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Full seroconversion in initial non-responders with higher antibody levels after heterologous COVID-19 vaccination schedule.

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ABSTRACT

Antibody testing after COVID-19 vaccination is generally not recommended. Here, we present the results of a retrospective study, in which we analyzed antibody levels before and after the first dose of the ChAdOx1 vector vaccine. We identified 5% non-responders (43.6 ± 10.6 years; females: 41%) and 3.4% low-responders (44.2 ± 10.1 years; females: 64%) after the first dose. Of these, 61 individuals received a timely second dose either with a homologous (ChAdOx1/ChAdOx1) or heterologous (ChAdOx1/mRNA-1273) schedule. All vaccinees achieved positive S1-specific IgG titers to the ancestral SARS-CoV-2 strain after the second dose, but antibody levels as well as neutralization titers against the ancestral SARS-CoV-2 strain were higher after the heterologous schedule. However, Omicron-specific neutralizing antibodies were not detectable after two doses in either group, indicating that a third vaccine dose is needed to enhance cross-reactive antibodies against currently circulating and emerging variants of concern.

1. Introduction

In 2021, COVID-19 vaccination programs have been widely established in order to combat the pandemic. Six different vaccines against COVID-19 are currently licensed in Europe, among those two mRNA-based vaccines and two viral vector-based vaccines which have all shown high efficacy in preventing symptomatic COVID-19 disease [1–3]. Since licensure studies indicated that all vaccines induced robust humoral and cellular immune responses in healthy individuals [4–6], SARS-CoV-2 spike-protein reactive antibody testing is not recommended in the healthy population. This recommendation was subjected to review by performing a retrospective study and analyzing the humoral immune response in a large population (n = 1255) of presumably healthy individuals undergoing voluntary COVID-19 vaccination. Therefore, we evaluated circulating antibody levels before the first and three to four weeks after the first as well as after the second dose.

2. Material and methods

2.1. Analysis of the humoral immune response

Anti-SARS-CoV-2 IgG antibodies directed against the subunit 1 of the spike protein (S1) were measured using Quantivac® (Euroimmune, Germany) before and three weeks after the first, as well as four weeks and six months after the second vaccine dose. Results are reported in relation to the WHO standard (NIBSC code 20/136) as binding antibody units (BAU)/ml. Results below 25.6 BAU/ml were considered as negative, between 25.7 and 35.2 BAU/ml as borderline and results above 35.2 BAU/ml as positive according to the manufacturer's instructions. The 50% neutralizing titers (NT50) against the ancestral SARS-CoV-2 D614G strain (isolate BetaCoV/Munich/BavPat1/2020 kindly provided by Prof. Christian Drosten, Charité, Berlin, Germany) were available from a subset of vaccinees at four weeks and six months after the second dose (n = 17). Additionally, neutralization test results with

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the Omicron BA.1 variant [7] (kindly provided by Prof. Karin Stiasny, Medical University of Vienna, Center for Virology, Vienna, Austria) were available from sera collected at four weeks and six month after the second dose were tested ($n = 14$ for each time point). Both neutralization assays were performed according to the protocol of Amanat et al. [8]. 600 TCID50 (half-maximal tissue culture infectious dose) of the virus, mixed (or not) with the serially diluted sera were used to infect Vero cells, and the infection rate was detected 48 h (the ancestral strain) or 72 h (Omicron) later by In-Cell ELISA, as previously described [9]. The sample was evaluated as positive if maximal neutralization capacity reached 50%, and in that case, also NT50 titers were calculated from the neutralization curves, as detailed in [9].

2.2. Study population and vaccination schedule

The data provided here are derived from a retrospective analysis of samples acquired during the routine vaccination program of 1255 employees of the Medical University of Vienna who received the vector vaccine ChAdOx1 (Vaxzevria, AstraZeneca) as a first dose. In total, antibody results of 914 employees were available from before the first dose and 1204 from three weeks after the first dose. Individuals with low or no antibody response (S1-specific IgG below 100 BAU/ml) after the first dose were offered to take the second dose already after 6–8 weeks with either ChAdOx1 or the full dose of mRNA-1273 (Spikevax, Moderna) on a case-by-case discussion. The threshold of 100 BAU/ml was chosen as most of the vaccinees (68.3%) had antibody levels well above this arbitrary threshold and at that time no cut-off level defined as protective existed.

Sixty-one vaccinees agreed to an earlier second dose at week 6–8; 35 individuals were revaccinated with ChAdOx1, while 26 with a heterologous schedule using mRNA-1273. Antibody quantification results were available from all three time points (prior to the first dose, 21 days post first and 28 days after second dose) of 52/61 individuals, 28 in the homologous and 24 in the heterologous group. Antibody response after the second dose were compared between those receiving a homologous second dose (ChAdOx1, $n = 35/1204$) or a heterologous second dose (mRNA-1273, $n = 26/1204$) at 6–8 weeks and those receiving a second homologous dose within 11–12 weeks (Fig. 1).

Additionally, we evaluated results from 19 individuals that had positive antibody results after the first dose with ChAdOx1 and received their second dose at 11–12 weeks as recommended at that time. All the included individuals were seronegative at baseline.

Neutralization test results against the ancestral D614G strain were available from a randomly selected set of 17 serum samples tested at four weeks after the second dose and neutralization test results against the Omicron BA.1 variant were available from 14 samples tested at four weeks and 6 months after the second dose.

2.3. Statistics

Geometric mean concentration (GMC) and fold-increase of anti-SARS-CoV-2-S antibodies were calculated and Kruskal-Wallis and Dunn's multiple comparisons test were used. Statistical analysis was performed with GraphPad Prim Version 7.

2.4. Ethics

The retrospective data analysis was approved by the Ethics Committee of the Medical University of Vienna (EK Nr1701/2021 and EK 2260/2021).

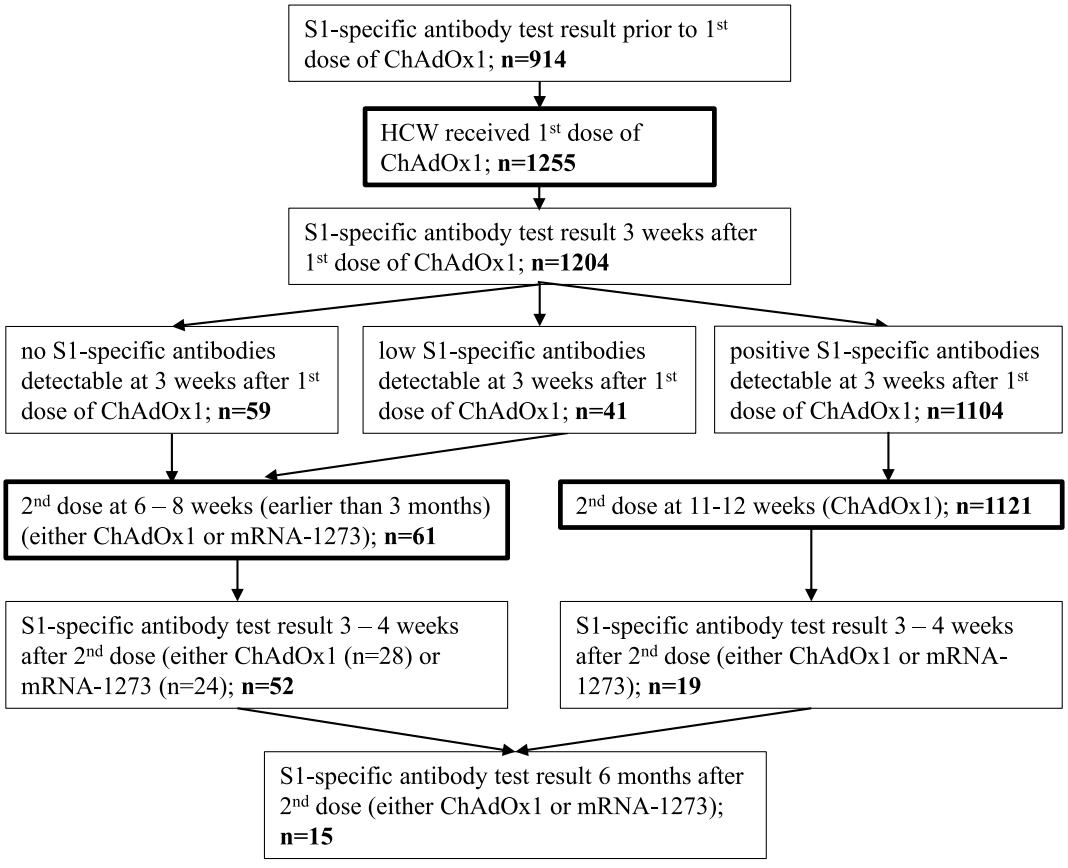

Fig. 1. Flowchart of participants included in the analysis.

3. Results

3.1. Analysis of antibody levels after the first dose of ChAdOx1

Anti-spike SARS-CoV-2 S1-specific IgG measurements prior to vaccination were available from 914/1255 (72.8%) vaccinees, revealing presence of anti-SARS-CoV-2 S1-specific IgG in 33/914 individuals (3.6%) indicative of prior infection. Antibody testing at week 3 to 4 after the first ChAdOx1 dose was performed in 1204 (95.6%) individuals and the GMC was 153 BAU/ml. In 1104/1204 (91.7%) individuals (mean age 39.6 ± 12.1; 61% females) anti-SARS-CoV-2 S1-specific IgG levels were positive. Only 41/1204 (3.4%) individuals (mean age 44.2 ± 10.1; 64% females) had levels in the borderline range and 59/1204 (4.9% with mean age 43.6 ± 10.6 years; 41.0% females) were negative.

3.2. Booster effect after a second dose of homologous or heterologous schedule

In the group with the homologous 6–8 week vaccination, mean age was 42.4 ± 10.7 years and 54% were female, in the heterologous 6–8 week group mean age was 46.5 ± 9.7 years and 37% were female, while in the homologous 11–12 week group mean age was 41.9 ± 9.9 years and 84% were female (Table 1). In the homologous 6–8 week group the GMC before the second vaccination was 44.3 BAU/ml (1/28 non-responder, 10/28 low-responder, 17/28 < 100 BAU/ml) and 16.2 BAU/ml (20/24 non-responders, 3/24 low-responders, 1/24 < 100 BAU/ml) in the heterologous 6–8 week group ($p<0.0001$) (Fig. 2A). In contrast, in the homologous 11–12 week group GMC before the second dose was 215.8 BAU/ml (0/19 non-responders, 1/19 <100 BAU/ml, 18/19 >100 BAU/ml). Four weeks after receiving the second dose at 6–8 weeks, a strong antibody response was observed in all 52/52 (100%) individuals, since an efficient antibody induction was achieved in all subjects upon the full vaccination protocol, even in those with low- or no initial response. Positive results with a 5.3 ± 6.3-fold increase ($p<0.0001$) of S1-specific IgG were measured following homologous (ChAdOx1/ChAdOx1) 6–8 week vaccination, and even higher, a 65.6 ± 88.6-fold increase after the heterologous (ChAdOx1/mRNA-1273) 6–8 week vaccination (Fig. 2A). However, GMCs were clearly lower (237.8 BAU/ml) following the homologous 6–8 week schedule compared to the heterologous 6–8 week schedule (768 ± 1 BAU/ml; $p<0.0001$) (Fig. 2A). In the homologous 11–12 week group, the fold increase was 1.9 ± 2.1 and GMCs reached 292.4 ± 2.2 BAU/ml, and were comparable to those in the homologous group with the shortened vaccination interval (6–8 week).

To see whether the higher GMCs measured in the heterologous schedule group translated to a higher neutralization capacity, and presumably, a better protection, the results from the neutralization test against the ancestral strain D614G and the Omicron BA.1 variant were analyzed. Indeed, all available results of individuals receiving the heterologous booster ($n = 5$) were able to completely (100%) neutralize the ancestral D614G virus, with the mean VN50 1:540. In contrast, only 6/12 sera of individuals belonging to the homologous 6–8 week group achieved 100% neutralization, and the maximal neutralization capacity of these six sera ranged between 53 and 94% translating to a lower mean VN50 1:58 ($p = 0.0039$). With regard to the ability to neutralize Omicron BA.1, neither sera of the homologous ($n = 7$) nor the heterologous ($n = 7$) group showed relevant neutralization since none achieved the required 50% maximal neutralization even at the lowest serum dilution of 1:10 (Fig. 2B and 2C).

To determine persistence or waning of the humoral response, the SARS-CoV-2-specific IgG antibodies were evaluated six months after receiving the second dose. Our data show that vaccinees with the homologous schedule with a 6–8 week interval (GMC 33.9 BAU/ml; $p = 0.0017$; $n = 7$) had lower antibody levels than those who received the heterologous schedule (GMC 198.9 BAU/ml; $n = 15$) (Fig. 2A).

Finally, also samples taken at six months after the second dose exhibited no relevant neutralization capacity against the Omicron BA.1 variant as the maximal neutralization capacity of all but one serum was well below 50%, and therefore NT50 either could not be determined or was ≤1:10 (Fig. 2B and 2C)). The only neutralizing sample of the heterologous group reached 70% maximal neutralization capacity against Omicron (corresponding to an NT50 1:18) (Fig. 2B and 2C)). The same sample had the highest IgG levels (1003 BAU/ml) against the ancestral strain compared to all other samples at six months after the second dose. In contrast, a positive control serum of a fully vaccinated individual recovered from the breakthrough infection was also included, and it was able to fully neutralize the virus with NT50 1:2601.

Table 1
Demographic data.

	Homologous 0/6–8 wk	Heterologous 0/6–8 wk	Homologous 0/11–12 wk
n=	28	24	19
Mean age (± SD); (age range)	42.4 (± 10.7) years; (25–58)	46.5 (± 9.7) years; (29–59)	41.9 (± 9.9) years; (25–60)
Sex: *n*= (%)	Female: 15 (54)	Female: 9 (37)	Female: 16 (84)

4. Discussion

It is widely accepted that SARS-CoV-2 vaccination is the keystone to ending the current pandemic. Sustained and durable protection against severe COVID-19 in the general population via induction of specific immune responses seems to be a prerequisite.

When the first immunization programs started, there was still a shortage of vaccines and the concomitant need to quickly provide protection to the population eligible for vaccination. Thus, the initial recommended interval between the two doses of the vector vaccine ChAdOx1 was extended from four to up to 12 weeks in several countries. We show a high vaccine seroconversion rate among more than 1000 presumably healthy individuals already after the first dose of the vector vaccine ChAdOx1 with 92% of the individuals positive for SARS-CoV-2 S1-specific IgG three weeks after administration. This is in line with slightly lower seroconversion rate at 82.1% after the first dose of ChAdOx1 in the general UK population, likely attributed to a higher proportion of elderly and individuals with underlying diseases [10]. In their study, the IgG levels induced by ChAdOx1 remained stable within the prolonged dosing interval. Thus, the data provided so far justifies the assumption that specific IgG antibodies induced by the first dose of ChAdOx1 are maintained until completing the second dose and protection against severe symptomatic COVID-19 disease could be assumed in the vast majority of the vaccines in our study already after the first dose. This hypothesis is now strengthened by data derived from the vaccination campaign of the UK showing a 60% reduction to become PCR positive and a 50% protection from hospitalization already after the first dose of a SARS-CoV-2 vaccine in an elderly population [11,12]. More recently, this finding could be confirmed also in the light of Omicron with a vaccine effectiveness of 52% against hospitalization already after the first dose [13].

We chose an arbitrary cut off at 100 BAU/ml to define individuals in our study who were considered as no- or low-responders after the first dose and for whom protection was therefore not assumed. This value was chosen since the majority of vaccinees had antibody levels well above this cut-off. These individuals had a discussion for a second dose applied earlier with either homologous ChAdOx1 or heterologous mRNA-1273 vaccination. We are aware of the off-label use upon combination of different vaccines, which is generally not recommended within a course of primary vaccination. However, these principles were challenged by vaccine supply shortage and due to safety concerns with specific vaccinees such as reports of vaccine-induced thrombotic thrombocytopenia after application of ChAdOx1 [14].

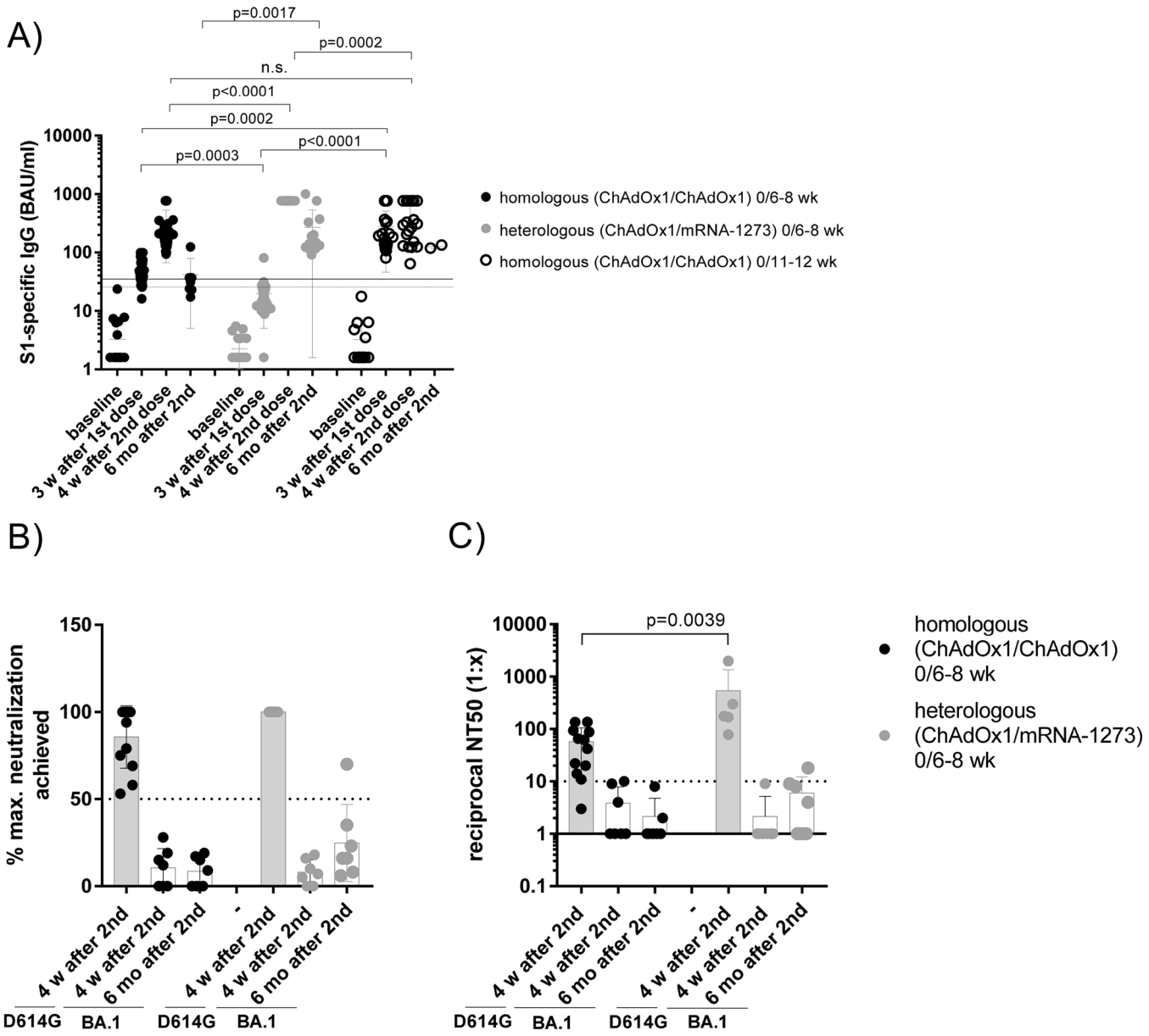

Fig. 2. SARS-CoV-2-specific IgG levels and neutralization capacity. (A) SARS-CoV-2-specific IgG levels at baseline, after the first and after the second dose according to the vaccination schedule. Results represent data from 71 individuals that had antibody results from all three time points (homologous 6–8 wk (n = 28) versus heterologous 6–8 wk (n = 24) and homologous 11–12 wk (n = 19)). Black line represents 35.2 BAU/ml cut-off level for positive values; dotted line represents 25.6 BAU/ml cut-off level for negative results, in between are borderline values. (B) Maximal neutralization capacity achieved and (C) neutralization titers (NT50) against the ancestral SARS-CoV-2 D614G strain (gray bars) determined four weeks after the second dose according to the vaccination schedule and against the Omicron BA.1 variant (white bars) at four weeks and six months after the second dose. Reciprocal titers are expressed as 1:x. Black circles represent results after homologous and gray circles after heterologous vaccination. Dotted line represents cut-offs for 50% maximal neutralization achieved (B) and NT50 1:10 (C).

Our data show that even in individuals with reduced immune response to a single dose of ChAdOx1, seroconversion based on S1 IgG levels was achieved after the second dose, independent of the vaccination strategy (early homologous or heterologous second dose). Thus, these data further show that antibody testing is generally not needed to confirm seroconversion and vaccine responsiveness after primary COVID-19 vaccination in healthy individuals. However, in individuals under immunosuppressive therapies antibody testing is useful to identify vaccine non-responders [15,16] and guide further prophylactic treatment options such as application of monoclonal antibodies.

Interestingly, antibody levels were not significantly higher when the second homologous dose was applied at 11–12 weeks compared to a shortened interval at 6–8 weeks, which is in contrast to a previous publication [17] and, therefore, indicates that an extended interval does not seem to be beneficial when vaccine supply is sufficient. In contrast, antibody levels four weeks after the completed two-dose heterologous schedule were higher compared to a homologous schedule with a 6–8 week interval. Thus, antibody levels after the first dose do not predict what antibody levels can be reached after the second dose, since even individuals with negative antibody results after the first dose will most likely seroconvert after the second dose with higher antibody levels after a heterologous schedule. Nevertheless, this may be different for the homologous mRNA vaccination schedule as it has been described that antibody levels after the first dose strongly correlated with the titer after the second dose in a homologous schedule with BNT162b2 [18].

With regard to persistence of antibody responses, vaccinees of the heterologous group maintained the higher antibody levels up to six months. The question, whether higher antibody levels after the

heterologous vaccination translates into better protection has been addressed by a recent study indeed describing a higher vaccine efficacy up to six months after a heterologous second dose with either BNT162b2 or mRNA-1273 after ChAdOx1 than homologous ChAdOx1, shown by a lower rate of breakthrough infections [19]. However, no cut-off levels or correlates of protection have been defined yet [20,21]. Although the WHO released an International Standard for anti-SARS-CoV-2 immunoglobulins to allow comparison of assays by using BAUs [22,23], there are still variations between the assays of the different providers. Thus, the clinical relevance of differences in antibody concentration induced by distinct vaccines or vaccination strategies is difficult to interpret. In particular, for Omicron even higher antibody levels seem to be required in order to keep neutralizing capacity [24,25]. In this respect, maintaining high antibody levels seems beneficial for protection. Therefore, the heterologous schedule appears favorable since neutralizing titers were higher after heterologous ChAdOx1/BNT162b compared to homologous vaccination with ChAdOx1 [26]. Nevertheless, our data show high neutralization against the ancestral D614G strain after the second dose with sera from the heterologous group reaching a higher NT50 compared to the homologous 6–8 week group, whereas no relevant neutralizing capacity against Omicron was found. In this regard, recent data clearly revealed that a third dose is needed to efficiently boost antibody responses and induce cross-neutralization against the new variants, such as Omicron [27]. Furthermore, regardless of the vaccine type (vector or mRNA) CD4 and CD8 T cell responses associated with protection from severe COVID-19 disease seem highly conserved for all variants of concern, including Omicron [28].

We are aware that our study has several limitations, such as the retrospective character, the small sample size, restriction to working-age participants and lack of clinical data to analyze protection in relation to the antibody levels.

5. Conclusion

We demonstrate that in individuals with insufficient antibody levels after the first dose of ChAdOx1, seroconversion can be achieved in all vaccinees upon a second dose – regardless of a homologous or heterologous strategy. This emphasizes that primary vaccination shall be performed without individual antibody testing. Since the heterologous schedule resulted in higher antibody levels, its preferential use should be discussed in the context of new emerging (escape) variants that require higher antibody levels for virus neutralization. Furthermore, our data strongly indicate that a booster dose is necessary to evoke cross-neutralizing antibodies against variants of concern, such as the currently circulating Omicron strain.

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Authors contributions

AW, KGP, UW contributed to conception and design of the study. AW organized the database. HS designed and oversaw the antibody measurements. AOR, HS, LG and GT designed, performed and analyzed neutralization assays. AW, MK and KGP performed the statistical analysis. AW and KGP wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Declaration of Competing Interest

None.

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