Regimen of COVID-19 vaccination influences extent and kinetics of antibody avidity

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Brief summary

The study found a significantly higher avidity of SARS-Cov-2 spike-specific IgG antibodies among subjects vaccinated with regimens that included at least one dose of the adenoviral vector vaccine ChAdOx1-S compared to two doses of the mRNA vaccine BNT162b2.

Abstract

We investigated antibody titers and avidity after heterologous versus homologous coronavirus disease 2019 (COVID-19) vaccination over six months following the second dose. We found a significantly higher avidity in regimens including at least one dose of the adenoviral vector vaccine ChAdOx1-S compared to two doses of the mRNA vaccine BNT162b2.

Key words: heterologous vaccination; SARS-CoV-2; antibody kinetics; avidity

Introduction

Since the onset of the coronavirus disease 2019 (COVID-19) pandemic in December 2019, antibody responses have been shown to play a crucial role in protective immunity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (1,2). Reflecting the quality of antibody responses, avidity is a measure of cumulative binding strength of antibodies to the target antigen (3). We and others previously demonstrated, that regardless of age, the avidity of antibodies increases over time following infection, while titers of binding and neutralizing antibodies wane (4–6). Comparable findings have been published after vaccination (1,7). However, little is known about dynamics of antibody avidity after vaccination with respect to different vaccination regimens. While first avidity data from individuals immunized with mRNA-based vaccine are available, differences to widely-used heterologous vaccination schedules (combination of an adenovirus vaccine with a mRNA vaccine) have not yet been investigated in a large study cohort over time (1,8). In this study, we therefore compared the dynamics of antibody avidity over a six-month period after the second vaccination in a large and partially randomized clinical trial, comprising homologous ChAdOx1-S (AZ, AstraZeneca) and BNT162b2 (BNT, Pfizer/BioNTech) vaccinees, as well as heterologous AZ/BNT vaccinated individuals.

Methods

Study population

For the present avidity study we analyzed plasma samples from the HEVACC (heterologous vaccination) clinical trial (ClincialTrails.gov Identifier: NCT04907331) (7). The local ethics committee (EC) of the Medical University of Innsbruck has approved the HEVACC study with EC number: EC 1191/2021. The HEVACC study is a three arm, partially randomized single-blinded multi-center clinical trial, where individuals received either two homologous AZ doses, two homologous BNT doses or a heterologous AZ/BNT regimen. Participants with a history of prior SARS-CoV-2 infection, indicated by antibodies against the nucleoprotein, or severe immune defects were excluded (7). Individuals who had been immunized with a first AZ dose, were randomized (matched for sex and study center) to receive a second vaccination either with AZ (referred to as AZ/AZ, homologous) or with BNT (referred to as AZ/BNT, heterologous). Additionally, plasma samples from an observational study cohort of two-dose BNT vaccinated individuals (referred to as BNT/BNT) were included. Number and age of the included study participants are shown in Supplementary Table 1.

Plasma Samples

Plasma samples from study participants were collected 30 days (± 3 days), 90 days (± 3 days), and 180 days (± 3 days) after the second vaccination. Plasma samples deviating from collection time points were excluded from the study. Participants who missed a blood collection were re-invited for the next time point of blood collection. Participants with a breakthrough infection (confirmed by PCR or anti-N seroconversion) or who received a booster vaccination were excluded from further study visits. In total, we analyzed 241 and 74 plasma samples from homologous AZ/AZ or BNT/BNT study cohorts, respectively, as well as further 329 plasma samples from the heterologous AZ/BNT group.

Details on numbers of plasma samples collected at each time point are shown in Supplementary Table 2.

Serological Testing

Plasma samples were analyzed for binding antibodies and antibody avidity using an Anti-SARS-CoV-2 enzyme-linked immunosorbent assay (ELISA; Euroimmun Ref. EI 2606-9601, Lübeck, Germany). Quantities of anti-S1 IgG binding antibodies were assessed using the fully automated 4-plate benchtop instrument Immunomat™ (Virion/Serion, Würzburg, Germany) and given as BAU/mL (binding antibody units per mL) with an assay lower limit of quantification of 3.2 BAU/mL. Thus, only samples with titers > 3.2 BAU/mL were included in the avidity analysis, which was performed as previously described (4). Briefly, after centrifugation for 5 min at 8,000 rpm, clarified plasma supernatant was diluted 1:401 in sample buffer and transferred in duplicate to a microtiterplate, precoated with the S1-domain of the SARS-CoV-2 ancestral spike protein. One well remained untreated whereas a duplicate was incubated with urea (5.5 M for 10 minutes). Plates were analyzed using a Tecan Sunrise absorbance plate reader (Tecan Austria GmbH, Groedig, Austria) at 620 nm (reference) and 450 nm (sample). Samples below (extinction < 0.3) or exceeding the linear range (extinction > 3.0) were diluted less (1:101) or more (1:1001), respectively. Antibody avidity was calculated as ratio of the absorbance of a sample in presence and absence of urea and was expressed as RAI (relative avidity index) in percentages.

Statistical analysis

Statistical differences were calculated with a non-parametric One-Way ANOVA followed by Kruskal-Wallis test with Dunn`s multiple comparison test using GraphPad Prism 9.0.1 (GraphPad Software, Inc., La Jolla, CA, USA). In order to account for the effect of major confounders on the level of avidity, we did hierarchical multivariable linear regression analysis adjusting for antibody concentration, age and sex (SPSS, Version 25.0. IBM Corp., Armonk, NY, USA).

Results

In all three study cohorts (Figure 1), in homologous AZ/AZ or BNT/BNT, as well as in heterologous AZ/BNT vaccinated individuals, titers of anti-S specific IgG antibodies declined over a period of six months after second vaccination (Figure 1A). Levels of binding antibodies significantly waned from a median of 193.6 BAU/mL to 59.3 BAU/mL in the AZ/AZ group, from 985.6 to 157.8 BAU/mL in the AZ/BNT group, and from 1430 to 163.7 BAU/mL in the BNT/BNT group, between D30 and D180. However, we found significantly higher titers in the heterologous AZ/BNT and the homologous BNT/BNT group compared to the homologous AZ/AZ group at almost all time points of plasma sampling. As an indicator of binding strength of antibodies and antibody functionality, we additionally investigated the avidity in collected samples (Figure 1B). We observed, that irrespective of sex and age of vaccinees (see Supplementary Figures 1 and 2), avidity significantly increased over time in all study cohorts to a median RAI of 74.48 % (95% CI 72.86-76.6) in the heterologous AZ/BNT, 72.7 % (median; 95% CI 70.40-74.28) in homologous AZ/AZ, and 65.57 % (median; 95% CI 62.35-69.40) in the homologous BNT/BNT group, 180 days after the second vaccination. Notably, when

performing this analysis, we discovered remarkable differences in the dynamics of avidity of the antibodies among the three study cohorts. Study participants pre-vaccinated with one dose of AZ, possessed significantly higher avidity as early as 30 days after their BNT boost vaccination, compared to individuals that received two doses of AZ or BNT (Figure 1B). Moreover, while individuals belonging to the homologous AZ/AZ or the heterologous AZ/BNT cohort appeared to have already reached the plateau of maximal avidity 90 days after their second vaccination (median 69.26%; 95% CI 66.77-71.25 or median 73.15%; 95% CI 70.99-74.68, respectively), this was significantly lower in the homologous BNT/BNT group. Maximum avidity in the homologous BNT/BNT study population was not detected until 6 months after the boost vaccination (median 65.57%; 95% CI 62.35-69.40). The observed association between vaccine regimen and avidity persisted even when we controlled for the effects of antibody concentration, age and sex.

Discussion

In this study, we investigated antibody responses after heterologous AZ/BNT vaccination compared to homologous AZ/AZ or BNT/BNT vaccination regimens over a six-month period after the second vaccine dose. In line with other studies examining immune responses following SARS-CoV-2 infection or vaccination, we found declining levels of IgG antibodies against SARS-CoV-2 and increasing antibody avidities in all examined study cohorts over time (1,4–7,9). In particular, we and others demonstrated that a second dose vaccination with an mRNA-based vaccine induces significantly higher titers of binding antibodies compared to a second dose with an adenoviral vector vaccine, irrespective of whether study participants had received primary immunization with AZ or BNT. However, a higher antibody avidity seems to correlate with the initial inclusion of an adenoviral vector vaccine, here AZ, within the vaccination regimen. After receiving their second vaccination with AZ or BNT, we found a remarkably higher avidity in study cohorts pre-vaccinated with AZ, irrespective of age and sex of study participants. By combining the advantages of early rise in avidity of antibodies after immunization with an adenoviral vector vaccine and higher levels of binding antibodies induced by at least one dose of an mRNA vaccine, a heterologous vaccination regimen therefore elicits a significant advantage to vaccinees over their homologous counterparts. We acknowledge that the effect size of the association between vaccine regimen and avidity was rather moderate as we adjusted for the effect of antibody concentration. The persistence of the statistical significance is, however, worthy of note and needs further elaboration. Both, elevated levels of binding antibodies as well as antibody avidity have recently been shown to correlate with virus neutralization (1,7,10). Noteworthy, antibody avidity after vaccination was associated with even broader recognition of SARS-CoV-2 epitopes and thus with an improved capacity to neutralize emerged SARS-CoV-2 variants of concern (1). Our study has the limitation of a small sample size in the homologous BNT/BNT group and sample size decreased in all study cohorts over time, since some study participants had already received their third vaccination outside the study or had a breakthrough infection and therefore had to be excluded. We recommend that future larger studies – also targeting risks of breakthrough infections across vaccine regimen – elaborate further on this finding. However, the observed statistical significance despite the small sample size delivered a strong insight into a potential impact of vaccination schedules on antibody avidity.

Hence, our study demonstrates the benefit of including at least one dose of an adenoviral vector in initial COVID-19 vaccination schemes to achieve a level of antibodies with significantly higher avidity.

However, further studies investigating the avidity differences after a third shot and other heterologous vaccination schedules would be of value.

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Conflict of interest statement

The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines which list Florian Krammer as co-inventor. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. Florian Krammer has consulted for Merck and Pfizer (before 2020), and is currently consulting for Pfizer, Seqirus, 3rd Rock Ventures and Avimex. The Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2. Reinhard Würzner received regular support from Pfizer to organize the annual Tyrolean Vaccination Day. All other authors declare no conflict of interest.

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Figure 1. Distinct dynamics of antibody responses after heterologous AZ/BNT vaccination compared to homologous AZ/AZ or BNT/BNT vaccination

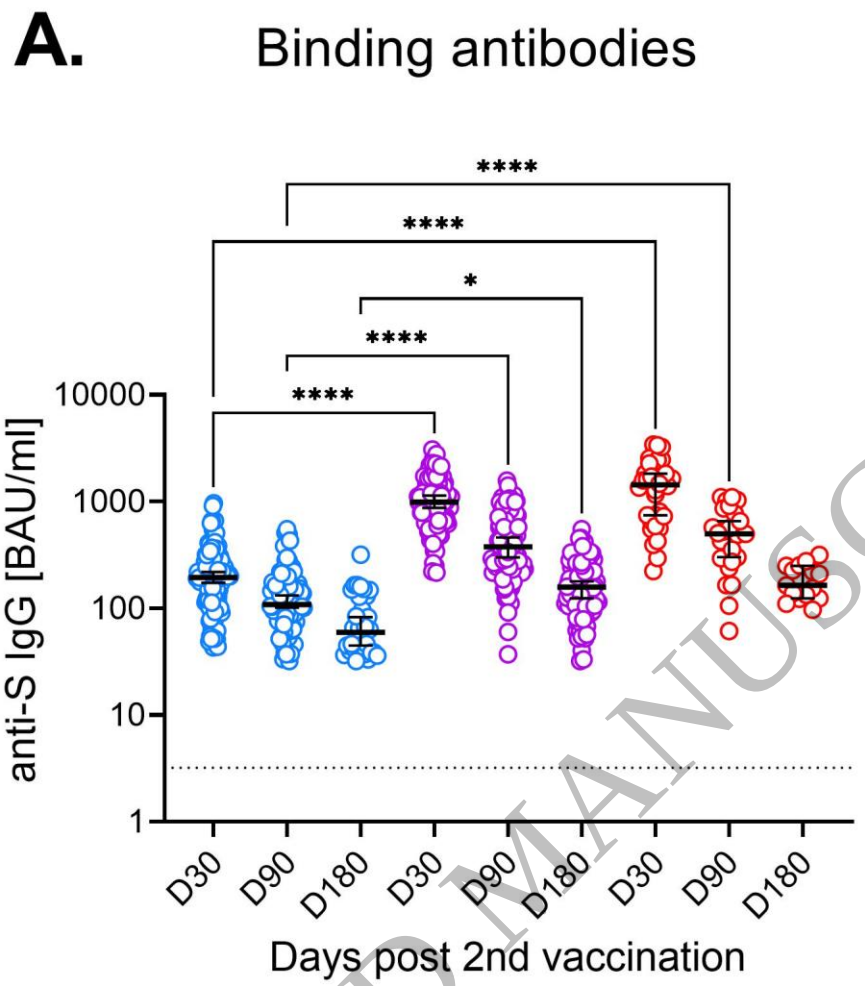
Titers of anti-S1 IgG binding antibodies (Panel A) and relative avidity indices (RAI, Panel B) were analyzed 30 days, 90 days and 180 days post second vaccination. Dotted line in panel A indicates lower limit of quantification (3.2 BAU/mL). Median, 95% confidence interval and individual values are shown. Open circles display individual values of study participants after homologous AZ/AZ (blue) or BNT/BNT (red) vaccination, respectively, or heterologous AZ/BNT (violet) vaccination regimen. A detailed summary of samples sizes is shown in Supplementary table 2. Statistical differences were calculated using non-parametric ANOVA followed by Kruskal-Wallis test and Dunn´s multiple comparisons test. *P*-values indicate statistical significance (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns=non-significant). Significant differences between study cohorts (AZ/AZ; AZ/BNT; BNT/BNT) at indicated time points are displayed by asterisk in the graphs, and significant differences between time points (D30 vs. D90; D90 vs D180; D30 vs D180) within study cohorts are shown in tables below graphs.

Figure S1. Dynamics of binding antibodies and antibody avidity in women and men

Titers of anti-S1 IgG binding antibodies (Panel A; BAUL/ml) and antibody avidity (Panel B; RAI in %) of female (▽) and male (△) study participants of the homologous AZ/AZ (blue symbols), the heterologous AZ/BNT (violet symbols) or the homologous BNT/BNT (red symbols) study cohorts were analysed using blood samples collected 30 days, 90 days and 180 days post second dose vaccination. Shown are Median, 95% confidence interval and individual values of female and male study participants.

Figure S2. Avidity relative to age of study participants

Correlation of relative avidity indices (RAI; %) and age of study participants of homologous AZ/AZ cohort (Panel A; blue symbols), heterologous AZ/BNT cohort (Panel B; violet symbols) and homologous BNT/BNT cohort (Panel C; red symbols) on Day 30, Day 90 and Day 180 post second dose vaccination. Individual results of study participants are shown as open circles and linear regression is indicated by solid lines.



	D30 vs D90	D90 vs D180	D30 vs D180
AZ/AZ	*	ns	***
AZ/BNT	****	****	****
BNT/BNT	ns	ns	****

	D30 vs D90
AZ/AZ	****
AZ/BNT	**
BNT/BNT	ns

Figure 1
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