Immunogenicity and reactogenicity of heterologous Ad26.COV.2 and BNT162b2 vaccination

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Abstract

Given increased rates of breakthrough infection, German and other national authorities recommend heterologous booster vaccinations for Ad26.COV.2 vaccinated individuals, although data on immunogenicity and reactogenicity are lacking. We therefore recruited fifteen individuals who received the Ad26.COV.2 vaccination followed by a heterologous booster vaccination using the mRNA-vaccine BNT162b2. Immune response included SARS-CoV-2 spike-specific IgG, neutralizing activity as well as Spike-specific CD4 and CD8 T cells. Reactogenicity was self-reported using a questionnaire. We show that induction of specific immunity was poor after one dose of Ad26.COV.2. Heterologous boosting with BNT162b2 led to a significant increase in spike-specific IgG, neutralizing antibodies and CD4 and CD8 T cells. Heterologous boosting was well tolerated with more frequent systemic adverse events after the first dose. Thus, our data support current recommendations for heterologous booster-vaccination to optimize specific immunity and protection against SARS-CoV-2 infection in Ad26.COV.2-vaccinated individuals.

Main Text

Among the authorized vaccines in Europe, the vector-based vaccine ChAdOx1 nCoV-19 and the mRNA-vaccines BNT162b2 and mRNA-1273 are given as dual-dose regimens, whereas the vector-vaccine Ad26.COV.2 is licensed as a single dose only. In general, mRNA-vaccines were shown to have a higher efficacy compared to vector-based regimens, and breakthrough infections are most frequently observed after single-dose Ad25.COV-2 administration[1–3]. This led German and other national authorities to recommend heterologous booster vaccinations for Ad26.COV.2 vaccinated individuals[3], although data on immunogenicity and reactogenicity are lacking. As heterologous mRNA-vaccine boosting after priming with the ChAdOx1-nCoV-19 vector vaccine led to an equivalent or superior antibody- and T-cell response as compared to homologous regimens[4–7], we hypothesized that heterologous mRNA-boosting may also be able to optimize specific immunity following Ad25.COV-2-priming.

We analyzed immunogenicity and reactogenicity of fifteen individuals who received heterologous BNT162b2-boosting 16.1 [13.0-19.3] weeks after Ad26.COV.2 vaccination. Blood-counts, vaccine-induced IgG, neutralizing activity using a surrogate assay, spike-specific CD4 and CD8 T cells and polyclonally activated T cells were quantified exactly as previously described[4]. Reactogenicity was self-reported within the first seven days after the first and the second vaccination. Eleven males and four females with a mean age of 29.3±8.6 years were included. Heparinized blood samples for immunological testing were obtained 21 (IQR 21-28) days after the first and 16 (IQR 14-23) days after the booster-vaccination.

As shown in figure 1A and Table 1, a single dose of Ad26.COV.2 led to a poor induction of spike-specific IgG (62.16 [46.87-112.2] BAU/ml). Accordingly, median neutralizing activity was low (29 [12.7-47.9] % inhibition) with positive results in only 2/15 individuals. Heterologous boosting with BNT162b2 led to a significant increase in median IgG-levels (3168 [1896-4985] BAU/ml, 39-fold, $p<0.0001$) and in neutralizing activity (median 100 [100-100] %, 8-fold, $p<0.0001$). Spike-specific CD4 and CD8 T cells were stimulated with overlapping peptides and identified based on upregulation of CD69 and induction of

IFNγ. As shown in Figure 1B, spike-specific CD4 T-cell levels after Ad26.COV.2 priming were overall low (0.032 [0.019-0.051] %) with only 8/15 individuals above detection limit. In contrast, median levels of spike-specific CD8 T cells were higher (0.405 [0.120-1.038] %). Although the booster effect on CD4 T cells was less pronounced (3-fold) than on CD8 T cells (7-fold), both T-cell subtypes showed a significant increase reaching median levels of 0.070 [0.049-0.157] % CD4 T cells and 1.077 [0.374-3.811] % CD8 T cells ($p=0.003$ and $p=0.002$, respectively, Figure 1B). All effects were spike-specific, as boosting had no effect on SEB-reactive CD4 or CD8 T-cell levels (Figure 1C). The heterologous vaccine regimen was well tolerated, and adverse events were reported more pronounced after the Ad26.COV.2 dose (figure 2A and B). Overall, both local and systemic adverse events were rather moderate with largely no difference between the two doses (Figure 2C and D). Among systemic adverse events, chills were the only events which were significantly more frequent after the first Ad26.COV.2 vaccination (figure 2D).

Table 1
Differential blood counts and immunological parameters after the first and the second vaccination

vaccine		Ad26.COV.2 first vaccine	BNT162b2 booster vaccine	p-value*
Differential blood-counts				
Leukocytes (cells/µl)	median [IQR]	5200 [4600-7500]	5800 [4900-7100]	p=0.552
Lymphocytes (cells/µl)	median [IQR]	2012 [1706-2446]	2011 [1835-2361]	p=0.454
Monocytes (cells/µl)	median [IQR]	470 [419-643]	538 [446-676]	p=0.303
Granulocytes (cells/µl)	median [IQR]	2580 [2106-4456]	3131 [2583-3593]	p=0.345
Thrombocytes (cells/µl)	median [IQR]	241000 [207000-282000]	273000 [205000-293000]	p=0.192
Immunological parameters				
IgG (BAU/ml)	median [IQR]	62 [47-112]	3168 [1896-4985]	p<0.0001
	% positive§	93% [14/15]	100% [15/15]	p>0.999#
Neutralizing activity (% inhibition)	median [IQR]	29.5 [12.7-47.9]	100.2 [100.0-100.6]	p<0.0001
	% positive§	13% [2/15]	100% [15/15]	p<0.0001#
Spike-specific CD4 T cells (%)	median [IQR]	0.032 [0.019-0.051]	0.070 [0.049-0.157]	p=0.003
	% positive§	53% [8/15]	93% [14/15]	p=0.035#

*p-values were calculated using the Wilcoxon matched-pairs test or #Fisher´s exact test. §refers to the detection limits (see methods).

vaccine		Ad26.COV.2 first vaccine	BNT162b2 booster vaccine	p-value*
Spike-specific CD8 T cells (%)	median [IQR]	0.405 [0.120-1.038]	1.077 [0.374-3.811]	p=0.002
	% positive§	87% [13/15]	100% [15/15]	p=0.483#
SEB-reactive CD4 T cells (%)	median [IQR]	4.834 [2.491-6.717]	4.179 [2.159-6.872]	p=0.934
SEB-reactive CD8 T cells (%)	median [IQR]	7.885 [5.116-13.22]	5.928 [9.004-10.420]	p=0.188

*p-values were calculated using the Wilcoxon matched-pairs test or #Fisher´s exact test. §refers to the detection limits (see methods).

Despite investigated in a small sample size, a heterologous boost with an mRNA vaccine led to a substantial increase in both humoral and cellular immunity in all tested individuals. The immunogenicity profile after vaccination with the Ad26.COV.2 vector shares similarities with primary vaccination with the ChAdOx1 nCoV-19 vector where spike-specific T cells were more strongly induced as compared to antibodies[8]. The increase after heterologous boosting is in a similar range as previously described for individuals after homologous BNT162b2 or heterologous ChAdOx1 nCoV-19/BNT162b2-vaccination[4]. Thus, together with evidence of a less profound booster-effect after homologous ChAdOx1 nCoV-19 vector-vaccination[4,7] and recent data on high effectiveness of heterologous ChAdOx1 nCoV-19/mRNA vaccination[9,10], our data support current recommendations for heterologous booster-vaccination to optimize specific immunity and protection against SARS-CoV-2 infection in Ad26.COV.2-vaccinated individuals. Given the moderate spectrum of adverse events, heterologous boosting appears safe and may raise confidence in following recommendations towards heterologous vaccination.

Methods

Study group

In this observational study, we prospectively recruited healthy immunocompetent individuals who received Ad26.COV.2 vaccination and underwent secondary vaccination with BNT162b2. Individuals did not have any history of SARS-CoV-2 infection. Blood samples were drawn once after the Ad26.COV.2 and once after the BNT162b2 vaccination. Reactogenicity was self-reported within the first seven days after the first and the second vaccination using a questionnaire. Ethical approval was obtained by the ethics

committee of the Ärztekammer des Saarlandes (reference 133/21) and all individuals gave written informed consent.

Immunological analyses

SARS-CoV-2 spike-specific IgG and neutralizing antibody activity were determined using ELISA assays according to the manufacturers instructions (Euroimmun, Lübeck, Germany). Thresholds for negative, intermediate and positive IgG-levels (<25.6 BAU/ml negative, ≥25.6 and <35.2 BAU/ml intermediate, ≥35.2 BAU/ml positive) were used as determined by the manufacturer. Neutralizing capacity was quantified using a surrogate assay and determined as percentage of inhibition (IH) by 1 minus the ratio of the extinction of the respective sample and the extinction of the blank value with threshold as defined by the manufacturer (IH<20% negative, IH≥20 to <35 intermediate, and IH≥35% positive). Spike-specific CD4 and CD8 T cells were quantified using flow-cytometry after specific stimulation with SARS-CoV-2 spike-derived overlapping peptides (JPT, Berlin, Germany) based on induction of CD69 and IFNγ exactly as described before[4]. Negative control stimulations (using peptide diluent) and positive control stimulation using *Staphylococcus aureus* enterotoxin B (SEB) were carried out in parallel. Spike-specific CD4 and CD8 T-cell levels were quantified after subtraction of the percentage of reactive cells after specific stimulation and negative control stimulation. Detection limits for SARS-CoV-2-specific CD4 and CD8 T cells were set at 0.03% as defined before[4].

Statistical analysis

Statistical analysis was performed using GraphPad Prism (Version 9.2.0). Comparison between continuous variables (spike-specific IgG, neutralizing activity, CD4 and CD8 T cells, and leukocyte subpopulations) was carried out using the Wilcoxon matched pairs test. Categorial variables (percentage of individuals above/below detection limits, adverse events) was carried out using Fisher´s exact test.

Declarations

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Author Contributions

V.K., A.V., B.C.G., T.M. and M.S. designed the study; V.K., T.S., U.S. and M.S. designed the experiments; V.K., and T.S. performed experiments; L.H., A.V., F.G., and T.M. contributed to study design, patient recruitment, and clinical data acquisition. V.K., T.S., M.S., and U.S. performed statistical analysis. V.K., T.S., U.S., and

M.S. supervised all parts of the study, performed analyses and wrote the letter. All authors approved the final version of the manuscript.

Competing interest statement

M.S. has received grant support from Astellas and Biotest to the organization Saarland University outside the submitted work, and honoraria for lectures from Biotest and Novartis. All other authors of this manuscript have no conflicts of interest to disclose.

Data availability

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

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Figures

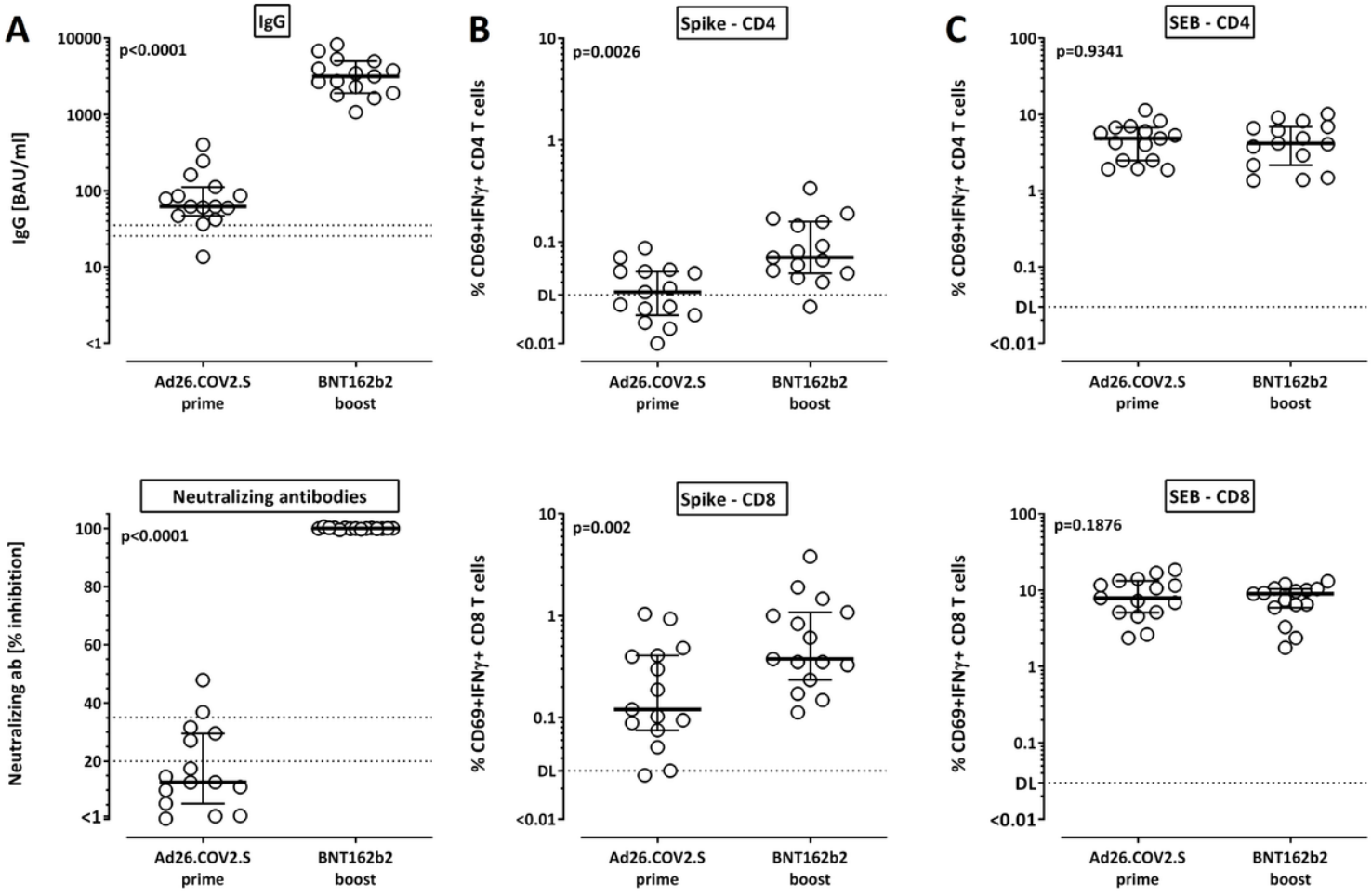

Figure 1

Antibody and T-cell responses against the SARS-CoV-2 spike protein after vaccination with Ad26.COV.2 followed by heterologous boosting with BNT162b2. Humoral and cellular immune parameters were analyzed 21 (IQR 21-28) days after Ad26.COV.2 priming and 16 (IQR 14-23) days after BNT162b2 boosting in fifteen immunocompetent individuals. (A) Spike-specific IgG were determined using an ELISA

and a surrogate neutralization assay was performed to quantify neutralizing antibodies. (B) SARS-CoV-2 specific CD4 and CD8 T cells were quantified after peptide-stimulation followed by intracellular cytokine staining of whole blood samples using flow-cytometry. Reactive cells were quantified based on co-expression of CD69 and IFNγ among CD4 or CD8 T cells and subtraction of reactivity of respective negative control stimulations. (C) As a control, Staphylococcus aureus Enterotoxin B (SEB)-reactive CD4 and CD8 T cells were determined. Bars indicate medians with interquartile ranges. Differences between immune responses after priming and boosting were calculated using the non-parametric Wilcoxon matched-pairs test. Dotted lines indicate thresholds to distinguish negative, intermediate and positive IgG-levels or levels of inhibitory activity, respectively, as per manufacturer's instructions. Detection limits for SARS-CoV-2-specific CD4 and CD8 T cells were set at 0.03% as defined before4. IFN, Interferon; SEB, Staphylococcus aureus enterotoxin B.

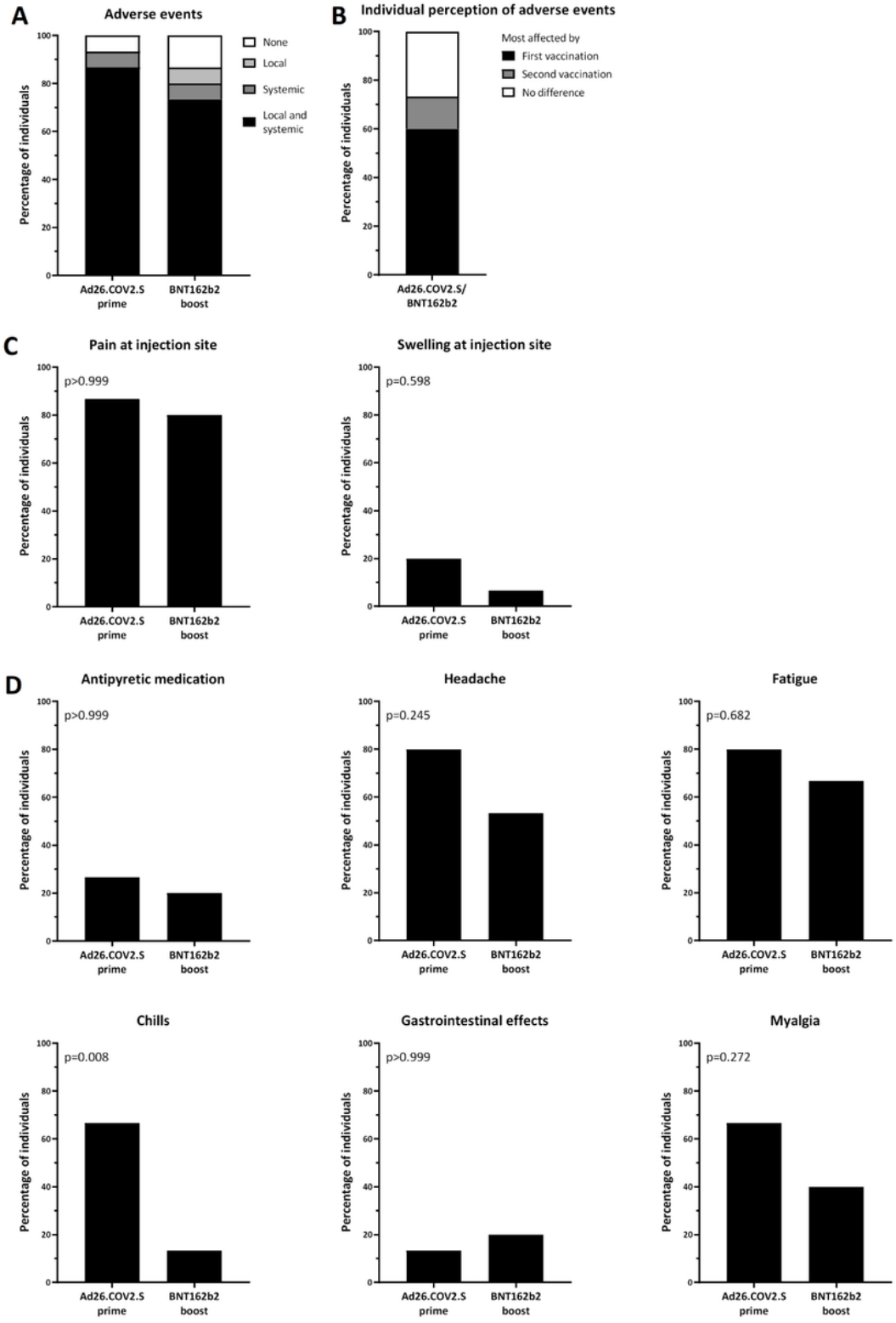

Figure 2

Reactogenicity after vaccination with Ad26.COV.2 followed by heterologous boosting with BNT162b2. Reactogenicity within the first week after Ad26.COV.2-priming and after BNT162b2-boosting, respectively, was self-reported using a standardized questionnaire. Presence of local or systemic adverse events (A) in general, and (B) individual perception of which of the two vaccinations affected more are shown. (C) Local or (D) systemic adverse events are further stratified. Chills were significantly more frequent after the

priming dose. Gastrointestinal symptoms include nausea, vomiting, and diarrhea. Fever and arthralgia was also included in the questionnaire, but was not reported by any of the participants. Comparisons between the groups were performed using Fisher´s exact test with p-values included in panels C and D.