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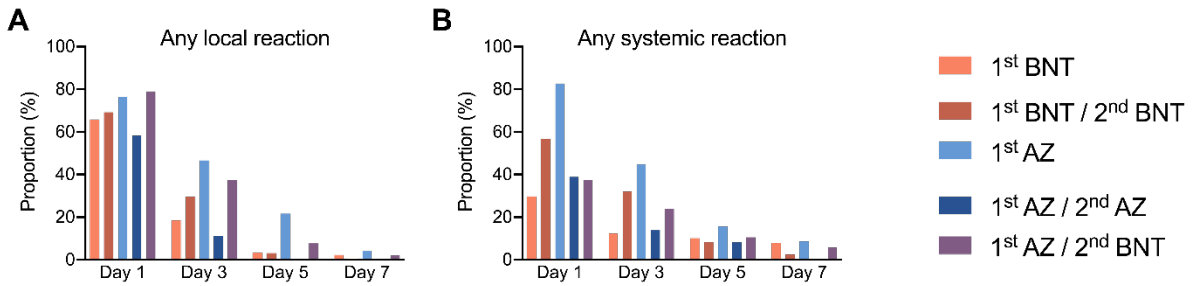
Supplementary appendix

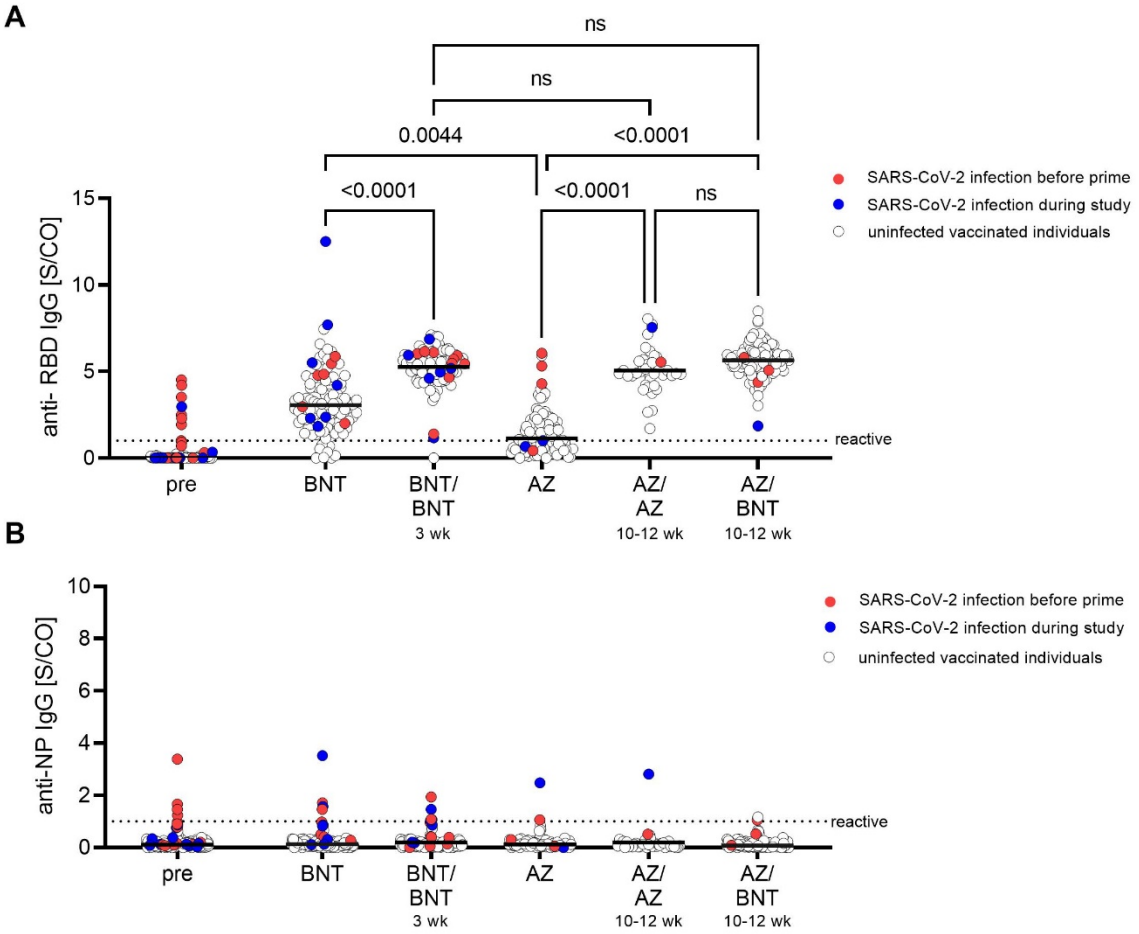
This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: 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; published online August 12. http://dx.doi.org/10.1016/S2213-2600(21)00357-X.

Supplementary Material



Supplementary figure 1: Reactogenicity of BNT or AZ prime immunisation and homologous or heterologous booster vaccination until day seven after vaccination.
(**A**) Local reactions (any severity) reported by day, over the course of seven days. (**B**) Systemic reactions (any severity) reported by day, over the course of seven days.



Supplementary figure 2: SARS-CoV-2 specific antibody response in SARS-CoV-2 infected and uninfected vaccinated individuals

Anti-RBD IgG (**A**) and anti-nucleocapsid IgG (**B**) in serum of uninfected vaccinated (white dots), previously SARS-CoV-2 infected vaccinated (red dots) and vaccinated during the study infected (blue dots) individuals measured by SeraSpot Anti-SARS-CoV-2 IgG assay. BNT: BNT162b2 / Comirnaty; AZ: ChAdOx1-nCoV19 / Vaxzevria; IgG: Immunoglobulin G; S/CO: signal-to-cutoff ratio, NP: nucleocapsid protein, ns: not significant, wk: week. Sampling time points: pre: pre-immune sample prior to first immunisation; prime: three weeks after first vaccination; boost: three to four weeks after boost vaccination. The dotted line indicates the manufacturer's pre-specified threshold (>1 S/Co). Lines indicate the median. P-values are indicated.

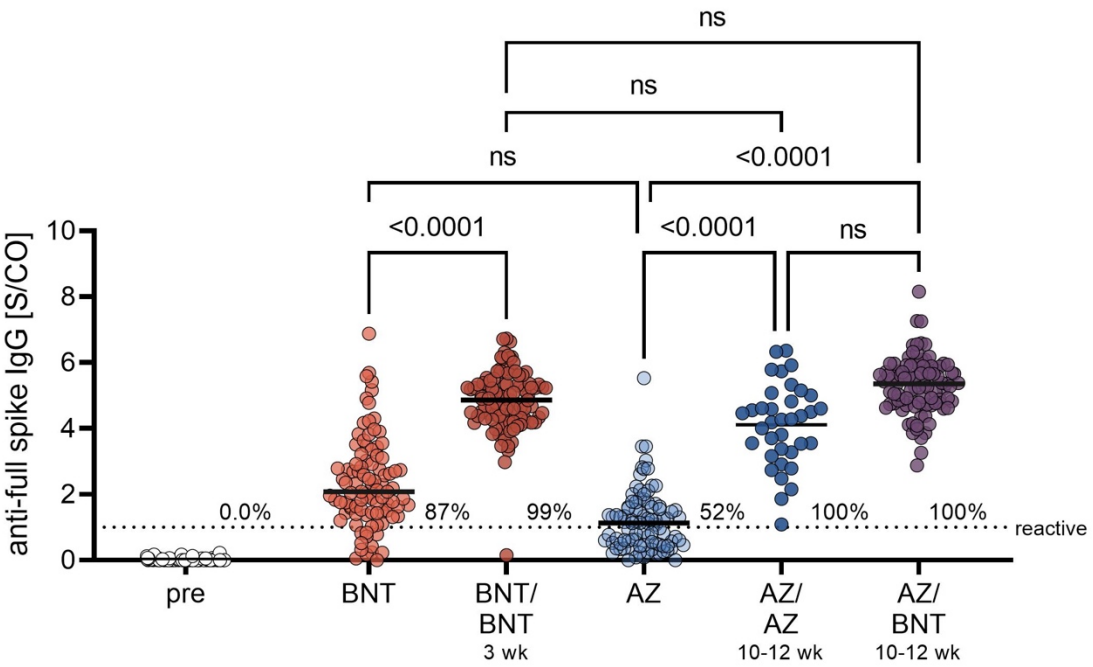


Figure S3: Serum anti-full spike IgG response after BNT or ChAdOx prime immunisations and homologous or heterologous booster vaccination. Anti-full spike- IgG in serum measured by SeraSpot Anti-SARS-CoV-2 IgG assay. BNT: BNT162b2 / Comirnaty; AZ: ChAdOx1-nCoV19 / Vaxzevria; IgG: Immunoglobulin G; S/CO: signal-to-cutoff ratio, ns: not significant, wk: week. Sampling time points: pre: pre-immune sample prior to first immunisation; prime: three weeks after first vaccination; boost: three to four weeks after boost vaccination. The dotted line indicates the manufacturer's pre-specified threshold (>1 S/Co). Lines indicate the median. P-values are indicated. BNT: BNT162b2 / Comirnaty; AZ: ChAdOx1-nCoV19 / Vaxzevria; IgG: Immunoglobulin G; S/CO: signal-to-cutoff ratio, ns: not significant, wk: week. Sampling time points: pre: pre-immune sample prior to first immunisation; prime: three weeks after first vaccination; boost: three to four weeks after boost vaccination. The dotted line indicates the manufacturer's pre-specified threshold (>1 S/Co). Lines indicate the median. P-values are indicated.

Prime and boost vaccination	**1st BNT, n=179**	**1st BNT / 2nd BNT n=174**	**1st AZ n=151**	**1st AZ / 2nd AZ, n=38**	**1st AZ / 2nd BNT, n=107**
Prime to boost interval, median days (IQR)	-	21 (21-21)	-	83 (71-84)	71 (70-73)
Serology data measured, n	107	116	98	38	99
Seropositivity for spike at baseline or NP at baseline or follow-up	7	8	2	2	1
Active SARS-CoV-2 infection	6	7	4	-	4

Table S1: Sample numbers for serology including individuals with seropositivity for spike at baseline or NP at baseline or follow-up and active SARS-CoV-2 infection.

Local reaction	1st BNT	1st BNT / 2nd BNT	1st AZ	1st AZ / 2nd AZ	1st AZ / 2nd BNT
Local reaction (any severity), %	69(62 - 75.4) [123/178]	74 (66.9 - 80.4) [118/159]	81 (74 - 86.6) [120/148]	58 (42.2 - 72.9) [21/36]	84 (75.4 - 89.5) [87/104]
Local reaction (only severe), %	2 (0.5 - 4.8) [3/178]	4 (1.7 - 8) [6/159]	3 (1.5 - 7.7) [5/148]	3 (0.1 - 14.2) [1/36]	7 (3.3 - 13.2) [7/104]
Pain at injection site (any severity), %	62 (55.1 - 69.1) [111/178]	70 (62.3 - 76.4) [111/159]	78 (70.3 - 83.7) [115/148]	53 (37 - 68) [19/36]	79 (70 - 85.6) [82/104]
Tenderness (any severity), %	69 (61.4 - 74.9) [122/178]	74 (66.2 - 79.8) [117/159]	80 (73.3 - 86) [119/148]	58 (42.2 - 72.9) [21/36]	83 (74.3 - 88.8) [86/104]
Swelling (any severity), %	4 (1.9 - 7.9) [7/178]	8 (4.4 - 12.7) [12/159]	3 (1.5 - 7.7) [5/148]	6 (1 - 18.1) [2/36]	6 (2.7 - 12) [6/104]
Redness (any severity), %	2 (0.9 - 5.6) [4/178]	2 (0.5 - 5.4) [3/159]	3 (1.1 - 6.7) [4/148]	3 (0.1 - 14.2) [1/36]	7 (3.3 - 13.2) [7/104]
Systemic reaction					
Systemic reaction (any severity), %	39 (31.9 - 46.1) [69/178]	65 (57.1 - 71.8) [103/159]	87 (80 - 91.1) [128/148]	39 (24.8 - 55.1) [14/36]	49 (39.6 - 58.5) [51/104]
Systemic reaction (only severe), %	1 (0 - 3.1) [1/178]	6 (3.5 - 11.2) [10/159]	23 (16.9 - 30.4) [34/148]	6 (1 - 18.1) [2/36]	2 (0.3 - 6.7) [2/104]
Headache (any severity), %	17 (12.5 - 23.7) [31/178]	39 (31.8 - 46.7) [62/159]	75 (67.5 - 81.3) [111/148]	22 (11.7 - 38.1) [8/36]	35 (26.2 - 44.2) [36/104]
Fatigue (any severity), %	24 (18.5 - 30.9) [43/178]	48 (40.2 - 55.5) [76/159]	73 (65.3 - 79.5) [108/148]	33 (20.2 - 49.7) [12/36]	36 (27 - 45.1) [37/104]
Feverishness & chills (any severity), %	9 (5.6 - 14.1) [16/178]	20 (14.1 - 26.3) [31/159]	70 (62.5 - 77) [104/148]	22 (11.7 - 38.1) [8/36]	18 (12 - 26.8) [19/104]
Myalgia (any severity), %	21 (15.5 - 27.3) [37/178]	34 (27.1 - 41.6) [54/159]	55 (46.7 - 62.5) [81/148]	14 (6.1 - 28.7) [5/36]	20 (13.6 - 28.9) [21/104]
Arthralgia (any severity), %	4 (1.9 - 7.9) [7/178]	10 (6.3 - 15.7) [16/159]	30 (23 - 37.5) [44/148]	19 (9.8 - 35) [7/36]	7 (3.3 - 13.2) [7/104]
Nausea & vomiting (any severity), %	4 (1.9 - 7.9) [7/178]	4 (2.1 - 8.8) [7/159]	16 (10.6 - 22.2) [23/148]	3 (0.1 - 14.2) [1/36]	6 (2.7 - 12) [6/104]
Diarrhoea (any severity), %	4 (1.9 - 7.9) [7/178]	6 (3.5 - 11.2) [10/159]	7 (4.2 - 12.8) [11/148]	6 (1 - 18.1) [2/36]	4 (1.5 - 9.5) [4/104]
Antipyretic medication					
Intake within first 24 hours, %	10 (6 - 14.8) [17/178]	23 (16.8 - 29.7) [36/159]	73 (65.3 - 79.5) [108/148]	19 (9.8 - 35) [7/36]	32 (23.6 - 41.2) [33/104]
Prophylactic intake, %	3 (1.2 - 6.4) [5/178]	3 (1.4 - 7.1) [5/159]	27 (20.5 - 34.7) [40/148]	3 (0.1 - 14.2) [1/36]	5 (2.1 - 10.8) [5/104]

Table S2: Local and systemic reactogenicity of BNT or AZ prime immunisations and homologous or heterologous boosting until day 7 after vaccination.

Proportion of participants reporting local and systemic reactions and intake of antipyretic medication per group (95% CI). 95% confidence intervals were calculated according to the Wilson and Brown method.

Detection of anti-SARS-CoV-2 Antibodies

SARS-CoV-2 specific IgG antibodies to the spike and nucleocapsid protein were measured by using a solid phase immunoassay as recommended by the manufacturer's instructions (SeraSpot®Anti-SARS-CoV-2 IgG, Seramun Diagnostica, Heidsee, Germany) and as described before [1] [2]. Briefly, four recombinant SARS-CoV-2 antigens (nucleocapsid, spike receptor binding domain (RBD), S1 domain and complete spike) and internal controls are printed as spots on the bottom of each well. Serum samples were diluted 1:101 with sample dilution buffer, placed to the well and incubated for 30 min at 37°C. After a washing step, horseradish peroxidase-labeled anti-human IgG was added to the bound antibodies and incubated for 30 min at 37°C. After a further washing step, each well was incubated for 15 min in the dark with 3′3,5,5-tetramethylbenzidine (TMB) solution. Following a stop solution step, colour intensity of each spot was measured by SpotSight plate scanner. Results were normalized as signal-to cutoff (S/Co) ratio by dividing the measured signal strength by that of an internal cutoff control. S/Co ratio above 1 was considered as reactive by the manufacturer.

Detection of IgG antibody avidity

Maturation of IgG avidity was analyzed in serum of 30 randomly picked AZ prime, BNT prime, homologous BNT/BNT boost, homologous AZ/AZ boost and heterologous AZ/BNT boost immunised participants as previously described [1]. Briefly, avidity testing was done utilizing an anti-SARS-CoV-2 ELISA Kit (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany). All serum samples were diluted 1:101 in sample buffer, pipetted to SARS-CoV-2 spike protein precoated plate, and incubated at 37°C for 60 minutes. Afterwards, the plate was washed once with washing buffer and 200 µl urea or PBS was placed into the well. Following incubation at 37°C for 10 min, the plate was washed three times, 100 µl of the conjugate solution was added, and incubated at 37°C for 30 minutes. After a further washing step, substrate solution was addedand incubated at room temperature for 30 minutes in the dark. Then stop solution was added into each well and the optical density (OD) at 450 nm and a reference wavelength at 620 nm was detected by plate reader Tecan Infinite 200pro (Tecan, Männedorf, Switzerland). Relative avidity index in percent was calculated by following formula: (OD of sample treated with urea / OD of sample treated without PBS) x 100. Avidity indices between 40-60% were considered as borderline avidity and above 60% as high avidity.

Detection of neutralizing antibodies by surrogate SARS-CoV-2 neutralization test (sVNT)

Neutralizing capacity was measured by a commercially available ELISA-based SARS-CoV-2 sVNT according to the manufacturer's instructions (cPass/GenScript, medac GmbH, Wedel, Germany) [1] [2] [3]. Briefly, internal positive, negative control and sera were diluted 1:10 with sample buffer and preincubated 1:1 with HRP-RBD solution for 30 min at 37°C. Afterwards, each mixture was pipetted to human ACE2 receptor protein (hACE2) coated microplates and incubated for 15 min at 37°C. Following a washing step, TMB solution was added and incubated in the dark for 15 min at room temperature. By adding the Stop solution the reaction was stopped and the absorbance was measured immediately by 450 nm using Tecan Infinite 200pro plate reader. ACE2/RBD binding inhibition was calculated as follows: (1-(OD value of sample / OD value of negative control) x 100 %. A signal inhibition >30% was considered as positive by the manufacturer.

Measuring serum neutralization titers using a SARS-CoV-2 pseudovirus neutralization assay

SARS-CoV-2 pseudovirus particles were generated by co-transfection of individual plasmids encoding HIV-Tat, HIV-Gag/Pol, HIV-Rev, firefly luciferase followed by an IRES and ZsGreen [4], and the SARS-CoV-2 spike protein into HEK 293T cells using the FuGENE® 6 Transfection Reagent (Promega). Spike sequences from the B.1.1.7 (alpha) variant and B.1.351 (beta) variants were used for testing serum neutralization as previously described [5]. Briefly, virus supernatants were harvested at 48 h and 72 h post transfection and stored at -80°C till use. The harvested pseudoviruses were titrated by infecting 293T cells expressing ACE2 (293T-ACE2), and after a 48-hour incubation at 37°C and 5% CO2, luciferase activity was determined by addition of luciferin/lysis buffer (10 mM MgCl2, 0.3 mM ATP, 0.5 mM Coenzyme A, 17 mM IGEPAL (all Sigma-Aldrich), and 1 mM D-Luciferin (GoldBio) in Tris-HCL) and measured on a microplate reader (Berthold). Pseudovirus dilutions resulting in relative luminescence units (RLU) of approximately 1000-fold in infected vs. non-infected cells were selected for neutralization assays. Serum samples were heat inactivated at 56°C for 45 min and serial dilutions of serum were co-incubated with pseudovirus supernatants for 1 h at 37°C, following which 293T-ACE-2 cells were added. After a 48-hour incubation at 37°C and 5% CO2, luciferase activity was determined by adding luciferin/lysis buffer. After subtracting background RLUs of non-infected cells, 50% inhibitory dilutions (ID50s) were calculated as the serum dilution resulting in a 50% reduction in RLU compared to the untreated virus control wells by plotting dose response curves in GraphPad Prism 7.0.

Detection of SARS-CoV-2 specific T cells by interferon-γ (IFN-γ) release assay (IGRA)

SARS-CoV-2 specific T cell responses were assessed by a commercially available Interferon-γ (IFN) Release assay (IGRA, Medizinische Labordiagnostika AG, Lübeck, Germany) as previously described [1 2]. Briefly, lithium-heparin blood samples were collected from 75 AZ prime, 34 AZ/AZ boost, 91 AZ/BNT boost and 66 BNT/BNT immunised individuals. For each participant, 0.5 ml of blood was added into each of the Euroimmun IGRA CoV-2 tubes containing SARS-CoV-2 spike S1 domain peptide pool, mitogen as a positive control and nil as negative control. Then the tubes were shaken six times and incubated by 37°C for 24 h. Afterwards, whole blood was centrifuged at 12.000g for 10 min to separate the supernatant. IFN-γ concentration in the supernatant was measured by ELISA using the fully Euroimmun Analyzer I. IFN-γ concentration in the negative control was used as a measure of patient IFN-γ background and was subtracted from the IFN-γ concentration in the stimulated mitogen and S1 domain tubes.

References

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