adult patients with Omicron-variant breakthrough infections and improve clinical outcomes.

1. Introduction

Since the World Health Organization (WHO) first designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant B.1.1.529 (Omicron) as a variant of concern (VOC) on November 26, 2021, Omicron has spread to the vast majority of countries worldwide and has quickly become the overwhelmingly dominant strain. Multiple studies have reported that the Omicron variant is becoming less pathogenic and less invasive to the lungs [1–3], but more transmissible and more easily able to evade vaccine-induced immunity, leading to an increased risk of breakthrough infection or reinfection [4,5]. Currently, widespread vaccination to establish a solid immune barrier in the population may be the most critical measure for protecting humans from SARS-CoV-2 variant infections. Multiple SARS-CoV-2 vaccines, including commonly used inactivated virus vaccines and mRNA vaccines globally, have shown efficacy in reducing the severity of coronavirus disease 2019 (COVID-19) [6,7]. Emerging studies have reported that booster vaccinations can significantly increase neutralizing antibody levels and their effectiveness in vaccinated individuals [8–16]. In addition, some studies have demonstrated differences in neutralizing antibody levels between homologous or heterologous inactivated virus vaccines or mRNA vaccines, suggesting the importance of booster vaccination regimens [11,12,17]. Moreover, a recent study found that adult participants had higher neutralizing antibody titers after their second booster (more than 3 months apart from the first booster) compared to those before the booster, regardless of previous SARS-CoV-2 infection. This indicates the need for repeated booster vaccinations [13].

Generally, the clinical outcome of an individual with a viral infection largely depends on the host's immunity, especially the production of specific antibodies. Therefore, booster vaccination should be given priority. However, little is known about the effect of the regimen and frequency of booster vaccination on the clinical outcomes of Omicron-variant breakthrough infections in the real-world and the durability of its effectiveness, which are worth further studies to improve the protection of vaccination. Our research team has been collecting clinical information from patients since December 2021 when the first case infected with the Omicron variant was admitted to the Shenzhen Third People's Hospital. In an in vivo study, we found that patients who received three full doses of homologous inactivated virus vaccine produced higher neutralizing antibodies against Omicron-variant breakthrough infections compared to those before booster vaccinations [14]. Therefore, this observational real-world cohort study may provide data for governments and health service systems to make timely decisions and implement appropriate prevention and control measures to combat emerging SARS-CoV-2 variants.

2. Materials and methods

2.1. Study oversight and data collection

The institutional COVID-19 database of Shenzhen Third People's Hospital, which was continuously updated until December 1, 2022, was used. An observational real-world cohort study was conducted following the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement. Our study balanced and controlled confounding factors through stratified design, logistic regression, propensity score matching (PSM), and multi-subgroup analysis with large sample size. Cases with missing key data were directly excluded. Our research team updates patient's information daily from the hospital's electronic medical records (EMRs), including laboratory, epidemiological, clinical, and radiological characteristics and outcomes. All clinical data were reviewed by two trained and experienced physicians. Case selection criteria and data collection forms were discussed and developed within the research team before the formal dataset was established, and each team member was trained to ensure data quality. The authors participating in the final data analysis were blinded to the grouping settings and conditions.

2.2. Inclusion criteria

(1) Until December 1, 2022, all discharged adult cases infected with SARS-CoV-2 Omicron variants who had clear vaccination information.

Exclusion criteria.

(1) Cases with missing key data such as vaccination regimen/frequency or COVID-19 severity.
(2) Previously infected cases.
(3) Cases with pre-existing immunodeficiency or autoimmune disease or those on long-term oral immunosuppressants.

2.3. Clinical definition and classification

Adults were defined as individuals aged 18 years or older. Laboratory confirmation of SARS-CoV-2 variant infection was conducted at least twice in two different institutions: the Chinese Center for Disease Control and Shenzhen Key Laboratory of Pathogen and Immunity (Shenzhen Third People's Hospital). Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) (GeneoDX CO., Ltd) was utilized to detect SARS-CoV-2 positivity and measure viral load in nasopharyngeal swab samples. A cycle threshold (Ct) value of $\geq$35 for the N gene or ORF1ab gene was considered negative. Whole-genome sequencing and bioinformatics analysis were used to confirm Omicron subvariant types. Following hospital admission, all patients underwent chest computed tomography scans, and relevant examinations were completed as routine procedures according to the Chinese Diagnosis and Treatment Guideline of COVID-19 (Ninth Trial Version). Fever was recognized when body temperature reached or exceeded 37.3 °C. Peak body temperature referred to the highest recorded temperature during fever episodes.

Vaccination status and frequency was classified as unvaccination, partial vaccination, regular vaccination, first booster, second booster. Inactivated virus vaccines included CoronaVac (Sinovac Biotech Co., Ltd.), BBIBP-CorV (Sinopharm Beijing Biological Co., Ltd.), WIBP (Sinopharm Wuhan Biological Co., Ltd.), IMBCAMS (Institute of Medical Biology, Chinese Academy of Medical Sciences) or KCONVAC (Shenzhen Kangtai Biological Co., Ltd.), and mRNA vaccines included BNT162b2 (Pfizer BioNTech) or mRNA-1273 (Moderna). The booster regimen was categorized as either homologous inactivated virus or mRNA vaccines, or heterologous inactivated plus mRNA vaccines. Referring to the interval (about five months) between the first and second boosters in a clinical trial funded by Moderna (NCT04927065) [13], and the recommended interval of at least six months for booster vaccination in China's epidemic prevention policies, we initially classified the interval from the last booster to onset as Month 0–6, Month 7- after vaccination.

2.4. Antibody production

The specific IgM, IgG against both the S1 and N proteins of SARS-CoV-2 in serum specimens were quantitatively determined using the Novel Coronavirus (2019-nCoV) Antibody Detection Kit based on chemiluminescence method according to the manufacturer's instructions (Medical & Biological Laboratories Co., Ltd., China). The first IgM and IgG titers were defined as the test values within the first day after admission. Given that the 95th percentile length of hospital stay for included Omicron-infected patients was 21 days, we selected seven time periods from T_1 to T_7 after admission to observe antibody dynamics. T_1 to T_7 represent the following: $T_1 \leq 1$; $1 < T_2 \leq 3$; $3 < T_3 \leq 7$; $7 < T_4 \leq 10$; $10 < T_5 \leq 14$; $14 < T_6 \leq 17$; $17 < T_7 \leq 21$ days.

2.5. COVID-19 severity

The severity of COVID-19 was clinically classified according to the Chinese Diagnosis and Treatment Guideline of COVID-19 (Ninth Trial Version) as follows: (1) Asymptomatic, no clinical symptoms; (2) Mild, mild clinical symptoms, and no pneumonia on imaging; (3) Moderate, obvious clinical symptoms, such as fever, respiratory tract symptoms, pneumonia on imaging; (4) Severe, dyspnea or respiratory distress or a respiratory rate $\geq$30 times/min at rest or oxygen saturation $\leq$93 % or a ratio of partial arterial oxygen pressure (PaO_2) to fraction of inspired oxygen (FiO_2) $\leq$ 300 mmHg (1 mmHg = 0.133 kPa) at standard atmospheric pressure.

2.6. Statistical analysis

Data were analyzed using of IBM Statistical Product and Service Solutions Version 26 (SPSS 26.0, IBM Inc, Chicago, IL), and figures were generated using GraphPad Prism 8.0 software. Continuous variables were summarized as median with interquartile range (IQR), mean with standard deviation (SD), median (IQR), or mean $\pm$ SD depending on whether their distributions were normal or not. Categorical variables were compared using frequency (relative number) and Pearson Chi-square test, or Fisher exact test. The parametric tests (independent sample Student t-test or One-way analysis of variance) or non-parametric tests (Mann-Whitney *U* test or Kruskal-Wallis test) were used to analyze continuous variables. *P* values were adjusted with Bonferroni correction for multiple tests. $P < 0.05$ was considered statistically significant in all tests if applied.

2.7. Propensity score matching (PSM) analysis

To reduce the impact of selection bias and potential confounding in an observational cohort study, we conducted PSM. The propensity scores were estimated using multivariate logistic regression models, with the Omicron subvariants BA.2 and BA.5/5.2 included as binary dependent variables. Due to significant differences in vaccination status between BA.2 and BA.5/5.2 infections, we performed stratified one-to-one matching based on five levels: unvaccination, partial vaccination, regular vaccination, first booster, second booster. Age, male gender, body mass index (BMI), hypertension comorbidity, hyperlipemia comorbidity, diabetes comorbidity, smoking status, alcohol consumption were included covariates. PSM was implemented using a one-to-one nearest neighbor algorithm.

3. Results

3.1. Baseline demographic and clinical characteristics pre-PSM or post-PSM

Until December 1, 2022, a total of 7428 adult cases were included in the study, of which 3910 (53 %) were male, and 2669 (36 %) or 4759 (64 %) infected with Omicron subvariant BA.2 or BA.5/5.2, respectively. The median (IQR) age was 39 (31–51) years. There were significant differences in age, male gender, BMI, vaccination status, hypertension comorbidity, diabetes comorbidity pre-PSM between the BA.2 and BA.5/5.2 infection groups. Stratified 1:1 matching yielded 2194 BA.2 cases and 2194 BA.5/5.2 cases. There was no statistical difference in all parameters post-PSM between the two groups. Detailed demographic and clinical characteristics were presented in Table 1.

3.2. Effect of subvariant type on clinical manifestations and outcomes

There was no patient who was severely ill. Compared to patients infected with BA.2 (n = 2194), patients infected with BA.5/5.2 (n = 2194) had a higher percentage of fever, muscle soreness, fatigue, headache; a lower percentage of cough, phlegm, dyspnea, chestpain, dysgeusia, olfactory disorder, myocardial injury, asymptomatic cases, pneumonia (Table 2); lower IgM titers (0.2 vs. 0.3 within T_1; 0.2 vs. 0.3 within T_2; 0.2 vs. 0.3 within T_3 AU/mL) (Fig. 1a, Fig. s3a); lower IgG titers (27 vs. 100 within T_1; 29 vs. 69 within T_2; 169 vs. 248 within T_3; 346 vs. 369 within T_4; 317 vs. 368 within T_5 AU/mL) (Fig. 1b, Fig. s3b); higher Ct values for N gene (27.4 vs. 26.7 at day 6; 30.1 vs. 29.5 at day 7); longer intervals of N gene negative conversion (13 vs. 12 days) (Fig. 1c, Fig. s3c); higher peak body temperatures (38.4 vs. 38.2 °C) (Table s1, Fig. 1d).

3.3. Demographic characteristics and vaccination profile in BA.5/5.2 infection

A total of 3516 vaccinated cases infected with Omicron subvariant BA.5/5.2, of which 1958 (56 %) were male, and 727 (21 %), 2679 (76 %), 110 (3.1 %) received regular vaccination, first booster, second booster, respectively. The median (IQR) age and length of hospital stay were 39 (30–50) years and 10 (7–13) days. There were 2469 (89 %), 169 (6.1 %), 151 (5.4 %) booster vaccinated patients with homologous inactivated virus vaccines, homologous mRNA vaccines, heterologous inactivated plus mRNA vaccines, respectively. The median (IQR) intervals from the first booster to onset and from the second booster to onset were 275 (216–331) and 107 (45–208) days, respectively. There were 453 (17 %), 2168 (83 %) or 74 (69 %), 34 (31 %) patients in Month 0–6, Month 7- after the first or second booster, respectively (Table 3).

Table 1
Demographic and clinical characteristics pre-PSM or post-PSM between the BA.2 and BA.5/5.2 infections.

Subvariant type	Pre-PSM			Post- PSM (stratified 1:1 matching)		
	BA.2 (n = 2669)	BA.5/5.2 (n = 4759)	*P* value	BA.2 (n = 2194)	BA.5/5.2 (n = 2194)	*P* value
Age (years)	40 (30–52)	39 (31–50)	0.01	40 (31–51)	39 (30–51)	0.47
≥18, <25	277 (10 %)	486 (10 %)		233 (11 %)	233 (11 %)	
≥25, <35	746 (28 %)	1345 (28 %)		630 (29 %)	634 (29 %)	
≥35, <45	594 (22 %)	1224 (26 %)		515 (24 %)	497 (23 %)	
≥45, <55	524 (20 %)	991 (21 %)		447 (20 %)	457 (21 %)	
≥55, <65	316 (12 %)	490 (10 %)		226 (10 %)	234 (11 %)	
≥65	212 (7.9 %)	223 (4.7 %)		143 (6.5 %)	139 (6.3 %)	
Male gender	1280 (48 %)	2630 (55 %)	<0.001	1087 (50 %)	1082 (49 %)	0.88
Vaccination status			<0.001			1.00
Unvaccination	218 (8.2 %)	723 (15 %)		211 (9.6 %)	211 (9.6 %)	
Partial vaccination	98 (3.7 %)	88 (1.8 %)		63 (2.9 %)	63 (2.9 %)	
Regular vaccination	1126 (42 %)	809 (17 %)		717 (33 %)	717 (33 %)	
First booster	1222 (46 %)	3007 (63 %)		1198 (55 %)	1198 (55 %)	
Second booster	5 (0.2 %)	132 (2.8 %)		5 (0.2 %)	5 (0.2 %)	
BMI (Kg/m^2)	23 (21–25)	23 (21–26)	0.047	23 (21–25)	23 (21–25)	0.31
<24	1658 (62 %)	2811 (59 %)		1327 (61 %)	1349 (62 %)	
≥24, <28	759 (28 %)	1447 (30 %)		666 (30 %)	654 (30 %)	
≥28, <30	139 (5.2 %)	268 (5.6 %)		111 (5.1 %)	94 (4.3 %)	
≥30	113 (4.2 %)	233 (4.9 %)		90 (4.1 %)	97 (4.4 %)	
Comorbidities						
Hypertension	238 (8.9 %)	319 (6.7 %)	<0.001	153 (7.0 %)	149 (6.8 %)	0.81
Hyperlipemia	19 (0.7 %)	38 (0.8 %)	0.69	8 (0.4 %)	8 (0.4 %)	1.00
Diabetes	102 (3.8 %)	127 (2.7 %)	0.005	52 (2.4 %)	53 (2.4 %)	0.92
Smoking status	219 (8.2 %)	429 (9.0 %)	0.24	159 (7.2 %)	189 (8.6 %)	0.09
Alcohol consumption	136 (5.1 %)	271 (5.7 %)	0.28	86 (3.9 %)	93 (4.2 %)	0.59

Baseline characteristics are presented as the number (%) for categorical variables and the median (IQR) for continuous variables. BMI, body mass index; IQR, interquartile range; PSM, propensity score matching.

Table 2
Clinical manifestations between the BA.2 and BA.5/5.2 infections matched by stratified 1:1 PSM

Subvariant type	Total (n = 4388)	BA.2 (n = 2194)	BA.5/5.2 (n = 2194)	*P value
Asymptomatic	1418 (32 %)	785 (36 %)	633 (29 %)	<0.001
Clinical symptoms				
Fever	1500 (34 %)	627 (29 %)	873 (40 %)	<0.001
Cough	1075 (25 %)	613 (28 %)	462 (21 %)	<0.001
Phlegm	236 (5.4 %)	170 (7.7 %)	66 (3.0 %)	<0.001
Dyspnea	13 (0.3 %)	13 (0.6 %)	0 (0 %)	<0.001
Muscle soreness	313 (7.1 %)	103 (4.7 %)	210 (9.6 %)	<0.001
Fatigue	537 (12 %)	223 (10 %)	314 (14 %)	<0.001
Running nose	179 (4.1 %)	97 (4.4 %)	82 (3.7 %)	0.25
Pharyngalgia	1091 (25 %)	566 (26 %)	525 (24 %)	0.15
Chestpain	29 (0.7 %)	21 (1 %)	8 (0.4 %)	0.02
Headache	158 (3.6 %)	41 (1.9 %)	117 (5.3 %)	<0.001
Gastrointestinal symptoms	79 (1.8 %)	31 (1.4 %)	48 (2.2 %)	0.054
Dysgeusia	53 (1.2 %)	40 (1.8 %)	13 (0.6 %)	<0.001
Olfactory disorder	28 (0.6 %)	20 (0.9 %)	8 (0.4 %)	0.02
Pneumonia	308 (7.0 %)	221 (10 %)	87 (4.0 %)	<0.001
Myocardial injury†	173 (3.9 %)	155 (7.1 %)	18 (0.8 %)	<0.001

Myocardial injury†, troponin I values exceeded the upper limit of the reference range (0–0.034 μg/L) during hospitalization. PSM, Propensity Score Matching. *P value, BA.2 vs. BA.5/5.2.

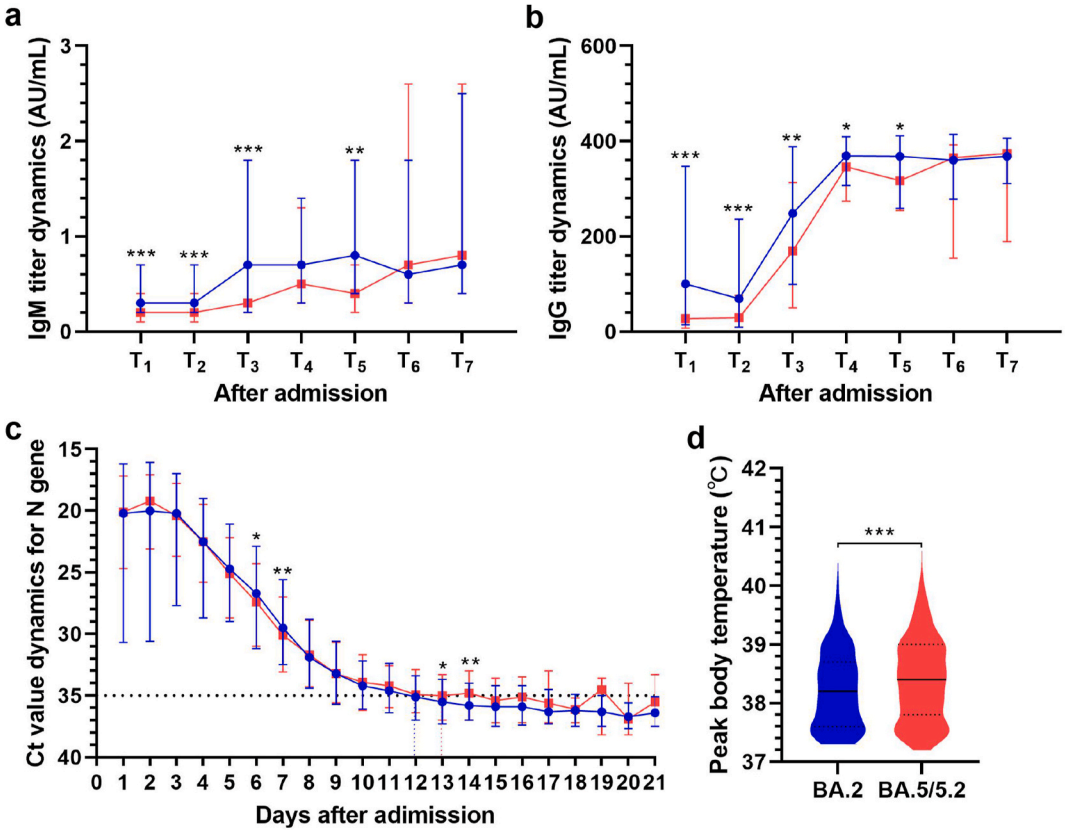

Fig. 1. Effect of subvariant type on antibody production and clinical outcomes of patients with Omicron-variant breakthrough infection. The symbols and error bars on the connecting lines in the line chart represent median and quartiles. Solid and dashed lines in the violin plot represent median and quartiles. Ct, cycle thredhold. T_1 to T_7 in (a) and (b) represent the following: $T_1 \leq 1$; $1 < T_2 \leq 3$; $3 < T_3 \leq 7$; $7 < T_4 \leq 10$; $10 < T_5 \leq 14$; $14 < T_6 \leq 17$; $17 < T_7 \leq 21$ days. Mann-Whitney U test was used for continuous variables. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (a) IgM titer dynamics. (b) IgG titer dynamics. (c) Ct value dynamics for N gene. (d) Peak body temperature, n = 627, 873 per condition.

Table 3
Demographic characteristics and vaccination profile of patients with Omicron subvariant BA.5/5.2 breakthrough infection.

Study population	N = 3516
Age (years)	39 (30–50)
Male gender	1958 (56 %)
Length of hospital stay (days)	10 (7–13)
Vaccination frequency	
Regular vaccination	727 (21 %)
First booster	2679 (76 %)
Second booster	110 (3.1 %)
Regular vaccination regimens	
Inactivated virus vaccines	677 (93 %)
mRNA vaccines	47 (6.5 %)
Inactivated virus plus mRNA vaccines	3 (0.4 %)
First booster regimens	
Homologous inactivated virus vaccines	2432/2679 (91 %)
Homologous mRNA vaccines	156/2679 (5.8 %)
Heterologous inactivated virus plus mRNA vaccines	91/2679 (3.4 %)
Second booster regimens	
Homologous inactivated virus vaccines	37/110 (34 %)
Homologous mRNA vaccines	13/110 (12 %)
Heterologous inactivated virus plus mRNA vaccines	60/110 (55 %)
Interval from the first booster to onset (days)	275 (216–331)
Month 0–6	453/2621 (17 %)
Month 7-	2168/2621 (83 %)
Interval from the second booster to onset (days)	107 (45–208)
Month 0–6	74/108 (69 %)
Month 7-	34/108 (31 %)

Data are presented as the number (%) for categorical variables and the median (IQR) for continuous variables. IQR, interquartile range.

3.4. Effect of vaccination frequency

Patients who received the second booster (n = 110) than the first booster (n = 2679) or regular vaccination (n = 727) had higher first IgM titers (0.4 vs. 0.2 or 0.2 AU/mL) (Fig. 2a, Fig. s4a); higher IgG titers (349 vs. 40 or 10 within T_1; 367 vs. 34 or 18 within T_2; 355 vs. 169 or 115 within T_3 AU/mL) (Fig. 2b, Fig. s4b); higher first Ct values on admission (22.6 vs. 19.9 or 20.6); shorter intervals of N gene negative conversion (10 vs. 12 or 14 days) (Fig. 2c, Fig. s4c); lower peak body temperatures (38 vs. 38.4 or 38.5 °C) (Fig. 2d); a lower percentage of fever (15 % vs. 41 % or 38 %) (Fig. 2e); a higher percentage of asymptomatic cases (45 % vs. 32 % or 27 %) (Table s2, Fig. 2f). Notably, these groups have included all different vaccination regimens and intervals.

3.5. Effect of booster vaccination regimen

There are 2432, 156, 91 or 37, 13, 60 patients who received the first or second booster vaccinated with homologous inactivated vaccines, homologous mRNA vaccines, heterologous inactivated plus mRNA vaccines, respectively. Patients who received the first booster vaccinated with homologous mRNA or heterologous inactivated plus mRNA vaccines than homologous inactivated vaccines had higher first IgM (Fig. 3a) and IgG titers (Fig. 3b); higher Ct values for N gene; shorter intervals of N gene negative conversion (10 or 11 vs. 12 days) (Fig. 3c, Fig. s5); a lower percentage of fever (13 % or 16 % vs. 43 %) (fig. 3e); a higher percentage of asymptomatic cases (44 % or 44 % vs. 25 %) (Fig. 3f, Table s3). Moreover, those who received the second booster vaccinated with homologous mRNA or heterologous inactivated plus mRNA vaccines than homologous inactivated vaccines had higher first IgG titers (Fig. s1, Table s5). Except for first Ct values for N gene, there was no significant difference between booster regimens of homologous mRNA vaccines and heterologous inactivated plus mRNA vaccines(Table s3). Notably, these groups have included all different vaccination intervals.

3.6. Effect of interval from the last booster to onset

The first IgG titer in patients who received the first booster decreased monthly from 3 months after vaccination, reaching and maintaining relatively low levels at about 6 months, but maintaining higher levels in patients who received the second booster (Fig. s2, Table s6). Patients in Month 7- than Month 0–6 after the first booster had lower first IgM (0.2 vs. 0.3 AU/mL) (Fig. 4a) and IgG (34 vs. 89 AU/mL) (Fig. 4b) titers and first Ct values for N gene (19.6 vs. 21.6) (Fig. 4c), a lower percentage of asymptomatic cases (25 % vs. 38 %) (Fig. 4f), and a higher percentage of fever (43 % vs. 30 %) (Fig. 4e); and only a higher percentage of pneumonia (12 % vs. 1.4 %) (Fig. 4f) after the second booster (Table s4). Notably, these groups have included all different vaccination regimens.

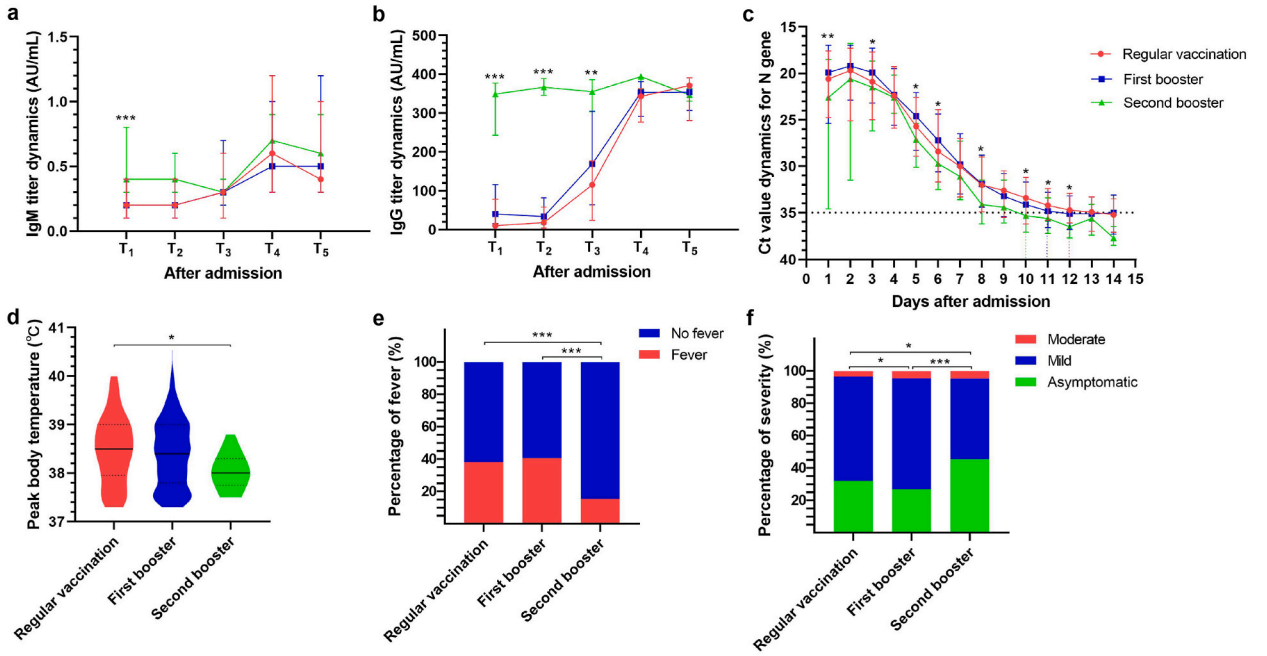

Fig. 2. Effect of vaccination frequency on antibody production and clinical outcomes of patients with Omicron subvariant BA.5/5.2 breakthrough infection. The symbols and error bars on the connecting lines in the line chart represent median and quartiles. Solid and dashed lines in the violin plot represent median and quartiles. Ct, cycle thredhold. T_1 to T_5 in (a) and (b) represent the following: $T_1 \leq 1$; $1 < T_2 \leq 3$; $3 < T_3 \leq 7$; $7 < T_4 \leq 10$; $10 < T_5 \leq 14$ days. Kruskal-Wallis test was used for continuous variables, and Pearson Chi-square test or Fisher exact test for categorical variables. *P* values were adjusted with Bonferroni correction for multiple comparisons. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (a) IgM titer dynamics. (b) IgG titer dynamics. (c) Ct value dynamics for N gene. (d) Peak body temperature, n = 278, 1088, 17 per condition. (e) Percentage of fever, 278 (38 %), 1088 (41 %), 17 (15 %) in patients received the regular vaccination, first booster, second booster vaccination presented with fever, respectively. (f) Percentage of COVID-19 severity, 233 (32 %), 718 (27 %), 50 (45 %) in patients received the regular vaccination, first booster, second booster vaccination were asymptomatic, respectively; 469 (65 %), 1842 (69 %), 55 (50 %) were mild; 25 (3.4 %), 119 (4.4 %), 5 (4.5 %) were moderate.

4. Discussion

Given the limitations of current clinical studies (such as the lack of large multicenter randomized clinical trials, significant heterogeneity of the cohort, insufficient sample size and representation, etc.), obtaining reliable evidence on the effect of booster vaccination on clinical outcomes of Omicron-variant breakthrough infection in real-world settings is challenging. This difficulty is particularly pronounced for the second booster since there are no existing reports in the literature. To our knowledge, this represents the first real-world cohort study focusing on the effect of booster vaccination regimen and frequency on clinical outcomes of Omicron-variant breakthrough infection in adults, as well as the durability of its effectiveness. Our observational study revealed several key findings: (1) BA.2 infection presented with more pulmonary symptoms, dysgeusia, olfactory disorder, pneumonia, and myocardial injury; whereas BA.5/5.2 infection presented with more influenza-like symptoms. (2) Among three different vaccination frequencies evaluated, the second booster showed the most significant improvement in clinical outcomes. (3) Both booster regimens of homologous mRNA vaccines or heterologous inactivated plus mRNA vaccines were superior to that of homologous inactivated virus vaccines. And heterologous inactivated plus mRNA vaccines were not inferior to homologous mRNA vaccines. (4) The durability of the effectiveness of booster vaccinations was approximately six months. Confounding factors were effectively balanced and controlled through stratified study design, logistic regression, PSM and multi-subgroup analysis involving a large sample size; thus ensuring robustness and reliability of our final results. These informative findings deepen our understanding of the importance of repeated booster vaccinations in preventing severe illness and improving clinical outcomes, which would contribute to the formulation of post-pandemic booster vaccination strategies.

The emergence of five main lineages of Omicron subvariants, including BA.1, BA.2, BA.3, BA.4, and BA.5, has raised new concerns about further escape from immunity induced by prior infection or vaccination and their different clinical characteristics [18]. In particular, BA.5 (first found in southern Africa in February 2022) displays higher transmissibility than other Omicron subvariants and rapidly replaces the previously circulating BA.1 and BA.2 while causing several waves of outbreaks worldwide [19]. Among the included cases, infections with BA.2 and BA.5/5.2 showed significantly different clinical features after adjusting for confounding factors such as age, male gender, vaccination status, BMI, hypertension comorbidity, and diabetes comorbidity. In addition to clinical symptoms, compared with BA.2 infection, BA.5/5.2 infection had lower first IgM and IgG titers; longer intervals of N gene negative conversion; higher peak body temperatures; a lower percentage of asymptomatic cases and pneumonia. Given that booster vaccinations were initiated clinically in mid-2021, and considering the sequential timing of the epidemics caused by BA.2 and BA.5/5.2, there

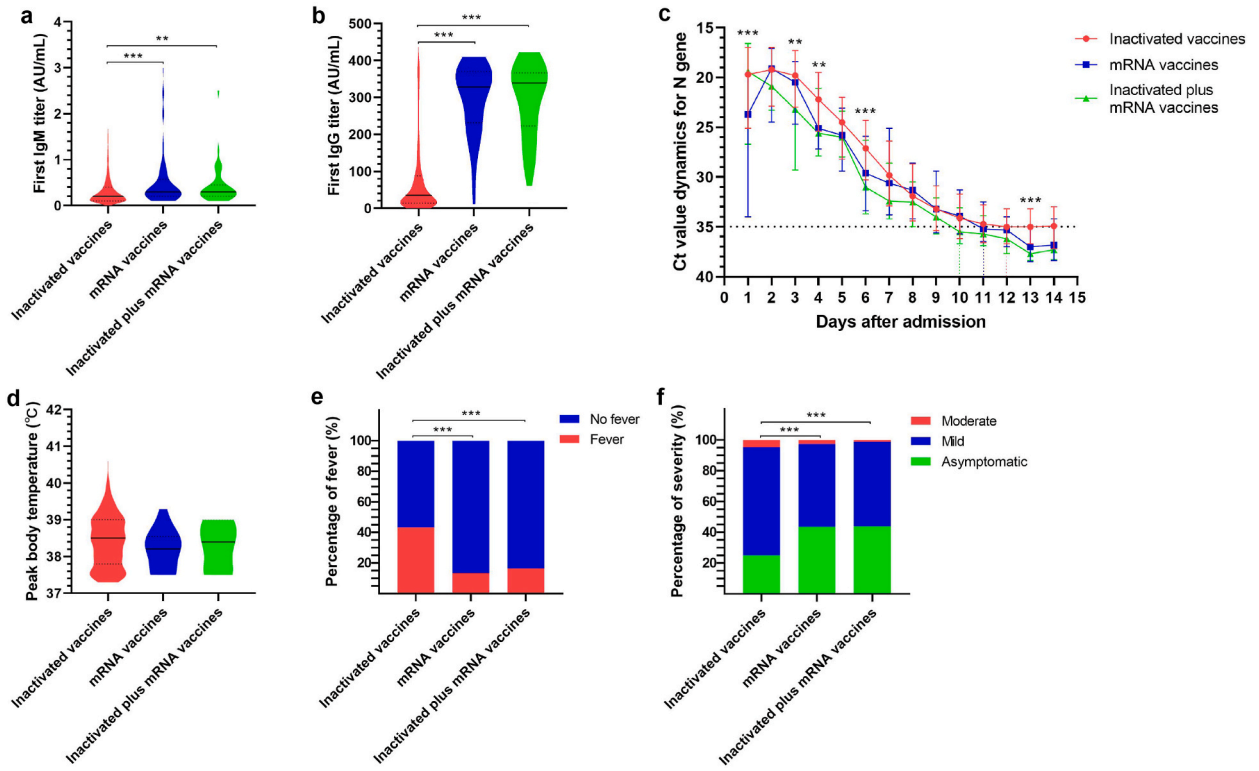

Fig. 3. Effect of first booster regimens on antibody production and clinical outcomes of patients with Omicron subvariant BA.5/5.2 breakthrough infection. The symbols and error bars on the connecting lines in the line chart represent median and quartiles. Solid and dashed lines in the violin plot represent median and quartiles. Ct, cycle thredhold. Kruskal-Wallis test was used for continuous variables, and Pearson Chi-square test or Fisher exact test for categorical variables. *P* values were adjusted with Bonferroni correction for multiple comparisons. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, n = 1423, 69, 42 per condition in (a) and (b). (a) First IgM titer. (b) First IgG titer. (c) Ct value dynamics for N gene. (d) Peak body temperature, n = 1052, 21, 15 per condition. (e) Percentage of fever, 1052 (43 %), 21 (13 %), 15 (16 %) in booster vaccinated patients with inactivated, mRNA, inactivated plus mRNA vaccines presented with fever, respectively. (f) Percentage of COVID-19 severity, 610 (25 %), 68 (44 %), 40 (44 %) in booster vaccinated patients with inactivated, mRNA, inactivated plus mRNA vaccines were asymptomatic, respectively; 1708 (70 %), 84 (54 %), 50 (55 %) were mild; 114 (4.7 %), 4 (2.6 %), 1 (1.1 %) were moderate.

were significant differences in the vaccination status among individuals infected with these subvariants. It should not be ignored that different booster vaccinations can have a complex impact on clinical characteristics and outcomes of Omicron-variant breakthrough infection. Our study found that individuals with BA.5/5.2 than BA.2 infection had a higher proportion of booster vaccination, especially the second booster. The data obtained by balancing this influencing factor by PSM might be more convincing than other studies [18,19]. These data also suggest that the Omicron subvariant BA.5/5.2 may exhibit further immune escape, delayed virus clearance, less pulmonary invasiveness, and a tendency to coexist more with the host. It is worth noting that several bivalent Omicron-containing vaccines have entered clinical use [13,19,20], which could have significant implications for future epidemic prevention and control.

The Omicron variant has multiple mutations in the antigenic site of the spike protein receptor binding domain (RBD), which could result in significant changes in antibody production after infection and evasion of vaccine-induced immunity [21]. RBD-specific SARS-CoV-2 antibodies typically reflect vaccine immunogenicity and effectiveness in preventing and combating infections [22,23]. Zhang et al. demonstrated that virus-specific IgG may play a crucial role in long-term immune protection [7]. Our study observed higher first IgG titers on admission among patients who received the first booster than regular vaccination, the second booster than the first booster. These findings suggest that the second booster can enhance IgG production more effectively after vaccination than both the first booster and regular vaccination alone, and that breakthrough infections with Omicron variants based on booster vaccinations can recall and elicit a stronger IgG response, which is basically consistent with previous studies [10,12–15]. Most importantly, patients who received the second booster had the shortest intervals of N gene negative conversion, the highest first Ct values for N gene on admission and percentage of asymptomatic cases, and the lowest percentage of fever and peak body temperatures. However, there was no significant difference between patients who received the first booster and regular vaccination. These results suggest that the second booster, rather than the first booster, may improve clinical outcomes by reducing escape from immunity induced by booster vaccination and accelerating viral clearance, highlighting the importance of repeated booster vaccinations against Omicron-variant breakthrough infections.

Despite some recent and important achievements in heterologous vaccination strategy involving mRNA vaccines as first or second boosters based on inactivated virus vaccines [8,9,20,24,25], there is still a lack of clinical research with a large sample size to

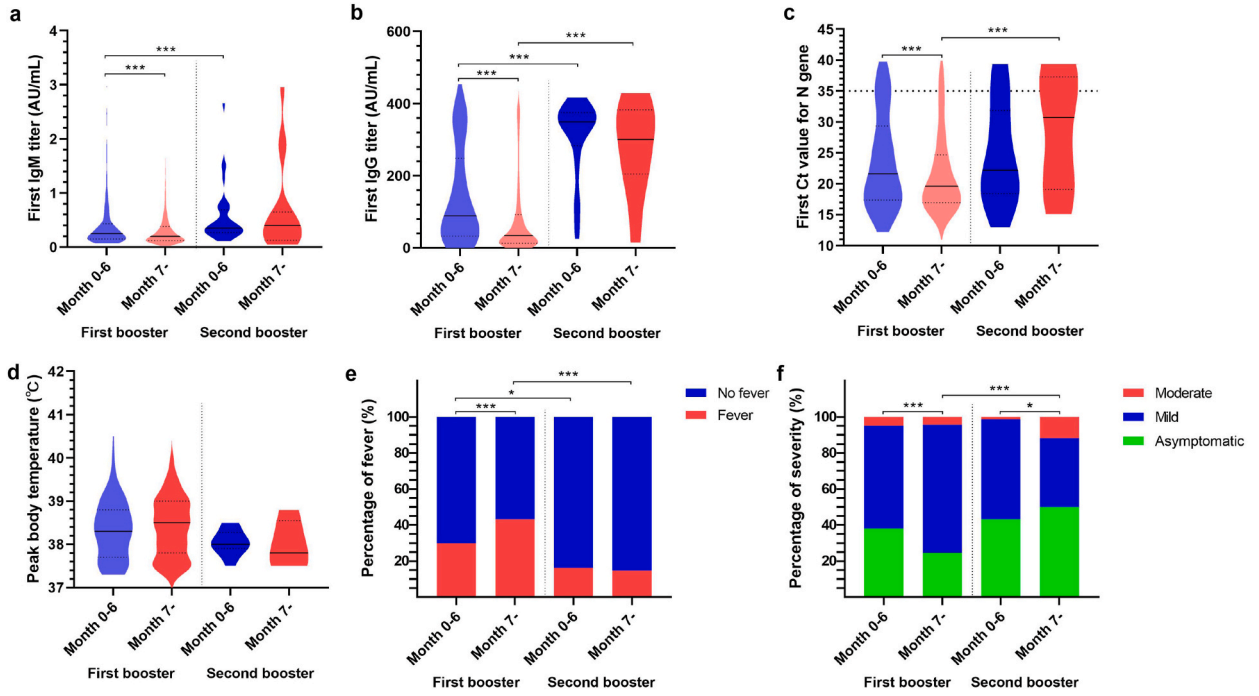

Fig. 4. Effect of interval from the last booster to onset on antibody production and clinical outcomes of patients with Omicron subvariant BA.5/5.2 breakthrough infection. Solid and dashed lines in the violin plot represent median and quartiles. Ct, cycle thredhold. Mann-Whitney *U* test was used for continuous variables, and Pearson Chi-square test or Fisher exact test for categorical variables. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, n = 255, 1517, 38, 15 per condition in (a) and (b). (a) First IgM titer. (b) First IgG titer. (c) First Ct value for N gene, n = 269, 1494, 41, 36 per condition. (d) Peak body temperature, n = 135, 936, 12, 5 per condition. (e) Percentage of fever, 135 (30 %), 936 (43 %) and 12 (16 %), 5 (15 %) in patients in Month 0–6, Month 7- after the first and second booster presented with fever, respectively. (f) Percentage of COVID-19 severity, 172 (38 %), 532 (25 %) and 32 (43 %), 17 (50 %) in patients in Month 0–6, Month 7- after the first and second booster were asymptomatic, respectively; 259 (57 %), 1541 (71 %) and 41 (55 %), 13 (38 %) were mild; 22 (4.9 %), 95 (4.4 %) and 1 (1.4 %), 4 (12 %) were moderate.

investigate the effects of different booster regimens on clinical outcomes of Omicron-variant breakthrough infections in real-world settings. In this study, we compared antibody production among booster vaccinated patients and found that those who received homologous mRNA or heterologous inactivated plus mRNA vaccines than homologous inactivated virus vaccines had higher first IgM and IgG titers, which is consistent with previous reports [11,12,26]. In addition, they had shorter intervals of N gene negative conversion, a lower percentages of fever, and a higher percentages of asymptomatic cases. These data indicate that both booster regimens of homologous mRNA or heterologous inactivated plus mRNA vaccines may be superior to that of homologous inactivated virus vaccines. However, there was no statistical difference between the two booster regimens of homologous mRNA vaccines and heterologous inactivated plus mRNA vaccines, suggesting that heterologous inactivated plus mRNA vaccines may not be inferior to homologous mRNA vaccines regarding their protective ability. Therefore, it may be reasonable for countries that previously primarily used inactivated virus vaccines to prioritize heterologous mRNA vaccine booster regimens.

To date, there have been few reports in the literature on the antibody dynamics after booster vaccination and the durability of immunological protection. In a follow-up cohort study evaluating antibody immunity to SARS-CoV-2 variants by vaccination, mean plasma RBD-specific IgG gradually decreased to low levels at month 7 after the second vaccination, but a third vaccination was able to recall and elicit a stronger IgG response [27]. We also observed similar antibody responses. The first IgM and IgG titers in patients in Month 7- than Month 0–6 after the first booster significantly decreased, but not after the second booster. Furthermore, patients in Month 7- than Month 0–6 after the first booster had lower first Ct values, a lower percentage of asymptomatic cases, and a higher percentage of fever; and only a higher percentage of pneumonia after the second booster. These data suggest that the effectiveness of booster vaccinations may last for about six months. Differences and inconsistencies in antibody production and clinical outcomes between after the first and second boosters have been hypothesized to be related to stronger immune protection and longer durability of effectiveness of the second booster, indicating a need for repeated boosters every six months in healthy vaccine recipients or another booster for patients recovering from COVID-19 six months after infection.

There are several limitations that warrant mention in our study. (1) Due to the single-center, observational, non-randomized nature of the study, there are certain confounding factors. (2) Due to particularly strict quarantine measures in China, some asymptomatic or mildly symptomatic patients infected with Omicron variants were admitted to the hospital during the very early stages of COVID-19. Clinical parameters were observed starting from hospital admission in this study, which is different from previous studies that measured parameters from symptom onset. This difference may have affected the first IgM and IgG titers on admission, as well as the Ct

value dynamics for N gene and the interval of N gene negative conversion. (3) Not all patients underwent testing for all parameters at every time point, leading to differences between calculated values and real values. For example, only a subset of patients who received the second booster were tested for antibodies; due to small sample size, statistical analysis may not accurately reflect real differences. (4) We did not include functional assays that could demonstrate the neutralization ability of these RBD-specific antibodies, and the titer may not adequately reflect the efficacy of the booster vaccination. Our study was retrospective and only a small number of patients had blood samples in the institutional biobank. Therefore, these functional assays may need to be performed in further prospective randomized clinical trials. Consequently, these results should be carefully interpreted and applied clinically due to potential selection bias and residual confounding.

5. Conclusions

Omicron subvariant BA.2 infection presented with more pulmonary symptoms, dysgeusia, olfactory disorder, pneumonia and myocardial injury. BA.5/5.2 infection presented with more influenza-like symptoms. Repeated booster vaccinations every six months, with priority given to heterologous mRNA vaccine booster regimens in countries previously primarily using inactivated vaccines, may provide protection for adult patients with Omicron-variant breakthrough infections and improve clinical outcomes.

Availability of data and materials

The datasets used and/or analyzed during the current study are all included in the article and the supplementary material. Further enquiries can be directed to the corresponding author.

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Institutional review board statement

This investigation involving human participants were reviewed and approved by the Ethics Committee of The Third People's Hospital of Shenzhen (approval number: 2022-029-03).

Informed consent statement

Written informed consent from patients participating in this study was waived in accordance with the national legislation and the institutional requirements. Patients' personal information will be strictly protected.

CRediT authorship contribution statement

Denggao Peng: Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Liuqing Yang:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cheng Jin:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jiaqi Feng:** Investigation, Formal analysis, Data curation. **Mengli Cao:** Investigation, Formal analysis, Data curation. **Yingxia Liu:** Writing – review & editing, Validation, Supervision, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Yingxia Liu reports financial support was provided by Shenzhen Fund for Guangdong Provincial High-level Clinical Key Specialties (SZGSP011). Denggao Peng reports financial support was provided by National Science and Technology Major Project (2022YFC0868800).

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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.2023.e23344.

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