Clinical Immunology 278 (2025) 110523

Contents lists available at ScienceDirect

Clinical Immunology

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

Full Length Article

The type of the first prime/boost vaccine against SARS-CoV-2 exerts long-term effects on the humoral immune response

Franz Mai [a], Emil C. Reisinger [b], Brigitte Müller-Hilke [a,*]

[a] Core Facility for Cell Sorting and Cell Analysis, Rostock University Medical Center, 18057 Rostock, Germany
[b] Division of Tropical Medicine and Infectious Diseases, Center of Internal Medicine II, Rostock University Medical Center, 18055 Rostock, Germany

ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19
BNT162b2
AZD1222
Immune imprinting
Breakthrough infection

ABSTRACT

The outbreak of COVID-19 spurred the development of different vaccines against SARS-CoV-2 however, recommendations on how to maintain long-term protection from COVID-19 remain elusive.

We here report on a cohort of 192 health care workers receiving their primary vaccination with either AZD1222 or BNT162b2. Over the course of three years, six blood samples were taken and analyzed for antibody dynamics against the receptor binding domain of the Spike protein and for function via surrogate virus neutralization.

Our results showed that higher anti SARS-CoV-2 S titers correlated with increased neutralizing capacity and ameliorated COVID-19 disease. The type of the first prime/boost vaccine exerted long term effects with a homologous BNT162b2 regimen outperforming AZD1222 in terms of antibody titers and neutralizing capacity. This deficit for AZD1222 was not compensated for by subsequent boosting with RNA vaccines, was still evident after three years, and is discussed in the context of immune imprinting.

1. Introduction

Following the outbreak of coronavirus disease (COVID)-19 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a number of different vaccines were developed using various strategies [1]. Among the first vaccines that were clinically tested and approved were the mRNA-based ones from Pfizer-BioNTech (Comirnaty, BNT162b2) or Moderna (Spikevax, mRNA-1273) as well as the adenoviral vector-based vaccine from AstraZeneca (Vaxzevria, AZD1222). All three were proven to be safe and effective [2–4]. They also protected against severe disease courses, hospitalization, and death [5]. Moreover, vaccination against SARS-CoV-2 also turned out to reduce the risk of the long-term sequelae long−/post-COVID [6,7]. However, vaccinations did not altogether prevent COVID-19 and so called breakthrough infections in fully vaccinated individuals have been observed early on [8]. Up until today they are ascribed to circulating variants of concern (VOC) of SARS-CoV-2 [9,10], but also to the speed of natural decline of antibodies among the vaccinated. Numerous studies from our own group and others have described the immune responses to the above-mentioned vaccines as well as differences between vaccine types and the waning of antibody titers up to two years after vaccination [11–19]. However, data on vaccine efficacy more than 24 months after primary immunization are scarce. While high binding antibody units and neutralizing capacity have from the start been understood as protective correlates [20–22], neither is routinely tested, nor is there any more need to verify SARS-CoV-2 in case of respiratory infections. As a consequence, individual vaccination status are unclear and recommendations for or against repeated booster immunizations remain unfounded. Moreover, the concept of immune imprinting – or original antigenic sin (OAS) – questions the benefit of multiple vaccinations with the same antigen [23–26]. According to OAS, the immune system repeatedly relies on the first cohort of B cells engaged by an antigenic stimulus and will not induce de novo responses upon encounter of related antigens [27]. The concerns raised in the context of COVID-19 are that repeated immunizations with the receptor binding domain (RBD) of SARS-CoV-2 may in the future prevent an adequate response to newly emerging and more aggressive variants of concern.

To investigate the phenomenon of immune imprinting and to analyze vaccine efficacies at three years after the first immunization, we here followed up on three cohorts of a total of 192 health care workers who had undergone different prime/boost regimen. We monitored binding antibody units and neutralizing capacity against the background of

* Corresponding author.
E-mail address: brigitte.mueller-hilke@med.uni-rostock.de (B. Müller-Hilke).

https://doi.org/10.1016/j.clim.2025.110523
Received 20 March 2025; Received in revised form 23 April 2025; Accepted 14 May 2025
Available online 15 May 2025

infection and vaccination histories.

2. Materials and methods

2.1. Study participants and blood sampling

Initially, 192 employees of Rostock University Medical Center from the medical and non-medical fields were recruited by the Coordination Center for Clinical Studies [28]. Personal data was only collected on age and sex. EDTA and serum blood samples were taken by venipuncture every six months at each visit. In addition, new vaccinations and results of a COVID-19 PCR or rapid test carried out in the last six months were documented, and the participants were asked about symptoms of a respiratory infection in the last six months. At the end of the study, each remaining subject was given a questionnaire to record the number and duration of symptoms (cough, runny nose, sore throat, fever, shortness of breath, severe feeling of illness, headache, aching limbs, loss of taste/odor, nausea/vomiting/diarrhea) of COVID-19 illness in the last six months. The samples were collected in the period from July 2021 to February 2024. Study participants were exclusively Caucasians except for two subjects of Asian and two of Syrian origins. Plasma and serum were processed via centrifugation at 1500*g* for 10 min and 2000 g for 10 min, respectively. Both were subsequently frozen at −80 °C for later use. The study was approved by the Ethics Committee of the University Medical Center Rostock under the number A 2020–0086. Written informed consent was obtained from all study participants.

2.2. Quantification of anti-SARS-CoV-2 S and anti-Nucleocapsid antibodies

To quantify antibodies against SARS-CoV-2, anti-SARS-CoV-2S and anti-SARS-CoV-2 N Elecsys® Assays (Roche Diagnostics, Mannheim, Germany) were performed according to the manufacturer's instructions and were run on a Cobas E411 (Roche Diagnostics, Mannheim, Germany). Results were compared to the WHO international standard for anti-SARS-CoV-2 immunoglobulin to obtain binding antibody units (BAU) [29]. Measured U/mL correlated strongly with the international WHO standard binding antibody units (BAU/mL) (U = 0.972 * BAU; Pearson $r = 0.99996$).

2.3. Neutralizing capacity against omicron BA.2

The SARS-CoV-2 Surrogate Virus Neutralizing Test (sVNT) kit (GenScript, Piscataway, NJ, USA) was used according to the manufacturer's instructions. In short, frozen plasma was thawed, centrifuged at 10,000*g* for five min to remove precipitates, and diluted 1:40 in dilution buffer. HRP peptide Omicron BA.2 (SARS-CoV-2 Spike protein RBD-HRP, BA.2 variant, His Tag) (GenScript) were diluted at 1:1000. Diluted plasma samples and diluted HRP peptide samples were mixed in equal parts. After 30 min of incubation at 37 °C, samples were pipetted onto an ACE2 coated ELISA capture plate and incubated for an additional 15 min at 37 °C. Then, substrate solution was added and incubated for another 15 min at room temperature before stop solution was added to terminate the reaction. Photometric measurements of the capture plate were performed at 450 nm using the InfiniteM200 (Tecan, Männeheim, Switzerland). Optical densities (OD) were used for calculation: Neutralizing Capacity $= (1 - OD_{sample}/OD_{NegCtrl}) \times 100$ %.

2.4. Statistics

Contingency table analyses were performed via chi-square test. Data were first tested for Gaussian distribution using the Shapiro-Wilk test. For unpaired comparisons, either the Mann-Whitney-*U* test was used for two groups or the Kruskal-Wallis test followed by Dunn's correction for multiple comparisons for three groups. Pearson was performed for correlation analyses of metric data and the Spearman rank correlation for metric-ordinal data. Statistical assays were performed with GraphPad InStat® version 3.10 for Windows (GraphPad Software, San Diego, CA, USA) or IBM SPSS Statistics Version 27 (IBM, Armonk, NY, USA). Graphs were created with R (Version 2024.04.2).

2.5. Ethic commitment

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Rostock University Medical Center (file number: A 2020–0086, date of approval: 16 June 2021). Written informed consent was obtained from all subjects involved in the study.

3. Results

3.1. Study design

We here monitored three cohorts of healthcare workers who had undergone either a homologous prime/boost regimen with BNT162b2 ($n = 66$) or AZD1222 ($n = 61$) or a heterologous regimen with AZD1222 followed by BNT162b2 ($n = 65$), respectively. Priming took place for all participants at the turn of 2021/2022 and the intervals between priming and first boost were recommended by the manufacturers to be four weeks for BNT162b2 and three months for AZD1222. For the heterologous prime/boost regimen, the local authorities recommended three months as well. Starting with the third vaccination, at about nine months after priming, all participants received mRNA vaccines. A detailed vaccination scheme is presented in the supplemental Fig. 1. All cohorts were followed up over three years with subsequent blood withdrawals at 6, 12, 18, 24, 30, and 36 months after priming. Study design plus overview of vaccination and infection processes are summarized in Table 1. A detailed overview of the vaccination schedule including the spike variant is provided in Supplemental Fig. 1.

3.2. The primary vaccination regime exerted longtime effects

Already at six months after the first vaccination, there were significant differences between the three cohorts regarding their anti-SARS-CoV-2 S antibody titers and their capacity to neutralize SARS-CoV-2 virus, with the heterologous vaccination regimen outperforming the other two (Fig. 1 and [28]). However, the different time intervals between prime and boost - depending on the vaccine used for priming – resulted in different time intervals between boost and blood withdrawal at six months. These were a mean of 160 days for those being primed with BNT162b2 and only 100 days for those being primed with AZD1222, allowing for a more pronounce waning of antibody titers in the BNT162b2 primed group. We therefore compared at six months only the homologous AZD1222 prime/boost regimen to the heterologous AZD1222/BNT162b2 one. Fig. 1 shows that the latter – priming with AZD1222 followed by a BNT162b2 boost – by far outperformed the homologous prime/boost regimen with AZD1222. Fig. 1 also shows that at 12 months – shortly after the third vaccination – anti-SARS-CoV-2 S titers looked comparable for the three cohorts and even though there was a trend, also the neutralizing capacities showed no significant differences. At later time points, those who had been homologously primed and boosted with BNT162b2 kept outperforming those homologously primed and boosted with AZD1222. These differences were statistically significant for the anti-SARS-CoV-2 S titers while only a trend was observed for the neutralizing capacities. However, statistics were more difficult to calculate for the neutralizing capacities as the test results quickly reached the upper limit of detection. To stay below this limit would have required plasma dilutions which in turn render a transformation into percent neutralizing capacity impossible. In summary, the homologous prime/boost regimen with AZD1222 exerted a longtime disadvantage over a regimen including BNT162b2, even after 36 months, and this disadvantage could not be compensated for by

Table 1
Summary of vaccinations and infection processes.

time point (blood sampling)	vaccination regimen	numbers of samples	numbers of vaccinations					numbers of infections				
		N	2	3	4	5	6	0	1	2	3	4
T1	AZD/AZD	61	61					61	0			
(6 Months)	AZD/BNT	65	65					63	2			
	BNT/BNT	66	66					66	0			
T2	AZD/AZD	57	3	54				54	3			
(12 Months)	AZD/BNT	63	4	59				60	3			
	BNT/BNT	61	4	57				58	3			
T3	AZD/AZD	53	0	52	1			25	28			
(18 Months)	AZD/BNT	63	1	60	2			18	45			
	BNT/BNT	60	0	55	5			22	38			
T4	AZD/AZD	53	0	44	9	0		12	29	12		
(24 Months)	AZD/BNT	63	1	50	12	0		6	40	16		
	BNT/BNT	59	0	49	8	2		6	39	14		
T5	AZD/AZD	50	0	41	9	0		7	21	20	2	
(30 Months)	AZD/BNT	56	0	44	12	0		5	22	20	9	
	BNT/BNT	54	0	44	7	3		3	26	19	6	
T6	AZD/AZD	48	0	38	8	2	0	3	10	24	10	1
(36 Months)	AZD/BNT	55	0	44	10	1	0	2	10	21	17	5
	BNT/BNT	49	0	38	5	3	3	2	7	23	14	3

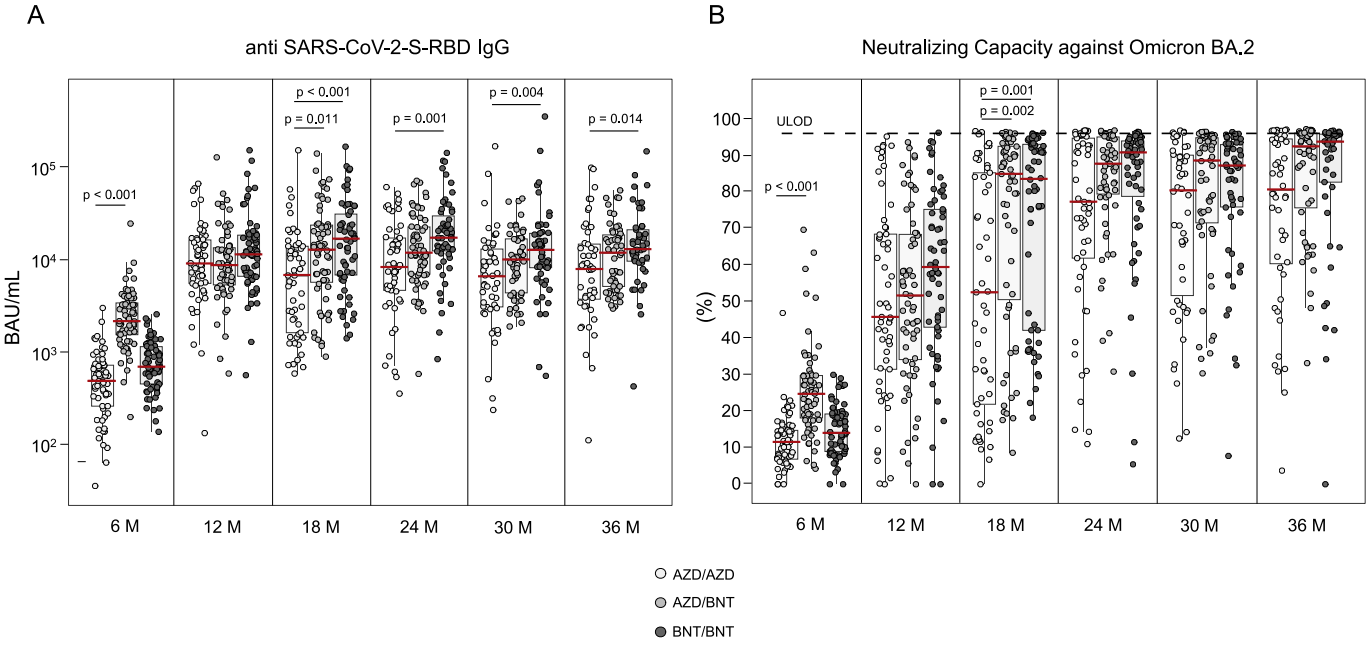

Fig. 1. The primary vaccination regime exerts longtime effects.
Dot plots overlaid by box-plots present anti-SARS-CoV-2 S titers measured in BAU/ml (A) and percentage neutralizing capacity against BA.2 (B) over time; each dot represents one study participant; boxes show median values; ULOD = upper limit of detection; all participants (N = 1036) are included: *p* values result from Kruskal-Wallis test followed by Dunn's correction for multiple comparisons: raw *p*-values are given.

introducing mRNA vaccines for third, fourth and possibly fifth vaccination.

3.3. Infection rates did not differ between the three vaccination cohorts

At every blood withdrawal, we requested information about previous SARS-CoV-2 infections. Self-disclosures were then either confirmed or complemented with antibody titers against the nucleocapsid so that we were able to monitor infection rates within the three vaccination cohorts. Fig. 2 summarizes these results and shows that despite the differences in anti-SARS-CoV-2 S titers and neutralizing capacities shown in Fig. 1, infections were distributed evenly over the three cohorts. Table 1 and Supplemental Table 1 complement these data by showing that also the numbers of vaccinations were distributed evenly over the three cohorts with the majority of individuals having received three vaccinations and recovered from two infections at 36 months. In addition, neither age nor sex had any impact on infection rates.

3.4. Higher anti-SARS-CoV-2 S titers and elevated neutralizing capacities reduced breakthrough infections and negatively correlated with duration and numbers of COVID-19 symptoms

As there is no consensus yet as to the level of anti-SARS-CoV-2 S titer or neutralizing capacity required to confer protection, we were intrigued whether the differences we observed held any biological meaning. To that extent, we compared the titers at any given blood withdrawal with the presence or absence of a SARS-CoV-2 infection within the last six months. Fig. 3A indeed shows a statistically significant difference in the

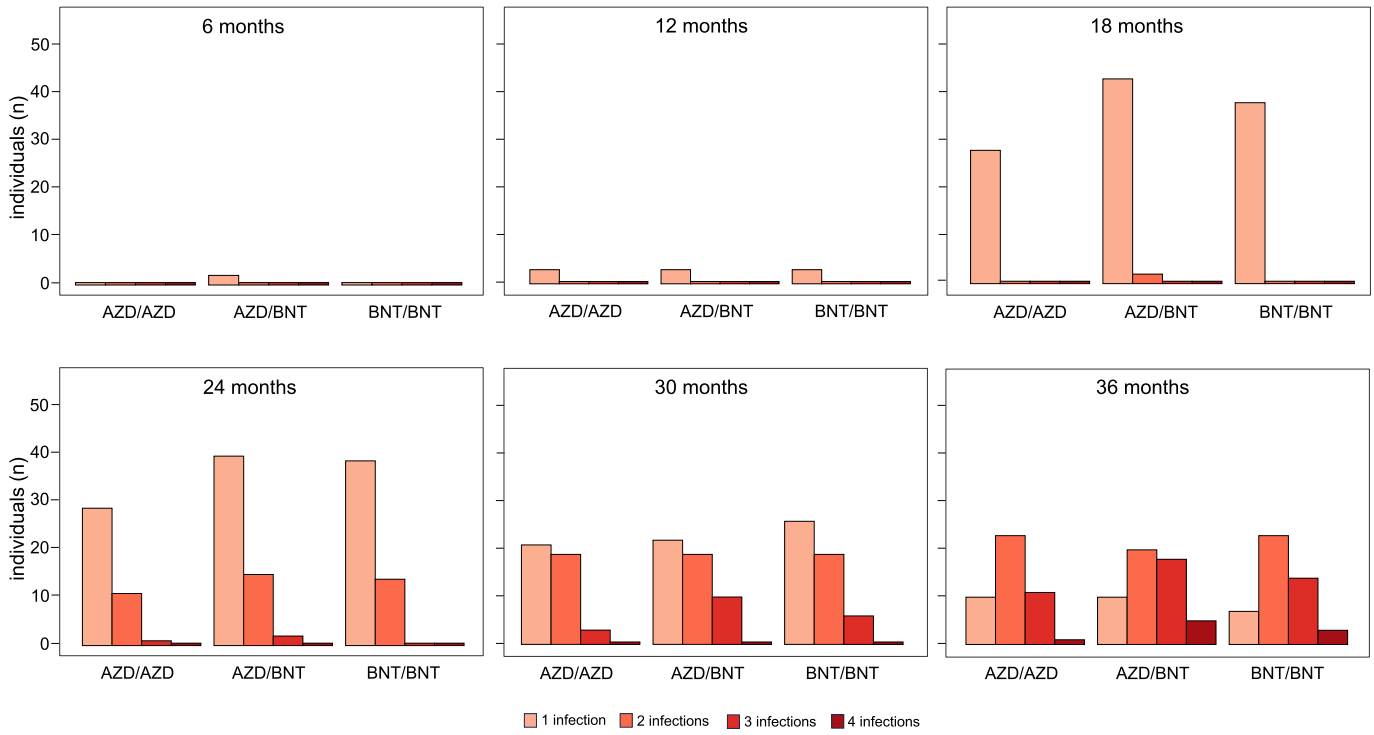

Fig. 2. Infection rates do not differ between the three vaccination cohorts.
Bar plots indicate - for the six time points and the three vaccination cohorts - the numbers of individuals who had experienced either one, two, three, or four SARS-CoV-2 infections; all participants ($N = 1036$) are included: Chi^2 tests negated statistically significant differences between the vaccination cohorts.

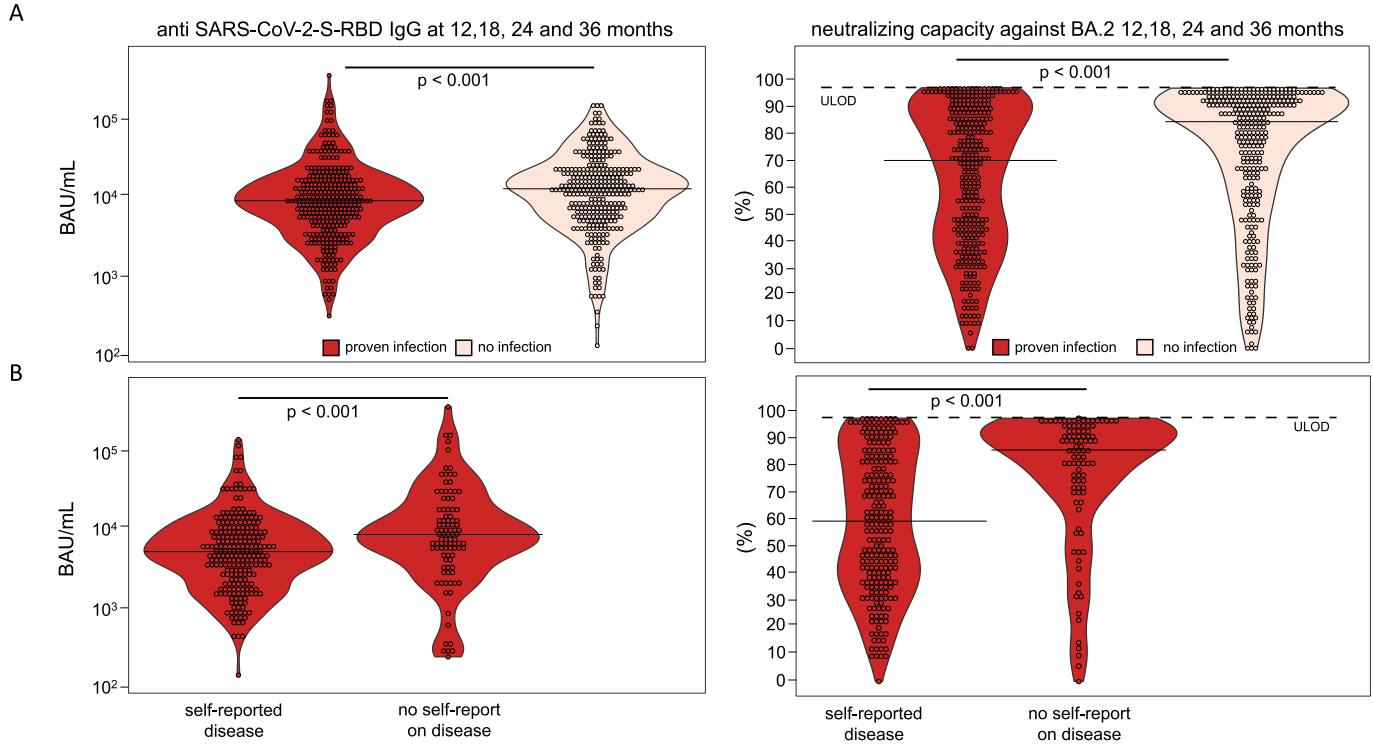

Fig. 3. Higher anti-SARS-CoV-2 S titers and elevated neutralizing capacities reduce break-through infections.
Dot plots overlaid with violin plots indicate anti SARS-CoV-2 S titers and neutralizing capacities at a given time point of blood collection and differentiate between those with a proven COVID-19-infection ($N = 334$) during the last six months and those without ($N = 322$) (A); The same results are shown in (B) yet differentiate the reconvalescents into those who were aware of an infection and self-reported it ($N = 237$) vs those who were unaware ($N = 97$); ULOD = upper limit of detection; each dot represents one study participant; plots show median values; *p* values result from Mann-Whitney-*U* test: raw *p*-values are given.

titers with those having experienced an infection presenting a median of 9188 BAU/ml as opposed to 13,027 BAU/ml for those who have not. Likewise, the median neutralizing capacity was 69·8 % for those who experienced an infection as opposed to 84·2 % for those who had not. Importantly, Fig. 3B shows that among those with a proven SARS-CoV-2 infection within the last six months, there were statistically significant differences in anti-SARS-CoV-2 S titers and neutralizing capacities between those who had noticed and reported their SARS-CoV-2 infection (median of 8010 BAU/ml and 59 % neutralizing capacity) – and those who had not (median of 12,635 BAU/ml and 85 % neutralizing capacity). Along these lines, we found negative correlations between anti-SARS-CoV-2 S titers and neutralizing capacities on the one hand and duration and numbers of symptoms on the other (Fig. 4). In summary, our results suggest that the higher the humoral immune response against the SARS-CoV-2 virus, the lower the numbers of infections and the milder the course of COVID-19.

3.5. Infections contribute better to neutralizing capacities than vaccinations

At the end of the observation period, we were curious whether it was infection or immunization that contributed most to high antibody titers and neutralizing capacities. Since all participants had taken at least three immunizations, we compared the titers and neutralizing capacities of those with three immunizations to those with four and more. Fig. 5 shows that for those who had not been infected with SARS-CoV-2 at all, a fourth or fifth immunization is beneficial as titers rose from a median of 427 BAU/ml to 6160. Among the convalescents, anti-SARS-CoV-2 S titers also increased from a median of 9948 BAU/ml to a median of 15,620 in case of one to two infections and from 12,815 to 15,694 BAU/ml in case of three to four infections. These differences did not though reach significance. Likewise, comparing one to two versus three to four infections after having taken either three or four and more immunizations, also showed trends, only. Along these lines, neutralizing capacities increased only mildly after additional immunizations, from medians of 89 % to 93 % in case of one to two infections and from 94 % to 96 % in case of three to four infections. However, against the background of three immunizations, three to four infections resulted in significantly higher neutralizing capacities than one to two with medians of 89 % and 94 %, respectively. Likewise, four immunizations and three to four infections led to medians of 96 % neutralizing capacity compared to only 93 % in the case of one to two infections. In summary both, infection and immunization contribute equally to anti-SARS-CoV-2 S titers. However, as for neutralizing capacities, infections seem to contribute better.

4. Discussion

We here report on a three years follow-up of 192 health care workers who had received different primary vaccination regimen against SARS-CoV-2 – either homologously primed and boosted with AZD1222, homologously primed and boosted with BNT162b2, or primed with AZD1222 and boosted with BNT162b2. Thereafter, all study participants had received at least one additional mRNA vaccine. Our results are in line with previous reports showing that, the various regimen are similarly efficient in that infection rates with SARS-CoV-2 are comparable among the three cohorts [2,4,30]. We also confirmed that the heterologous prime/boost regimen was superior to the homologous one including AZD1222 and that was true for all time points analyzed.

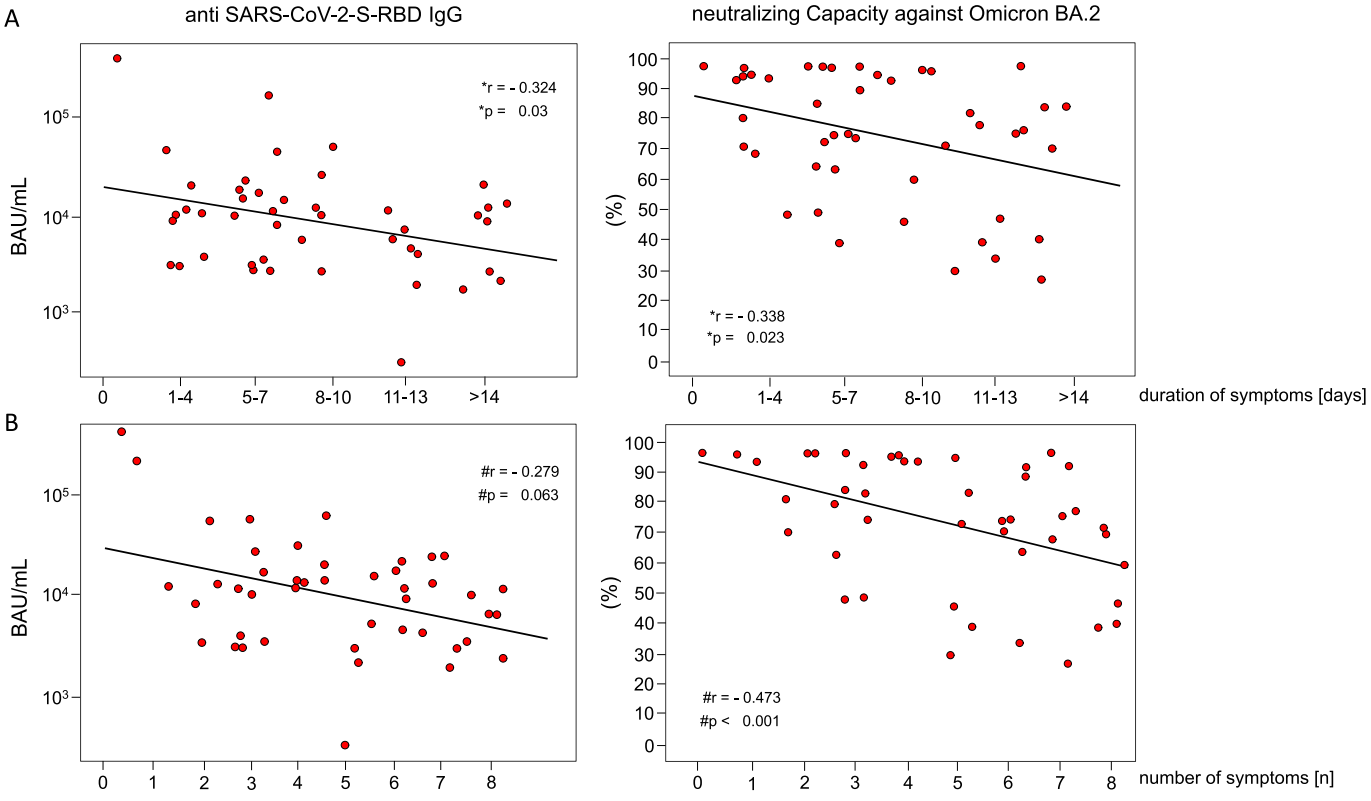

Fig. 4. Higher anti-SARS-CoV-2 S titers and elevated neutralizing capacities negatively correlate with duration and numbers of COVID-19 symptoms.
At the end of the observation period (36 months), participants were asked about previous SARS-CoV-2 infections. (A) Dot plots and regression curves indicate negative correlations between the duration of symptoms resulting from infection and anti-SARS-CoV-2 S titers as well as neutralizing capacities; (B) shows a negative correlation between the numbers of symptoms experienced during infection and neutralizing capacities against Omicron BA.2. For anti-SARS-CoV-2 S titers, there was only a trend of a negative correlation; each dot represents one study participant; All participants whose self-reported disease was confirmed by increase in nucleocapsid antibodies in the last six months were included ($N = 45$): *p values result from Pearson correlation or #Spearman rank correlation: raw p-values are given.

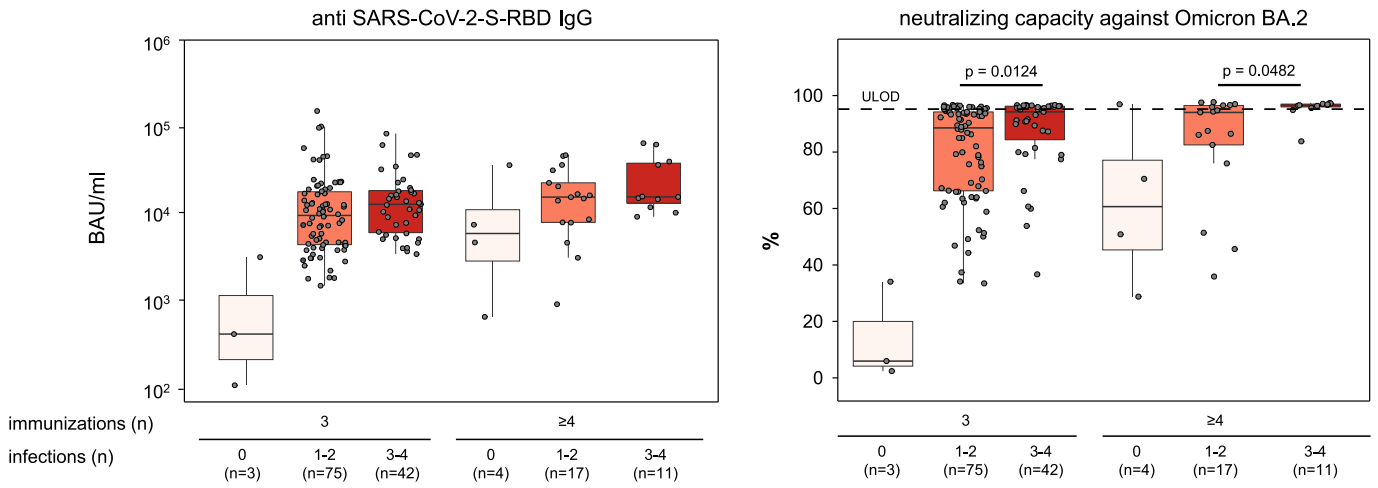

Fig. 5. Infections contribute better to neutralizing capacities than vaccinations.
Dot plots overlaid with box-plots present anti-SARS-CoV-2 S titers and neutralizing capacities in the context of three or four and more immunizations and increasing numbers of proven infections; ULOD = upper limit of detection; each dot represents one study participant; number of included samples are shown: p values result from Mann-Whitney-*U* test: raw p-values are given.

However, differences between the heterologous and the homologous prime/boost regimen including BNT162b2 need to be viewed critically – certainly at 12 months - as different time intervals between boost and blood sampling may have impacted on the results [31,28,32,33]. Indeed, a systematic review confirmed comparable anti-SARS-CoV-2 S antibody titers for the homologous prime/boost regimen with BNT162b2 and the heterologous one [34].

In the long run though, antibody titers against the spike protein and capacities to neutralize SARS-CoV-2 were highest in those having received the homologous prime/boost regimen with BNT162b2, and were lowest in those homologously primed and boosted with AZD1222. Importantly, this effect was independent of age and sex, was continuously observed over three years and could not be compensated for by an additional third, fourth, or fifth vaccination with an mRNA vaccine, nor by infection with the virus. Our results therefore imply long-term constraints on the humoral immune response after primary immunization with the adenoviral vaccine. Due to previous observations on significant differences in the very early immune responses to AZD1222 or BNT162b2, we would like to discuss our recent findings in the context of immune imprinting. We previously showed that compared to BNT162b2, the AZD1222 cohort showed transient leukopenia and a transient increase in pro-inflammatory monocytes on day 2 after priming, a significant bout of plasma blasts on day 6, followed by again a significant increase of late memory B cells on days 13 and 20 [12]. However, on day 20 the BNT162b2 cohort performed significantly better in virus neutralization and had already significantly higher titers of anti-spike IgG and IgA antibodies. In short, even though the SARS-CoV-2 antigens presented to the immune system should be similar if not identical, both vaccines induced disparate immune reactions. Along these lines, it was shown by others that the context of the spike antigen – either as mRNA vaccine or SARS-CoV-2 itself – led to different retention periods in germinal centers, with the vaccine encoded antigen outlasting the virus encoded one [35,36]. We therefore would like to speculate that the mRNA vaccine – either due to specific adjuvant effects and/or prolonged retention of the antigen in germinal centers led to either a larger pool of memory- and long-lived plasma cells or to higher affinity antibodies. Both would lead to higher reads when assessing antibody titers or neutralization capacities. However, the fact that subsequent immunizations with mRNA vaccine in the AZD1222 cohort did not compensate for early deficits is reminiscent of immune imprinting. Immune imprinting was first described as original antigenic sin, summarizing a propensity of the immune system to rely repeatedly on the first cohort of B cells engaged by an antigenic stimulus - in detriment to the induction of de novo responses upon encounter of related antigens [27,37,38]. As the presence of imprinting correlates with the extent of sequence similarity among immunogens and as both, BNT162b2 and AZD1222 encode the wild type RBD of the SARS-CoV-2 spike protein [39,2,4] it does not come as a surprise that boosting the AZD1222 primed cohort with an mRNA vaccine merely led to a re-stimulation of the first cohort of B cells engaged. Along these lines, a decreased response to variant-specific epitopes in vaccinated individuals compared to unvaccinated ones has previously been discussed to result from imprinting [36]. These observations question the benefit of repeated boosting as adequate responses to a newly emerging and aggressive variant of concern may be hampered. Our findings of infections contributing better to increased neutralizing capacity than vaccinations suggests that breakthrough infections, which are generally mild, may offer sufficient protection against current and upcoming VOCs [26]. However, relying on this protection puts vulnerable groups at risk and comes also at the risk of long/post-COVID. To complicate matters, pre-boost antibody titers against the RBD of the original spike protein inversely correlated with post-boost antibody reactivity against VOC, indicating that high antibody titers against the original strain resulted in reduced immunogenicity of the variant protein [40]. Against this background, it is imperative to define target ranges of anti-SARS-CoV-2 S antibody titers that allow for a well-founded recommendation for or against a repeated boost.

As higher antibody titers and better neutralizing capacities in our study correlated with fewer infections and milder courses of COVID-19, we would like to speculate that, a stronger humoral response may also contain long and post-COVID. A detailed investigation into the various vaccine platforms is therefore imperative in order to optimize future vaccines. Strategies to optimize immunity without exacerbating immune imprinting have already been suggested and include among others the utilization of different adjuvants, change of vaccine platforms, alternating intramuscular and intradermal immunizations, removal of conserved domains in the immunogen, increasing antigen dosages and, as indicated above, optimizing vaccination intervals [38,41,40].

There are limitations to our study that need to be pointed out: i) our heterologous prime/ boost regimen included priming with AZD1222 and boosting with BNT162b2 only. We therefore cannot rule out that a vice versa set-up may ameliorate immune imprinting. ii) the age group analyzed here included the working population only, and therefore does not allow for any extrapolation towards the elderly or children. iii) we here concentrated on the humoral immune response, only. As there is evidence that different vaccines also induce different cellular immune

responses [42–47,31], T cell memory will have to be assessed before final recommendations for or against a repeated boost are passed.

In summary, we are the first to provide a three year follow up on anti-SARS-CoV-2 S titers and neutralizing capacities following three different vaccination regimens. We show that a homologous prime/boost regimen with BNT162b2 is superior to AZD1222 and speculate that imprinting prevents mRNA boosts from clearing out any differences. Future research therefore needs to improve strategies to optimize immunity without exacerbating immune imprinting.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2025.110523.

Data sharing statement

Further information and requests for resources should be directed to the corresponding author: brigitte.mueller-hilke@med.uni-rostock.de. The data is available upon request.

CRediT authorship contribution statement

Franz Mai: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Emil C. Reisinger:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Brigitte Müller-Hilke:** Writing – review & editing, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

No AI-assisted technologies were used during the preparation of this work.

Funding

This study was financially supported by the Federal State of Mecklenburg-Western Pomerania, the Ministry of Science, Culture, Federal and European Affairs via the "Sondervermögen des MV Schutzfonds, Säule Gesundheit" GW-20-0004. This work was also funded on the basis of a resolution of the German Bundestag by the German government (project „COVICare - M-V"funding code ZMII2-2524FSB031).

The source of funding had no influence on the study. Neither in study design, collection, analysis, interpretation of the data, writing of the report, decision to submit for publication, or other.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We would like to thank the staff of the Coordination Center for Clinical Studies (KKS) of the University Medical Center Rostock for organizing blood collection appointments. Finally, we are grateful to all study participants who have supported us over the years and to Johann Aleith and Marcel Kordt for fruitful discussions.

Data availability

Data will be made available on request.

References

[1] M. Jeyanathan, S. Afkhami, F. Smaill, M.S. Miller, B.D. Lichty, Z. Xing, Immunological considerations for COVID-19 vaccine strategies, Nat. Rev. Immunol. 20 (10) (2020) 615–632.
[2] F.P. Polack, S.J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, et al., Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine, N. Engl. J. Med. 383 (27) (2020) 2603–2615.
[3] L.R. Baden, H.M. El Sahly, B. Essink, K. Kotloff, S. Frey, R. Novak, et al., Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine, N. Engl. J. Med. 384 (5) (2021) 403–416.
[4] M. Voysey, S.A.C. Clemens, S.A. Madhi, L.Y. Weckx, P.M. Folegatti, P.K. Aley, et al., Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK, Lancet 397 (10,269) (2021) 99–111.
[5] W. Külper-Schiek, V. Piechotta, A. Pilic, M. Batke, L.-S. Dreveton, B. Geurts, et al., Facing the omicron variant-how well do vaccines protect against mild and severe COVID-19? Third interim analysis of a living systematic review, Front. Immunol. 13 (2022) 940562.
[6] Y. Xie, T. Choi, Z. Al-Aly, Postacute sequelae of SARS-CoV-2 infection in the Pre-Delta, Delta, and omicron eras, N. Engl. J. Med. 391 (6) (2024) 515–525.
[7] Z. Al-Aly, H. Davis, L. McCorkell, L. Soares, S. Wulf-Hanson, A. Iwasaki, et al., Long COVID science, research and policy, Nat. Med. 30 (8) (2024) 2148–2164.
[8] C. Ledda, C. Costantino, G. Motta, R. Cunsolo, P. Stracquadanio, G. Liberti, et al., SARS-CoV-2 mRNA vaccine breakthrough infections in fully vaccinated healthcare personnel: a systematic review, Trop. Med. Infect. Dis. 7 (1) (2022).
[9] J. Shastri, S. Parikh, V. Aggarwal, S. Agrawal, N. Chatterjee, R. Shah, et al., Severe SARS-CoV-2 breakthrough reinfection with Delta variant after recovery from breakthrough infection by alpha variant in a fully vaccinated health worker, Front. Med. 8 (2021) 737007.
[10] E. Hacisuleyman, C. Hale, Y. Saito, N.E. Blachere, M. Bergh, E.G. Conlon, et al., Vaccine breakthrough infections with SARS-CoV-2 variants, N. Engl. J. Med. 384 (23) (2021) 2212–2218.
[11] J.Y. Kim, S. Bae, S. Park, J.-S. Kwon, S.Y. Lim, J.Y. Park, et al., Comparison of antibody and T cell responses induced by single doses of ChAdOx1 nCoV-19 and BNT162b2 vaccines, Immune Netw. 21 (4) (2021) e29.
[12] M. Müller, J. Volzke, B. Subin, S. Müller, M. Sombetzki, E.C. Reisinger, et al., Single-dose SARS-CoV-2 vaccinations with either BNT162b2 or AZD1222 induce disparate Th1 responses and IgA production, BMC Med. 20 (1) (2022) 29.
[13] F. Mai, J. Volzke, E.C. Reisinger, B. Müller-Hilke, Vaccine-induced T-cell and antibody responses at 12 months after full vaccination differ with respect to omicron recognition, Vaccines (Basel) 10 (9) (2022).
[14] F. Mai, M. Kordt, W. Bergmann-Ewert, E.C. Reisinger, B. Müller-Hilke, NVX-CoV2373 induces humoral and cellular immune responses that are functionally comparable to vector and mRNA-based vaccines, Front. Immunol. 15 (2024) 1359475.
[15] F. Mai, W. Bergmann, E.C. Reisinger, B. Müller-Hilke, The varying extent of humoral and cellular immune responses to either vector- or RNA-based SARS-CoV-2 vaccines persists for at least 18 months and is independent of infection, J. Virol. 98 (4) (2024) e0191223.
[16] L. Fernández-Ciriza, Á. González, J.L. Del Pozo, A. Fernández-Montero, F. Carmona-Torre, S. Carlos, et al., Humoral and cellular immune response over 9 months of mRNA-1273, BNT162b2 and ChAdOx1 vaccination in a University Hospital in Spain, Sci. Rep. 12 (1) (2022) 15606.
[17] G.M.N. Behrens, J. Barros-Martins, A. Cossmann, G.M. Ramos, M.V. Stankov, I. Odak, et al., BNT162b2-boosted immune responses six months after heterologous or homologous ChAdOx1nCoV-19/BNT162b2 vaccination against COVID-19, Nat. Commun. 13 (1) (2022) 4872.
[18] N. Heinen, C.S. Marheinecke, C. Bessen, A. Blazquez-Navarro, T. Roch, U. Stervbo, et al., In-depth analysis of T cell immunity and antibody responses in heterologous prime-boost-boost vaccine regimens against SARS-CoV-2 and omicron variant, Front. Immunol. 13 (2022) 1062210.
[19] L. Ruhl, J.F. Kühne, K. Beushausen, J. Keil, S. Christoph, J. Sauer, et al., Third SARS-CoV-2 vaccination and breakthrough infections enhance humoral and cellular immunity against variants of concern, Front. Immunol. 14 (2023) 1120010.
[20] D.S. Khoury, D. Cromer, A. Reynaldi, T.E. Schlub, A.K. Wheatley, J.A. Juno, et al., Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection, Nat. Med. 27 (7) (2021) 1205–1211.
[21] S. Feng, D.J. Phillips, T. White, H. Sayal, P.K. Aley, S. Bibi, et al., Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection, Nat. Med. 27 (11) (2021) 2032–2040.
[22] P.B. Gilbert, D.C. Montefiori, A.B. McDermott, Y. Fong, D. Benkeser, W. Deng, et al., Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial, Science 375 (6576) (2022) 43–50.
[23] J. Pušnik, J. Zorn, W.O. Monzon-Posadas, K. Peters, E. Osypchuk, S. Blaschke, et al., Vaccination impairs de novo immune response to omicron breakthrough infection, a precondition for the original antigenic sin, Nat. Commun. 15 (1) (2024) 3102.
[24] T.S. Johnston, S.H. Li, M.M. Painter, R.K. Atkinson, N.R. Douek, D.B. Reeg, et al., Immunological imprinting shapes the specificity of human antibody responses against SARS-CoV-2 variants, Immunity 57 (4) (2024) 912–925.e4.
[25] B. Ju, Q. Fan, M. Wang, X. Liao, H. Guo, H. Wang, et al., Antigenic sin of wild-type SARS-CoV-2 vaccine shapes poor cross-neutralization of BA.4/5/2.75 subvariants in BA.2 breakthrough infections, Nat. Commun. 13 (1) (2022) 7120.

[26] M. Aguilar-Bretones, R. Fouchier, M.P. Koopmans, G.P. van Nierop, Impact of antigenic evolution and original antigenic sin on SARS-CoV-2 immunity, J. Clin. Invest. 133 (1) (2023).
[27] T. Francis, On the Doctrine of Original Antigenic Sin, Available from: URL, https://www.jstor.org/stable/985534, 1960.
[28] B. Müller-Hilke, F. Mai, M. Müller, J. Volzke, E.C. Reisinger, Higher SARS-CoV-2 spike binding antibody levels and neutralization capacity 6 months after heterologous vaccination with AZD1222 and BNT162b2, Vaccines (Basel) 10 (2) (2022).
[29] P.A. Kristiansen, M. Page, V. Bernasconi, G. Mattiuzzo, P. Dull, K. Makar, et al., WHO international standard for anti-SARS-CoV-2 immunoglobulin, Lancet 397 (10,282) (2021) 1347–1348.
[30] Y. Liu, Q. Ye, Safety and efficacy of the common vaccines against COVID-19, Vaccines (Basel) 10 (4) (2022).
[31] Y.J. Baek, W.-J. Kim, J.-H. Ko, Y.-J. Lee, J.Y. Ahn, J.H. Kim, et al., A heterologous AZD1222 priming and BNT162b2 boosting regimen more efficiently elicits neutralizing antibodies, but not memory T cells, than the homologous BNT162b2 regimen, Vaccine 41 (10) (2023) 1694–1702.
[32] C. Orlandi, G. Stefanetti, S. Barocci, G. Buffi, A. Diotallevi, E. Rocchi, et al., Comparing heterologous and homologous COVID-19 vaccination: a longitudinal study of antibody decay, Viruses 15 (5) (2023).
[33] D.-I. Kim, S.J. Lee, S. Park, P. Kim, S.M. Lee, N. Lee, et al., Immunogenicity and durability of antibody responses to homologous and heterologous vaccinations with BNT162b2 and ChAdOx1 vaccines for COVID-19, Vaccines (Basel) 10 (11) (2022).
[34] J. Lv, H. Wu, J. Xu, J. Liu, Immunogenicity and safety of heterologous versus homologous prime-boost schedules with an adenoviral vectored and mRNA COVID-19 vaccine: a systematic review, Infect. Dis. Pover. 11 (1) (2022) 53.
[35] J.S. Turner, J.A. O'Halloran, E. Kalaidina, W. Kim, A.J. Schmitz, J.Q. Zhou, et al., SARS-CoV-2 mRNA vaccines induce persistent human germinal centre responses, Nature 596 (7870) (2021) 109–113.
[36] K. Röltgen, S.C.A. Nielsen, O. Silva, S.F. Younes, M. Zaslavsky, C. Costales, et al., Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination, Cell 185 (6) (2022) 1025–1040.e14.
[37] A. Schiepers, van't Wout MFL, A.J. Greaney, T. Zang, H. Muramatsu, Lin PJC, et al., Molecular fate-mapping of serum antibody responses to repeat immunization, Nature 615 (7952) (2023) 482–489.
[38] X. Ding, F. Zhao, Z. Liu, J. Yao, H. Yu, X. Zhang, Original antigenic sin: a potential double-edged effect for vaccine improvement, Biomed. Pharmacother. 178 (2024) 117187.
[39] S. Fish, E. Zenowich, M. Fleming, T. Manser, Molecular analysis of original antigenic sin. I. Clonal selection, somatic mutation, and isotype switching during a memory B cell response, J. Exp. Med. 170 (4) (1989) 1191–1209.
[40] R.R. Goel, M.M. Painter, K.A. Lundgreen, S.A. Apostolidis, A.E. Baxter, J.R. Giles, et al., Efficient recall of omicron-reactive B cell memory after a third dose of SARS-CoV-2 mRNA vaccine, Cell 185 (11) (2022) 1875–1887.e8.
[41] J.W. Yewdell, J.J.S. Santos, Original antigenic sin: how original? How sinful? Cold Spring Harb. Perspect. Med. 11 (5) (2021).
[42] D. Hillus, T. Schwarz, P. Tober-Lau, K. Vanshylla, H. Hastor, C. Thibeault, 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. 9 (11) (2021) 1255–1265.
[43] M. Nam, S.G. Yun, S.-W. Kim, C.G. Kim, J.H. Cha, C. Lee, et al., Humoral and cellular immune responses to vector, mix-and-match, or mRNA vaccines against SARS-CoV-2 and the relationship between the two immune responses, Microbiol. Spectr. 10 (4) (2022) e0249521.
[44] J. Barros-Martins, S.I. Hammerschmidt, A. Cossmann, I. Odak, M.V. Stankov, G. Morillas Ramos, et al., Immune responses against SARS-CoV-2 variants after heterologous and homologous ChAdOx1 nCoV-19/BNT162b2 vaccination, Nat. Med. 27 (9) (2021) 1525–1529.
[45] M.M. Hollstein, L. Münsterkötter, M.P. Schön, A. Bergmann, T.M. Husar, A. Abratis, et al., Interdependencies of cellular and humoral immune responses in heterologous and homologous SARS-CoV-2 vaccination, Allergy 77 (8) (2022) 2381–2392.
[46] S. Assawakosri, S. Kanokudom, J. Chansaenroj, N. Suntronwong, C. Auphimai, P. Nilyanimit, et al., Persistence of immunity against omicron BA.1 and BA.2 variants following homologous and heterologous COVID-19 booster vaccines in healthy adults after a two-dose AZD1222 vaccination, Int. J. Infect. Dis. 122 (2022) 793–801.
[47] J.Y.L. Fu, M.H. Pukhari, M.K. Bador, I.-C. Sam, Y.F. Chan, Humoral and T cell immune responses against SARS-CoV-2 after primary and homologous or heterologous booster vaccinations and breakthrough infection: a longitudinal cohort study in Malaysia, Viruses 15 (4) (2023), https://doi.org/10.1101/cshperspect.a038786.