ORIGINAL ARTICLE

Immunogenicity and Reactogenicity of Vaccine Boosters after Ad26.COV2.S Priming

Roos S.G. Sablerolles, M.D., Wim J.R. Rietdijk, Ph.D., Abraham Goorhuis, M.D., Ph.D., Douwe F. Postma, M.D., Ph.D., Leo G. Visser, M.D., Ph.D., Daryl Geers, M.Sc., Katharina S. Schmitz, M.Sc., Hannah M. Garcia Garrido, M.D., Marion P.G. Koopmans, D.V.M., Ph.D., Virgil A.S.H. Dalm, M.D., Ph.D., Neeltje A. Kootstra, Ph.D., Anke L.W. Huckriede, Ph.D., Melvin Lafeber, M.D., Ph.D., Debbie van Baarle, Ph.D., Corine H. GeurtsvanKessel, M.D., Ph.D., Rory D. de Vries, Ph.D., and P. Hugo M. van der Kuy, Pharm.D., Ph.D., for the SWITCH Research Group*

ABSTRACT

BACKGROUND

The Ad26.COV2.S vaccine, which was approved as a single-shot immunization regimen, has been shown to be effective against severe coronavirus disease 2019. However, this vaccine induces lower severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein (S)–specific antibody levels than those induced by messenger RNA (mRNA)–based vaccines. The immunogenicity and reactogenicity of a homologous or heterologous booster in persons who have received an Ad26.COV2.S priming dose are unclear.

METHODS

In this single-blind, multicenter, randomized, controlled trial involving health care workers who had received a priming dose of Ad26.COV2.S vaccine, we assessed immunogenicity and reactogenicity 28 days after a homologous or heterologous booster vaccination. The participants were assigned to receive no booster, an Ad26.COV2.S booster, an mRNA-1273 booster, or a BNT162b2 booster. The primary end point was the level of S-specific binding antibodies, and the secondary end points were the levels of neutralizing antibodies, S-specific T-cell responses, and reactogenicity. A post hoc analysis was performed to compare mRNA-1273 boosting with BNT162b2 boosting.

RESULTS

Homologous or heterologous booster vaccination in 434 participants resulted in higher levels of S-specific binding antibodies, neutralizing antibodies, and T-cell responses than a single Ad26.COV2.S vaccination. The increase in binding antibodies was significantly larger with heterologous regimens that included mRNA-based vaccines than with the homologous booster. The mRNA-1273 booster was most immunogenic and was associated with higher reactogenicity than the BNT162b2 and Ad26.COV2.S boosters. Local and systemic reactions were generally mild to moderate in the first 2 days after booster administration.

CONCLUSIONS

The Ad26.COV2.S and mRNA boosters had an acceptable safety profile and were immunogenic in health care workers who had received a priming dose of Ad26.COV2.S vaccine. The strongest responses occurred after boosting with mRNA-based vaccines. Boosting with any available vaccine was better than not boosting. (Funded by the Netherlands Organization for Health Research and Development ZonMw; SWITCH ClinicalTrials.gov number, NCT04927936.)

The authors' affiliations are listed in the Appendix. Dr. van der Kuy can be contacted at h.vanderkuy@erasmusmc.nl or at the Department of Hospital Pharmacy, Erasmus Medical Center, Dr. Molewaterplein 40, 3015 GD, Rotterdam, the Netherlands.

*A full list of the SWITCH Research Group investigators is provided in the Supplementary Appendix, available at NEJM.org.

Drs. GeurtsvanKessel, de Vries, and van der Kuy contributed equally to this article.

This article was published on January 19, 2022, at NEJM.org.

DOI: 10.1056/NEJMoa2116747

Copyright © 2022 Massachusetts Medical Society.

The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.

Four vaccines are authorized for use in the European Union to prevent coronavirus disease 2019 (Covid-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). These vaccines include two messenger RNA (mRNA)–based vaccines (BNT162b2 [Pfizer–BioNTech] and mRNA-1273 [Moderna]) and two adenovirus vector–based vaccines (ChAdOx1 nCoV-19 [Oxford–AstraZeneca] and Ad26.COV2.S [Johnson & Johnson–Janssen]). These vaccines have been shown to be highly efficacious in preventing mild-to-severe Covid-19.[1-4]

The original regimens of BNT162b2, mRNA-1273, and ChAdOx1 nCoV-19 vaccines were homologous prime–boost regimens, whereas the original regimen of Ad26.COV2.S vaccine was a single-shot regimen. The durability of protection and potential need for boosting doses (a third vaccination in the case of BNT162b2, mRNA-1273, and ChAdOx1 nCoV-19 and a second vaccination in the case of Ad26.COV2.S) is under continuous assessment.

The Ad26.COV2.S vaccine is advantageous because it can be administered in a single dose.[3] In addition, Ad26.COV2.S vaccination induces both humoral and cellular immune responses that persist up to 8 months.[5,6] However, in head-to-head comparisons, the mRNA-based vaccines induced higher levels of SARS-CoV-2 spike protein (S)–specific antibodies than the Ad26.COV2.S vaccine.[7,8] The difference between Ad26.COV2.S and mRNA-based vaccination with respect to efficacy against hospitalization has been less evident, probably in part because of T-cell responses. A recent study of homologous Ad26.COV2.S boosters showed that they increased binding antibody levels,[6] but this study did not evaluate the effects of boosters on T-cell immunity.

"Mixing and matching" of Covid-19 vaccines enhances the flexibility of vaccination campaigns[9] and may induce broader immune responses.[10-14] Heterologous vaccination regimens with ChAdOx1 nCoV-19 followed by BNT162b2 have been shown to have an acceptable safety profile and to have induced immune responses that were similar or even superior to those of homologous regimens.[15-18] Complete immunologic and safety assessments of the effect of mRNA boosters in persons who have received a priming dose of Ad26.COV2.S vaccine are under way,[19] and they are highly relevant because millions of persons have been immunized with Ad26.COV2.S vaccine. To support decision making regarding boosters in persons who have received a priming dose of Ad26.COV2.S vaccine, we performed the SWITCH trial, a head-to-head comparison of homologous and heterologous boosters administered to health care workers.

METHODS

TRIAL OVERSIGHT

This single-blind, multicenter, randomized, controlled trial involved health care workers from four academic hospitals in the Netherlands (see the protocol, available with the full text of this article at NEJM.org).[9] The trial adhered to the principles of the Declaration of Helsinki and was approved by the medical ethics review committee of Erasmus Medical Center and the local review boards of the participating centers. All the participants provided written informed consent before enrollment. Qiagen provided QuantiFERON SARS-CoV-2 assay kits (starter packs and extended packs for research use only) but had no role in the trial design, data acquisition, or analysis. The authors vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol.

PARTICIPANTS AND RANDOMIZATION

Health care workers were eligible to participate if they were between 18 and 65 years of age and did not have severe coexisting factors or conditions (e.g., receipt of treatment for cancer, use of immunosuppressant agents, dependence on dialysis, or receipt of a solid-organ or bone marrow transplant) or a history of SARS-CoV-2 infection (either laboratory-confirmed or reported by the participant).[9] A list of the inclusion and exclusion criteria is provided in the protocol. The representativeness of the trial population is described in Table S1 of the Supplementary Appendix, available at NEJM.org.

Participants had been vaccinated with Ad26.COV2.S 3 months before enrollment and were randomly assigned, in a 1:1:1:1 ratio, to not receive a booster or to receive an Ad26.COV2.S booster, an mRNA-1273 booster, or a BNT162b2 booster. The prespecified prime–boost interval was 84 days (interquartile range, –7 to 21). Randomization was stratified according to trial site

The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.

after written informed consent was obtained from the participants. In addition, half the participants in each group were randomly selected for analyses of the S-specific T-cell response.

TRIAL DESIGN

At the first trial visit, the participants received a booster by injection into the deltoid muscle. The volume and appearance of the assigned vaccines were concealed from the participants in order to maintain blinding. The vaccine doses were administered according to the summary of product characteristics for Ad26.COV2.S ($\geq 8.92 \times 10^{10}$ viral particles), mRNA-1273 (100 μg), and BNT162b2 (30 μg).

Participants who were randomly assigned to the nonbooster group were informed of their assignment at the first trial visit, and they did not receive an injection of placebo because of ethical concerns. Blood samples were collected at the first and second trial visits (at 0 and 28 days). Booster assignments were unblinded 8 days after the boosters were administered, after the participants had completed a questionnaire about reactogenicity.

REACTOGENICITY

Safety assessments included monitoring of reactions reported by the participants after the Ad26.COV2.S priming dose and after the boosters. Perceived severity was assessed with the use of a modified 4-point Food and Drug Administration toxicity grading scale (on which 0 indicates no symptoms, 1 mild symptoms that do not interfere with daily activities, 2 moderate symptoms that interfere with daily activities, and 3 severe symptoms that prohibit daily activities).[20]

In addition, the participants reported whether the adverse events were present each day from the day of injection until 7 days after the injection. Adverse events were reported by means of an electronic questionnaire that the participants completed 8 days after they received a booster. Adverse events that had occurred after the previously administered priming dose were reported at enrollment (approximately 3 months after the priming injection) and were subject to potential recall bias. Other serious adverse events and solicited local or systemic reactions were reported by the participants in a questionnaire, by email, or by telephone. Safety monitoring (blood biochemical testing and a hematologic assessment) was performed at days 0 and 28.

IMMUNOGENICITY

The analysis of humoral and cellular immune responses is described in the Supplementary Methods section in the Supplementary Appendix. Briefly, in order to confirm that the participants had not been exposed to SARS-CoV-2, SARS-CoV-2 nucleocapsid (N)–specific antibodies were measured in all samples at baseline and in samples obtained from a selection of participants in the nonbooster group who had unexpected responses at day 28. At days 0 and 28 after booster vaccination, S-specific binding antibodies were measured with the use of a quantitative anti-spike IgG assay (Liaison SARS-CoV-2 TrimericS IgG assay, DiaSorin).[21,22] Neutralizing antibodies against infectious SARS-CoV-2 D614G (Global Initiative on Sharing All Influenza Data sequence, hCov-19/Netherlands/ZH-EMC-2498) were assessed with a plaque reduction neutralization test (PRNT) in Vero E6 cells. S-specific T-cell responses were assessed with an interferon-γ–release assay (QuantiFERON, Qiagen) at days 0 and 28 after booster vaccination, as previously described.[23]

STATISTICAL ANALYSIS

The sample size was determined on the basis of available data.[9,15,17] We calculated that 108 participants per group (432 total) would provide the trial with 80% power at a one-sided 2.5% significance level to detect a log-transformed difference of 0.2 in antibody levels among the groups, with 25% SARS-CoV-2 seropositivity at baseline and an anticipated 25% loss to follow-up.

The baseline characteristics in each group, including immune responses, are described. Continuous variables at baseline are presented as medians and interquartile ranges. Median differences across the four groups were compared with the use of the Kruskal–Wallis test. Categorical variables are presented as numbers and percentages, and between-group differences were compared with the use of Fisher's exact test.

The primary end point was the log-transformed level of S-specific IgG binding antibodies 28 days after booster vaccination. We used Mann–Whitney U tests to assess the differences in log-transformed titer values for the following

The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.

three comparisons: Ad26.COV2.S booster with no booster, Ad26.COV2.S booster with BNT162b2 booster, and Ad26.COV2.S booster with mRNA-1273 booster. In a post hoc analysis, we also compared the BNT162b2 booster with an mRNA-1273 booster. Effect sizes (beta coefficients) and 98.3% confidence intervals were estimated with the use of quantile regression in which we varied the reference category to estimate each contrast.

The prespecified secondary end points were levels of neutralizing antibodies, S-specific T-cell responses, and reactogenicity. Furthermore, we analyzed the following variables in a post hoc manner. We classified participants as having a response or no response on the basis of a prespecified cutoff value (according to the manufacturers' instructions or an external validation cohort for each assay), and we compared responses across groups with the use of Fisher's exact test. In addition, in each group, to correct for baseline values, we assessed differences in the median factor change in $\log_{10}$-transformed values for S-specific IgG binding antibody levels, neutralizing antibody levels, and S-specific T-cell responses before the booster, as compared with after the booster. The Spearman's correlation coefficient and linear regression were calculated to examine the association between binding antibody levels and neutralizing antibody levels, and between binding antibody levels and S-specific T-cell responses, in samples obtained before and after booster vaccination. Linear regressions accompany the beta coefficients and 95% confidence intervals. These analyses do not control for multiple comparisons, and the inferences may not be reproducible.

To assess the comparability of the trial groups with adjustment for baseline titer values, we performed a quantile regression on the log-transformed S-specific IgG binding antibody levels 28 days after booster vaccination, with group, recruiting center, and log-transformed baseline titer value as covariates. For the secondary end points, we analyzed the database on pairwise deletion without imputation.

Statistical analyses were performed with GraphPad Prism software, version 9.1.2, and RStudio software, version 4.0.5. We prespecified that a P value of less than 0.017 was considered to indicate statistical significance (with the application of Bonferroni correction at the 0.05 level to the three comparisons for the prespecified primary end point).

RESULTS

BASELINE CHARACTERISTICS OF THE PARTICIPANTS

Of 697 health care workers who were screened for eligibility, 461 underwent randomization and 236 were excluded; of those who were excluded, 78 did not meet inclusion criteria, 18 declined to participate after reading the patient information, and 140 were not included for logistic reasons (e.g., they were not able to adhere to the trial schedule or did not reply to the screening questionnaire). For the per-protocol analyses, we excluded 27 participants (Fig. 1). All 434 participants who were included in the per-protocol analysis adhered to the timing between trial visits (Table 1). The median interval between the Ad26.COV2.S priming dose and the booster was 94 days (interquartile range, 86 to 98).

Baseline characteristics did not differ considerably among the groups (Table 1). In particular, no major differences among the groups were noted in S-specific binding antibody levels, neutralizing antibody levels, and S-specific T-cell responses at baseline (Table 1 and Fig. 2A, 2C, and 2E).

S-SPECIFIC BINDING ANTIBODIES AFTER A BOOSTER

The primary end point, the level of IgG against the S1 subunit of the spike protein (anti-S1) binding antibodies after booster vaccination, was determined by means of quantitative assay (Fig. 2A). We found significantly more binding antibodies in participants who had received homologous Ad26.COV2.S booster vaccination than among those who had not received a booster (beta coefficient, 0.64; 98.3% confidence interval [CI], 0.41 to 0.81; P<0.001). Likewise, as compared with the homologous Ad26.COV2.S vaccine regimen, there were more binding antibodies after the Ad26.COV2.S–BNT162b2 booster regimen (beta coefficient, 0.73; 98.3% CI, 0.57 to 0.90; P<0.001) and after the Ad26.COV2.S–mRNA-1273 booster regimen (beta coefficient, 0.94; 98.3% CI, 0.85 to 1.12; P<0.001). Finally, our post hoc analysis showed that the mRNA-1273 booster increased binding antibodies to higher levels than the BNT162b2 booster (beta coefficient, 0.21; 98.3% CI, 0.13 to 0.37). No

The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.

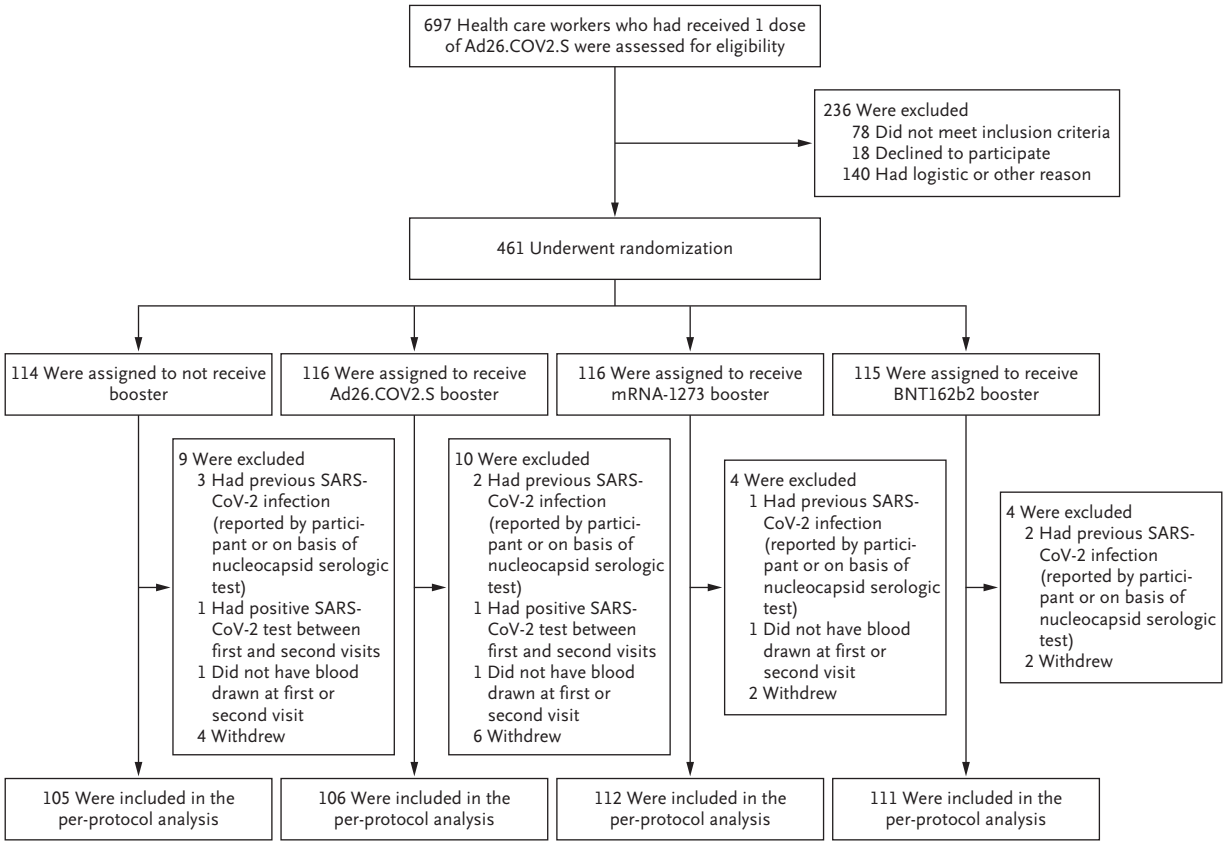


Figure 1. Screening, Randomization, and Analysis.

Between trial enrollment and randomization, 236 health care workers were excluded, including 78 who did not meet the inclusion criteria, 18 who declined to participate after reading the patient information form, and 140 who could not adhere to the strict schedule of the trial or did not reply to the screening questionnaire. After randomization, the numbers of participants who were lost to follow-up did not differ significantly among the groups. SARS-CoV-2 denotes severe acute respiratory syndrome coronavirus 2.

imputation was required because no data on baseline characteristics or for the primary end point were missing. Similar results were found in a quantile regression controlling for group, recruiting center, and log-transformed baseline titer value. Thus, differences in the primary end point could not be attributed to differences at baseline (Table S2).

RAPID RECALL OF S-SPECIFIC BINDING ANTIBODIES AFTER A BOOSTER

A cutoff value of 33.8 binding antibody units per milliliter was used to define test positivity, according to the manufacturer's instructions. On the basis of this criterion, 389 of 434 participants (89.6%) who had received a priming dose of Ad26.COV2.S vaccine had detectable S-specific binding antibodies at baseline (Fig. 2A). In the three groups that received boosters, booster vaccination led to levels of binding antibodies that were higher than those at baseline (Table S3). The effect sizes and confidence intervals are presented in Table S4. Increases in S-specific binding antibody levels after the administration of the booster were assessed (Fig. 2A). In addition, to correct for baseline levels, we assessed increases in antibody levels in terms of the factor change per participant, and similar differences in factor changes among the groups were observed (Fig. 2B). Heterologous mRNA-based booster vaccinations resulted in significantly higher binding antibody levels than homologous

The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.

Table 1. Baseline Characteristics of the Trial Participants, Who Had Received a Priming Dose of Ad26.COV2.S Vaccine.*

Characteristic	No Booster (N=105)	Ad26.COV2.S Booster (N=106)	mRNA-1273 Booster (N=112)	BNT162b2 Booster (N=111)	Total (N=434)
Median age (IQR) — yr	41.0 (30.0 to 51.0)	41.0 (31.0 to 51.0)	40.5 (30.8 to 49.0)	38.0 (29.0 to 47.0)	40.5 (30.0 to 50.0)
Sex — no. (%)					
Male	35 (33)	36 (34)	37 (33)	45 (41)	152 (35)
Female	70 (67)	70 (66)	75 (67)	66 (59)	281 (65)
Median BMI (IQR)†	24.2 (22.0 to 27.5)	23.4 (21.2 to 26.2)	24.1 (21.7 to 26.5)	23.8 (21.9 to 26.0)	23.9 (21.6 to 26.6)
Ancestry — no. (%)‡					
African	1 (1)	0	0	0	1 (<1)
Asian	3 (3)	4 (4)	4 (4)	4 (4)	15 (3)
European	95 (90)	98 (92)	105 (94)	105 (95)	403 (93)
North American	1 (1)	1 (1)	0	0	2 (<1)
South American	1 (1)	1 (1)	1 (1)	0	3 (1)
Other	4 (4)	2 (2)	2 (2)	2 (2)	10 (2)
Occupation in hospital — no. (%)					
Administrative worker or policy-maker	18 (17)	22 (21)	24 (21)	16 (14)	80 (18)
Medical doctor	3 (3)	4 (4)	4 (4)	9 (8)	20 (5)
Facility services staff member	4 (4)	6 (6)	4 (4)	5 (5)	19 (4)
Manager	13 (12)	10 (9)	10 (9)	9 (8)	42 (10)
Support staff					
Clinic or emergency department	1 (1)	1 (1)	1 (1)	1 (1)	4 (1)
Outpatient clinic	0	2 (2)	0	0	2 (<1)
Researcher	42 (40)	39 (37)	43 (38)	38 (34)	162 (37)
Nurse	2 (2)	1 (1)	4 (4)	2 (2)	9 (2)
Other	22 (21)	21 (20)	22 (20)	31 (28)	96 (22)
Coexisting condition — no. (%)					
Cardiovascular disease	4 (4)	0	2 (2)	3 (3)	9 (2)
Pulmonary disease	4 (4)	2 (2)	1 (1)	4 (4)	11 (3)
Diabetes mellitus	0	1 (1)	1 (1)	0	2 (<1)
Liver disease	0	1 (1)	1 (1)	0	2 (<1)
Kidney disease	0	2 (2)	0	0	2 (<1)
Immune response on day 0§					
Median $\log_{10}$-transformed level of SARS-CoV-2 spike protein (S)–specific binding antibodies (IQR) — BAU/ml	2.0 (1.7 to 2.4)	2.1 (1.8 to 2.4)	2.0 (1.8 to 2.2)	2.1 (1.8 to 2.4)	2.0 (1.8 to 2.3)
Median $\log_{10}$-transformed level of neutralizing antibodies (IQR) — IU/ml	1.9 (1.5 to 2.0)	1.6 (1.4 to 2.0)	1.7 (1.5 to 1.9)	1.7 (1.4 to 2.1)	1.7 (1.5 to 2.0)
Median $\log_{10}$-transformed S-specific T-cell response (IQR) — IU/ml	–0.6 (–0.9 to –0.2)	–0.6 (–1.1 to –0.1)	–0.6 (–1.2 to –0.2)	–0.6 (–0.9 to –0.2)	–0.6 (–1.0 to –0.2)
Testing — no. (%)					

The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.

Table 1. (Continued.)

Characteristic	No Booster (N=105)	Ad26.COV2.S Booster (N=106)	mRNA-1273 Booster (N=112)	BNT162b2 Booster (N=111)	Total (N=434)
Before first trial visit	32 (30)	45 (42)	36 (32)	39 (35)	152 (35)
Between first and second trial visits	11 (10)	10 (9)	6 (5)	19 (17)	46 (11)
Median time between first vaccination and first trial visit (IQR) — days	91 (86 to 99)	95 (88 to 97)	96 (86 to 98)	89 (85 to 96)	94 (86 to 98)
Median time between first and second trial visits (IQR) — days	28 (28 to 28)	28 (28 to 28)	28 (28 to 28)	28 (28 to 28)	28 (28 to 28)
Current use of prescription medication — no. (%)	26 (25)	36 (34)	28 (25)	37 (33)	127 (29)

* The first trial visit occurred before the booster, and the second trial visit occurred 28 days after administration of the booster. IQR denotes interquartile range, and SARS-CoV-2 severe acute respiratory syndrome coronavirus 2.
† The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters.
‡ Categories were reported by the participants. The categories shown are those used by the investigators to denote ancestry.
§ In all four groups, blood was drawn on day 0, which was the day of the first trial visit. Day 0 was also the day of the booster in the three booster groups.

boosters with Ad26.COV2.S vaccine, and boosting with mRNA-1273 vaccine performed better than boosting with BNT162b2 vaccine.

NEUTRALIZING ANTIBODY LEVELS AFTER A BOOSTER

For all the secondary end points, missing values were minimal. To assess antibody functionality, levels of S-specific neutralizing antibodies were measured by means of a PRNT for infectious virus in 213 participants at Erasmus Medical Center (Figs. 2C and 2D and S1). On the basis of a PRNT with a 50% cutoff value for positivity ($PRNT_{50}$) of 28.6 IU per milliliter (corresponding to a serum dilution of 1:40), 158 of 213 participants (74.2%) who had received an Ad26.COV2.S priming dose had levels of neutralizing antibodies that were higher than those at baseline. In all the groups that received a booster, booster vaccination led to higher levels of neutralizing antibodies than baseline levels (Fig. 2C). Overall, heterologous mRNA-based booster vaccinations increased neutralizing antibody levels to a greater extent than the Ad26.COV2.S booster; this increase was assessed in terms of both antibody levels and factor change per participant (Fig. 2C and 2D).

CORRELATIONS BETWEEN S-SPECIFIC BINDING ANTIBODIES AND NEUTRALIZING ANTIBODIES

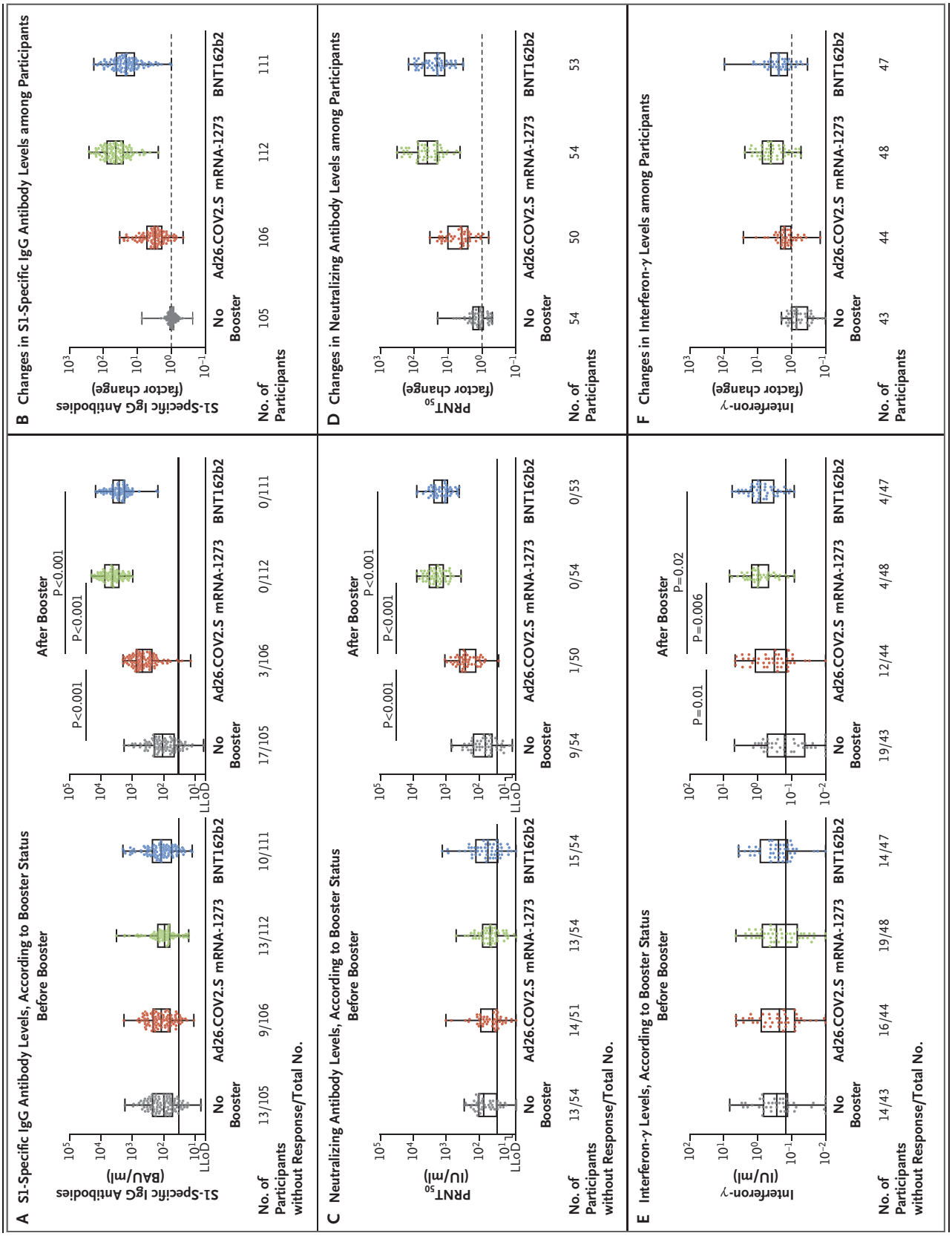
To determine correlations between levels of S1-binding antibodies and levels of neutralizing antibodies, we performed linear regression analyses on log-transformed data. We found a positive correlation between levels of binding antibodies and levels of neutralizing antibodies in both the before-booster and after-booster serum samples (Spearman's rank correlation coefficients, 0.82 at baseline and 0.93 after booster vaccination) (Fig. 3).

RAPID RECALL OF S-SPECIFIC T-CELL RESPONSES AFTER A BOOSTER

To detect S-specific T-cell responses, levels of interferon-γ were measured after stimulation of whole blood from a random selection of samples obtained before and after booster vaccination from 182 participants at three participating centers (Figs. 2E, 2F, and S2). We used a peptide pool covering the S protein (Ag2, Qiagen) and a cutoff value for test positivity for interferon-γ of 0.15 IU per milliliter (according to the manufacturer's instructions) to determine that 119 of 182 participants (65.4%) who had received an Ad26.COV2.S priming dose had detectable T-cell responses at baseline (Fig. 2E). In all the groups that received a booster, booster vaccinations led to a rapid recall of T-cell responses, as compared with baseline levels.

We assessed T-cell responses according to interferon-γ levels after booster vaccination (Fig. 2E) and according to factor change in each participant (Fig. 2F), and we found that T-cell responses were higher in the group of patients who received Ad26.COV2.S booster vaccination

The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.



The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.

Figure 2 (facing page). SARS-CoV-2 S–Specific Immune Responses.

Panel A shows levels of SARS-CoV-2 spike protein (S)–specific IgG antibodies at baseline (before booster vaccination) and after booster vaccination in the four groups. The lower limit of detection (LLoD) was 4.81 binding antibody units (BAU) per milliliter. The cutoff value for response was 33.8 BAU per milliliter (horizontal line). Panel B shows the per-participant factor changes that were calculated by dividing the after-booster response by the before-booster response for S-specific binding antibodies. The dashed line indicates a factor change of 1 (no increase or decrease). Panel C shows the levels of neutralizing antibodies at baseline (before booster vaccination) and after booster vaccination, as assessed with a plaque reduction neutralization test with a 50% cutoff ($PRNT_{50}$) in the four groups. The LLoD was 7.7 IU per milliliter. The cutoff value for response was 28.6 IU per milliliter (corresponding to a serum dilution of 1:40; horizontal line). Panel D shows the per-participant factor changes that were calculated by dividing the after-booster response by the before-booster response for neutralizing antibodies. The dashed line indicates a factor change of 1 (no increase or decrease). Panel E shows interferon-γ levels in plasma after stimulation of whole blood with a peptide pool spanning the S protein at baseline (before booster) and after booster vaccination in the four groups. The LLoD was 0.01 IU per milliliter. The cutoff value for response was 0.15 IU per milliliter (horizontal line). Panel F shows per-participant factor changes calculated by dividing the after-booster response by the before-booster response for interferon-γ levels in plasma. The dashed line indicates a factor change of 1 (no increase or decrease). All data are presented in box-and-whisker plots. The whiskers indicate the range, the top and bottom of the boxes indicate the interquartile range, and the horizontal line within each box indicates the median. P values are reported for prespecified primary and secondary end points only (on the basis of Mann–Whitney tests). Comparisons between mRNA-1273 and BNT162b2 boosters (Panels A, C, and E, right panel) and comparisons among the groups on the basis of factor changes (Panels B, D, and F) were performed as post hoc analyses, and estimated effect sizes are reported in Table S4. Each dot in the figure represents an individual participant.

than in those in the nonbooster group. The mRNA-1273 and BNT162b2 boosters led to higher T-cell responses than the Ad26.COV2.S booster. The response was 91.7% with the mRNA-1273 booster and 91.5% with the BNT162b2 booster; both performed better than the homologous booster (response, 72.7%). Similar trends were observed with two other peptide pools. S-specific T-cell responses were positively correlated with the presence of S-specific binding antibodies (Fig. S3).

REACTOGENICITY

Retrospective reactogenicity data collected after the participants had received a priming dose of Ad26.COV2.S vaccine are presented in Tables S5 and S6. Prospective reactogenicity data collected within 7 days after the booster vaccination indicated a greater perceived severity and longer duration of local reactions (pain, redness, and swelling at the injection site) and systemic reactions (chills, fever, and muscle aches) after the mRNA-1273 booster than after the other vaccination regimens (Figs. 4 and S4 and Tables S7 and S8). The difference in the prevalence of reactions among regimens was most prominent on the day of the booster and on day 1 after vaccination. All the adverse events were mild to moderate and did not lead to hospitalization, and the symptoms generally resolved within 48 hours.

DISCUSSION

In this trial, we examined the immunogenicity and reactogenicity of homologous and heterologous boosters in health care workers who had received a priming dose of Ad26.COV2.S Covid-19 vaccine. We evaluated these outcomes 28 days after booster vaccination. Both homologous and heterologous boosters led to an increase in levels of S-specific binding antibodies and neutralizing antibodies and an increase in T-cell responses, but these increases were highest in participants who received heterologous regimens with mRNA-based Covid-19 vaccines.

The currently approved single-shot regimen of Ad26.COV2.S vaccine protects against severe Covid-19,[3] and durable immune responses can be detected up to 8 months after vaccination.[5] However, previous studies showed that antibody responses induced by Ad26.COV2.S vaccine were lower than those induced by mRNA-based vaccines.[7,8] This finding raises the question of whether booster vaccinations are necessary to protect against emerging circulating SARS-CoV-2 variants of concern such as the beta (B.1.351), delta (B.1.617.2), and omicron (B.1.1.529) variants that partially evade antibody responses.[7,8,24]

Data from phase 3 clinical trials of heterologous mixing and matching of vaccines are lacking, so the safety and reactogenicity of these regimens should be evaluated in postlicensure studies. We did not observe any serious adverse events in this trial; however, the sample size and

The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.

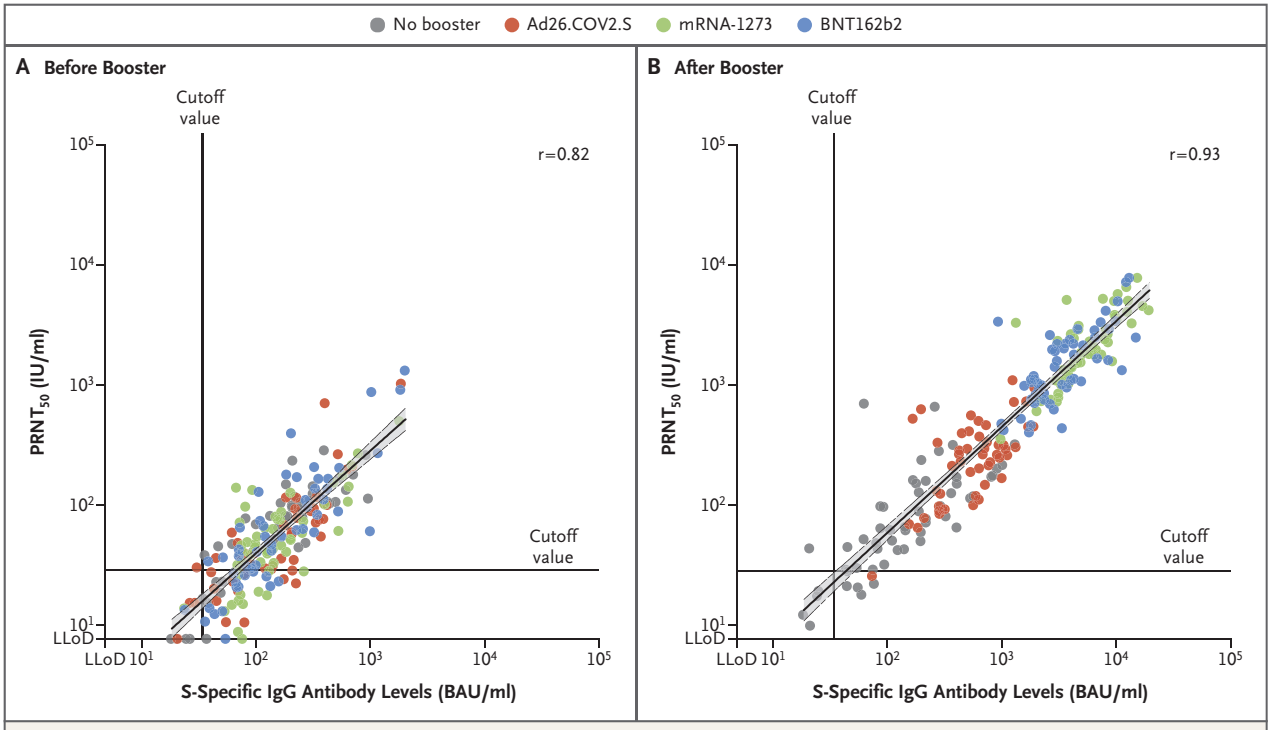


Figure 3. Correlation between S-Specific IgG Antibodies and Neutralizing Antibodies.

Panel A shows the correlation between S-specific IgG antibody levels and neutralizing antibody levels for the before-booster data, as assessed with $PRNT_{50}$ (Spearman's rank correlation coefficient, 0.82). Linear regression (diagonal lines) on log-transformed data was performed (beta coefficient, 0.90; 95% CI, 0.82 to 0.98). The gray shaded areas indicate the 95% CI of the best-fit line. Panel B shows the correlation between S-specific IgG antibody levels and neutralizing antibody levels for the after-booster data (Spearman's rank correlation coefficient, 0.93). Linear regression (diagonal lines) on log-transformed data was performed (beta coefficient, 0.72; 95% CI, 0.68 to 0.76). The gray shaded areas indicate the 95% CI of the best-fit line. Each dot in the figure represents an individual participant.

trial period were too limited to observe rare adverse events. Participants who received the mRNA-1273 booster reported more local and systemic reactions than those who received the other types of boosters; these reactions may have correlated with a stronger boosting of immune responses. In general, we found that mRNA-based vaccine boosters after a priming dose of Ad26.COV2.S vaccine had an acceptable safety profile, as previously described.[18,19]

Well-defined correlates of protection against SARS-CoV-2 infection have not yet been determined,[25] but neutralizing antibodies play an important role.[26] Unfortunately, assays to measure levels of neutralizing antibodies are not standardized, so the results of individual studies are difficult to compare. In the current trial, we measured the neutralization of D614G, which is similar to the virus used in the development of the approved adenovirus- and mRNA-based vaccines, to specifically focus on vaccine-induced responses. Higher neutralizing-antibody titers might be required in order to cross-protect or protect against SARS-CoV-2 variants.[27,28] Nonneutralizing antibodies could also play a key role in protection against severe Covid-19.[29]

In addition, the induction and boosting of S-specific T cells may play a crucial role in protection.[30] In our trial, S-specific T cells were detectable in 65.4% of the participants who had received a priming dose of Ad26.COV2.S vaccine 3 months previously. Boosting with mRNA-based vaccines led to a rapid increase in S-specific T-cell responses, which indicates that priming-induced immunity was rapidly recalled. S-specific T cells are capable of recognizing different variants,[31,32] and therefore the induction of T cells may be important in the face of waning antibody levels and the circulation of SARS-CoV-2 variants. In-depth phenotyping of T-cell responses may be helpful in delineating responses to boosters.

The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.

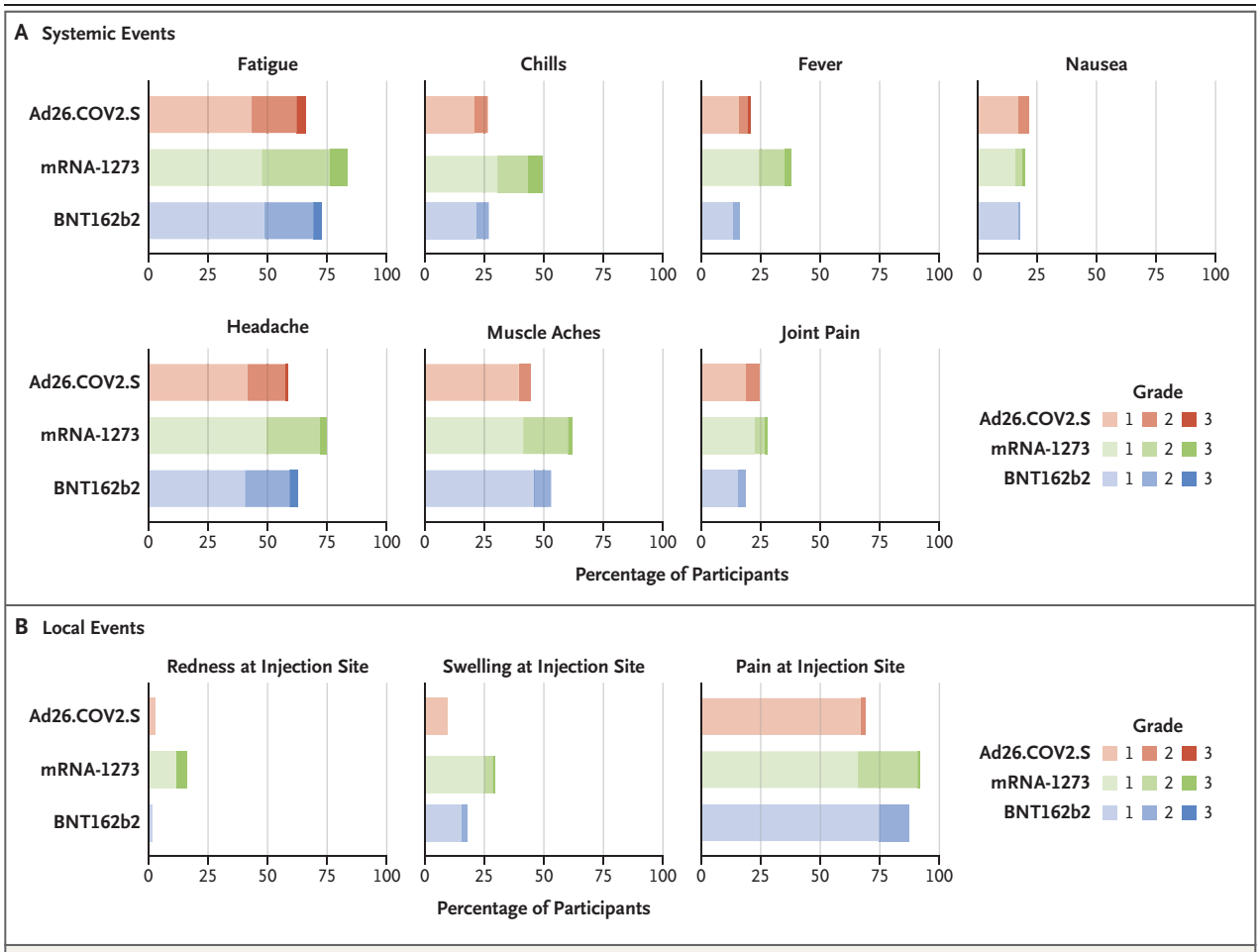


Figure 4. Severity of Systemic and Local Reactions after Booster Vaccination.

Panel A shows the percentage of participants with systemic reactions (fatigue, chills, fever, nausea, headache, muscle aches, or joint pain), and Panel B shows the percentage of participants with local reactions (redness, swelling, or pain at the injection site) after booster vaccination. These reactions were monitored in the 7 days after the administration of the booster. P values for the between-group differences in the distribution of adverse events are shown in Table S7.

A potential limitation of our trial is that we evaluated boosters in health care workers who were generally younger than participants in other studies of homologous vaccination regimens, and our participants did not have severe coexisting conditions. However, previous studies of homologous vaccination regimens showed similar immunogenicity in younger adults (18 to 55 years of age) and older adults (>55 years).[33-35] Furthermore, we evaluated booster vaccinations that were administered 3 months after the priming vaccination, as previously described in heterologous regimens,[36] but the most appropriate prime–boost interval remains to be determined. Results of trials of Ad26.COV2.S vaccine that were published after the inception of our trial showed that prime–boost intervals in homologous regimens varied from 2 to 6 months[6,37]; these findings suggest that late boosting might be more effective than early boosting. Finally, we assessed immunogenicity 28 days after the administration of a booster, but the further development of immune responses, as previously described,[5,37] and the longevity of responses remain to be evaluated.

Single-shot Ad26.COV2.S vaccination adequately primes the immune system. We found that in the face of waning immunity and circulation of SARS-CoV-2 variants, these responses were boosted most efficiently with mRNA-based

The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.

vaccines. Boosters probably increase vaccine effectiveness against infection with and transmission of SARS-CoV-2; however, study data on their added effectiveness against severe disease are limited. In discussions regarding the use of boosters, the prime–boost interval target population, level of SARS CoV-2 circulation, and global inequity in access to vaccines should be considered.

Supported by a grant (10430072110001) from the Netherlands Organization for Health Research and Development ZonMw.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

APPENDIX

The authors' affiliations are as follows: the Departments of Internal Medicine (R.S.G.S., M.L.), Hospital Pharmacy (R.S.G.S., W.J.R.R., P.H.M.K.), and Viroscience (D.G., K.S.S., M.P.G.K., C.H.G., R.D.V.) and the Department of Internal Medicine, Division of Allergy and Clinical Immunology, and Department of Immunology (V.A.S.H.D.), Erasmus University Medical Center, Rotterdam, the Center of Tropical Medicine and Travel Medicine, Department of Infectious Diseases (A.G., H.M.G.G.), and the Department of Experimental Immunology, Amsterdam University Medical Centers, Amsterdam Institute for Infection and Immunity, University of Amsterdam (N.A.K.), Amsterdam, the Department of Internal Medicine and Infectious Diseases (D.F.P.), and the Department of Medical Microbiology and Infection Prevention, University Medical Center Groningen, University of Groningen (A.L.W.H., D.B.), Groningen, the Department of Infectious Diseases, Leiden University Medical Center, Leiden (L.G.V.), and the Center for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven (D.B.) — all in the Netherlands.

REFERENCES

1. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med 2020;383:2603-15.

2. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med 2021; 384:403-16.

3. Sadoff J, Gray G, Vandebosch A, et al. Safety and efficacy of single-dose Ad26. COV2.S vaccine against Covid-19. N Engl J Med 2021;384:2187-201.

4. Voysey M, Clemens SAC, Madhi SA, 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 2021;397:99-111.

5. Barouch DH, Stephenson KE, Sadoff J, et al. Durable humoral and cellular immune responses 8 months after Ad26. COV2.S vaccination. N Engl J Med 2021; 385:951-3.

6. Sadoff J, Le Gars M, Cardenas V, et al. Durability of antibody responses elicited by a single dose of Ad26.COV2.S and substantial increase following late boosting. August 26, 2021 (https://www.medrxiv.org/content/10.1101/2021.08.25.21262569v1). preprint.

7. van Gils MJ, Lavell AHA, van der Straten K, et al. Four SARS-CoV-2 vaccines induce quantitatively different antibody responses against SARS-CoV-2 variants. September 28, 2021 (https://www.medrxiv.org/content/10.1101/2021.09.27.21264163v1). preprint.

8. Collier A-RY, Yu J, McMahan K, et al. Differential kinetics of immune responses elicited by Covid-19 vaccines. N Engl J Med 2021;385:2010-2.

9. Sablerolles RSG, Goorhuis A, Geurtsvan-Kessel CH, et al. Heterologous Ad26.COV2.S prime and mRNA-based boost COVID-19 vaccination regimens: the SWITCH Trial protocol. Front Immunol 2021;12:753319.

10. Spencer AJ, McKay PF, Belij-Rammerstorfer S, et al. Heterologous vaccination regimens with self-amplifying RNA and adenoviral COVID vaccines induce robust immune responses in mice. Nat Commun 2021;12:2893.

11. He Q, Mao Q, An C, et al. Heterologous prime-boost: breaking the protective immune response bottleneck of COVID-19 vaccine candidates. Emerg Microbes Infect 2021;10:629-37.

12. Duarte-Salles T, Prieto-Alhambra D. Heterologous vaccine regimens against COVID-19. Lancet 2021;398:94-5.

13. Hacisuleyman E, Hale C, Saito Y, et al. Vaccine breakthrough infections with SARS-CoV-2 variants. N Engl J Med 2021; 384:2212-8.

14. Krause PR, Fleming TR, Longini IM, et al. SARS-CoV-2 variants and vaccines. N Engl J Med 2021;385:179-86.

15. Liu X, Shaw RH, Stuart ASV, et al. Safety and immunogenicity of heterologous versus homologous prime-boost schedules with an adenoviral vectored and mRNA COVID-19 vaccine (Com-COV): a single-blind, randomised, non-inferiority trial. Lancet 2021;398:856-69.

16. Hillus D, Schwarz T, Tober-Lau P, 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 2021;9:1255-65.

17. Borobia AM, Carcas AJ, Pérez-Olmeda M, et al. Immunogenicity and reactogenicity of BNT162b2 booster in ChAdOx1-S-primed participants (CombiVacS): a multicentre, open-label, randomised, controlled, phase 2 trial. Lancet 2021;398:121-30.

18. Shaw RH, Stuart A, Greenland M, Liu X, Nguyen Van-Tam JS, Snape MD. Heterologous prime-boost COVID-19 vaccination: initial reactogenicity data. Lancet 2021;397:2043-6.

19. Atmar RL, Lyke KE, Deming ME, et al. Heterologous SARS-CoV-2 booster vaccinations — preliminary report. October 15, 2021 (https://www.medrxiv.org/content/10.1101/2021.10.10.21264827v2). preprint.

20. Food and Drug Administration. Toxicity grading scale for healthy adults and adolescent volunteers enrolled in preventive vaccine clinical trials. Guidance for industry. September 2007 (https://www.fda.gov/media/73679/download).

21. Mahmoud SA, Ganesan S, Bissar S, Zamil I, Zaher WA. Evaluation of serological tests for detecting SARS-CoV-2 antibodies: implementation in assessing post vaccination status. April 30, 2021 (https://www.medrxiv.org/content/10.1101/2021.04.27.21256205v1). preprint.

22. Leuzinger K, Osthoff M, Dräger S, et al. Comparing immunoassays for SARS-CoV-2 antibody detection in patients with and without laboratory-confirmed SARS-CoV-2 infection. J Clin Microbiol 2021;59: e0138121.

23. Sanders J-SF, Bemelman FJ, Messchendorp AL, et al. The RECOVAC Immune-Response Study: the immunogenicity, tolerability and safety of COVID-19 vaccination in patients with chronic kidney disease, on dialysis, or living with a kidney transplant. Transplantation 2021 November 19 (Epub ahead of print).

24. Goel RR, Painter MM, Apostolidis SA, et al. mRNA vaccines induce durable immune memory to SARS-CoV-2 and variants of concern. Science 2021;374:abm0829.

The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.

25. Krammer F. A correlate of protection for SARS-CoV-2 vaccines is urgently needed. Nat Med 2021;27:1147-8.
26. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med 2021;27:1205-11.
27. Mlcochova P, Kemp SA, Dhar MS, et al. SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. Nature 2021;599:114-9.
28. Planas D, Veyer D, Baidaliuk A, et al. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. Nature 2021;596:276-80.
29. Alter G, Yu J, Liu J, et al. Immunogenicity of Ad26.COV2.S vaccine against SARS-CoV-2 variants in humans. Nature 2021;596:268-72.
30. Tan AT, Lim JM, Le Bert N, et al. Rapid measurement of SARS-CoV-2 spike T cells in whole blood from vaccinated and naturally infected individuals. J Clin Invest 2021;131:131.
31. Geers D, Shamier MC, Bogers S, et al. SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. Sci Immunol 2021;6:eabj1750.
32. GeurtsvanKessel CH, Geers D, Schmitz KS, et al. Divergent SARS CoV-2 omicron-specific T- and B-cell responses in COVID-19 vaccine recipients. December 29, 2021 (https://www.medrxiv.org/content/10.1101/2021.12.27.21268416v1). preprint.
33. Anderson EJ, Rouphael NG, Widge AT, et al. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. N Engl J Med 2020;383:2427-38.
34. Walsh EE, Frenck RW Jr, Falsey AR, et al. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. N Engl J Med 2020;383:2439-50.
35. Ramasamy MN, Minassian AM, Ewer KJ, et al. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. Lancet 2021;396:1979-93.
36. Voysey M, Costa Clemens SA, Madhi SA, et al. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. Lancet 2021;397:881-91.
37. Stephenson KE, Le Gars M, Sadoff J, et al. Immunogenicity of the Ad26.COV2.S vaccine for COVID-19. JAMA 2021;325:1535-44.
Copyright © 2022 Massachusetts Medical Society.

The New England Journal of Medicine
Downloaded from nejm.org on January 21, 2022. For personal use only. No other uses without permission.
Copyright © 2022 Massachusetts Medical Society. All rights reserved.