Vaccine xxx (xxxx) xxx

Contents lists available at ScienceDirect

Vaccine

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

Comparable antibody levels in heterologous and homologous mRNA COVID-19 vaccination, with superior neutralizing and IgA antibody responses in mRNA homologous boosting

Salma Younes [a,b], Eleonora Nicolai [c], Nadin Younes [a,b], Massimo Pieri [c,d], Sergio Bernardini [c,d], Parveen B. Nizamuddin [a,b], Duaa W. Al-Sadeq [e], Hanin I. Daas [f], Ahmed Ismail [g], Hadi M. Yassine [a,b], Laith J. Abu-Raddad [h,i,j], Gheyath K. Nasrallah [a,b,*]

[a] Biomedical Research Center, Qatar University, Doha, P.O. Box 2713, Qatar
[b] Biomedical Sciences Department, College of Health Sciences, Qatar University, Doha, P.O. Box 2713, Qatar
[c] Department of Experimental Medicine, University of Rome Tor Vergata, Via Montpellier 1, 00133 Rome, Italy
[d] Clinical Biochemistry, Tor Vergata University Hospital, 00133 Rome, Italy
[e] Department of Basic Medical Sciences, College of Medicine, QU Health, Qatar University, PO Box 2713, Doha, Qatar
[f] College of Dental Medicine, QU Health, Qatar University, Doha, P.O. Box 2713, Qatar
[g] Laboratory Section, Medical Commission Department, Ministry of Public Health, Doha, Qatar
[h] Infectious Disease Epidemiology Group, Weill Cornell Medicine–Qatar, Cornell University, Qatar Foundation – Education City, Doha, Qatar
[i] World Health Organization Collaborating Centre for Disease Epidemiology Analytics on HIV/AIDS, Sexually Transmitted Infections, and Viral Hepatitis, Weill Cornell Medicine–Qatar, Cornell University, Qatar Foundation, Education City, Doha, Qatar
[j] Department of Healthcare Policy and Research, Weill Cornell Medicine, Cornell University, NY, USA

ARTICLE INFO

Keywords:
AZD1222 (Oxford-AstraZeneca, ChAd)
Homologous
Heterologous
mRNA vaccination
Neutralizing antibody
Anti-S1 IgA

ABSTRACT

Background: Priming with two doses of AZD1222 (Oxford-AstraZeneca; ChAd) followed by a third mRNA vaccine boosting is considered in several countries, yet comparisons between heterologous and homologous booster efficacy remain unexplored.
Aim: To evaluate and contrast the immunogenicity of homologous and heterologous boosting regimens.
Method: The study examined antibody responses in 1113 subjects, comprising 895 vaccine-naïve individuals across different vaccination strategies (partial, primary series, heterologous booster, homologous booster) and 218 unvaccinated, naturally infected individuals. Assessments included neutralizing total antibodies (NTAbs), total antibodies (TAbs), anti-S-RBD IgG, and anti-S1 IgA levels.
Results: The study found mRNA vaccines to exhibit superior immunogenicity in primary series vaccination compared to ChAd, with mRNA-1273 significantly enhancing NTAbs, TAbs, anti-S-RBD IgG, and anti-S1 IgA levels ($p < 0.001$). Both booster types improved antibody levels beyond primary outcomes, with no significant difference in TAbs and anti-S-RBD IgG levels between regimens. However, homologous mRNA boosters significantly outperformed heterologous boosters in enhancing NTAbs and anti-S1 IgA levels, with the BNT/BNT/BNT regimen yielding particularly higher enhancements ($p < 0.05$).
Conclusion: The study concludes that although TAbs and anti-S-RBD IgG antibody levels are similar for both regimens, homologous mRNA boosting outperform heterologous regimen by enhancing anti-S1 IgA and neutralizing antibody levels.

1. Introduction

The SARS-CoV-2 pandemic has inflicted severe global impacts, recording over 800 million infections and 6.5 million deaths by December 10, 2023, a count likely underestimated due to case underreporting [1]. In response, over 13.33 billion vaccine doses have been administered globally [2], with vaccines like Oxford-AstraZeneca (ChAd), Pfizer-BioNTech (BNT), and Moderna (m1273) receiving

* Corresponding author at: Department of Biomedical Science, College of Health Sciences, Qatar University, Doha 2713, Qatar.
E-mail address: gheyath.nasrallah@qu.edu.qa (G.K. Nasrallah).

https://doi.org/10.1016/j.vaccine.2024.06.010
Received 7 April 2024; Received in revised form 1 June 2024; Accepted 1 June 2024

Table 1
Demographic and clinical characteristics of samples collected from the vaccinated naïve (VN) cohort (n = 895) and unvaccinated SARS-CoV-2 naturally infected (NI) cohort (n = 218).

A. Vaccinated Naïve (VN) (n = 895)

	Partially vaccinated (n = 65)						Primary series (n = 766)						Primary series plus one heterologous booster dose (n = 19)				Primary series plus one homologous booster dose (n = 45)				Total (n=895)	
	ChAd		m1273		BNT		ChAd/ChAd		m1273/ m1273		BNT/BNT		ChAd/ChAd/ m1273		ChAd/ChAd+ BNT		m1273/m1273/ m1273		BNT/BNT/BNT			
	(n = 40)		(n = 11)		(n = 14)		(n = 40)		(n = 131)		(n = 595)		(n=10)		(n=9)		(n=7)		(n=38)			
Median age (IQR)	57	54-59	22	20-38	26	21-41	56	54-59	23	20-32	32	22-44	55	52-58	59	57-61	22	21-45	24	21-50	32	21-46
Gender																						
	30	75	5	45	9	64	30	75	70	53	288	48%	7	70	8	89	3	43	18	47	468	52
Male, n (%)	10	25	5	45	2	14	10	25	60	46	268	45%	3	30	1	11	4	57	11	29	374	42
Unspecified, n (%)	0	0	1	9	3	21	0	0	1	1	39	7%	0	0	0	0	0	0	9	24	53	6
No. of days after administration of 1st dose, median (IQR)	46	36-75	19	16-24	19	7.5-37	111	104-205	121	65-168	131	69-191	311	280-323	286	275-300	360	303-364	351	313-381	124	66-200
No. of months after administration of 1st dose, median (IQR)	1.5	1.18-2.47	0.63	0.53-0.8	0.63	0.25-1.23	3.63	3.42-6.73	4.02	2.18-5.58	4.37	2.3-6.37	10.21	9.21-10.6	9.4	9.03-9.85	11.98	10.12-12.12	11.68	10.42-12.68	4.13	2.2-6.61

B. Unvaccinated Naturally Infected (NI) (n = 218)

	Symptomatic		Pauci-symptomatic		Asymptomatic		Unkown		Total	
	(n=51)		(n=20)		(n=135)		(n=12)		(n = 218)	
Median age (IQR)	33	28–40	27	13–35	37	31–46	43	34.25–49	36	29–43.25
Gender										
Female, n (%)	18	35.3	6	30	4	3	2	17	30	14
Male, n (%)	33	64.7	14	70	131	97	10	83	188	86
Unspecified, n (%)	0	0	0	0	0	0	0	0	0	0
No. of days post-COVID-19 infection, median (IQR)	63	44–146	63	43–149	70	48–86	124	62–156	69	46–100
No. of months post-COVID-19 infection, median (IQR)	2.07	1.43–4.80	2.05	1.40–4.85	2.3	1.57–2.83	4.08	2.03–5.12	2.27	1.53–3.30

ChAd: ChAdOx1 nCoV-19 (AstraZeneca) vaccine. m1273: mRNA-1273 (Moderna) vaccine. BNT: BNT162b2 (Pfizer-BioNTech) vaccine. Partially vaccinated: one dose. Partially vaccinated individuals have received only one dose of the vaccine. The primary series refers to the initial series of vaccinations, which includes two doses required to achieve full vaccination status. A primary series plus one heterologous booster dose involves an additional booster dose using a different vaccine from the primary series. In contrast, a primary series plus one homologous booster dose involves a booster dose using the same vaccine as the primary series. Median age (IQR): The median age of participants, with the interquartile range in parentheses. Gender: Distribution of gender among participants, with the number and percentage in parentheses. No. of days after administration of 1st dose, median (IQR): The median number of days since the first dose, with the interquartile range in parentheses. No. of months after administration of 1st dose, median (IQR): The median number of months since the first dose, with the interquartile range in parentheses.

Demographic and Clinical Characteristics of Study Participants. This table presents a breakdown of the study cohort, comprising 1113 participants, into two main groups: Vaccinated Naïve (VN) and Unvaccinated Naturally Infected (NI). **A. Vaccinated Naïve (VN) Group (n = 895):** This subgroup includes individuals who received COVID-19 vaccinations but had no prior confirmed SARS-CoV-2 infection. The VN group is further categorized based on their vaccination schedules: **Partially Vaccinated (n = 65):** Participants who received a single dose of a COVID-19 vaccine, specified by vaccine type as ChAd, m1273, or BNT. **Primary Series (n = 766):** Individuals completing a two-dose regimen with the same vaccine type, labeled as ChAd, m1273, or BNT. **Primary Series plus One Heterologous Booster Dose (n = 19):** Participants initially receiving two doses of ChAd, followed by a booster dose of a different mRNA vaccine, either m1273 or BNT. These regimens are denoted as ChAd/ChAd/m1273 and ChAd/ChAd/BNT. **Primary Series plus One Homologous Booster Dose (n = 45):** Individuals who received three doses of the same type of mRNA vaccine, either all m1273 or all BNT, marked as m1273/m1273/m1273 or BNT/BNT/BNT. **B. Unvaccinated Naturally Infected (NI) Group (n = 218):** Participants in this subgroup had a confirmed diagnosis of SARS-CoV-2 infection and did not receive any COVID-19 vaccine prior to their inclusion in the study. Based on clinical manifestations post-infection, they are divided into: **Symptomatic (n = 51):** Individuals who exhibited common COVID-19 symptoms. **Pauci-symptomatic (n = 20):** Participants who showed very few or mild symptoms. **Asymptomatic (n = 135):** Individuals with no symptoms post-infection. **Unknown (n = 12):** Participants whose symptom status post-infection was not recorded or was unclear. For each subgroup, the table details median age with interquartile range (IQR), gender distribution (male, female, unspecified), and the median number of days and months post-administration of the first vaccine dose for the VN group, and post-COVID-19 infection for the NI group, with IQR.

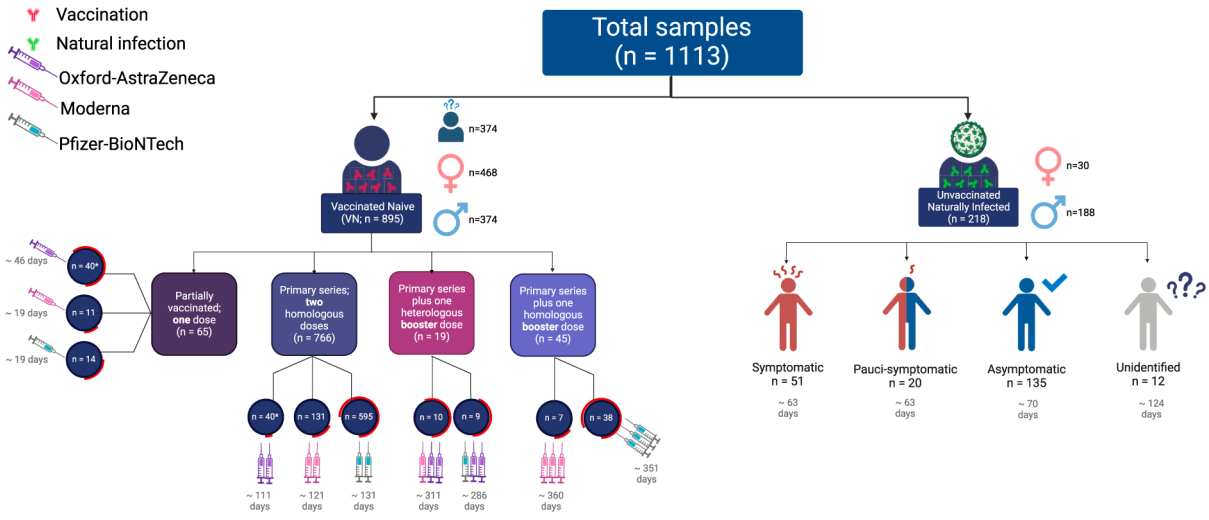

Fig. 1. Summary of the study cohort. A total of 1113 subjects were classified study subjects into two mutually exclusive groups: 1- vaccinated naïve (VN; n = 895), and 2- unvaccinated naturally infected (NI; n = 218). The VN group was further classified to four subgroups; 1- partially vaccinated group included samples collected post-one dose of either ChAd, m1273, or BNT. The primary series group included samples collected post-two homologous doses of either ChAd, m1273, or BNT. The primary series plus one heterologous booster dose group included samples collected post-two doses of ChAd, followed by a heterologous booster shot of either m1273 or BNT. The primary series plus one homologous booster dose group included samples collected post-three homologous doses of either m1273 or BNT. The NI group was further classified to symptomatic, pauci-symptomatic, and asymptomatic. Grey text indicates the number of days post-administration of the 1st dose. The syringes are color-coded by vaccine type. The number of doses is indicated by the number of syringes.

emergency use authorization and have been widely distributed across various regions [3].

As the COVID-19 pandemic progresses into 2024, the emergence of newer SARS-CoV-2 variants post-Omicron highlights the critical need for effective vaccination strategies. Initial vaccine regimens have shown declining effectiveness over time [4–8], particularly against milder disease manifestations and in combating novel variants [9,10]. This decline in effectiveness is further influenced by factors such as age, sex, and underlying comorbidities [11–14]. Consequently, the importance of booster doses has become evident [9,15–17]. The scientific and medical communities have rigorously evaluated booster vaccination strategies, focusing on both homologous and heterologous approaches. Homologous booster vaccinations, involving three consecutive doses of either BNT or m1273 vaccines, have been foundational in maintaining high levels of immunity and offering continued protection against severe COVID-19.

Conversely, the heterologous booster approach, particularly two doses of the ChAd vaccine followed by an mRNA booster, has emerged as a focal point of interest. Initially sparked by concerns about rare adverse events and optimizing immune responses [18–20], the heterologous strategy aims to harness the unique immunogenic properties of both vaccine types [21,22], potentially offering a broader and more durable immune response, and have been used in previous vaccine studies [23,24].

The inquiry into the comparative efficacy of homologous versus heterologous booster strategies against SARS-CoV-2 variants is crucial for global health policies and vaccine distribution. Despite multiple investigations, rigorous studies directly comparing antibody responses for homologous and heterologous vaccination regimens are lacking. Most research has primarily focused on NTAb, TAbs, and anti-S-RBD IgG. Our study stands out by also examining the less-studied anti-S1 IgA response, providing a more holistic view of the immune response. Additionally, we analyzed anti-N antibodies, which are crucial for identifying individuals who were asymptomatically infected before immunization. To address these gaps, we prospectively enrolled two matched cohorts, comprising vaccinated naïve (VN) and naturally infected (NI) individuals. We studied the immunogenicity of two heterologous and two homologous mRNA-vaccination regimens, comprehensively assessing antibody responses.

2. Methodology

2.1. Ethical approval and sample collection

The Qatar University Institutional Review Board (QU-IRB 1537-FBA/21) and Ethical Committee of the Tor Vergata University Hospital of Rome (protocol no. R.S.44.20) approved this study. Before sample collection, participants completed an informed consent form, which included questions about their demographics and any previous diseases they may have had.

Between January 2021 and April 2022, we collected a comprehensive set of 1113 peripheral blood samples from participants at Qatar University, including two primary study groups: unvaccinated naturally infected (NI; n = 218) and vaccinated naïve (VN; n = 895).

The NI group consisted of individuals who had confirmed SARS-CoV-2 infections, with samples taken at a median of 67 days post-diagnosis (n = 218). This group was subdivided based on the presence and extent of COVID-19 symptoms into four categories: those who experienced full symptoms (n = 51), those with very few symptoms (pauci-symptomatic, n = 20), those without any symptoms (asymptomatic, n = 135), and those for whom the symptomatology was not recorded or was unclear (unknown manifestations, n = 12).

The VN group consisted of samples collected from vaccinated subjects (~115 days from the first dose) with no previous history of infection, confirmed to be anti-N negative (n = 895). Within the VN group, the subgroups were delineated based on the number of doses administered: 1. Partially vaccinated (n = 65, samples collected ~37 days post first dose), 2. Primary series (n = 766, samples collected ~128 days post first dose), 3. Primary series plus one heterologous booster dose (n = 19, samples collected ~296 days post first dose), and 4. Primary series plus one homologous booster dose (n = 45, samples collected ~286 days post first dose).

2.2. Serology testing and immunoassays

2.2.1. Antibody assessments

After collection, whole blood samples were centrifuged to separate plasma for testing: [1] neutralizing Antibodies (NTAbs), [2] total antibodies (TAbs), [3] anti-S-RBD IgG, [4] Anti-S1 anti-S1 IgA.

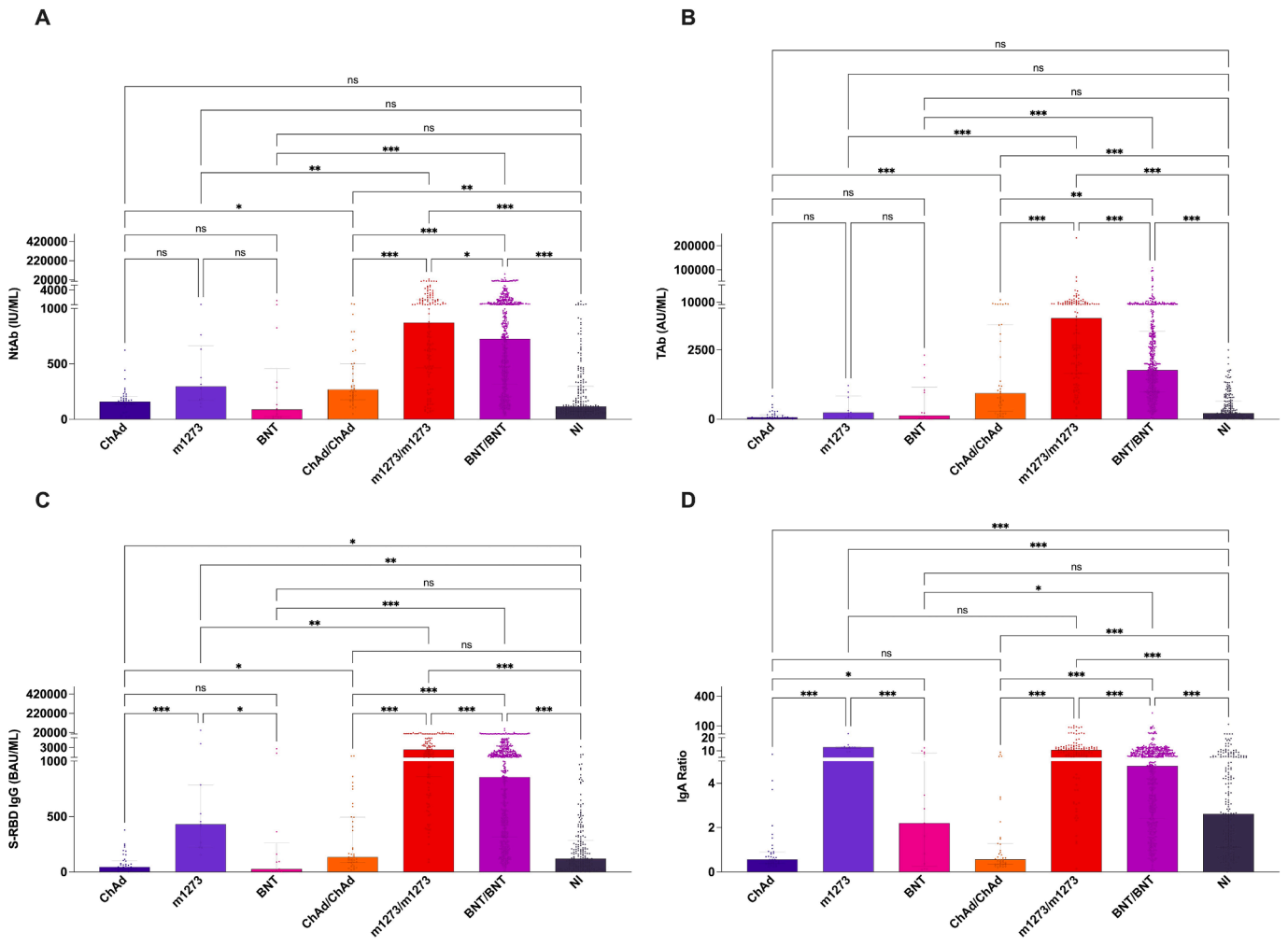

Fig. 2. Comparative Analysis of Antibody Responses Following Vaccination with Oxford-AstraZeneca (ChAd), Pfizer-BioNTech (BNT), and Moderna (m1273) COVID-19 Vaccines. Panels A to C depict the concentrations of neutralizing antibodies (NTAbs), total antibodies (TAbs), and antibodies specific to the spike-receptor binding domain IgG (anti-S-RBD IgG), respectively. Panel D shows the ratio of anti-S-RBD IgA post-vaccination. Statistical significance is denoted by asterisks (*p < 0.05, **p < 0.01, ***p < 0.001). Comparisons that did not reach statistical significance are marked with "ns". Box plots display the median and interquartile range, with outliers represented as individual points.

The SARS-CoV-2 Neutralizing Antibody chemiluminescent immunoassay (CLIA) (Cat No. SARS-CoV-2 Neutralizing Antibody 121, Mindray, China) was utilized to measure NTAbs. This assay has a measuring range of 2.0–400.0 AU/ml, with results ≥10.0 AU/ml considered reactive for NTAbs. Samples exceeding 400 AU/ml were diluted with phosphate-buffered saline (PBS) and re-analyzed. Results were standardized using the WHO conversion factor (1 AU = 3.31 IU/mL).

Total Antibodies (TAbs), including anti-S-RBD IgG, anti-S1 IgA, and IgM, were quantified using the CL-900i® CLIA assay (Cat No. SARS-CoV-2 Total 91 Antibodies 121, Mindray, China). The assay features a measuring and linearity range from 3 to 2000 AU/ml. Samples with values >2000 AU/ml were diluted in PBS and re-analyzed. According to the manufacturer's criteria, readings <10 AU/ml indicated a negative result, and those ≥10 AU/ml indicated positivity for TAbs to SARS-CoV-2.

Anti-S-RBD IgG levels were measured using the SARS-CoV-2 S-RBD IgG CLIA assay (Cat No. SARS-CoV-2 Anti-S-RBD IgG122, Mindray, China). This assay has a range of 3.0–1000.0 AU/ml, with results ≥10.0 AU/ml considered positive for S-RBD IgG. Samples exceeding 1000.0 AU/ml were diluted and re-analyzed. Results were standardized to 1.15 BAU/mL using WHO guidelines.

The Euroimmun Anti-SARS-CoV-2 anti-S1 IgA assay (Euroimmun, Germany; Cat. No. EI 2606-9601 A) was performed according to the manufacturer's instructions. The computed ratios were interpreted in accordance with the manufacturer's recommendations. A ratio <0.8 was designated negative, ≥ 0.8 to <1.1 was considered borderline, and ≥1.1 was considered positive [25].

Samples were screened for past SARS-CoV-2 infection using the Architect automated chemiluminescent assay (Abbott Laboratories, USA) and Euroimmun ELISA (catalog no. El 2606-9601-2 G). This detected SARS-CoV-2 anti-nucleoprotein anti-S-RBD IgG antibodies (anti-N), distinguishing them from anti-S-RBD IgG antibodies generated against the spike protein's RBD. Positive anti-N results indicate prior exposure to the whole virus [26], leading to exclusion from the VN group.

2.3. Statistical analysis

Data were analyzed using GraphPad Prism 9.2.0. (San Diego, CA, USA). The gathered dataset was evaluated for normality using the Shapiro–Wilk normality test. Due to the lack of a normal distribution, nonparametric tests using the Kruskal-Wallis test for the differences between independent samples were conducted. In the bar charts, the horizontal bar line represents the median titer, and the error bars represent the interquartile range (IQR). Using the Spearman rank correlation test, the correlation between NTAbs/anti-S-RBD IgG and

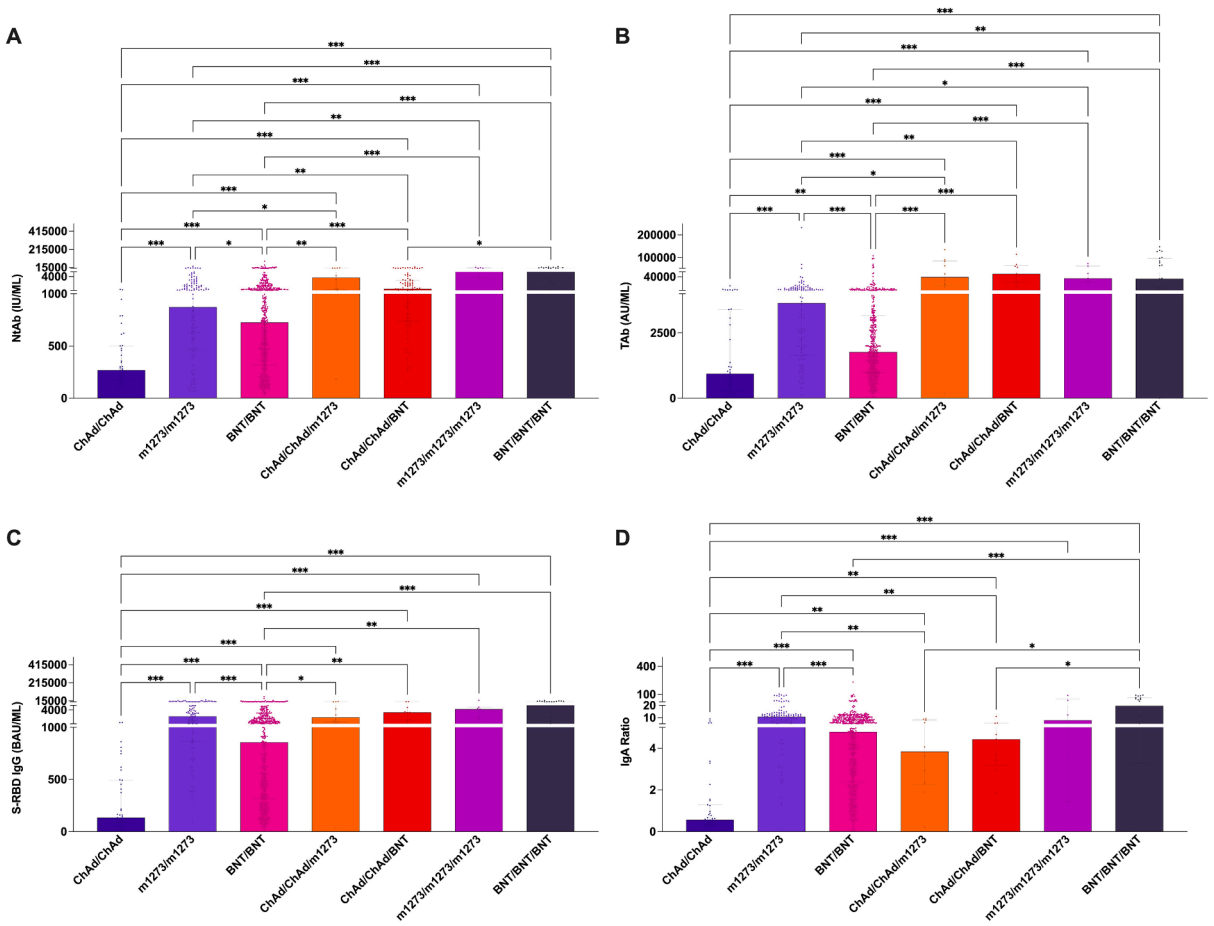

Fig. 3. Immunogenicity Profile of Homologous and Heterologous COVID-19 Vaccination Schedules. Panels A to D illustrate the concentrations of neutralizing antibodies (NTAbs), total antibodies (TAbs), antibodies specific to the spike-receptor binding domain IgG (anti-S-RBD IgG), and antibodies specific to the S1 subunit IgA (anti-S1 IgA) post-vaccination. Statistical significance is marked by asterisks (*p < 0.05, **p < 0.01, ***p < 0.001). For clarity and emphasis on relevant findings, only significant comparisons are illustrated in the figure.Box plots represent the median and interquartile range, with outliers shown as separate points.

NTAbs/Anti-S1 IgA levels was analyzed. A scatterplot was used to illustrate the direction, form, and magnitude of the correlation. The significance level was set at $P < 0.05$.

3. Results

3.1. Descriptive statistics and participant characteristics

A total of 1113 samples were included in this study, comprising samples collected from VN (n = 895) and NI (n = 218) individuals (Table 1 [13], Fig. 1). In the VN group, samples were collected at median: 115 days (~3.8 months) after receiving the first dose of either BNT, m1273 or ChAd vaccines. The VN group comprised 52 % females, 42 % males, and 6 % of unspecified gender.

In the VN group (n = 895), the partially vaccinated group (n = 65) included samples collected post-one dose of either ChAd (61.5 %), m1273 (16.9 %), or BNT (21.5 %). The primary series group (n = 766) included samples collected post-two homologous doses of either ChAd/ChAd (5.2 %), m1273/m1273 (17.1 %), or BNT/BNT (77.7 %). The heterologous regimen: primary series plus one booster dose group (n = 19) included samples collected post-two homologous doses of ChAd, followed by a heterologous booster shot of either ChAd/ChAd/m1273 (52.6 %), or ChAd/ChAd/BNT (47.4 %). The homologous regimen: primary series plus one booster dose of the same mRNA vaccine used in the primary series, (n = 45), included samples collected post-three homologous doses of either m1273/m1273/m1273 (15.6 %) or BNT/BNT/BNT (84.4 %).

Samples were collected from individuals in the NI group at a median of approximately 2.2 months (67 days) after SARS-CoV-2 infection. The NI group consisted of 86.2 % males and 13.8 % females as indicated in Table 1. Out of the 218 individuals in the NI group, 23 % were symptomatic (n = 51), 9.2 % were pauci-symptomatic (n = 20), 61.9 % were asymptomatic (n = 135), and 5.5 % were of unidentified manifestations (n = 12).

3.2. Comprehensive analysis of antibody response (NTAb, TAb, anti-S-RBD IgG, and S-RBD anti-S1 IgA) among the different study groups

3.2.1. Primary series mRNA vaccination regimens induce strong antibody responses compared to vector virus vaccine

The comparison between primary series vaccination regimens and partial vaccination regimens revealed significant enhancements in antibody responses. Individuals who completed the primary vaccination series exhibited notably higher levels of NTAb, TAb, and anti-S-RBD IgG compared to those who received only one dose of the same vaccine type ($p < 0.05$) as shown in Fig. 2 A-C. Conversely, the difference in anti-S1 IgA levels between primary and partial vaccination were significant only for the BNT ($P < 0.05$), but not with ChAd or m1273 (Fig. 2D).

Among primary series vaccination regimens, ChAd/ChAd elicited weaker immune responses (NTAb, TAb, anti-S-RBD IgG, and anti-S1 IgA) compared to BNT/BNT or m1273/m1273 ($P < 0.05$). Additionally, ChAd/ChAd resulted in approximately 4.6 times less anti-S1 IgA response compared to natural infection ($P < 0.001$). mRNA vaccination regimens induced robust antibody responses compared to ChAd/ChAd

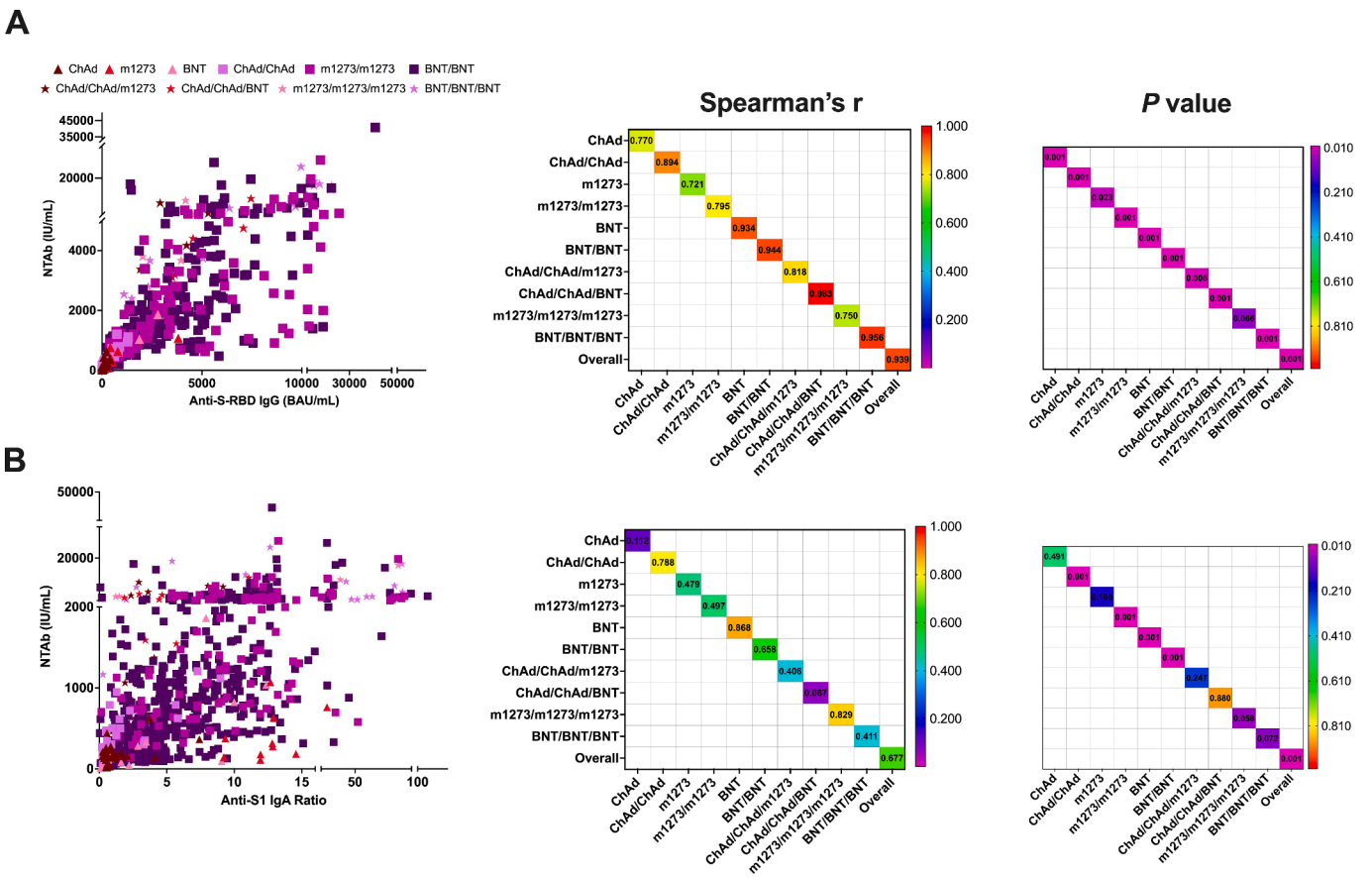

Fig. 4. Correlation between Neutralizing Antibodies and Anti-Spike Protein Antibodies Post-Vaccination in Vaccinated Naïve (VN) Individuals. Panels A and B showcase scatter plots and correlation analyses for neutralizing antibodies (NTAbs) against antibodies specific to the spike-receptor binding domain IgG (anti-S-RBD IgG) and antibodies specific to the S1 subunit IgA (anti-S1 IgA), respectively. Spearman's correlation coefficients (r) and corresponding p-values are provided for each vaccine regimen. p-values < 0.001 being represented as 0.001.

($P < 0.05$), with m1273/m1273 showing the strongest enhancement, including a 3.2-fold increase in NTAbs ($p < 0.001$), 3.9-fold rise in TAbs ($p < 0.001$), 19.6-fold increase in anti-S-RBD IgG levels ($p < 0.001$), and an 18.9-fold elevation in anti-S1 IgA ratios (Fig. 2).

3.2.2. *Homologous mRNA vaccination demonstrated a significantly superior capacity to elicit robust NTAb and anti-S1 IgA responses when compared to heterologous regimens*

The data in Fig. 3 demonstrate the immunogenic profile post-vaccination with primary series and booster regimens against SARS-CoV-2. m1273/m1273 exhibits the strongest response across all antibody measures (NTAb, TAb, anti-S-RBD IgG), followed by BNT/BNT, while ChAd/ChAd shows lower responses ($P < 0.05$). mRNA vaccines consistently outperform the ChAd/ChAd regimen in NTAbs, Tabs, anti-S-RBD IgG, and anti-S1 IgA ($p < 0.001$). Boosters with heterologous regimens (ChAd/ChAd/m1273 or ChAd/ChAd/BNT) enhance antibody levels compared to primary ChAd/ChAd. No significant differences were observed between homologous (m1273/m1273/m1273 or BNT/BNT/BNT) and heterologous (ChAd/ChAd/m1273 or ChAd/ChAd/BNT) boosting regimens for Tabs and anti-S-RBD IgG. However, mRNA homologous BNT/BNT/BNT shows significantly superior capacity for NTAb and S-RBD anti-S1 IgA responses, by 4.3-fold and 4.4-fold, respectively ($p < 0.05$), compared to the heterologous boosting regimen ChAd/ChAd/BNT.

3.2.3. *Predominant role of Anti-S-RBD IgG in virus neutralization among VN individuals*

In the evaluation of post-vaccination neutralizing potency, the correlation between NTAbs/anti-S-RBD IgG and NTAbs/anti-S1 IgA among VN subjects was examined (Fig. 4). Anti-S-RBD IgG demonstrated a more pronounced role in virus neutralization compared to anti-S1 IgA, with particularly strong correlations noted in ChAd/ChAd/BNT VN individuals ($r = 0.983$). Consistently strong to very strong correlations were observed between NTAbs and anti-S-RBD IgG across all VN groups, while the correlation with anti-S1 IgA, although significant, was less robust. The overall correlation between NTAbs and anti-S1 IgA varied among different groups.

3.2.4. *Anti-anti-S-RBD IgG and anti-S1 IgA significantly contribute to virus neutralization among NI individuals*

In the investigation of post-SARS-CoV-2 infection serological dynamics and neutralizing potency, the correlation between NTAbs/anti-S-RBD IgG and NTAbs/anti-S1 IgA among NI subjects ($n = 218$) was analyzed (Fig. 5). The NI group displayed significant correlations between NTAbs and both anti-S-RBD IgG and anti-S1 IgA ($p < 0.001$). Notably, NTAbs/anti-S-RBD IgG exhibited a very strong overall correlation ($r = 0.809$, $p < 0.001$) compared to NTAbs/anti-S1 IgA, which showed significant but moderate correlations ($r = 0.501$, $p < 0.001$). Stratification by clinical manifestations revealed significant correlations among symptomatic, asymptomatic, and pauci-symptomatic groups, with the strongest observed for NTAbs/anti-S-RBD IgG in pauci-symptomatic and symptomatic individuals ($r = 0.949$, $p < 0.001$, and 0.835, $p < 0.001$, respectively).

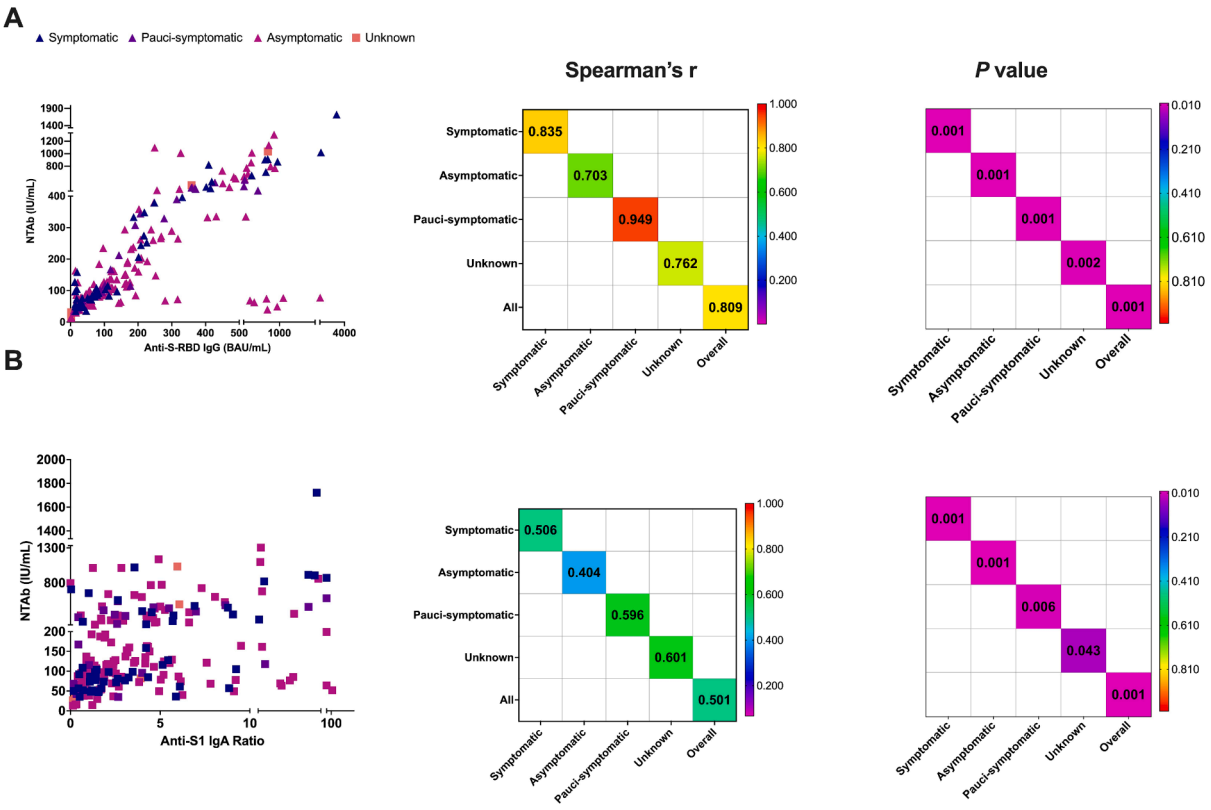

Fig. 5. Correlation between Neutralizing Antibodies and Anti-Spike Protein Antibodies in Naturally Infected (NI) Individuals. Panels A and B present scatter plots depicting the relationship between neutralizing antibodies (NTAbs) and antibodies specific to the spike-receptor binding domain IgG (anti-S-RBD IgG), and antibodies specific to the S1 subunit IgA (anti-S1 IgA), across different clinical manifestations. Spearman's correlation coefficients (r) and p-values are provided for each clinical group. p-values < 0.001 being represented as 0.001.

4. Discussion

The study presents a comprehensive comparison of immune responses triggered by various COVID-19 vaccination strategies and natural SARS-CoV-2 infections. Analyzing 1113 samples from VN and NI individuals (Fig. 1), the findings suggest that while heterologous mRNA boosters offer improvement over primary vaccination, homologous mRNA boosting regimens demonstrate potential benefits in enhancing anti-S1 IgA antibody levels and neutralizing capacity.

Significant differences were observed in immune responses between mRNA vaccines and the vector-based ChAd vaccine. mRNA vaccines demonstrated a pronounced superiority in inducing comprehensive antibody responses, including NTAbs, TAb, and anti-S-RBD IgG levels (Fig. 2). Notably, m1273 emerged as the most potent in stimulating these immune markers, outperforming both BNT and ChAd (Fig. 2). This is consistent with the literature, which has highlighted the robust antibody responses triggered by mRNA vaccines over vector-based vaccines [27–29].

Additionally, primary vaccination with BNT showed notable increases in anti-S1 IgA levels (Fig. 2), highlighting its unique ability to engage mucosal immunity. This finding is particularly interesting, given the role of anti-S1 IgA in neutralizing pathogens at mucosal sites [30].

Investigation into booster vaccination strategies revealed that heterologous boosting, starting with ChAd followed by an mRNA vaccine booster, improved antibody levels compared to the primary ChAd regimen alone. This observation suggests that booster vaccines may not necessarily need to align with the vaccines used for the primary series, as indicated by previous research [31]. Conversely, homologous mRNA boosting, particularly with BNT, proved more effective in bolstering both neutralizing antibodies and anti-S1 IgA levels (Fig. 3). This underscores the strategic preference for homologous mRNA boosters to optimize both neutralizing and mucosal immune defenses.

Assessing neutralizing antibody levels on an individual basis is crucial for determining the necessity of additional doses and avoiding unnecessary vaccinations [32]. The analysis also highlighted the distinct contributions of anti-S-RBD IgG and anti-S1 IgA to virus neutralization, with vaccinated individuals relying more on anti-S-RBD IgG, while both isotypes played significant roles in the NI group, particularly in symptomatic individuals.

While the study had limitations, including not adressing confounding factors, the absence of data on antibody responses against different variants and the predominantly asymptomatic nature of the NI group, which may not fully represent the range of responses seen in individuals with more severe infections [33], it also possesses several notable strengths. First, the study design facilitates a direct comparison of vaccination regimens. This is critical to inform public health policies and vaccination strategies, especially as the world continues to grapple with the pandemic and the emergence of new variants. Moreover, unlike most research focusing on NTAb, TAbs, and anti-S-RBD IgG, our study also examines the less-studied anti-S1 IgA response. Second, in this research, we analyzed anti-N antibodies, which are essential to identify people who were infected with a virus but had no symptoms before immunization.

5. Conclusion

The study conclusively demonstrates that mRNA-based COVID-19 vaccines significantly outperform vector-based counterparts in eliciting a robust immunological profile. While heterologous boosting effectively enhanced antibody levels, homologous mRNA boosting, particularly with BNT, significantly outperformed heterologous boosting in enhancing neutralizing and anti-S1 IgA antibody titers, emphasizing the

immunological superiority of a uniform vaccine platform. Furthermore, correlation analyses revealed that both, anti-S-RBD IgG and anti-S1 IgA substantially contribute to viral neutralization in NI individuals, whereas, among VN individuals, anti-S-RBD IgG was the main contributor to virus neutralization. These findings strongly support the strategic emphasis on homologous mRNA vaccine regimens as a crucial component of effective public health strategies to combat COVID-19.

Funding

GKN would like to acknowledge the funding from WHO grant number COVID-19-22-43 and Qatar University grant No. QUCG-BRC-23/24-170. This work was also made possible by grants number UREP29-026-3-004 and UREP28-173-3-057 from the Qatar National Research Fund (a member of Qatar Foundation). The statements made herein are solely the responsibility of the authors.

Author contributions

Supervision: G.K.N. First draft writing: S.Y and G.K.N. Participant recruitment and demographic data collection: HID, E.N., M.P., S.B., S.Y., N.Y., P.B.N., N.Z., M.A., and G.K.N. Laboratory testing: E.N., M.P., P.B. N., N.Z., M.A., A.I. Review and editing: S.Y., E.N., N.Y., M.P., S.B., P.B. N., N.Z., M.A., H.M.Y., L.J.A.-R., A.I., and G.K.N. Funding: G.K.N. All authors have read and agreed to the published version of the manuscript.

CRediT authorship contribution statement

Salma Younes: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Eleonora Nicolai:** Writing – review & editing, Investigation, Data curation. **Nadin Younes:** Writing – review & editing, Methodology, Data curation. **Massimo Pieri:** Writing – review & editing, Data curation. **Sergio Bernardini:** Writing – review & editing, Data curation. **Parveen B. Nizamuddin:** Writing – review & editing, Investigation, Data curation. **Duaa W. Al-Sadeq:** Writing – review & editing, Data curation. **Hanin I. Daas:** Writing – review & editing, Data curation. **Ahmed Ismail:** Writing – review & editing, Data curation. **Hadi M. Yassine:** . **Laith J. Abu-Raddad:** . **Gheyath K. Nasrallah:** .

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: We would like to declare that all Mindray kits used in this paper were provided as in-kind support for GKN lab.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgment

We thank the laboratory technologists at the Medical Commission Laboratory Section, Ministry of Public Health, Qatar, for their help in performing the Architect® immunoassay.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2024.06.010.

References

[1] WHO. WHO Coronavirus (COVID-19) Dashboard [Internet]. <https://covid19.who.int/>.
[2] Total COVID-19 vaccine doses administered - Our World in Data. <https://ourworldindata.org/grapher/cumulative-covid-vaccinations?country=~OWID_WRL> [accessed 16 Mar 2023].
[3] Centers for Disease Control and Prevention. Understanding mrna COVID-19 vaccines. Centers for Disease Control and Prevention; 2021. <https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/mrna.html>.
[4] Goldberg Y, Mandel M, Bar-On YM, Bodenheimer O, Freedman L, Haas EJ, et al. Waning immunity after the BNT162b2 vaccine in Israel. N Engl J Med 2021;385(24):e85.
[5] Chemaitelly H, Tang P, Hasan MR, AlMukdad S, Yassine HM, Benslimane FM, et al. Waning of BNT162b2 vaccine protection against SARS-CoV-2 infection in Qatar. N Engl J Med 2021;385(24):e83.
[6] Tartof SY, Slezak JM, Fischer H, Hong V, Ackerson BK, Ranasinghe ON, et al. Effectiveness of mRNA BNT162b2 COVID-19 vaccine up to 6 months in a large integrated health system in the USA: a retrospective cohort study. Lancet 2021;398(10309):1407–16.
[7] Abu-Raddad LJ, Chemaitelly H, National Study Group for COVID-19 Vaccination. Waning mRNA-1273 vaccine effectiveness against SARS-CoV-2 infection in Qatar. N Engl J Med 2022;386(11):1091–3. https://doi.org/10.1056/NEJMc2119432.
[8] Abou-Saleh H, Abo-Halawa BY, Younes S, Younes N, Al-Sadeq DW, Shurrab FM, et al. Neutralizing antibodies against SARS-CoV-2 are higher but decline faster in mRNA vaccinees compared to individuals with natural infection. J Travel Med 2022;29(8):taac130. https://doi.org/10.1093/jtm/taac130.
[9] 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(4):139–45.
[10] Altarawneh HN, Chemaitelly H, Ayoub HH, Tang P, Hasan MR, Yassine HM, et al. Effects of previous infection and vaccination on symptomatic omicron infections. N Engl J Med 2022;387(1):21–34.
[11] Notarte KI, Catahay JA, Peligro PJ, Velasco JV, Ver AT, Guerrero JJ, et al. Humoral response in hemodialysis patients post-SARS-CoV-2 mRNA vaccination: a systematic review of literature. Vaccines (Basel) 2023;11(4).
[12] Notarte KI, Guerrero-Arguero I, Velasco JV, Ver AT, Santos de Oliveira MH, Catahay JA, et al. Characterization of the significant decline in humoral immune response six months post-SARS-CoV-2 mRNA vaccination: a systematic review. J Med Virol 2022;94(7):2939–61.
[13] Notarte KI, Ver AT, Velasco JV, Pastrana A, Catahay JA, Salvagno GL, et al. Effects of age, sex, serostatus, and underlying comorbidities on humoral response post-SARS-CoV-2 Pfizer-BioNTech mRNA vaccination: a systematic review. Crit Rev Clin Lab Sci 2022;59(6):373–90.
[14] Shurrab FM, Al-Sadeq DW, Abou-Saleh H, Al-Dewik N, Elsharafi AE, Hamaydeh FM, et al. Assessment of the neutralizing antibody response of BNT162b2 and mRNA-1273 SARS-CoV-2 vaccines in naïve and previously infected individuals: a comparative study. Vaccines (Basel) 2022;10(2).
[15] Ferdinands JM, Rao S, Dixon BE, Mitchell PK, DeSilva MB, Irving SA, et al. Waning 2-dose and 3-dose effectiveness 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(7):255–63.
[16] Barda N, Dagan N, Cohen C, Hernán MA, Lipsitch M, Kohane IS, et al. Effectiveness of a third dose of the BNT162b2 mRNA COVID-19 vaccine for preventing severe outcomes in Israel: an observational study. Lancet 2021;398(10316):2093–100.
[17] Abu-Raddad LJ, Chemaitelly H, Ayoub HH, AlMukdad S, Yassine HM, Al-Khatib HA, et al. Effect of mRNA vaccine boosters against SARS-CoV-2 omicron infection in Qatar. N Engl J Med 2022;386(19):1804–16.
[18] Agency EM. EMA and ECDC recommendations on heterologous vaccination courses against COVID-19: 'mix-and-match' approach can be used for both initial courses and boosters; 2021 <https://www.ema.europa.eu/en/news/ema-ecdc-recommendations-heterologous-vaccination-courses-against-covid-19-mix-match-approach-can-be>.
[19] Greinacher A, et al. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. N Engl J Med 2021; 384: 2092–2101.Return to ref 1 in article.
[20] Scully M, et al. Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination. N Engl J Med 2021;384:2202–11.
[21] Munro APS, Janani L, Cornelius V, Aley PK, Babbage G, Baxter D, et al. Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicentre, randomised, controlled, phase 2 trial. Lancet 2021;398(10318):2258–76.
[22] JCVI statement regarding a COVID-19 booster vaccine programme for winter 2021 to 2022 (Department of Health and Social Care, 2021).
[23] Tapia MD, Sow SO, Lyke KE, Haidara FC, Diallo F, Doumbia M, et al. Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: A phase 1, single-blind, randomised trial, a phase 1b, open-label and double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial. Lancet Infect Dis 2016;16:31–42. https://doi.org/10.1016/s1473-3099(15)00362-x.

[24] Brown SA, Surman SL, Sealy R, et al. Heterologous prime-boost HIV-1 vaccination regimens in pre-clinical and clinical trials. Viruses 2010;2:435–67. https://doi.org/10.3390/v2020435.

[25] Gededzha MP, Mampeule N, Jugwanth S, Zwane N, David A, Burgers WA, et al. Performance of the EUROIMMUN Anti-SARS-CoV-2 ELISA Assay for detection of IgA and IgG antibodies in South Africa. PLoS One 2021;16(6):e0252317.

[26] Ismail A, Shurrab FM, Al-Jighefee HT, Al-Sadeq DW, Qotba H, Al-Shaar IA, et al. Can commercial automated immunoassays be utilized to predict neutralizing antibodies after SARS-CoV-2 infection? A comparative study between three different assays; 2021.

[27] Fabricius D, Ludwig C, Scholz J, Rode I, Tsamadou C, Jacobsen EM, et al. mRNA vaccines enhance neutralizing immunity against SARS-CoV-2 variants in convalescent and ChAdOx1-primed subjects. Vaccines (Basel) 2021;9(8).

[28] Adjobimey T, Meyer J, Sollberg L, Bawolt M, Berens C, Kovačević P, et al. Comparison of IgA, IgG, and neutralizing antibody responses following immunization with Moderna, BioNTech, AstraZeneca, Sputnik-V, Johnson and Johnson, and Sinopharm's COVID-19 Vaccines. Front Immunol 2022;13:917905. https://doi.org/10.3389/fimmu.2022.917905.

[29] Gareth I. Covid-19: Antibodies after AstraZeneca and Pfizer vaccines decrease with age and are higher in women, data show. BMJ 2022;376:o428.

[30] Azzi L, Dalla Gasperina D, Veronesi G, Shallak M, Ietto G, Iovino D, et al. Mucosal immune response in BNT162b2 COVID-19 vaccine recipients. EBioMedicine 2022;75:103788.

[31] Michael B, Daniel M, Selma T, Daniela S, Maximilian K, Peter M, et al. Additional heterologous versus homologous booster vaccination in immunosuppressed patients without SARS-CoV-2 antibody seroconversion after primary mRNA vaccination: a randomised controlled trial. Ann Rheum Dis 2022;81(5):687.

[32] Pieri M, Nicolai E, Ciotti M, Nuccetelli M, Sarubbi S, Pelagalli M, et al. Antibody response to COVID-19 vaccine: a point of view that can help to optimize dose distribution. Int Immunopharmacol 2022;102:108406. https://doi.org/10.1016/j.intimp.2021.108406.

[33] Long Q-X, Tang X-J, Shi Q-L, Li Q, Deng H-J, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 2020;26(8):1200–4.