International Journal of Infectious Diseases 128 (2023) 166–175

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Heterologous prime-boost immunization with ChAdOx1-S and BNT162b2: reactogenicity and immunogenicity in a prospective cohort study

Niko Kohmer[1,*], Shivana Stein[1], Barbara Schenk[1], Katharina Grikscheit[1], Melinda Metzler[1], Holger F. Rabenau[1], Marek Widera[1], Eva Herrmann[2], Sabine Wicker[3], Sandra Ciesek[1,4,5,**]

[1] Institute for Medical Virology, University Hospital, Goethe University Frankfurt am Main, Frankfurt, Germany
[2] Institute of Biostatistics and Mathematical Modelling, Goethe University Frankfurt, Frankfurt, Germany
[3] Occupational Health Service, University Hospital, Goethe University Frankfurt am Main, Frankfurt, Germany
[4] German Centre for Infection Research, External Partner Site, Frankfurt, Germany
[5] Fraunhofer Institute for Translational Medicine and Pharmacology, Frankfurt, Germany

ARTICLE INFO

Article history:
Received 23 August 2022
Revised 21 November 2022
Accepted 23 December 2022

Keywords:
Heterologous prime-boost
ChAdOx1-S
BNT162b2
Immunogenicity
Reactogenicity

ABSTRACT

Objectives: Regarding reactogenicity and immunogenicity, heterologous COVID-19 vaccination regimens are considered as an alternative to conventional immunization schemes.
Methods: Individuals receiving either heterologous (ChAdOx1-S [AstraZeneca, Cambridge, UK]/BNT162b2 [Pfizer-BioNTech, Mainz, Germany]; n = 306) or homologous (messenger RNA [mRNA]-1273 [Moderna, Cambridge, Massachusetts, USA]; n = 139) vaccination were asked to participate when receiving their second dose. Reactogenicity was assessed after 1 month, immunogenicity after 1, 3, and/or 6 months, including a third dose, through SARS-CoV-2 antispike immunoglobulin G, surrogate virus neutralization test, and a plaque reduction neutralization test against the Delta (B.1.167.2) and Omicron (B.1.1.529; BA.1) variants of concern.
Results: The overall reactogenicity was lower after heterologous vaccination. In both cohorts, SARS-CoV-2 antispike immunoglobulin G concentrations waned over time with the heterologous vaccination demonstrating higher neutralizing activity than homologous mRNA vaccination after 3 months to low neutralizing levels in the Delta plaque reduction neutralization test after 6 months. At this point, 3.2% of the heterologous and 11.4% of the homologous cohort yielded low neutralizing activity against Omicron. After a third dose of an mRNA vaccine, ≥99% of vaccinees demonstrated positive neutralizing activity against Delta. Depending on the vaccination scheme and against Omicron, 60% to 87.5% of vaccinees demonstrated positive neutralizing activity.
Conclusion: ChAdOx1-S/BNT162b2 vaccination demonstrated an acceptable reactogenicity and immunogenicity profile. A third dose of an mRNA vaccine is necessary to maintain neutralizing activity against SARS-CoV-2. However, variants of concern-adapted versions of the vaccines would be desirable.

1. Introduction

At the beginning of the vaccination campaign during the COVID-19 pandemic, the ChAdOx1-S vaccine (AstraZeneca, Cambridge, UK) was available in Europe. The recommendation for the use in Germany was given by the Standing Committee on Vaccination (STIKO) for individuals aged 18-64 years on January 29, 2021. The shortage of vaccine doses at this time point led to a prioritization of the ChAdOx1-S vaccine mainly to individuals with a high risk for an infection with SARS-CoV-2, including health care workers at the front line. After a series of blood clotting events in Europe, in particular, severe sinus vein thrombosis in young individuals [1], this recommendation was adjusted in April 2021 to the effect that a messenger RNA (mRNA) vaccine instead of the vector vaccine ChAdOx1-S was recommended to people aged below 60 years [2]. Consequently, a heterologous vaccination scheme with a mRNA vaccine (BNT162b2 [BioNTech/Pfizer, Mainz, Ger-

* Corresponding authors: Niko Kohmer; Tel: +49 696301 6454
** Sandra Ciesek: Tel: +49 696301 5219
E-mail addresses: niko.kohmer@kgu.de (N. Kohmer), sandra.ciesek@kgu.de (S. Ciesek).

https://doi.org/10.1016/j.ijid.2022.12.034

many/New York, NY, USA]/mRNA-1273 [Moderna, Cambridge, Massachusetts, USA]) was considered for individuals having received their first dose with ChAdOx1-S [3]. Data regarding reactogenicity and immunogenicity concerning this regimen gained importance. Several studies indicate that the heterologous vector/mRNA vaccine scheme is associated with a tolerable reactogenicity profile [4,5] and is not inferior to a homologous scheme in terms of immunogenicity [6–8]. The purpose of the presented study is to determine the reactogenicity and immunogenicity of the heterologous vaccination (ChAdOx1-S/BNT162b2) scheme. To achieve this, employees of the University Hospital Frankfurt having received their routine COVID-19 vaccination were asked to participate in our study. A homologous mRNA-1273 vaccinated cohort was used as a control. As the humoral mediated immune response serves as a surrogate for immunity, we focused our analysis on the SARS-CoV-2 antispike immunoglobulin (Ig) G and neutralizing antibody response for up to 6 months after basic immunization. As the Delta (B.1.167.2) and Omicron (B.1.1.529) variants of concern (VOCs) became dominant in the second half of 2021 and spring 2022 in Germany, respectively, neutralizing capacity was measured by plaque reduction neutralization test (PRNT) against these variants. When the STIKO recommended a third vaccine dose in November 2021 [9], we decided to include participants receiving the booster dose as well.

2. Materials and methods

2.1. Study design

Employees of the University Hospital Frankfurt (18-59 years of age) receiving their routine COVID-19 immunization according to the guidelines of the STIKO were asked to participate in our study. On the date of receiving the second dose, informed written consent was obtained together with baseline demographic and health (focus on immunodeficiency or immunosuppression) data and blood for immunological analyses. The heterologous cohort received their second dose with 30 µg of BNT162b2 (mRNA-vaccine), further called “BNT”, within 9-12 weeks after the first dose of ChAdOx1-S vaccine (vector vaccine), further called “AZ” (heterologous scheme: AZ/BNT). The homologous cohort received their second dose of mRNA-1273 (mRNA-vaccine), further called “Moderna” 6 weeks after the first dose (100 µg each; homologous scheme: two × Moderna). Dosages for individuals receiving a third dose were: 30 µg for BNT and 50 µg for Moderna. There were three follow-up visits about 1 month (follow-up I), 3 months (follow-up II), and 6 months (follow-up III) after the second dose. For individuals receiving a third dose 6 months after the second dose, the follow-up III examination was about 14 days after the third dose. On every visit, blood was drawn and participants were asked whether there was a polymerase chain reaction (PCR)-confirmed SARS-CoV-2 infection. Enrolled participants not showing up to a study visit were reinvited to the next visit. The study design is depicted in Figure 1.

2.2. Assessment of reactogenicity and safety

A month after the second dose (follow-up I) participants were asked for subjective local or systemic reactions (mild/moderate/severe) and the timing of occurrence after receiving the vaccine by using a questionnaire. Participants could provide additional data through a free text field.

2.3. Assessment of immunogenicity

The presence of SARS-CoV-2 specific antispike (receptor binding domain) and antinucleocapsid protein IgG antibodies was assessed using the Abbott SARS-CoV-2 IgG II (cutoff for positivity ≥7.1 binding antibody units [BAU]/ml) and SARS-CoV-2 IgG on the Abbott Alinity i platform (Abbott GmbH, Wiesbaden, Germany), according to the manufacturers’ recommendation. The first assay was used to measure the vaccine-induced humoral mediated immune response, the latter to detect individuals with convalescent SARS-CoV-2 infection. To assess the functional neutralization capacity of antibodies**,** we used the GenScript SARS-CoV-2 Surrogate Virus Neutralization Test Kit, further referred as surrogate virus neutralization test (sVNT; GenScript Biotech, Piscataway Township, USA) according to the manufacturer’s specification. The manufacturer’s cutoff for positivity is set to ≥30% inhibition (INH%). This assay works according to the principle of competitive binding: anti-SARS-CoV-2 neutralizing antibodies block an enzyme-labeled S-receptor binding domain protein from binding its natural ligand, the angiotensin-converting enzyme 2, precoated on a microtiter plate. In addition, a cell culture-based PRNT was performed to determine the neutralization capacity against SARS-CoV-2 Delta (B.1.617.2) 3 months and against Delta (B.1.617.2) and Omicron (B.1.1.529; BA.1) about 6 months after basic immunization, including individuals receiving a third dose. A titer of 1 : 10 was defined as equivocal and ≥1 : 20 as positive test result. Further details about the conducted PRNT can be found in the supplemental material.

2.4. Statistical analysis

Unpaired two-tailed *t*-tests, Kruskal-Wallis with Dunn multiple comparisons test, Mann-Whitney U test, Wilcoxon test, three-way analysis of variance (ANOVA), and Spearman correlation analyses were performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). Correlation coefficients were interpreted according to Cohen. *P*-values <0.05 were considered significant.

2.5. Study design

The study design is depicted in Figure 1. From June 2 to 17, 2021, 453 employees of the University Hospital Frankfurt received their second dose of COVID-19 vaccine, either as ChAdOx1-S (AZ) and BNT162b2 (BNT) or two × mRNA-1273 (Moderna) vaccination; 16 individuals were excluded from further analysis because of positive PCR and/or SARS-CoV-2 nucleocapsid results. Exclusion criteria were an incomplete questionnaire, self-reported SARS-CoV-2 PCR-confirmed infection, and an initially positive SARS-CoV-2 nucleocapsid result. Enrolled participants not showing up to a study visit were reinvited to the next visit. One newly detected SARS-CoV-2 PCR-confirmed infection was reported in the AZ and BNT vaccination cohort after follow-up II (3 months), the individual was excluded from further analysis. At follow-up III (6 months), nine individuals were excluded from further analysis due to missing information on the questionnaire (n = 8) or due to SARS-CoV-2 PCR-confirmed infection (n = 1) in the AZ/BNT cohort. In the two × Moderna-vaccinated cohort at follow-up III (6 months), one individual was excluded due to missing information on the questionnaire.

3. Results

Baseline characteristics are shown in Table 1. According to baseline health data, all participants turned out to be immunocompetent.

3.1. Reactogenicity

Subjective reactogenicity was reported by 98.8% (244/247) of participants who attended the follow-up I (1 month) examination

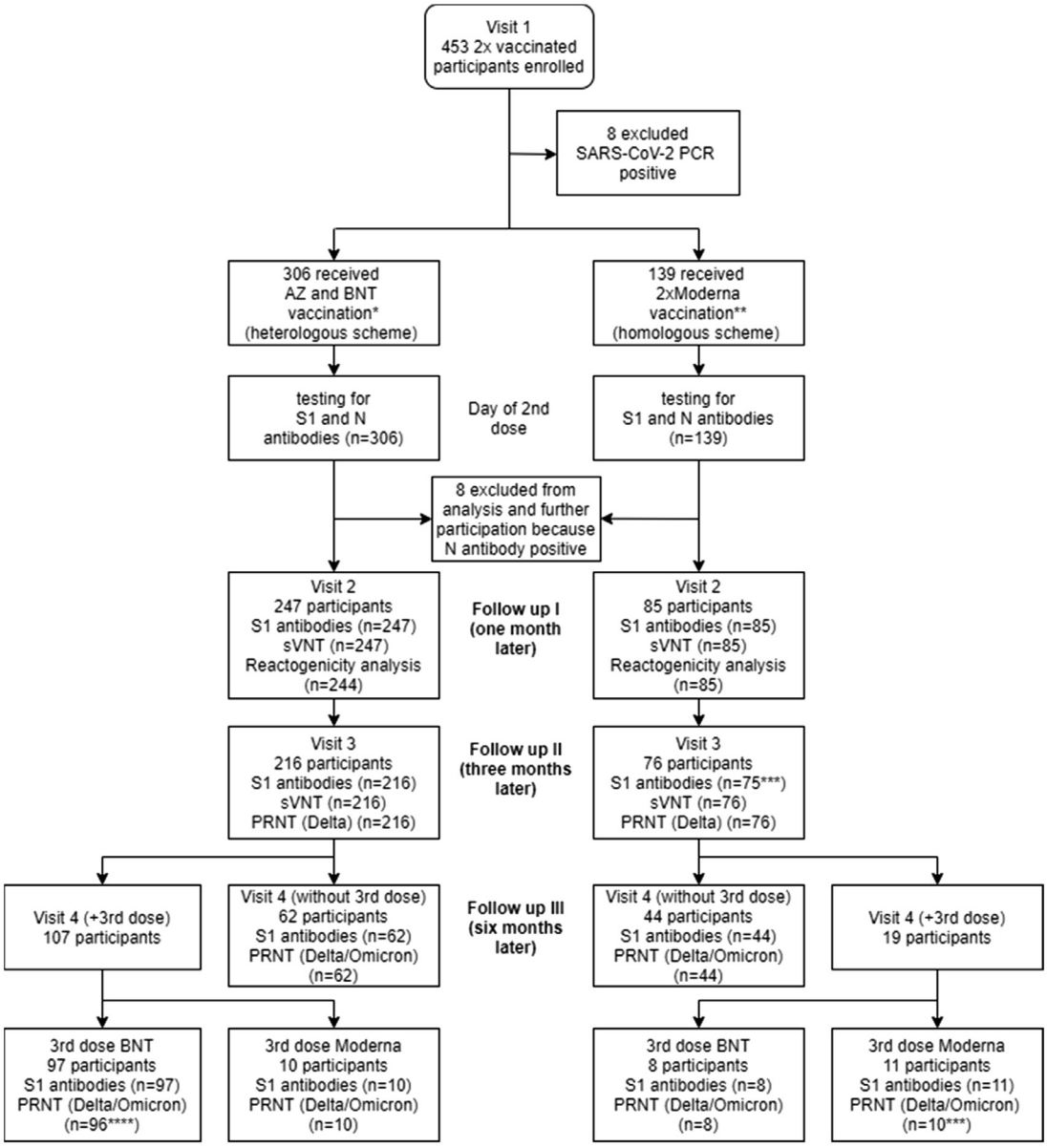

Figure 1. Study design.
AZ, ChAdOx1-S; BNT, BNT162b2; Delta, B.1.617.2; Ig, immunoglobulin; N antibody(ies), SARS-CoV-2 anti-Nucleocapsid IgG; Omicron, B.1.1.529; PCR, polymerase chain reaction; PRNT, cell culture-based plaque reduction neutralization test; S1 antibodies, SARS-CoV-2 antispike IgG antibodies; sVNT, surrogate virus neutralization test.
*About 12 weeks distance according to heterologous vaccination scheme
**About 4 weeks distance according to homologous vaccination scheme
***For one participant not enough sample volume for the assay
****For one participant not enough sample volume for the cell culture-based neutralization assay against SARS-CoV-2 Omicron VOC

Table 1
Baseline characteristics of the study cohort.

	AZ and BNT vaccination (n = 300)		2 x Moderna vaccination (n = 137)	
Age, years (median)	36 (19-59)		35 (19-59)	
Sex[a]				
Female	213 (71%)		91 (66.4%)	
Male	86 (28.7%)		46 (33.6%)	
Vaccination to sampling interval, days (median)	29 (22-37) follow-up-I (n = 247) 91 (85-98) follow-up-II (n = 216)		28 (27-35) follow-up-I (n = 85) 92 (85-93) follow-up-II (n = 76)	
	181.5 (168-190) follow-up-III without third dose (n = 62)	14 (7-24) follow-up-III additional third dose[b] (n = 107)	182 (172-183) follow-up-III without third dose (n = 44)	12 (10-21) follow-up-III additional third dose[b] (n = 19)

AZ, ChAdOx1-S; BNT, BNT162b2.
[a] One participant in the AZ and BNT vaccination cohort stated to be diverse.
[b] About 6 months after second dose with either BNT or Moderna.

Table 2
Proportions (%) of subjectively reported symptoms after boosting according to the heterologous or homologous vaccination scheme. The 95% confidence intervals according to Wilson and Brown are shown in brackets.

Reactogenicity (any severity), %	ChAdOx1-S and BNT162b2 vaccination	2 x Moderna vaccination
Headache	61.1 (54.8-67) [149/244]	56.5 (45.9-66.5) [48/85]
Limb pain	40.6 (34.6-46.8) [99/244]	64.7 (54.1-74) [55/85]
Fever (including elevated temperature)	19.7 (15.2-25.1) [48/244]	43.5 (33.5-54.1) [37/85]
Injection site pain	81.6 (76.2-85.9) [199/244]	82.4 (72.9-89) [70/85]
Injection site erythema	12.3 (8.8-17) [30/244]	27.7 (16.8-34.8) [21/85]
Injection site pruritus	7.8 (5-11.8) [19/244]	16.5 (10.1-25.8) [14/85]

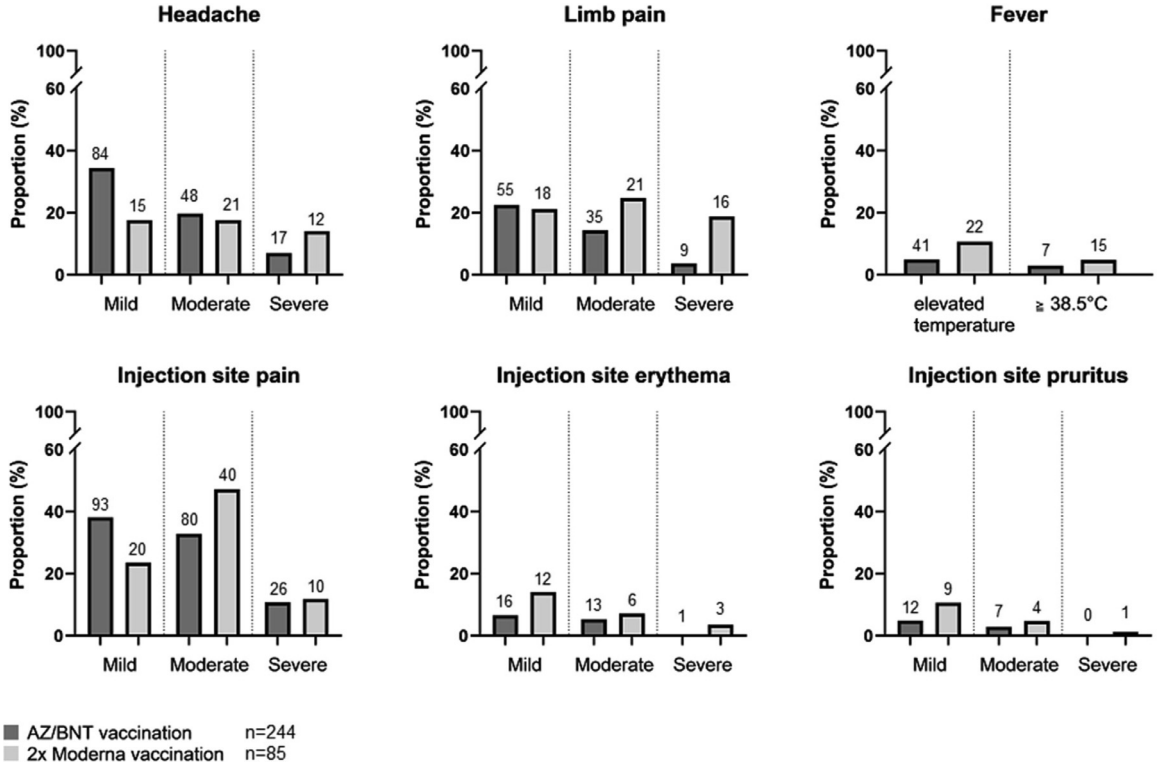

Figure 2. Proportions (%) and number of subjectively reported symptoms after receiving the second dose according to the heterologous or homologous vaccination scheme grouped by severity.
AZ, ChAdOx1-S; BNT, BNT162b2.

after heterologous (AZ/BNT) and by 100% (85/85) of participants after homologous (two × Moderna) vaccination (Table 2). The majority of symptoms were reported to have appeared within 12 hours after receiving the second dose.

Without differentiating by severity, headache and local injection site pain were reported to a comparable degree in both cohorts. Injection site erythema, pruritus, fever, and limb pain were observed about 3-, 2-, and 1.5-fold more frequently by participants vaccinated according to the homologous scheme. The overall reactogenicity was lower in the heterologous (AZ/BNT) immunized cohort than in the homologous (two × Moderna) immunized group. Proportions of reported symptoms grouped by severity are shown in Figure 2.

With the exception of severe limb pain and fever (≥38.5°C), which were reported 10-fold more frequently in the homologous immunized cohort, reported symptoms grouped by severity were comparable between both cohorts. In addition, no potential life-threatening reactions have been reported.

3.2. Immunogenicity

Data on immunogenicity were obtained on the day of receiving the second dose and 1, 3, and 6 months after receiving the second dose (Figure 3/Table 3). Individuals receiving a third dose about 6 months after the second dose were analyzed separately. On the day of receiving the second dose, 96% (288/300) of individuals receiving AZ and 100% (137/137) receiving Moderna as their first dose had detectable SARS-CoV-2 antispike IgG antibodies. All seronegative individuals attending the follow-up I examination (n = 9) demonstrated seroconversion after having received their second dose.

Individuals receiving their second dose according to the homologous (two × Moderna) vaccination scheme revealed significant (P <0.0001) higher mean SARS-CoV-2 antispike IgG antibody levels at the follow-up I, II, and III examinations than the heterologous (AZ/BNT) vaccinated cohort.

In addition, SARS-CoV-2 antispike IgG antibody levels decreased significantly in a Wilcoxon test (P <0.0001) within each cohort from 1 to 3 and 6 months after receiving their second dose.

Significant differences in antibody concentrations were also observed through three-way ANOVA for the confounding factors of sex and age (P <0.0001).

In the Spearman correlation analysis, weak negative correlations between the age of the heterologous (AZ/BNT) immunized participants and levels of SARS-CoV-2 antispike IgG antibodies were observed at the follow-up I and II examinations; however, the

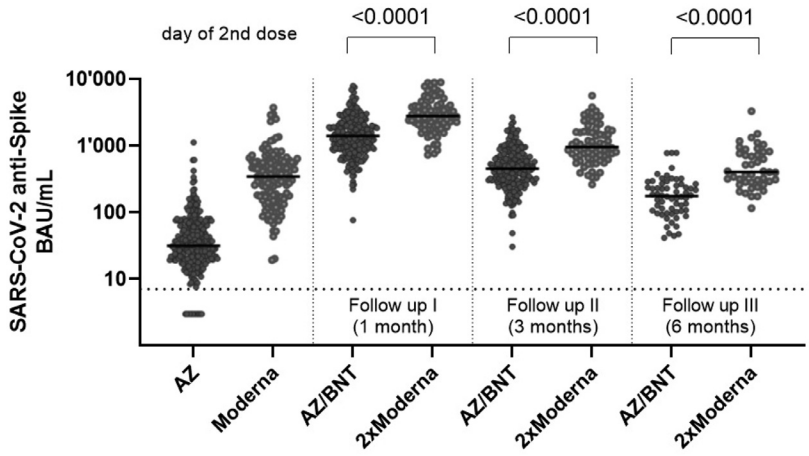

Figure 3. Levels of SARS-CoV-2 antispike IgG in BAU/ml and median on the day of receiving the second dose and 1, 3 and 6 months after either heterologous or homologous basic vaccination (without a history of infection or having received a third dose). The cutoff to positivity is shown as dotted horizontal line. *P*-values of an unpaired two-tailed *t*-test between both cohorts are shown for the follow-up data.
AZ, ChAdOx1-S; BAU, binding antibody units; BNT, BNT162b2; Ig, immunoglobulin.

Table 3
Mean SARS-CoV-2 antispike IgG levels (BAU/ml) and 95% CI at the follow-up-I to III examinations of individuals vaccinated with either AZ/BNT or 2 x Moderna.

	Follow-up-I (1 month)	Follow-up-II (3 months)	Follow-up-III (6 months)	
	Mean SARS-CoV-2 antispike IgG BAU/mL			
AZ/BNT	**1721.3**	**570.5**	**210.4**	P <0.0001[b]
	(1564.4-1878.2 95% CI)	(513.9-627.1 95% CI)	(169.8-250.9 95% CI)	
	(n = 247)	(n = 216)	(n = 62)	
2xModerna	**3347.2**	**1294.4**	**596.7**	
	(2927.4-3767 95% CI)	(1074.8-1514 95% CI)	(423.2-761.1 95% CI)	
	(n = 85)	(n = 75)	(n = 44)	
	P <0.0001[a]			

AZ, ChAdOx1-S; BAU, binding antibody units; BNT, BNT162b2; CI, confidence interval; Ig, immunoglobulin.
[a] Within each vaccination scheme between each follow-up visit
[b] Between both vaccination schemes for each follow-up visit

correlation was only significant at follow-up I (r = -0.1622 [-0.2847 to -0.03446 95% confidence interval (CI)], P = 0.0107) and weakly but not significantly positive at follow-up III (Table S1). For the homologous (two × Moderna) immunized participants, weak negative correlations between age and levels of SARS-CoV-2 antispike IgG antibodies were also observed for all follow-up examinations. Correlations, however, were only significant at follow-up I (r = -0.2229 [-0.4216 to -0.003912 95% CI], P = 0.0403) and at follow-up II (r = -0.2997 [-0.4983 to -0.07124 - 95% CI], P = 0.0090).

Mean SARS-CoV-2 antispike IgG antibody levels correlating with positivity in the PRNT (titer ≥1 : 20) against Delta (B.1.617.2) at follow-up II (3 months) were 488.7 BAU/ml (381.9-595.4 BAU/ml 95% CI) in the AZ/BNT (n = 39) and 1311.1 BAU/ml (1032.9-1589.4 BAU/ml 95% CI) in the two × Moderna (n = 23) cohort. The mean follow-up SARS-CoV-2 antispike IgG antibody levels were comparable between both sexes; male participants yielded slightly higher BAU levels than female participants in the heterologous (AZ/BNT) vaccinated cohort and female participants had slightly higher levels than male participants in the homologous (two × Moderna) vaccinated cohort for most of the cases (Table S2). Differences, however, were only significant (P = 0.0162) in the heterologous (AZ/BNT) vaccinated cohort at follow-up III (6 months).

3.3. Surrogate virus neutralization test

Regarding potential neutralizing antibodies measured by sVNT against SARS-CoV-2 (wild type), at the follow-up I (1 month) examination, there was no significant difference in neutralizing activity of the AZ/BNT (mean of 95.46 % inhibition INH%, n = 247) compared with the two × Moderna (mean of 95.66 INH%, n = 85)-vaccinated cohort (Figure 4).

At the follow-up II (3 months) examination, the homologous vaccinated cohort yielded significantly (P <0.0001) higher neutralizing activity (mean of 95.95 INH%, n = 76) than the heterologous vaccinated cohort (mean of 93.18 INH%, n = 216, including one negative tested sample). The neutralizing activity, however, was high (>80% INH) for the majority of the cohorts at both time points.

3.4. PRNT

In a cell culture-based neutralization assay against Delta (B.1.617.2) conducted at the follow-up II (3 months) examination, the AZ/BNT-vaccinated cohort (n = 216, including four negative tested samples) demonstrated significantly higher (P = 0.0085) serum neutralizing activity than the two × Moderna-vaccinated cohort (n = 76, including one negative tested sample) (Figure 5). Titer distributions can also be found in the supplemental material (Table S3).

In the heterologous vaccinated cohort and in the homologous vaccinated cohort, 88.9% (192/216) and 88.2% (67/76), respectively, of the vaccinees demonstrated positive serum neutralizing activity (titer ≥1 : 20). At follow-up III (6 months) and also against Delta (B.1.617.2), 24.2% (15/62) of the AZ/BNT and 52.3% (23/44) of the two × Moderna-vaccinated individuals yielded a titer of ≥1 : 20. The serum neutralizing activity was significantly higher (P <0.0001) in the two x Moderna-vaccinated cohort. In a PRNT against Omicron (B.1.1.529; BA.1) conducted at follow-up III (6

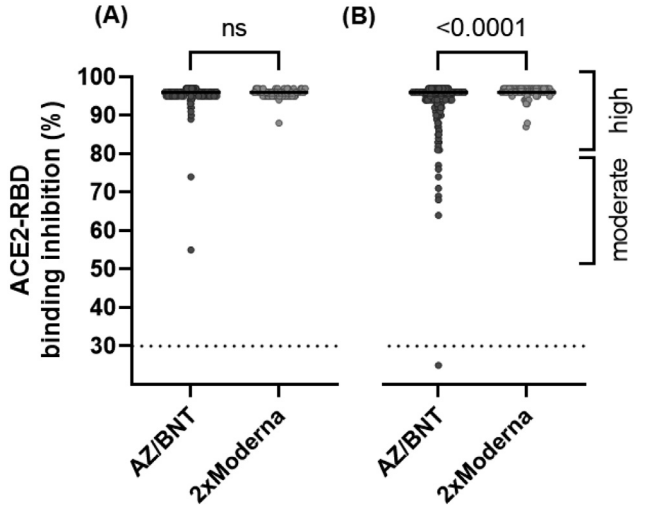

Figure 4. Levels of ACE2-RBD binding inhibition and mean are shown for participants of the 1 month (a) and/or 3 months (b) follow-up visit after heterologous or homologous second dose, including median, 95% confidence interval and *P*-value of a conducted Mann-Whitney U test between both cohorts. The cutoff to positivity is shown as dotted horizontal line.
ACE2, angiotensin-converting enzyme 2; AZ, ChAdOx1-S; BNT, BNT162b2; ns, not significant; RBD, receptor binding domain.

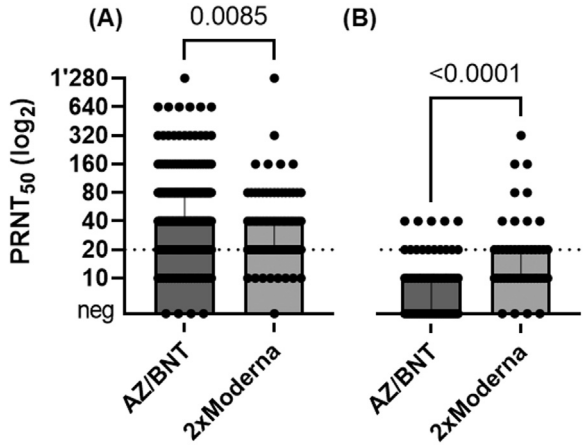

Figure 5. Results of a PRNT against the Delta (B.1.617.2) VOC conducted for participants of the 3 (a) and 6 (b) months follow-up visit after second dose (not having received a third dose), including median, 95% confidence interval of titers and the *P*-value of a conducted Mann-Whitney U test. Dotted lines indicate cutoff to positivity.
AZ, ChAdOx1-S; BNT, BNT162b2; neg, negative; PRNT, plaque reduction neutralization test.

months), only a minority demonstrated serum neutralizing activity: AZ/BNT-vaccinated 3.2% (2/62), with a titer of 1 : 10 each; two × Moderna-vaccinated 11.4% (5/44), with a titer of 1 : 10 (n = 3) and 1 : 20 (n = 2).

3.5. Immunogenicity after the third dose

For individuals having received a third dose of either BNT (30 µg) or Moderna (50 µg) about 6 months after basic immunization (two doses), SARS-CoV-2 antispike IgG antibody levels were measured about 14 days after vaccination. Individuals having received three doses of Moderna yielded the highest mean SARS-CoV-2 antispike IgG antibody concentration of 6045.4 BAU/ml, followed by the homologous vaccinated cohort with a third dose of BNT of 5375.7 BAU/ml, the heterologous vaccinated cohort with a third dose of Moderna of 3821.4 BAU/ml, and the heterologous vaccinated cohort with a third dose of BNT of 2912.8 BAU/ml (Table 4).

Table 4
Mean SARS-CoV-2 antispike IgG antibody levels (BAU/ml) and 95% CI of individuals having received either a third dose of BNT or Moderna.

Vaccination scheme	SARS-CoV-2 antispike IgG BAU/ml
3 x Moderna (n = 11)[a]	**6045.4** (3885.8-8205.1 95% CI)
2 x Moderna/BNT (n = 8)	**5375.7** (2463.5-8287.9 95%CI)
AZ/BNT/Moderna (n = 10)	**3821.4** (1600.4-6042.5 95% CI)
AZ/2 x BNT (n = 97)[a]	**2912.8** (2488-3337.7 95% CI)

AZ, ChAdOx1-S; BAU, binding antibody units; BNT, BNT162b2; CI, confidence interval; Ig, immunoglobulin.
[a] Significantly different using the conducted Kruskal-Wallis and *post hoc* Dunn multiple comparisons test: $P = 0.0043$

Only the mean SARS-CoV-2 antispike IgG antibody levels between individuals receiving three doses of Moderna and the heterologous vaccinated individuals receiving a third dose of BNT were significantly different ($P = 0.0043$) in a conducted Kruskal-Wallis and *post hoc* Dunn multiple comparisons test (Figure S1). Other *P*-values were as follows: AZ/2xBNT versus two × Moderna/BNT, $P = 0.2713$; AZ/BNT/Moderna versus two × Moderna/BNT, $P > 0.9999$; AZ/two × BNT versus AZ/BNT/Moderna, $P > 0.9999$; AZ/BNT/Moderna versus three × Moderna, $P = 0.4678$; three × Moderna versus two × Moderna/BNT, $P > 0.9999$. The median age among the cohorts was comparable, with a predominant proportion of women: aged 39 (19-59) years, female (n = 78) and male (n = 19; AZ/two × BNT); 40 (29-57) years, female (n = 6) and male (n = 4; AZ/BNT/Moderna); 48 (31-59) years, female (n = 6) and male (n = 2; two × Moderna/BNT); and 41 (29-59) years, female (n = 7) and male (n = 4) (three × Moderna).

The Wilcoxon tests revealed significant higher SARS-CoV-2 antispike IgG levels after receiving a third dose than the antibody levels at the follow-up II (3 months) examination in all cohorts (two × Moderna/BNT-vaccinated [n = 8], $P = 0.0078$; three × Moderna-vaccinated [n = 10], $P = 0.0020$; AZ/two × BNT-vaccinated [n = 85], $P < 0.0001$; AZ/BNT/Moderna-vaccinated [n = 10], $P = 0.0020$).

In addition to SARS-CoV-2 antispike IgG analyses, the neutralization capacity was assessed by PRNT against SARS-CoV-2 Delta (B.1.617.2) and Omicron (B.1.1.529; BA.1). Within each vaccination regime and compared with Delta (B.1.617.2), significantly lower serum neutralizing activity was observed against Omicron (B.1.1.529; BA.1) using the Wilcoxon test (Figure 6).

Regarding each VOC specific neutralization, individuals who were vaccinated with three × Moderna reached significantly higher serum neutralizing activity than the AZ/2xBNT vaccinated individuals ($P = 0.0427$) in a conducted Mann-Whitney U test. Other differences in neutralizing antibody levels were not significant. A total of ≥99% of the vaccinees demonstrated positive serum neutralizing activity (titer ≥1 : 20) against Delta (B.1.617.2). Against Omicron (B.1.1.529; BA.1), 60% (6/10) of AZ/BNT/Moderna, 70.8% (68/96, excluding one sample with not enough sample volume) of AZ/two × BNT, 80% (8/10, excluding one sample with not enough sample volume) of three × Moderna, and 87.5% (7/8) of two × Moderna/BNT-vaccinated individuals demonstrated positive serum neutralizing activity (titer ≥1 : 20). No serum neutralizing activity against Omicron (B.1.1.529; BA.1) was observed for 8.3% (8/96) of individuals in the AZ/two × BNT, 20% (2/10) in the AZ/BNT/Moderna, 10% (1/10) in the three × Moderna, and 12.5% (1/8) in the two × Moderna/BNT cohort. Titer distributions can also be found in the supplemental material (Table S4).

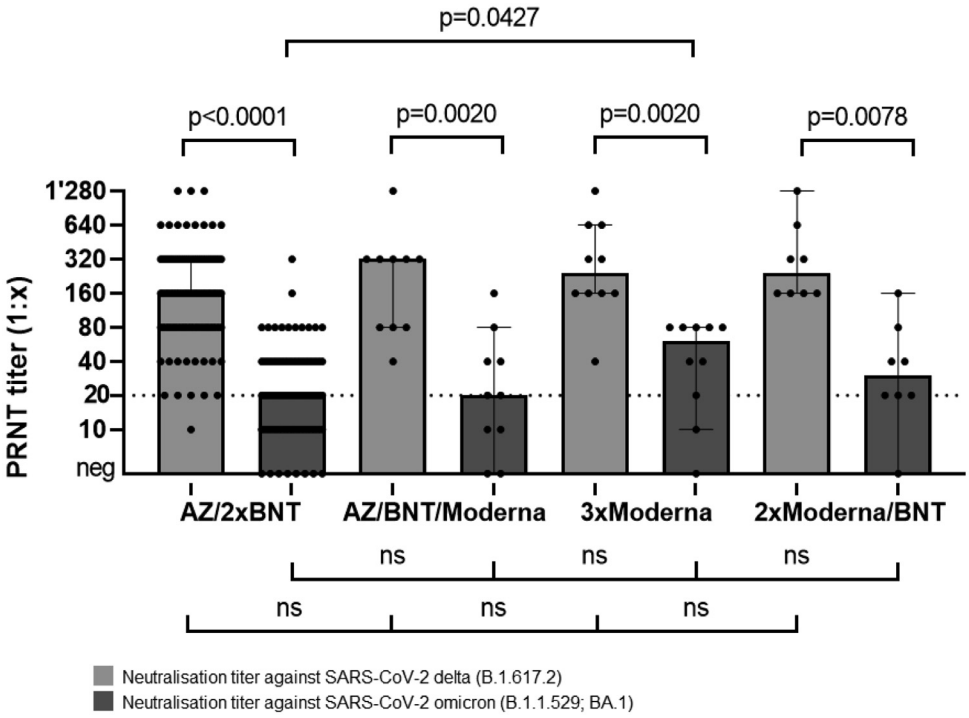

Figure 6. Results of the PRNT regarding the serum neutralization activity of individuals 2 weeks after having received either a third dose of BNT or Moderna including median and 95% confidence interval, significant differences (*P*-values) and cutoff to positivity (dotted line).
AZ, ChAdOx1-S; BNT, BNT162b2; ns, not significant; neg, negative; PRNT, plaque reduction neutralization test.

4. Discussion

In our study, we compared the reactogenicity and immunogenicity in COVID-19 vaccinated immunocompetent adults according to a heterologous (AZ/BNT) and a homologous (two × Moderna) vaccination scheme. Furthermore, we analyzed the elicited humoral immune response in individuals receiving a third dose of an mRNA-COVID-19 vaccine (BNT or Moderna).

Overall and based on the findings of our study using a small to medium sample size, both vaccination schemes yielded an acceptable and manageable reactogenicity profile, which was tendentially lower in the heterologous vaccinated cohort. This is in concordance with findings of similar studies, in which no major differences between both vaccination schemes were observed [7,10,11]. However, when the interval between prime and heterologous mRNA boost was reduced to about 4 weeks, higher reactogenicity could be observed [12–14]. In our study, most of the reported symptoms were rather expected and consisted of local reactions, headache, and limb pain. Slightly more side effects were reported in the homologous vaccinated cohort, which might be attributed to a higher dosage in the Moderna (100 µg) vaccine than BNT (30 µg) and a shorter vaccination interval. No potential life-threatening reactions have been reported.

In terms of immunogenicity, individuals in the two-dose homologous vaccinated cohort (two × Moderna) yielded significantly higher mean SARS-CoV-2 antispike IgG antibody levels than the two-dose heterologous vaccinated cohort (AZ/BNT) at all time points. This was also observed in comparable studies [10,15,16]. In addition, SARS-CoV-2 antispike IgG antibodies in both cohorts waned over time in a similar manner, as described elsewhere [10,16–18]. Interestingly, about 2.5 higher levels of SARS-CoV-2 antispike IgG antibody levels were required in the homologous cohort to achieve positive neutralizing activity against Delta (B.1.617.2) after 3 months, evolving into a rather low reactivity after 6 months. This phenomenon suggests a higher neutralizing capacity of antibodies generated in the heterologous vaccinated cohort three months after the second dose. Literature also shows that immunity after heterologous (AZ/mRNA) vaccination is equivalent to or more pronounced than homologous mRNA or vector-based regimens [5,19–21]. For individuals receiving a third dose, solely individuals vaccinated with Moderna yielded highest mean SARS-CoV-2 antispike IgG antibody levels compared with the other cohorts. Regarding data on immunogenicity after a third dose, in a study by Herzberg *et al.*, 4 weeks after a third dose, a heterologous vaccinated (AZ/BNT) cohort receiving a third dose of BNT yielded significantly lower SARS-CoV-2 antispike IgG levels than a homologous vaccinated cohort receiving three doses of BNT but higher concentrations than the two × AZ/BNT-vaccinated cohort [22]. A third dose after 6 months also augmented waning antispike IgG in heterologous vaccinated individuals in a study by Behrens *et al.*; however, the neutralizing activity against Omicron (B.1.1.529) remained severely impaired [23].

We could observe a weak negative correlation between age and the SARS-CoV-2 antispike IgG antibodies in the heterologous cohort at follow-up I (1 month after second vaccination) and follow-up I and II (3 months after second vaccination) in the homologous group. A prospective multicenter study from Taiwan with 353 participants in a homologous mRNA-1273 vaccinated group found no age-related variation in groups from 20-40, 40-60, and >60 years related to antibody production and neutralizing ability 1 month after receiving the second vaccination [15]. We did not observe significant differences in the SARS-CoV-2 antispike IgG antibody levels between different sexes, which is in concordance to the findings of a comparable study conducted by Benning *et al.* [6]. At present, sex-disaggregated data about immunogenicity and reactogenicity after COVID-19 vaccination are sparse.

The significant difference in neutralizing activity between homologous and heterologous vaccinated individuals in the sVNT (SARS-CoV-2 wild type) at follow-up II (3 months) is rather negligible because the majority of both cohorts yielded activity within

a high neutralizing range (>80% inhibition) [24]. These findings are in concordance with studies using a comparable study profile [7,25].

Regarding SARS-CoV-2 Delta (B.1.617.2) specific neutralization as assessed by PRNT after 3 and 6 months, the heterologous vaccinated cohort showed significantly higher neutralizing activity than the homologous cohort after 3 months, however after 6 months neutralizing activity was more pronounced in the latter Literature shows that B cell memory compartments continue to evolve after infection and enhance the serologic response after boosting with an mRNA vaccine. Perhaps this process is also involved here [26,27].

Concerning the neutralizing activity against SARS-CoV-2 Delta (B.1.617.2) and Omicron (B.1.1.529; BA.1) after 6 months, the majority of vaccinees still demonstrated neutralizing efficacy against Delta but not against Omicron. In the group of individuals having received a third dose, individuals vaccinated with three × Moderna reached significantly higher serum neutralizing activity than the individuals vaccinated with AZ/two × BNT. Other differences in the neutralizing activity between the different regimens were comparable. Nevertheless, the neutralizing activity against Omicron is rather low even in the three × Moderna cohort. These findings can be related to the description from the UK Health Security Agency from December 2021, demonstrating a reduced efficacy of the AZ/BNT vaccination regime against SARS-CoV-2 Delta (B.1.617.2) and Omicron (B.1.1.529) [28] and neutralizing activity studies including individuals with a third dose of COVID-19 vaccine [29,30]. In these studies, neutralization against Omicron was lower than against wild type or Delta but as also shown in our study, was generally higher in individuals who recently received their third (booster) dose. Furthermore, it supports the molecular findings of strong immune escape mutations in Omicron [31–33]. This underlines the need for a third dose after basic immunization. Because the local SARS-CoV-2 incidence was low when the study was conducted (only two PCR-confirmed infection were reported by the study participants during our study period), we cannot provide data for vaccine effectiveness. But it can be assumed that neutralizing antibody levels are highly predictive of immune protection from the severe clinical course of COVID-19 [34] and that there is a positive correlation between serum neutralizing activity and cross-protection against VOCs as a meta-analysis from 24 studies on *in vitro* neutralization and clinical protection showed [35]. In addition, immunoglobulins are used as therapeutics [36]; however, their neutralizing capability should continuously be assessed as mutations in the spike protein of SARS-CoV-2 might significantly lower neutralizing efficiency [37].

A recent study with a large cohort of elderly individuals in Finland, before the emergence of Omicron, showed a vaccine effectiveness against COVID-19-related hospitalization of 95%, 14-60 days after homologous and heterologous three dose series for the period from December 2020 to March 2022 [38]. Vaccine effectiveness against severe COVID-19 by Omicron is estimated at 50-90% after two doses and about 90% after having received a third dose (including heterologous prime-boost schemes). More research is needed to determine how long the protection will last [39–41].

Statistical methods were used to describe (significant) differences between antibody levels and neutralizing activity. Whether they also translate into clinical observable differences in immunity is not clear. To date, there is no specified threshold of antibody or *in vitro* neutralization levels for protective immunity against SARS-CoV-2.

T cell-mediated immune response, although not analyzed in our study, plays a role in SARS-CoV-2 infection [42,43]. Early data suggest that besides poor humoral elicited immune response in COVID-19-vaccinated and convalescent individuals, T cell response is cross-reactive against Omicron [44–46]. In several studies, heterologous AZ/BNT vaccination led to a strong T cell response [7,11,16,19]. In a study conducted in Austria, the frequency and multifunctionality of spike-specific T cell response of AZ/BNT-vaccinated was comparable to individuals vaccinated with homologous BNT/BNT [47]. This was also the case in a study from Germany where a stable and polyfunctional T cell response was generated after heterologous AZ/BNT vaccination. The mechanism of immune memory, however, is complex, and the source of SARS-CoV-2 long-term protective immunity is not defined in humans.

One limitation of our study is that symptoms after the second dose were asked subjectively and not standardized. There has been no blinding to the participants and no placebo was involved, so it cannot be ruled out that some participants were guided by expectations or experiences from the first vaccination. In addition, our study size was too small to capture rare adverse events, such as anaphylaxis. Reactogenicity after receiving a third dose was not analyzed. In addition, we did not ask if the vaccinees took any prophylactic antipyretic medication to prevent or limit the occurrence of vaccine related reactions. A study performed between December 2020 and July 2021 in 380 health care workers in Berlin (Germany) compared the reactogenicity of a homologous BNT162b2 and heterologous ChAdOx1-S/BNT162b2 vaccination regime and showed that the prophylactic intake of antipyretics did not affect adverse reactions [7]. Furthermore, reactogenicity and immunogenicity results might be biased due to dropout and differences in cohort size throughout the study. The cohort sizes of individuals having received a third dose are too small to draw further specific and generally applicable recommendations. There was no sample available directly before the third dose to compare the effect of the booster dose. Because the sampling was conducted 14 days after the third dose, we do not know about long-term effects of the booster dose. In addition, vaccination to sample intervals were slightly different among the examined cohorts. We would have preferred to include individuals receiving BNT as the homologous mRNA vaccinated control cohort; however, when the study was performed, only individuals vaccinated with Moderna were available and compatible with the study schedule.

In conclusion and based on the findings of our study, the heterologous (AZ/BNT) vaccination regime demonstrated an acceptable reactogenicity and immunogenicity profile. Heterologous (AZ/BNT) vaccination temporarily led to higher neutralizing activity than homologous mRNA vaccination. Administering a third dose of an mRNA vaccine is necessary to maintain neutralizing activity against SARS-CoV-2, especially against Omicron (B.1.1.529). Further research is needed to confirm our findings, identify optimal combinations, doses, intervals and determine how immunogenicity translates into protection against SARS-CoV-2. If vaccine efficacy could be improved, for instance, by optimizing neutralizing activity against dominant circulating SARS-CoV-2 variants, VOC adapted versions of the vaccines would be desirable.

Declaration of competing interest

SC received honorarium for serving on a clinical advisory board for BioNTech. All other authors declare no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval

The study was conducted according to the guidelines of Good Clinical Practice and the Declaration of Helsinki and approved by

the ethics committee of the Medical Faculty of Goethe University Frankfurt, Germany (Vote No. 2021-201).

Author contributions

NK, SW and SC conceptualized and designed the study. NK, SS, BS, KG, MM and MW contributed to the acquisition of data. NK and SS performed data analysis and the writing of the original draft. EH supported data analysis. SW, HR and SC supervised the study. All authors contributed to the manuscript development and approved the final version.

Acknowledgments

We would like to thank all participants and supporters of the study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.12.034.

References

[1] European Medicines Agency. Signal assessment report on embolic and thrombotic events (SMQ) with COVID-19 Vaccine (ChAdOx1-S [recombinant]) – Vaxzevria (previously COVID-19 Vaccine AstraZeneca) (Other viral vaccines), https://www.ema.europa.eu/en/documents/prac-recommendation/signal-assessment-report-embolic-thrombotic-events-smq-covid-19-vaccine-chadox1-s-recombinant_en.pdf; 2021 [accessed 19 August 2022].
[2] Robert Koch Institut. Beschluss der STIKO zur 4. Aktualisierung der COVID-19 Impfempfehlung, https://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2021/Ausgaben/16_21.pdf?__blob=publicationFile; 2021 [accessed 19 August 2022].
[3] Robert Koch Institut. COVID-19-Zielimpfquote: STIKO: 8. Aktualisierung der COVID-19-Impfempfehlung VRE-Jahresbericht, https://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2021/Ausgaben/27_21.pdf?__blob=publicationFile; 2021 [accessed 19 August 2022].
[4] Cheng H, Peng Z, Si S, Alifu X, Zhou H, Chi P, et al. Immunogenicity and safety of homologous and heterologous prime-boost immunization with COVID-19 vaccine: systematic review and meta-analysis. *Vaccines* 2022;**10**:798. doi:10.3390/vaccines10050798.
[5] Chiu NC, Chi H, Tu YK, Huang YN, Tai YL, Weng SL, et al. To mix or not to mix? A rapid systematic review of heterologous prime-boost Covid-19 vaccination. *Expert Rev Vaccines* 2021;**20**:1211–20. doi:10.1080/14760584.2021.1971522.
[6] Benning L, Töllner M, Hidmark A, Schaier M, Nusshag C, Kälble F, et al. Heterologous ChAdOx1 nCoV-19/BNT162b2 prime-boost vaccination induces strong humoral responses among health care workers. *Vaccines* 2021;**9**:857. doi:10.3390/vaccines9080857.
[7] Hillus D, Schwarz T, Tober-Lau P, Vanshylla K, Hastor H, Thibeault C, et al. Safety, reactogenicity, and immunogenicity of homologous and heterologous prime-boost immunisation with ChAdOx1 nCoV-19 and BNT162b2: a prospective cohort study. *Lancet Respir Med* 2021;**9**:1255–65. doi:10.1016/S2213-2600(21)00357-X.
[8] Liu X, Shaw RH, Stuart ASV, Greenland M, Aley PK, Andrews NJ, et al. Safety and immunogenicity of heterologous versus homologous prime-boost schedules with an adenoviral vectored and mRNA COVID-19 vaccine (Com-COV): a single-blind, randomised, non-inferiority trial. *Lancet* 2021;**398**:856–69. doi:10.1016/S0140-6736(21)01694-9.
[9] Robert Koch Institut. STIKO: 14. Aktualisierung der COVID-19 Impfempfehlung. Reiseassoziierte Infektionskrankheiten 2020, https://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2021/Ausgaben/48_21.pdf?__blob=publicationFile; 2021 [accessed 19 August 2022].
[10] Bonnet B, Chabrolles H, Archimbaud C, Brebion A, Godignon M, Dutheil F, et al. Comparative T and B immune responses of four different anti-COVID-19 vaccine strategies 6 months after vaccination. *J Infect* 2022;**84**:e45–7. doi:10.1016/j.jinf.2022.03.006.
[11] Borobia AM, Carcas AJ, Pérez-Olmeda M, Castaño L, Bertran MJ, García-Pérez J, et al. Immunogenicity and reactogenicity of BNT162b2 booster in ChAdOx1-S-primed participants (CombiVacS): a multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet* 2021;**398**:121–30. doi:10.1016/S0140-6736(21)01420-3.
[12] Powell AA, Power L, Westrop S, McOwat K, Campbell H, Simmons R, et al. Real-world data shows increased reactogenicity in adults after heterologous compared to homologous prime-boost COVID-19 vaccination, March–June 2021, England. *Euro Surveill* 2021;**26**:2100634. doi:10.2807/1560-7917.ES.2021.26.28.2100634.
[13] Shaw RH, Stuart A, Greenland M, Liu X, Nguyen Van-Tam JS, Snape MD. Com-COV study group. heterologous prime-boost COVID-19 vaccination: initial reactogenicity data. *Lancet* 2021;**397**:2043–6. doi:10.1016/S0140-6736(21)01115-6.
[14] Warkentin L, Zeschick N, Kühlein T, Steininger P, Überla K, Kaiser I, et al. Reactogenicity after heterologous and homologous COVID-19 prime-boost vaccination regimens: descriptive interim results of a comparative observational cohort study. *BMC Infect Dis* 2022;**22**:504. doi:10.1186/s12879-022-07443-x.
[15] Kang CM, Lee NY, Lin CH, Hsu YS, Chang YC, Chung MY, et al. Immunogenicity and safety of homologous and heterologous ChAdOx1-S and mRNA-1273 vaccinations in healthy adults in Taiwan. *J Clin Virol* 2022:150–1 105156. doi:10.1016/j.jcv.2022.105156.
[16] Schmidt T, Klemis V, Schub D, Mihm J, Hielscher F, Marx S, et al. Immunogenicity and reactogenicity of heterologous ChAdOx1 nCoV-19/mRNA vaccination. *Nat Med* 2021;**27**:1530–5. doi:10.1038/s41591-021-01464-w.
[17] Atmar RL, Lyke KE, Deming ME, Jackson LA, Branche AR, El Sahly HM, et al. Homologous and heterologous Covid-19 booster vaccinations. *N Engl J Med* 2022;**386**:1046–57. doi:10.1056/NEJMoa2116414.
[18] Doria-Rose N, Suthar MS, Makowski M, O'Connell S, McDermott AB, Flach B, et al. Antibody persistence through 6 months after the second dose of mRNA-1273 vaccine for Covid-19. *N Engl J Med* 2021;**384**:2259–61. doi:10.1056/NEJMc2103916.
[19] Klemis V, Schmidt T, Schub D, Mihm J, Marx S, Abu-Omar A, et al. Comparative immunogenicity and reactogenicity of heterologous ChAdOx1-nCoV-19-priming and BNT162b2 or mRNA-1273-boosting with homologous COVID-19 vaccine regimens. *Nat Commun* 2022;**13**:4710. doi:10.1038/s41467-022-32321-0.
[20] Nguyen TT, Quach THT, Tran TM, Phuoc HN, Nguyen HT, Vo TK, et al. Reactogenicity and immunogenicity of heterologous prime-boost immunization with COVID-19 vaccine. *Biomed Pharmacother* 2022;**147**:112650. doi:10.1016/j.biopha.2022.112650.
[21] Westrop SJ, Whitaker HJ, Powell AA, Power L, Whillock C, Campbell H, et al. Real-world data on immune responses following heterologous prime-boost COVID-19 vaccination schedule with Pfizer and AstraZeneca vaccines in England. *J Infect* 2022;**84**:692–700. doi:10.1016/j.jinf.2022.01.038.
[22] Herzberg J, Fischer B, Becher H, Becker AK, Honarpisheh H, Guraya SY, et al. Cellular and humoral immune response to a third dose of BNT162b2 COVID-19 vaccine – a prospective observational study. *Front Immunol* 2022;**13**:896151. doi:10.3389/fimmu.2022.896151.
[23] Behrens GMN, Barros-Martins J, Cossmann A, Ramos GM, Stankov MV, Odak I, et al. BNT162b2-boosted immune responses six months after heterologous or homologous ChAdOx1nCoV-19/BNT162b2 vaccination against COVID-19. *Nat Commun* 2022;**13**:4872. doi:10.1038/s41467-022-32527-2.
[24] Kohmer N, Rühl C, Ciesek S, Rabenau HF. Utility of different surrogate enzyme-linked immunosorbent assays (sELISAs) for detection of SARS-CoV-2 neutralizing antibodies. *J Clin Med* 2021;**10**:2128. doi:10.3390/jcm10102128.
[25] Rose R, Neumann F, Grobe O, Lorentz T, Fickenscher H, Krumbholz A. Humoral immune response after different SARS-CoV-2 vaccination regimens. *BMC Med* 2022;**20**:31. doi:10.1186/s12916-021-02231-x.
[26] Cohen KW, Linderman SL, Moodie Z, Czartoski J, Lai L, Mantus G, et al. Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells. *Cell Rep Med* 2021;**2**:100354. doi:10.1016/j.xcrm.2021.100354.
[27] Wang Z, Muecksch F, Schaefer-Babajew D, Finkin S, Viant C, Gaebler C, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature* 2021;**595**:426–31. doi:10.1038/s41586-021-03696-9.
[28] UK Health Security Agency. SARS-CoV-2 variants of concern and variants under investigation in England: technical Briefing 34, https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1050236/technical-briefing-34-14-january-2022.pdf; 2022 (accessed 19 August 2022).
[29] Pajon R, Doria-Rose NA, Shen X, Schmidt SD, O'Dell S, McDanal C, et al. SARS-CoV-2 omicron variant neutralization after mRNA-1273 booster vaccination. *N Engl J Med* 2022;**386**:1088–91. doi:10.1056/NEJMc2119912.
[30] Wilhelm A, Widera M, Grikscheit K, Toptan T, Schenk B, Pallas C, et al. Limited neutralisation of the SARS-CoV-2 Omicron subvariants BA.1 and BA.2 by convalescent and vaccine serum and monoclonal antibodies. *eBioMedicine* 2022;**82**:104158. doi:10.1016/j.ebiom.2022.104158.
[31] Hu J, Peng P, Cao X, Wu K, Chen J, Wang K, et al. Increased immune escape of the new SARS-CoV-2 variant of concern Omicron. *Cell Mol Immunol* 2022;**19**:293–5. doi:10.1038/s41423-021-00836-z.
[32] Liu L, Iketani S, Guo Y, Chan JF-W, Wang M, Liu L, et al. Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. *Nature* 2022;**602**:676–81. doi:10.1038/s41586-021-04388-0.
[33] Planas D, Saunders N, Maes P, Guivel-Benhassine F, Planchais C, Buchrieser J, et al. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. *Nature* 2022;**602**:671–5. doi:10.1038/s41586-021-04389-z.
[34] Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021;**27**:1205–11. doi:10.1038/s41591-021-01377-8.
[35] Cromer D, Steain M, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: a meta-analysis. *Lancet Microbe* 2022;**3**:e52–61. doi:10.1016/S2666-5247(21)00267-6.
[36] Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhore R, et al. REGN-COV2, a neutralizing antibody cocktail, in outpatients with Covid-19. *N Engl J Med* 2021;**384**:238–51. doi:10.1056/NEJMoa2035002.
[37] Widera M, Wilhelm A, Hoehl S, Pallas C, Kohmer N, Wolf T, et al. Limited neutralization of authentic severe acute respiratory syndrome coronavirus 2 vari-

ants carrying E484K in vitro. *J Infect Dis* 2021;**224**:1109–14. doi:10.1093/infdis/jiab355.

[38] Baum U, Poukka E, Leino T, Kilpi T, Nohynek H, Palmu AA. High vaccine effectiveness against severe COVID-19 in the elderly in Finland before and after the emergence of Omicron. *BMC Infect Dis* 2022;**22**:816. doi:10.1186/s12879-022-07814-4.

[39] Buchan SA, Chung H, Brown KA, Austin PC, Fell DB, Gubbay JB, et al. Effectiveness of COVID-19 vaccines against Omicron or Delta symptomatic infection and severe outcomes. *JAMA Netw Open* 2022;**5**:e2232760. doi:10.1001/jamanetworkopen.2022.32760.

[40] Chemaitelly H, Ayoub HH, AlMukdad S, Coyle P, Tang P, Yassine HM, et al. Duration of mRNA vaccine protection against SARS-CoV-2 Omicron BA.1 and BA.2 subvariants in Qatar. *Nat Commun* 2022;**13**:3082. doi:10.1038/s41467-022-30895-3.

[41] Thompson MG, Natarajan K, Irving SA, Rowley EA, Griggs EP, Gaglani M, et al. Effectiveness of a third dose of mRNA vaccines against COVID-19-associated emergency department and urgent care encounters and hospitalizations among adults during periods of delta and omicron variant predominance - VISION network, 10 states, August 2021–January 2022. *MMWR Morb Mortal Wkly Rep* 2022;**71**:139–45. doi:10.15585/mmwr.mm7104e3.

[42] Rydyznski Moderbacher C, Ramirez SI, Dan JM, Grifoni A, Hastie KM, Weiskopf D, et al. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. *Cell* 2020;**183**:996–1012 e19. doi:10.1016/j.cell.2020.09.038.

[43] Tan AT, Linster M, Tan CW, Le Bert N, Chia WN, Kunasegaran K, et al. Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. *Cell Rep* 2021;**34**:108728. doi:10.1016/j.celrep.2021.108728.

[44] Gao Y, Cai C, Grifoni A, Müller TR, Niessl J, Olofsson A, et al. Ancestral SARS-CoV-2-specific T cells cross-recognize the Omicron variant. *Nat Med* 2022;**28**:472–6. doi:10.1038/s41591-022-01700-x.

[45] GeurtsvanKessel CH, Geers D, Schmitz KS, Mykytyn AZ, Lamers MM, Bogers S, et al. Divergent SARS-CoV-2 Omicron-reactive T and B cell responses in COVID-19 vaccine recipients. *Sci Immunol* 2022;**7** eabo2202. doi:10.1126/sciimmunol.abo2202.

[46] Keeton R, Tincho MB, Ngomti A, Baguma R, Benede N, Suzuki A, et al. T cell responses to SARS-CoV-2 spike cross-recognize Omicron. *Nature* 2022;**603**:488–92. doi:10.1038/s41586-022-04460-3.

[47] Bánki Z, Mateus J, Rössler A, Schäfer H, Bante D, Riepler L, et al. Heterologous ChAdOx1/BNT162b2 vaccination induces stronger immune response than homologous ChAdOx1 vaccination: the pragmatic, multi-center, three-arm, partially randomized HEVACC trial. *EBiomedicine* 2022;**80**:104073. https://www.sciencedirect.com/science/article/pii/S2352396422002547. doi:10.1016/j.ebiom.2022.104073.