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Supplementary appendix

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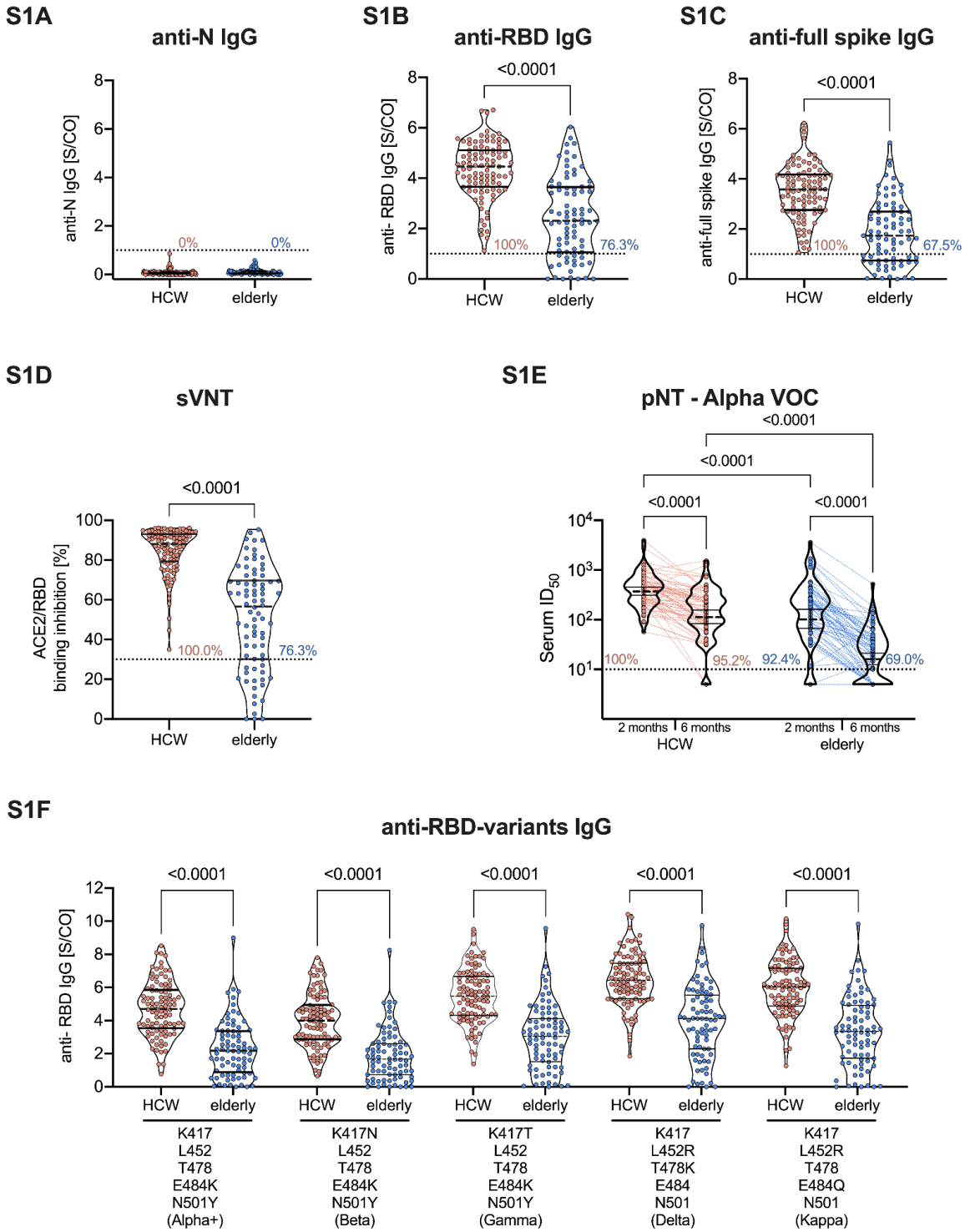
Supplement to: Tober-Lau P, Schwarz T, Vanshylla K, et al. Long-term immunogenicity of BNT162b2 vaccination in older people and younger health-care workers. *Lancet Respir Med* 2021; published online Oct 20. http://dx.doi.org/10.1016/S2213-2600(21)00456-2.

Appendix

Figure S1



Tober-Lau, Schwarz, Vanshylla, Hillus et al. (2021), Long-term immunogenicity of BNT162b2 vaccination in the elderly and in younger health care workers

Figure S1: Anti-SARS-CoV-2 nucleocapsid protein, RBD, and full spike IgG response at sixth-month follow-up in healthcare workers (HCW) and elderly persons vaccinated with BNT162b2. Study participants were enrolled at Charité - Universitätsmedizin Berlin (HCW, n=107) and an assisted living facility in Berlin (elderly, n=82), Germany. Twelve participants with a history of infection with SARS-CoV-2 (10 HCW and 2 elderly) were excluded from the analysis. (**A**) Anti-SARS-CoV-2 N, (**B**) RBD- (**C**) and full-spike IgG measured in the serum of BNT162b2 vaccinated HCW and elderly persons at six-month follow-up, 5 months after completion of the two-dose regimen. (**D**) Neutralizing capacity was measured by sVNT and (**E**) serum neutralization against Alpha (B.1.1.7) VOC detected by pNT in vaccinated HCW and elderly persons at six-month follow-up. (**F**) Binding capacity of serum IgG against six different RBDs of SARS-CoV-2 variants carrying the indicated mutations in HCW and elderly, measured by ELISA. Dotted lines indicate the manufacturer's threshold values: for anti-N, anti-RBD, and anti-full spike IgG ≥1 S/Co, for sVNT >30%, and the lower limit of detection (1:10 dilution) for pNT. Lines indicate the median and interquartile range except for pNT, where the geometric mean and 95% confidence interval are shown. P values were calculated by the non-parametric Mann Whitney *U* test or Kruskal-Wallis test with Dunn's multiple comparisons test. S/Co: signal-to-cutoff, N: nucleocapsid protein, RBD: receptor-binding domain, sVNT: surrogate virus neutralization test, ACE2: angiotensin-converting enzyme 2, ID_{50}: 50% inhibition dilution.

Appendix Table 1

	no infection				history of SARS-CoV-2 infection*			
	HCW		Elderly		HCW		Elderly	
Number of participants	97		80		10		2	
Sex								
Female (n, %)	59	60.8%	59	73.8%	6	60%	2	100%
Male (n, %)	38	39.2%	21	26.3%	4	40%	0	0%
Age	34	30-49	82	78-87	36	30-39	87	84-89
Included in analysis (n, %)	97	100%	80	100%	0	0%	0	0%
Dosing interval (days)	21	21-21	21	21-21	21	21-21	21	21-21
Sampling interval 2 months post 1st vaccination (days)	49	48-52	47	46-48	49	48-58	46	46-46
Sampling interval 6 months post 1st vaccination (days)	166	161-168	174	172-175	165	161-169	174	174-174
Comorbidities								
Cardiovascular disease (n, %)	14	14,4%	64	80%	1	10%	2	100%
Type 2 diabetes (n, %)	3	3.1%	16	20%	0	0%	1	50%
Respiratory disease (n, %)	16	16.5%	17	21.3%	0	0%	0	0%
Active solid malignancy (n, %)	0	0%	5	6.3%	0	0%	0	0%
Active haematological malignancy (n, %)	0	0%	4	5%	0	0%	0	0%
Immunodeficiency/ -suppression (n, %)	3	3.1%	5	6.3%	1	10%	0	0%

Characteristics of the elderly and healthcare workers (HCW) cohorts who completed six-month follow-up visits. All data are presented as median (IQR), unless indicated otherwise. *History of SARS-CoV-2 infection at baseline or before 2nd vaccination, determined by serology or PCR test. These patients were excluded from immunogenicity analysis.

Appendix Table 2

		Proportion of positive outcome (95%CI)			Outcome values median [IQR]		
time point	**test system**	**HCW**	**elderly**	**p value***	**HCW**	**elderly**	**p value #**
6-month follow-up	anti-S1 (S/CO)	97.9 (92.8-99.7) [2/97]	60.0 (48.4-70.8) [48/80]	<0.0001	3.2 (2.4-4.1)	1.2 (0.5-2.2)	<0.0001
	anti-RBD (S/CO)	100 (96.3-100) [97/97]	76.3 (65.4-85.1) [61/80]	<0.0001	4.5 (3.7-5.1)	2.3 (1.1-3.6)	<0.0001
	anti-spike full (S/CO)	100 (96.3-100) [97/97]	67.5 (56.1-77.6) [54/80]	<0.0001	3.6 (2.8-4.2)	1.7 (0.7-2.7)	<0.0001
	anti-N (S/CO)	0.0 (0.0-3.7) [0/97]	0.0 (0.0-4.5) [0/80]	-	0.1 (0.0-0.1)	0.1 (0.0-0.1)	-
	pNT delta (ID_{50})	95.2 (88.1-98.7) [79/83]	60.6 (48.3-72.0) [43/71]	<0.0001	72.7 (58.7-89.9) §	14.5 (11.5-18.2) §	<0.0001≠
	pNT alpha (ID_{50})	95.2 (88.1-98.7) [79/83]	69.0 (56.9-79.5) [49/71]	<0.0001	134.4 (104.2-173.4) §	20.2 (15.3-26.5) §	<0.0001≠
	sVNT (%)	100 (96.3-100) [97/97]	76.3 (65.4-85.1) [61/80]	<0.0001	88.1 (79.3-93.1)	56.6(30.1-59.6)	<0.0001
	anti-RBD alpha+ (S/CO)	-	-	-	4.7 (3.5-5.9)	2.2 (0.9-3.4)	<0.0001
	anti-RBD beta (S/CO)	-	-	-	4.0 (2.9-4.9)	1.7 (0.7-2.6)	<0.0001
	anti-RBD gamma (S/CO)	-	-	-	5.5 (4.3-6.7)	3.1 (1.5-4.1)	<0.0001
	anti-RBD delta (S/CO)	-	-	-	6.4 (5.3-7.5)	4.1 (2.3-5.5)	<0.0001
	anti-RBD kappa (S/CO)	-	-	-	6.0 (4.9-7.2)	3.3 (1.7-4.9)	<0.0001
	IGRA (mIU/ml)	-	-	-	1198.0 (593.9-2534.0)	261.6 (141.5-828.6)	<0.0001
2-month follow-up	pNT delta (ID_{50})	100 (94.7-100) [69/69]	90.9 (81.3-96.6) [60/66]	0.0121	382.1 (308.5-473.3) §	90.6 (63.9-128.2) §	<0.0001≠
	pNT alpha (ID_{50})	100 (94.9-100) [70/70]	95.2 (88.1-98.7) [61/66]	0.0248	390.7 (323.7-471.5) §	135.0 (93.9-194.1) §	<0.0001≠

Proportion of positive outcome and outcome values in the different test systems. S/CO: signal-to-cutoff ratio, S1: spike subdomain 1, RBD: receptor-binding domain, N: nucleocapsid protein, pNT: pseudovirus neutralization assay, IC_{50}: 50% inhibitory dilution, ACE2: angiotensin-coverting enzyme 2, IGRA: interferon-gamma release assay, IU: international units

* p-value was calculated by Fisher's exact test

p-value was calculated by the nonparametric Mann Whitney U test

§ geometric mean and 95%CI are shown

≠ p-value was calculated by the nonparametric Kruskal-Wallis test with Dunn's multiple comparisons test

Appendix Table 3

	HCW	elderly	p value
pNT delta	239.2 (143.4-347.8)	58.3 (25.2-154.5)	<0.0001
pNT alpha	323.7 (174.2-463.5)	103.8 (37.8-191.4)	<0.0001

Change in neutralization activity against SARS-CoV-2 delta and alpha based on area under the curve (AUC) calculations. p-value was calculated by the nonparametric Mann Whitney U test.

EICOV/COVIM Study group

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Supplementary Note

In this prospective cohort study, we investigated the durability of serological responses to COVID-19 mRNA vaccine BNT162b2 in a cohort of elderly persons and compared them to young HCW. We observed markedly reduced serum IgG responses to SARS-CoV-2 Spike and RBD, reduced sVNT titers, and reduced neutralizing titers against the Alpha and Delta VOCs, as measured by pNT (**Fig. 1, Appendix Fig. 1**). In addition, we assessed spike-specific T cells by Interferon gamma release assay (IGRA) and found a markedly reduced response in the elderly compared to HCW (**Fig. 1C**). However, memory B cells as well as memory T cells may be present despite reduced antibody levels,[5] and these were not investigated in this study. Circulating antigen-specific memory B cells are rare, their detection is technically challenging and the measurements are therefore usually restricted to smaller exploratory studies. The durability of vaccine-induced memory B cell and T cell responses in the elderly needs to be investigated in detail. Epidemiological evidence of waning protection, particularly in the elderly[6], may suggest insufficient immune memory to the two-dose vaccine regimen in this population.[5] The occurrence of Delta VOC has clearly contributed to increased rates of breakthrough infections.[7] However, there is clear epidemiological evidence demonstrating an increase of breakthrough infection rates with the time elapsed since vaccination[6,8,9], indicating that both the occurrence of Delta VOC, and time-dependent waning of immunity contribute to declining vaccine effectiveness. Our study complements the observation of time-dependent waning of protection, particularly in the elderly, supporting additional booster vaccinations for this risk population.

Methods

Study participants

Study participants were recruited in the EICOV, COVIMMUNIZE, and COVIM studies, three prospective cohort studies conducted under the auspices of Charité - Universitätsmedizin Berlin, Germany, in accordance with the Declaration of Helsinki and Good Clinical Practice.[1,2] EICOV and COVIMMUNIZE studies were approved by the local ethics committee of Charité - Universitätsmedizin Berlin (EA4/244/20, EA4/245/20), and COVIM was approved by the Federal Institute for Vaccines and Biomedicines (Paul Ehrlich Institute) and by the ethics committee of the state of Berlin (EudraCT-2021–001512–28). Written informed consent was obtained by all participants, according to local and national regulations.
HCW were recruited at Charité - Universitätsmedizin Berlin hospital, Berlin, Germany. Participants were eligible for inclusion if they were a) >18 years of age, b) employed at Charité - Universitätsmedizin Berlin, and c) received 2 doses of COVID-19 vaccine BNT162b2. Elderly cohort study participants were enrolled at an assisted-living facility in Berlin, Germany. Participants were eligible for inclusion if they were >70 years of age. All participants were largely self-sustained, and there were no cases of dementia or bedridden participants.
Blood sampling was conducted before 1st vaccination, 4±1 weeks after the 1st and 2nd vaccinations, respectively, and 6±1 months after the 1st vaccination. SARS-CoV-2 reverse transcription PCR (RT-PCR) of oropharyngeal swabs were performed during study visits in order to detect concomitant infections. Study participants were eligible for analysis of immunogenicity if they had completed a course of two vaccinations with BNT162b2 and the first dose was administered at least 22 weeks before the 6-month visit.
Ten HCW and two elderly participants were excluded from the analysis due to pre-existing anti-SARS-CoV-2 spike or nucleocapsid antibodies before 1st vaccination (n=6) or presence of anti-nucleocapsid antibodies 4±1 weeks after the 1st and before the 2nd vaccination (n=2). Additionally, 4 HCW presented with PCR-confirmed SARS-CoV-2 infection before 2nd vaccination. In the remaining 97 HCW and 80 elderly participants, no evidence of a SARS-CoV-2 infection was detected by PCR or antibody tests during the observation period.

Antibody assessment and Interferon-γ Release of SARS-CoV-2–Specific T Cells

Blood samples were tested for anti-SARS-CoV-2 antibodies, neutralizing capacity, and T cell reactivity as previously described.[2] In brief, SARS-CoV-2 specific antibodies were quantified using the commercially available SeraSpot® Anti-SARS-CoV-2 IgG microarray-based immunoassay including nucleocapsid and spike as antigens (Seramun Diagnostica GmbH, https://www.seramun.com), allowing for differentiation between infection and vaccine-induced immune responses. Functional neutralization capacity was investigated using a commercially available ELISA-based SARS-CoV-2 RBD-ACE2 binding inhibition assay (surrogate SARS-CoV-2 neutralization test (sVNT) cPass (medac GmbH, https://international.medac.de). A lentivirus-based SARS-CoV-2 pseudovirus neutralization assay (pNT) was employed to determine serum 50% inhibitory dilutions (ID_{50}) against Alpha (B.1.1.7) and Delta (B.1.617.2) variants of concern.[3] SARS-CoV-2 specific T cell responses were measured by an interferon-**γ** release assay (IGRA) of S1 stimulated T cells in whole blood using a commercially available kit (EUROIMMUN AG, https://www.euroimmun.de). SARS-CoV-2 RBD variants antibody testing was performed using RBD proteins (provided by InVivo BioTech Services GmbH, Hennigsdorf, Germany). Different RBD proteins were printed in an array format in a similar

manner as done for the screening SeraSpot® Anti-SARS-CoV-2 IgG microarray (Seramun Diagnostica GmbH, https://www.seramun.com) and tested the same way.

Statistical analysis

Statistical analysis was done by GraphPad PRISM, version 9.1.2, or JMP Pro, version 15.2.0. Group comparisons were analyzed in a univariate analysis utilizing Fisher's exact test, nonparametric Mann Whitney U test or nonparametric Kruskal-Wallis test with Dunn's multiple comparisons test. 95% CIs were calculated according to Clopper and Pearson.[4] Unless otherwise specified, values are given as medians with interquartile ranges [IQR]. For IC50 in pNT geometric mean and 95%CI are depicted. Reduction of neutralizing antibodies against delta and alpha VOC between follow-up 2 and 6 were measured by the area under the curve (AUC). P-values below 0.05 were considered significant.

Supplementary References

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