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Evaluation of immune response to SARS-CoV-2 Omicron sublineages six months after different vaccination regimens in Italy

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ABSTRACT

The Omicron variant is the most divergent, displaying more mutations than previous SARS-CoV-2 variants, particularly in the gene that encodes the spike protein. This study aimed to assess the persistence of neutralizing antibodies towards the SARS-CoV-2 Omicron sublineages (BA.2, BA.5, BQ.1, XBB and XBB1.5) six months after the third dose in different vaccination regimens. Subjects who received 3 doses of mRNA vaccine retained their neutralization activity against BA.2 and BA.5, even though 56.3% and 66.7% showed a $\geq$ 2-fold reduction in the neutralizing antibody titre, respectively. Subjects who had received the adenovirus-based vaccine plus a booster dose of mRNA vaccine retained their neutralization activity especially against BA.2. With regard to BQ.1, XBB and XBB.1.5, the majority of the subjects showed a $\geq$ 2-fold reduction in neutralizing antibody titre, with the greatest evasion being observed in the case of XBB. Overall, our results provide further evidence that triple homologous/heterologous vaccination and hybrid immunity result in detectable neutralizing antibodies against the ancestral virus; however, emerging Omicron sublineages, such as XBB and XBB.1.5, show a great evasive capacity, which compromises the effectiveness of current COVID-19 vaccines.

1. Introduction

Over approximately the past 3 years, the COVID-19 pandemic has caused 770,875,433 confirmed cases and 6959,316 deaths worldwide, as reported by the World Health Organization (WHO) on 27 September 2023 (World Health Organization, 2023). Since 2020, the SARS-CoV-2 virus has mutated, giving rise to several variants, some of which have been defined by the WHO as variants of concern (VOCs). To be classified as a VOC, a variant must increase the severity of clinical disease, or change the epidemiology of COVID-19, or significantly reduce the ability of available vaccines to protect against severe disease (World Health Organization, 2023). The Omicron variant (Pango lineage B.1.1.529) is the latest to emerge. It was first reported in South Africa and Botswana in November 2021, and since then has become the dominant circulating variant worldwide (World Health Organization, 2022). The Omicron variant has been divided into sublineages: BA.1, BA.2, BA.3, BA.4 and BA.5. As of 21 September 2023, no SARS-CoV-2 circulating variants meet the VOC criteria (European Centre for Disease Prevention and Control, 2023). BA.2 seems to be more transmissible than BA.1; their genetic sequences display some differences, including some amino acid differences in the Spike (S) and other proteins. BA.4 and BA.5 have identical S sequences and are usually referred to as BA.4/5; in comparison with BA.2, they display 4 additional modifications. BQ.1 is a sublineage of BA.5 and carries S mutations at some

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key antigenic sites. As of 30 January 2023, it had a prevalence of 31.5 %, reduced to only 0.4 % as of 25 May 2023, and had been detected in 149 countries (World Health Organization, 2023; World Health Organization, 2023). XBB is a recombination of two BA.2 sublineages: BJ.1 and BA.2.75; its S protein displays 14 mutations in addition to those found in BA.2. As of 7 May 2023, XBB had a global prevalence of 10.8 % and had been detected in 127 countries (World Health Organization, 2023; Firouzabadi et al., 2023; World Health Organization, 2022, Ke et al., 2022; Planas et al., 2023; World Health Organization, 2022; Wang et al., 2023). As of 29 September 2023, XBB prevalence is reduced to 4.1 %, but it is still considered a variant under monitoring by the WHO (World Health Organization, 2023). Another Omicron sublineage, XBB.1.5, is a descendant of XBB. It seems to be just as immune-evasive as XBB.1 and is one of the sublineages with the highest immune escape (World Health Organization, 2023). As of 7 May 2023, XBB.1.5 had a global prevalence of 41.57 % and had been detected in 113 countries (World Health Organization 2023). As of 29 September 2023, XBB.1.5 is still considered a variant of interest and has a prevalence of 8.6 % (World Health Organization, 2023). Currently, the Omicron variant is the most divergent, displaying more mutations than previous SARS-CoV-2 variants, particularly in the gene that encodes the S protein of the virus (Alam, 2023, Food and Administration, 2023). The S protein plays an essential role in viral attachment, fusion, entry and transmission, and is the primary target of the current vaccines, which induce the production of neutralizing antibodies (Martinez-Flores et al., 2021; Trombetta et al., 2022). The mutations of the Omicron variant result in higher binding affinity, enhanced transmissibility, and higher rates of antibody escape (Firouzabadi et al., 2023). Table 1 summarizes the S mutations of interest (defined as changes to S protein residues 319–541 (receptor binding domain, RBD) and 613–705 (the S1 part and a small stretch of S2 of the S1/S2 junction), and any other unusual variant-specific changes) for Omicron sublineages included in the present study, as reported by European Centre for Disease Prevention and Control (ECDC) (European Centre for Disease Prevention and Control, 2023).

In Italy, four COVID-19 vaccines (Ministero della Salute, 2023), based on different technologies, have been used for global vaccination, and most of them are based on the S protein of the ancestral wild-type SARS-CoV-2 virus. The BNT162b2 and mRNA-1273 vaccines were developed by the mRNA platform technology, and are manufactured by Pfizer-BioNTech and Moderna, respectively. Together with the monovalent vaccine, an adapted bivalent vaccine, which targets the Omicron sublineages BA.4 and BA.5 in addition to the original strain of SARS-CoV-2, was authorized in late 2022 (European Medicines Agency, 2022). The other two are adenovirus vectored vaccines (Ad26.COV2.S and ChAdOx1-S) manufactured by Janssen/Johnson & Johnson and AstraZeneca.

The Omicron variant has proved to be capable of escaping the immune response elicited by two vaccine doses, and a third dose is useful in order to enhance antibody-based cross-protection and protect against some emerging Omicron variants (Trombetta et al., 2022; Trombetta et al., 2022; Willett et al., 2022; Garcia-Beltran et al., 2022; United Nations in Western Europe, 2022). Notably, booster dose seems to provide protection against severe disease and death, while to have a decreased ability to prevent infection (Berec et al., 2022; Chi et al., 2022; Feikin et al., 2022; Yang et al., 2023).

This study aimed to assess the persistence of neutralising antibodies towards the SARS-CoV-2 Omicron sublineages (BA.2, BA.5, BQ.1, XBB and XBB1.5) six months after the third dose in different vaccination regimens.

Table 1
Omicron sublineages and their spike mutations of interest as reported by European Centre for Disease Prevention and Control (European Centre for Disease Prevention and Control 2023).

Omicron sublineage	Spike mutations
BA.2	G142D, N211I, Δ212, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
BA.5	L452R, F486V, R493Q
BQ.1	K444T, N460K
XBB	N460K, F490S
XBB.1.5	N460K, S486P, F490S

2. Materials and methods

2.1. Serum samples

We analysed 48 serum samples from healthcare workers employed at the Bari correctional facility (Apulia, Italy) collected a mean of six months after the 3rd dose (booster dose) of mRNA vaccine (mRNA-1273 and/or BNT162b2) (hereafter called homologous vaccination) (Stufano et al., 2022). Their median age was 48.0 years (interquartile range (IQR) 34.2–54.4 years), 16 were male and 32 were female. We also analysed 27 serum samples from employees of the University of Bari who had received two doses of adenovirus-based vaccine plus a booster dose of mRNA vaccine (hereafter called heterologous vaccination). No information on the age or sex of these subjects was available. The research protocol was approved by the Ethics Committee of the University Hospital of Bari (n. 6955, prot. N. 0,067,544–02,082,021). The serum survey was conducted in accordance with ethical principles (Declaration of Helsinki), and written informed consent was obtained from all the participants.

All serum samples were tested by the virus neutralization (VN) assay, by ELISA for the detection of antibodies against the nucleocapsid protein (NP), which are indicative of previous infection, and by rapid immunochromatographic testing to detect antibodies against the S protein.

2.2. Virus neutralization assay

Serum samples were assayed in duplicate by the VN assay using authentic live SARS-CoV-2 viruses. Ancestral wild-type SARS-CoV-2 2019 (2019-nCov/Italy-INMI1 strain) virus (hereafter called ancestral virus) was purchased from the European Virus Archive goes Global (EVAg, Spallanzani Institute, Rome), as well as the SARS-CoV-2 Omicron XBB sublineage (SKU: 005–04,937); the Omicron BA.2, BA.5, BQ.1 and XBB.1.5 sublineages were provided from Rega Institute (Leuven). The VN assay was performed as previously described (Manenti et al., 2020). Briefly, 2-fold serial dilutions (serum starting dilution 1:10) of heat-inactivated serum samples were mixed with an equal volume of SARS-CoV-2 viral solution containing 100 tissue culture infective dose 50% ($TCID_{50}$) of each virus (serum final starting dilution 1:20). After 1 hour of incubation at 37 °C in 5 % CO_2, 100 μL of virus–serum mixture was added to a 96-well plate containing an 80 % confluent Vero E6 (ATCC CRL-1586) cell monolayer. Plates were incubated for 72 hours (ancestral virus) and 96 hours (Omicron sublineages) at 37 °C, 5 % CO_2 in a humidified atmosphere and then checked for the presence/absence of cytopathic effect (CPE) by an inverted optical microscope. A CPE higher than 50 % indicated infection.

The VN titre was expressed as the reciprocal of the highest serum dilution that showed protection from viral infection and CPE. The lower limit of detection (LOD) of the VN assay was 20. For calculation purposes, samples with VN titre below the LOD were arbitrarily assigned a VN titre of 10 (i.e. half the detection limit).

2.3. ELISA

All samples were tested by commercial ELISA (Aeskulisa® SARS-CoV-2 NP IgG, Aesku.Diagnostics, Wendelsheim, Germany) for the detection of IgG antibodies against the NP, which are indicative of previous natural infection.

In accordance with the manufacturer's instructions, quantitative analysis was performed by using a 4-parameter logistic standard curve obtained by plotting the optical density (OD) values measured for 4 calibrators against their antibody activity (U/ml) using logarithmic/linear coordinates. The antibody activities of the samples were quantified from the OD values by using the curve generated, and were considered positive if > 12 U/ml.

2.4. Rapid immunochromatographic test

All samples were tested by a rapid immunochromatographic test (iRapid SARS-CoV-2 Quant "Neutralizing" Ab, DIESSE, Siena, Italy) for the semi-quantitative determination of IgG antibodies anti-S1 (RBD) SARS-CoV-2.

In accordance with the manufacturer's instructions, the colour intensity of the test line is proportional to the antibody concentration, which is measured in Binding Antibody Units (BAU/ml).

2.5. Statistical analysis

Median VN titres were calculated along with their IQR. For each Omicron sublineage, a decrease in median VN titres in relation to the ancestral virus was calculated as the reduction factor (RF), as follows: VN titre ancestral virus/VN titre variant. The VN titres were evaluated for normal distribution by D'Agostino and Pearson, Shapiro-Wilk and Kolmogorov-Smirnov normality tests. VN titres were log transformed and, since they were still not normally distributed even after the log transformation, comparison between median VN titres was performed by the non-parametric Friedman test (for paired determination) or Kruskal-Wallis test (for non-paired determination) with Dunn's correction for multiple comparisons. Statistical significance was set at $p < 0.05$, two-tailed. All statistical analyses were performed by means of GraphPad Prism version 9.0 for Windows (GraphPad Software, San Diego, California, USA).

3. Results

In samples collected from healthcare workers who had received homologous vaccination, the median VN titre against the ancestral virus was 546.3 (IQR 113.1–1810.0), whereas the median VN titres against BA.2 and BA.5 were 339.4 (IQR 40.0–1280.0) and 273.1 (IQR 31.2–640.0), respectively. Median VN titres against BQ.1, XBB and XBB.1.5 were 56.6 (IQR 10.0–148.3), 10.0 (IQR 10.0–56.6) and 28.3 (IQR 10.0–148.3), respectively (Table 2). In three samples, the VN titre against the ancestral virus was below the LOD; similarly, VN titres were below the LOD against BA.2 in 7 samples, BA.5 in 5 samples, BQ.1 in 16, XBB in 25 and XBB.1.5 in 17 (Fig. 1).

Of the 45 subjects with a VN titre above the LOD against the ancestral virus, 91.1 % proved positive on the rapid test. In comparison with the ancestral virus, the median VN titre displayed an RF of 1.6 toward BA.2, with 56.3 % of subjects showing a ≥ 2-fold reduction in the median VN titre, and an RF of 2.0 toward BA.5, with 66.7 % of subjects showing a ≥ 2-fold reduction in the median VN titre. The median VN titre showed an RF of 9.65 toward BQ.1, of 54.6 toward XBB and of 19.3 toward XBB.1.5 in comparison with the ancestral virus, with 93.75 % of subjects showing a ≥ 2-fold reduction in the median VN titre against all three of these sublineages.

Differences between the VN titres against the ancestral virus and the five Omicron sublineages were statistically significant in the cases of BA.5 (Friedman test, $p = 0.0193$), BQ.1, XBB and XBB.1.5 (Friedman test, $p < 0.0001$ for these last three). No significant differences in median VN titres were observed between BA.2 and BA.5 or amongst BQ.1, XBB and XBB.1.5, while the titres against these last three were significantly lower than those against BA.2 and BA.5 (Friedman test, $p < 0.0001$ for both).

The samples were then divided according to the reported history of COVID-19 and/or positivity to IgG against NP ($N = 22$). VN titres were significantly higher in samples from subjects who had experienced SARS-CoV-2 infection, both on comparing titres against the ancestral virus (Kruskal-Wallis test, $p = 0.0164$) and, even more so, on comparing titres against BA.2 (Kruskal-Wallis test, $p = 0.0002$) and BA.5 (Kruskal-Wallis test, $p = 0.0006$); in the case of the other Omicron sublineages, however, no significant difference emerged.

No statistically significant differences were found amongst subjects of different age, sex, or body mass index (BMI).

In samples collected from subjects who had received heterologous vaccination, the median VN titre against the ancestral virus was 113.1 (IQR 40.0–905.1), whereas against BA.2 and BA.5 it was 226.3 (IQR 56.6–905.1) and 56.6 (IQR 10.0–226.3), respectively. Median VN titres against BQ.1, XBB and XBB.1.5 were 10.0 (IQR 10.0–40.0), 10.0 (IQR 10.0–28.3) and 10.0 (IQR 10.0–28.3), respectively (Table 2). The VN titre was below the LOD against both the ancestral virus and BA.2 in 2 samples, against BA.5 in 8 samples, against BQ.1 in 15, XBB in 17 and XBB.1.5 in 16 (Fig. 2).

Of the 25 subjects with a VN titre above the LOD against the ancestral virus, 96.0 % proved positive on the rapid test. In comparison with the ancestral virus, the median VN titre displayed an RF of 0.5 toward BA.2, with 81.5 % of subjects showing the same median VN titre and 18.5 % showing a ≥ 2-fold reduction in the median VN titre; the RF toward BA.5 was 2.0, with 74.1 % of subjects showing a ≥ 2-fold reduction in the median VN titre. Median VN titres showed RF of 11.3 toward BQ.1, XBB and XBB.1.5 in comparison with the ancestral virus, with 92.6 % of subjects showing a ≥ 2-fold reduction in the median VN titre against all three sublineages.

Differences between VN titres against the ancestral virus and the Omicron sublineages were statistically significant in the cases of BQ.1, XBB and XBB.1.5 (Friedman test, $p < 0.0001$ for all three), but not BA.2 or BA.5. Moreover, significant differences were also found between BA.2 and BA.5 (Friedman test, $p = 0.0048$) and amongst BQ.1, XBB and XBB.1.5 (Friedman test, $p < 0.0001$ for all three).

The samples were then divided according to positivity to IgG against NP ($N = 9$). VN titres were significantly higher in subjects who had experienced SARS-CoV-2 infection on comparing titres against the Omicron sublineages BA.5 (Kruskal-Wallis test, $p = 0.0197$) and XBB.1.5 (Kruskal-Wallis test, $p = 0.0419$), but not on comparing titres against the ancestral virus or the other Omicron sublineages.

Table 2
Number of subjects and median virus neutralization (VN) titres (Interquartile range, IQR) against ancestral SARS-CoV-2 virus and Omicron sublineages, by cohort.

Cohort	Number of subjects	Ancestral virus median VN titre (IQR)	Omicron BA.2 median VN titre (IQR)	Omicron BA.5 median VN titre (IQR)	Omicron BQ.1 median VN titre (IQR)	Omicron XBB median VN titre (IQR)	Omicron XBB.1.5 median VN titre (IQR)
3x mRNA vaccine (Homologous vaccination)	48	546.3 (113.1–1810.0)	339.4 (40.0–1280.0)	273.1 (31.2–640.0)	56.6 (10.0–148.3)	10.0 (10.0–56.6)	28.3 (10.0–148.3)
2x adenovirus-based plus 1x mRNA vaccine (Heterologous vaccination)	27	113.1 (40.0–905.1)	226.3 (56.6–905.1)	56.6 (10.0–226.3)	10.0 (10.0–40.0)	10.0 (10.0–28.3)	10.0 (10.0–28.3)

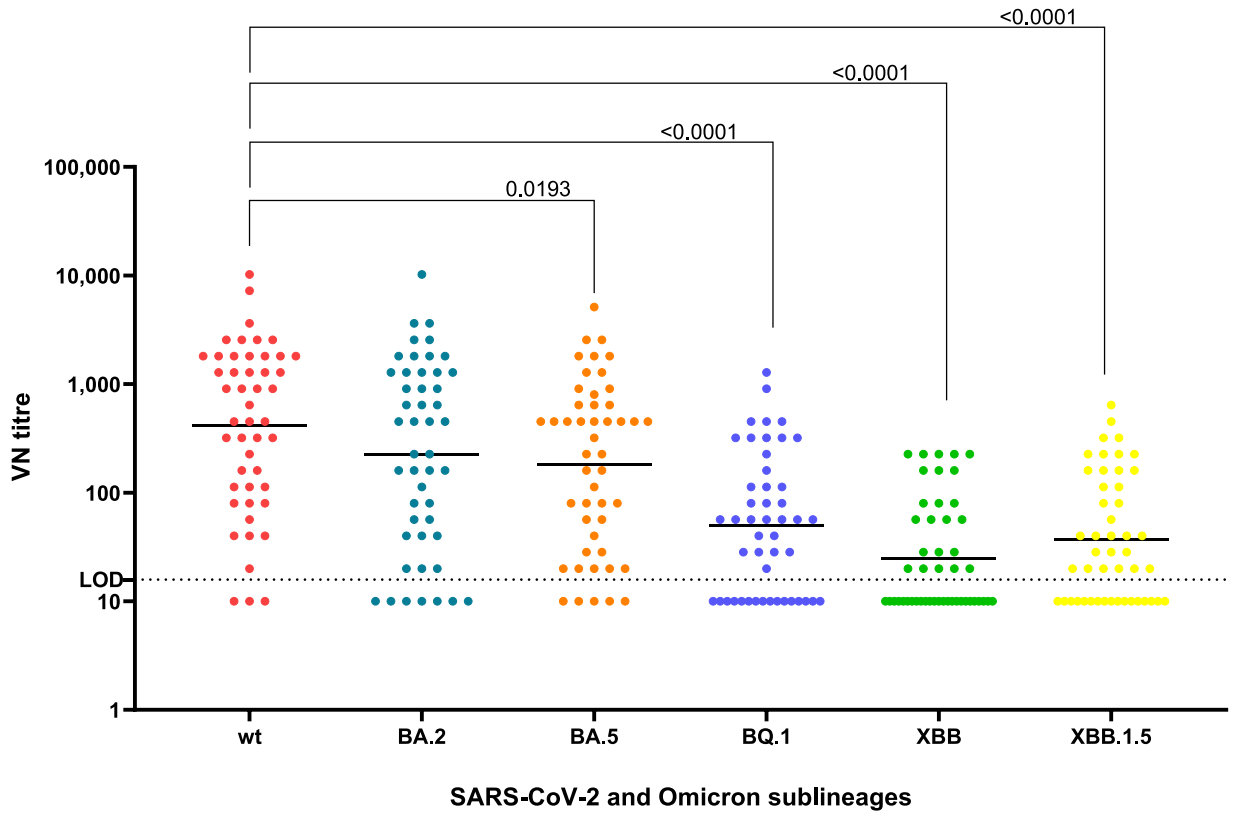

Fig. 1. Virus Neutralization (VN) titres against SARS-CoV-2 ancestral virus (wt) and Omicron sublineages in healthcare workers who had received three doses of mRNA vaccine (homologous vaccination, $N = 48$). Black lines indicate median VN titres against wt or Omicron sublineages. The horizontal dashed line represents the Lower Limit of Detection (LOD) of the VN assay. Statistically significant differences with respect to wt were reported as p-values.

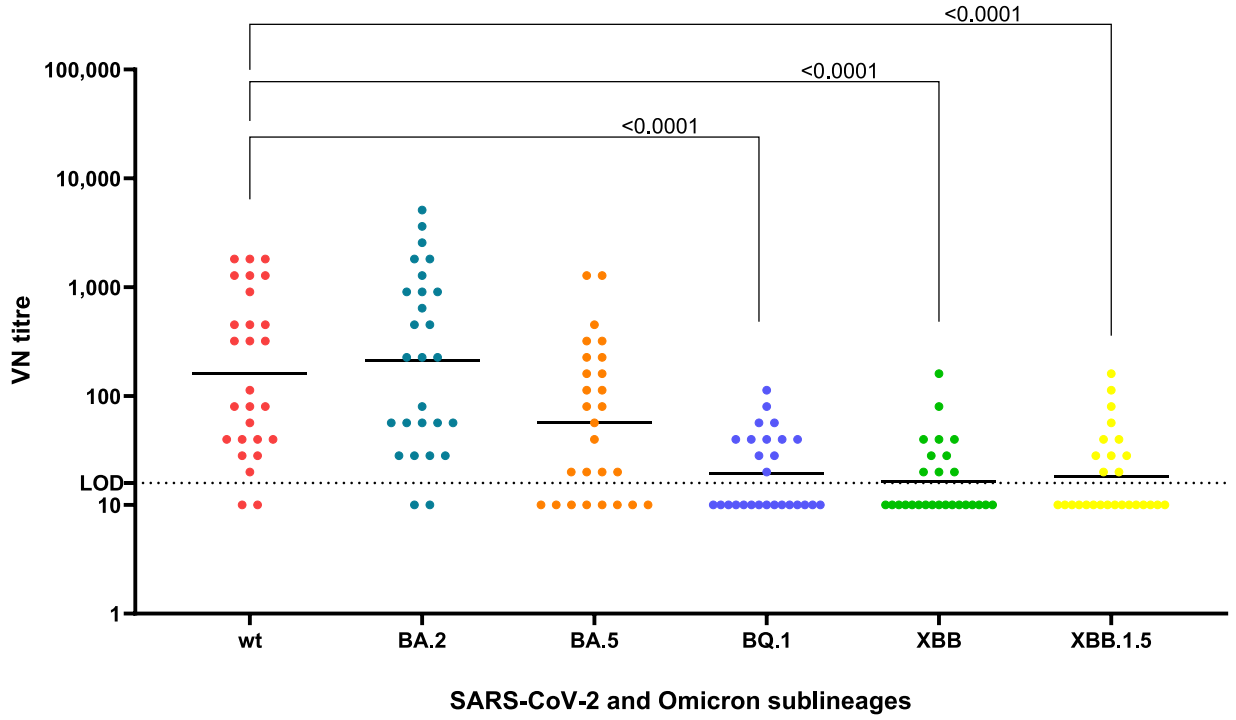

Fig. 2. Virus Neutralization (VN) titres against SARS-CoV-2 ancestral virus (wt) and Omicron sublineages in subjects who received two doses of adenoviral vaccine plus a booster dose of mRNA vaccine (heterologous vaccination, $N = 27$). Black lines indicate median VN titres against wt or Omicron sublineages. The horizontal dashed line represents the Lower Limit of Detection (LOD) of the VN assay. Statistically significant differences with respect to wt were reported as p-values.

No differences were found on comparing VN titres from both cohorts.

4. Discussion

The public health implications of the emergence of some Omicron sublineages, specifically XBB and BQ.1 and their sublineages (World Health Organization, 2022), is raising concerns regarding vaccine effectiveness and waning immunity.

Neutralizing antibodies are considered to be a correlate of protection, and the VN assay with live viruses is currently considered the gold standard for assessing antibody-mediated protection in naturally infected and vaccinated subjects (Ministero della Salute, 2023; European Medicines Agency, 2022).

We found that all but three subjects who had received three doses of mRNA vaccine had detectable neutralizing antibodies against ancestral SARS-CoV-2 virus six months after the 3rd vaccine dose; this is in agreement with other studies (Pajon et al., 2022; Xia et al., 2022). Notably, subjects with a history of COVID-19 and/or positivity to NP had significantly higher neutralizing antibody titres than uninfected subjects, as previously reported (Trombetta et al., 2022; Trombetta et al., 2022; Sette and Crotty, 2022; Anichini et al., 2021), thus suggesting that hybrid immunity may substantially increase both the potency and breadth of the humoral response against SARS-CoV-2 (Sette and Crotty, 2022; Collatuzzo et al., 2022).

We next assessed the neutralization activity against Omicron sublineages by comparing neutralization titres against the ancestral virus and Omicron BA.2 and BA.5. BA.5 has two additional mutations in the RBD, which are of most concern regarding antibody escape. The majority of subjects retained their neutralization activity against BA.2, even though 56.3 % showed a $\geq$ 2-fold reduction in the median VN titre. The same trend was noted for BA.5, but the decrease was more marked; 66.7 % of subjects showed a $\geq$ 2-fold reduction in the median VN titre, indicating the ability of these variants to partially escape the neutralizing antibodies induced by vaccination and/or hybrid immunity. These data are in line with those from other studies (Arora et al., 2022; Dapporto et al., 2022; Hachmann et al., 2022; Quandt et al., 2022).

Regarding heterologous vaccination, we found that all but two subjects who had received the adenovirus-based vaccine plus a booster dose of mRNA vaccine had detectable neutralizing antibodies against the ancestral SARS-CoV-2 virus six months after the booster dose. Surprisingly, 81.5 % of subjects showed the same VN titre against BA.2. One explanation could be that these subjects may have been infected by this latter variant. Another is that other branches of immunity may be involved. Indeed, it has been reported that heterologous vaccination provides an innate and adaptive immune response that exceeds homologous vaccination (Lee and al., 2022). An Austrian study (Jager et al., 2023) reported that subjects vaccinated twice with an adenoviral vaccine (ChAdOx1) displayed very robust T-cell responses and neutralizing activity against BA.1 six months after the second dose, while no lasting neutralization of BA.2 was detected. Moreover, a study evaluating the neutralizing response against Omicron BA.1 and BA.2, although inferior to that elicited against the ancestral virus, found a higher cross-reactive response to BA.2 than BA.1, particularly in subjects vaccinated with 3 heterologous doses (Dapporto et al., 2022), as also observed by Liu et al. (2021).

With regard to BQ.1, XBB and XBB.1.5, 93.75 % of subjects who received homologous vaccination showed a $\geq$ 2-fold reduction in the median VN titre, with the greatest evasion being observed in the case of XBB. Our results are consistent with those of another study, which reported that neutralizing activity was lowest towards XBB, even in subjects who had received the BA.5-containing bivalent booster, although they showed better neutralization activity than those who had received either one or two monovalent booster doses (Davis-Gardner et al., 2023). Another study reported that subjects who underwent monovalent vaccination showed dramatically lower neutralization activity, with roughly 80 % of vaccinees failing to neutralize XBB and XBB.1.5; these findings indicate that recent mutations in the S protein have enhanced the immune-evasive properties of new sublineages (Devasundaram et al., 2023). Strong neutralization resistance has also been detected in the case of the BQ.1 variant; this is largely driven by the N460K mutation, which is shared with BQ.1.1 (Wang et al., 2023; Qu et al., 2023). The loss of neutralizing activity against BQ.1, XBB and XBB.1.5 was also observed in subjects who received heterologous vaccination, with 92.6% of them showing a $\geq$ 2-fold reduction in the median VN titre.

Previous studies have showed how the third dose was able to elicit high neutralizing ability and IgG avidity against the Omicron variant (Richterman et al., 2021; Bar-On et al., 2021; Dapporto et al., 2022), supporting the recommendation for a supplementary dose in order to maintain protection against emerging variants.

A strength of this study was the use of authentic live SARS-CoV-2 viruses for VN assay, instead of a surrogate neutralization assay. In addition, the long incubation time (three to four days) of the virus-sample mixture in cell cultures allowed to identify more accurately the antibody titre that better correlates with protection, as this titre is based on the complete inhibition of CPE in the cell monolayer.

The present study has several limitations. First, the number of subjects enroled was relatively small, no information on the age or sex of subjects who received heterologous vaccination was available. The lack of follow-up samples and neutralizing activity following the third vaccine. Furthermore, we did not evaluate other branches of immunity, such as T-cell responses, which could contribute to protection even when neutralizing antibodies are absent or reduced. Lastly, we did not evaluate the antibody responses elicited by the bivalent vaccine.

Overall, our results provide further evidence that triple homologous/heterologous vaccination and hybrid immunity result in detectable neutralizing antibodies against the ancestral virus, while emerging Omicron sublineages, such as XBB and XBB.1.5, show a great evasive capacity, which compromises the effectiveness of current COVID-19 vaccines. Globally, there has been a steady rise in the prevalence of XBB.1.16. This variant has a similar profile to XBB.1.5, but displays additional changes in the S protein and shows greater infectivity and potentially increased pathogenicity (Schnirring, 2023; World Health Organization, 2023). Duration of vaccine-induced immunity is decisive for the formulation of vaccine policies, including the recommendation on the population target and timing of an additional booster dose. Considering that in Italy 31.42 % of eligible subjects have received a second booster dose (Ministero della Salute, 2023), most of the Italian population, and other populations (Tartof et al., 2022), may have low levels of vaccine-derived immunity. A booster programme may be a crucial in enhancing the effectiveness of COVID-19 vaccines against newly divergent emerging variants. The administration of a booster dose with the most recent S variants, especially for vulnerable and high-risk groups, should be considered, and efforts should be made to develop broadly protective vaccines.

Ethics statement

The research protocol was approved by the Ethics Committee of the University Hospital of Bari (n. 6955, prot. N. 0,067,544–02,082,021). The serum survey was conducted in accordance with ethical principles (Declaration of Helsinki), and written informed consent was obtained from all the participants.

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CRediT authorship contribution statement

Claudia Maria Trombetta: Conceptualization, Resources, Writing – original draft, Supervision, Project administration, Funding acquisition. **Serena Marchi:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration. **Margherita Leonardi:** Conceptualization, Investigation, Writing – review & editing. **Chiara Coppola:** Investigation, Writing – review & editing. **Linda Benincasa:** Investigation, Writing – review & editing. **Maria Giovanna Marotta:** Investigation, Writing – review & editing. **Nicola Buonvino:** Resources, Writing – review & editing. **Piet Maes:** Resources, Writing – review & editing. **Angela Stufano:** Resources, Writing – review & editing. **Daniela Pontrelli:** Resources, Writing – review & editing. **Violetta Iris Vasinioti:** Writing – review & editing. **Alessandro Manenti:** Writing – review & editing. **Michele Camero:** Writing – review & editing. **Emanuele Montomoli:** Writing – review & editing. **Nicola Decaro:** Resources, Writing – review & editing. **Piero Lovreglio:** Supervision, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: C. M.T. is an external consultant of VisMederi Research srl. M.L. and L.B. are employees of VisMederi Research srl. A.M. is an employee of VisMederi srl. E.M. is founder and Chief Scientific Officer of VisMederi srl and VisMederi Research srl. The other authors declare no competing interests.

Data availability

Data will be made available on request.

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