

Delayed Antibody and T-Cell Response to BNT162b2 Vaccination in the Elderly, Germany

Tatjana Schwarz,¹ Pinkus Tober-Lau,¹ David Hillus, Elisa T. Helbig, Lena J. Lippert, Charlotte Thibeault, Willi Koch, Irmgard Landgraf, Janine Michel, Leon Bergfeld, Daniela Niemeyer, Barbara Mühlemann, Claudia Conrad, Chantip Dang-Heine, Stefanie Kasper, Friederike Münn, Kai Kappert, Andreas Nitsche, Rudolf Tauber, Sein Schmidt, Piotr Kopankiewicz, Harald Bias, Joachim Seybold, Christof von Kalle, Terry C. Jones, Norbert Suttrop, Christian Drosten, Leif Erik Sander,² Victor M. Corman,² Florian Kurth²

We detected delayed and reduced antibody and T-cell responses after BNT162b2 vaccination in 71 elderly persons (median age 81 years) compared with 123 healthcare workers (median age 34 years) in Germany. These data emphasize that nonpharmaceutical interventions for coronavirus disease remain crucial and that additional immunizations for the elderly might become necessary.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has led to an urgent need for vaccines, particularly among persons at high risk for severe disease and death, such as the elderly (1). Efficacy against severe coronavirus disease (COVID-19) of mRNA vaccine BNT162b2 (Pfizer-BioNTech, <https://www.pfizer.com>) is reported to be >90% starting 7 days after the second vaccination; robust antibody and T-cell response has been demonstrated

consistently across age groups (2–4). However, only 4.3% of participants in the BNT162b2 efficacy trial were ≥75 years of age (4). Given the elderly generally have weaker immune responses after vaccination, more detailed investigation is necessary (4,5).

The Study

In a prospective observational cohort study, we investigated SARS-CoV-2-specific antibodies, maturation of IgG avidity, and interferon-γ (IFN-γ) release of SARS-CoV-2-specific T cells in 2 cohorts of young and elderly BNT162b2-vaccinated persons (Table). Participants were recruited from 2 studies conducted at Charité–Universitätsmedizin Berlin, both conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e-6-r2-guideline-good-clinical-practice-step-5_en.pdf) and approved by the local ethics committee (EA4/244/20 and EA4/245/20).

The first cohort consisted of 123 healthcare workers; median age was 34 (interquartile range [IQR] 20–64) years. The second cohort consisted of 71 elderly residents of an assisted living facility; median age was 81 (IQR 70–96) years. Blood samples were taken before the first vaccination (week 0), just before the second vaccination (week 3), and 4 weeks after the second vaccination (week 7). To discriminate between vaccine-induced antibody response and convalescent SARS-CoV-2 infection, we used the SeraSpot Anti-SARS-CoV-2 IgG microarray-based immunoassay including nucleocapsid and spike as antigens (Seramun Diagnostica GmbH, <https://www.seramun.com>) (Appendix, <https://wwwnc.cdc.gov/EID/article/27/8/21-1145->

Author affiliations: Charité–Universitätsmedizin Berlin, Berlin, Germany (T. Schwarz, P. Tober-Lau, D. Hillus, E.T. Helbig, L.J. Lippert, C. Thibeault, W. Koch, L. Bergfeld, D. Niemeyer, B. Mühlemann, C. Conrad, C. Dang-Heine, S. Kasper, F. Münn, K. Kappert, R. Tauber, S. Schmidt, P. Kopankiewicz, H. Bias, J. Seybold, C. von Kalle, T.C. Jones, N. Suttrop, C. Drosten, L.E. Sander, V.M. Corman, F. Kurth); Hausarztpraxis am Agaplesion Bethanien Sophienhaus, Berlin (I. Landgraf); Robert Koch Institute, Berlin (J. Michel, A. Nitsche); German Centre for Infection Research, Berlin (D. Niemeyer, B. Mühlemann, T.C. Jones, C. Drosten, V.M. Corman); Berlin Institute of Health, Berlin (C. Dang-Heine, K. Kappert, R. Tauber, S. Schmidt, J. Seybold, C. von Kalle); Labor Berlin–Charité Vivantes GmbH, Berlin (K. Kappert, R. Tauber); University of Cambridge, Cambridge, UK (T.C. Jones); German Center for Lung Research, Gießen, Germany (N. Suttrop, L.E. Sander, F. Kurth); Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany (F. Kurth); University Medical Centre Hamburg-Eppendorf, Hamburg (F. Kurth)

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¹These authors contributed equally to this article.

²These senior authors contributed equally to this article.

App1.pdf). Ten of 123 healthcare workers and 1 of 71 elderly participants showed reactive anti-nucleocapsid or anti-spike IgG before the first vaccination and were excluded from further analyses.

At week 3, in the younger cohort, 93/107 (86.9%, 95% CI 79.2%–92.0%) participants showed reactive SARS-CoV-2 receptor-binding domain (RBD) IgG, compared with only 16/52 elderly participants (30.8%, 95% CI 19.9%–44.3%). At week 7, the antibody response rate had increased in both cohorts, to 112/113 in younger participants (99.1%, 95% CI 95.2%–100.0%) and 64/70 in the elderly cohort (91.4%, 95% CI 82.5%–96.0%) (Figure, panel A; Appendix Table). The comparison of SARS-CoV-2 RBD IgG levels demonstrated a significant difference in the 2 cohorts at both week 3 ($p < 0.0001$) and week 7 ($p = 0.0003$) (Appendix Table), indicating a substantial delay and overall reduced antibody response in elderly participants. We observed similar kinetics and differences between cohorts for antibody responses to 2 further SARS-CoV-2 spike antigens: the S1 subdomain and the full spike protein (Appendix Table, Figure).

We further confirmed the delayed and reduced antibody response in the elderly by measurement of the functional neutralization capacity using the surrogate virus neutralization test (sVNT) cPass (medac GmbH, <https://international.medac.de>) (Appendix) (6). At week 3, only 24/52 elderly participants (46.2%, 95% CI 33.3%–59.5%) had neutralizing capacity in serum, compared with 97/107 younger participants (90.7%, 95% CI 83.7%–94.8%; $p < 0.0001$) (Figure). In addition, the median sVNT titer for elderly participants was significantly lower than the young cohort (26.4% [IQR 6.8%–40.9%] vs. 60.2% [IQR 45.0%–76.4%]; $p < 0.0001$) (Appendix Table). Although the neutralizing antibody response rate increased to 63/70 (90.0%, 95% CI 80.8%–95.1%) in elderly participants by week 7, the median sVNT titer remained significantly lower than in the younger cohort (89.6% [IQR 70.9%–95.2%] vs. 96.7% [IQR 95.6%–97.2%]; $p < 0.0001$) (Appendix Table).

To characterize the maturation of IgG avidity in all 16 elderly and 30 randomly selected younger participants who were seroreactive at week 3, we applied a modified SARS-CoV-2 S1 IgG ELISA (Euroimmun, <https://www.euroimmun.com>) (Appendix Figure 2). The delayed antibody response in the elderly is reflected in results: at week 7, only 8/16 elderly (50.0%, 95% CI 28.0%–72.0%) exhibited high S1 IgG avidity indices (≥ 60) compared with 28/30 young participants (93.3%, 95% CI 78.7%–98.8%) (Figure, panel C). Consequently, the median relative avidity index of IgG was significantly higher in

Table. Cohort characteristics in study of delayed antibody and T-cell response to BNT162b2 vaccination in the elderly, Germany*

Characteristic	Healthcare workers	Elderly
No. patients	123	71
Sex		
F	65 (52.9)	54 (76.1)
M	58 (47.2)	17 (23.9)
Median age, y (IQR)	34 (20–64)	81 (70–96)
Underlying conditions		
Cardiovascular disease	15 (12.2)	56 (78.9)
Type 2 diabetes	1 (0.8)	13 (18.3)
Respiratory disease	16 (13.0)	11 (15.5)
Dyslipidemia	5 (4.1)	21 (29.6)
Thyroid dysfunction	0	16 (22.5)
Chronic kidney disease	0	12 (16.9)
Chronic liver or GI disease	2 (1.6)	18 (25.4)
Rheumatic disease	6 (4.9)	7 (9.9)
Active solid malignancy	2 (1.6)	4 (5.6)
Active hematological malignancy	0	4 (5.6)
Neurologic disease	1 (0.8)	18 (25.4)
Immunodeficiency	1 (0.8)	0
Others	9 (7.3)	29 (40.9)
Outpatient medication		
No	79 (64.2)	5 (7.0)
Yes	39 (31.7)	64 (90.1)
Unknown	5 (4.1)	2 (2.8)

*Values are no. (%) except as indicated. BNT162b2 is manufactured by Pfizer-BioNTech (<https://www.pfizer.com>). GI, gastrointestinal; IQR, interquartile range.

the younger cohort than the elderly cohort (76.2% [IQR 67.6%–82.9%] vs. 59.3% [IQR 55.3%–68.9%]; $p = 0.0002$) (Figure, panel C).

In addition to antibody responses, we assessed SARS-CoV-2 spike-specific T cell responses by an IFN- γ release assay (IGRA) (Euroimmun) (Appendix) of S1 peptide-stimulated T cells at week 7. The proportion of persons with IGRA results above the defined threshold (Appendix) was significantly lower in the elderly than in younger participants (51.2% [95% CI 36.8%–65.4%] vs. 84.5% [95% CI 74.4%–91.1%]; $p = 0.0002$). Accordingly, median S1-induced IFN- γ release was significantly decreased in the elderly compared to younger participants (707.3 mIU/mL [IQR 216–1,392] vs. 2184 mIU/mL [IQR 1,274–2,484]; $p < 0.0001$) (Figure, panel D). In contrast, we detected no significant difference in IFN- γ release after mitogen stimulation between the 2 cohorts, indicating no general impairment of IFN- γ responses in the elderly ($p = 0.77$) (Figure, panel D; Appendix Table).

In summary, vaccination with BNT162b2 induces both arms of adaptive immunity: SARS-CoV-2-specific antibodies and SARS-CoV-2-specific T cells. However, we observed delayed and less robust cellular and humoral immune response among the elderly than among younger adults. A limitation of our study is the lack of data on other COVID-19 vaccines. Furthermore, we cannot exclude that underlying diseases or medications, which are more common in the

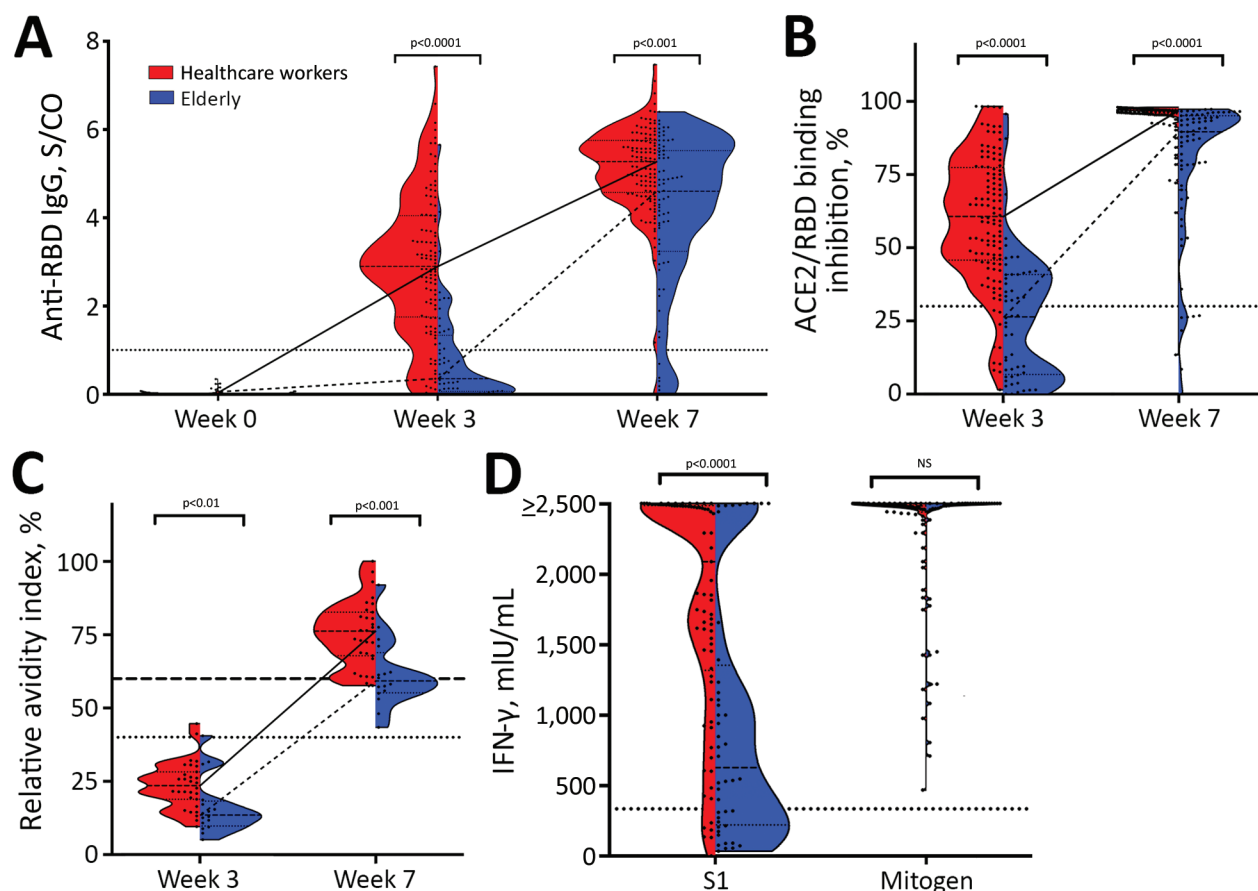


Figure. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–specific antibody and T-cell response after vaccination with BNT162b2 (Pfizer-BioNTech, <https://www.pfizer.com>) in the elderly, Germany. A) SARS-CoV-2 RBD IgG measured in serum of BNT162b2-vaccinated younger participants (healthcare workers) before the first vaccination ($n = 100$, week 0), 3 weeks after the first vaccination ($n = 107$, week 3), and 4 weeks after the second vaccination ($n = 113$, week 7) and from elderly participants at week 0 ($n = 70$), week 3 ($n = 52$), and week 7 ($n = 70$) using the SeraSpot Anti-SARS-CoV-2 IgG assay (Seramun Diagnostica GmbH, <https://www.seramun.com>). B) Neutralizing capacity of antibodies measured at week 3 and 7 in the young and elderly cohorts using the ELISA-based surrogate virus neutralization test (sVNT) cPass (medac GmbH, <https://international.medac.de>). C) SARS-CoV-2 spike IgG avidity analyzed in the healthcare workers cohort ($n = 30$) and elderly cohort ($n = 16$) at week 3 and 7. D) At week 7, whole blood from vaccinated elderly participants ($n = 43$) and young participants ($n = 71$) was stimulated *ex vivo* with components of the S1 domain of the spike protein for 24 h, and IFN- γ concentration in the supernatant was detected by ELISA. Dotted lines indicate the manufacturer's specified threshold for RBD IgG >1 S/Co, for sVNT $>30\%$, and for avidity 40–60% borderline avidity and $>60\%$ high avidity. For IGRA, we defined an arbitrary threshold at 334.2 mIU/mL. p value was calculated by the nonparametric Mann Whitney U test, and the median and interquartile range are depicted. ACE2, angiotensin-converting enzyme 2; IFN- γ , interferon- γ ; IU, international units; NS, not significant; RBD, receptor-binding domain; S/CO, signal-to-cutoff ratio; sVNT, surrogate virus neutralization test.

elderly (Table), might impair the vaccine-induced immune response. For example, patients on dialysis have significantly lower antibody response than vaccinated same-age patients not on dialysis (E. Schrezenmeier et al., unpub. data, <http://medrxiv.org/lookup/doi/10.1101/2021.03.31.21254683>).

Our data are supported by other real-world observations suggesting a delayed and reduced immunogenicity of BNT162b2 in the elderly (5,7; D.A. Collier et al., unpub. data, <http://medrxiv.org/lookup/doi/10.1101/2021.02.03.21251054>). In line with our observations for BNT162b2, an effect of age-dependent decrease of

immune function, referred to as immunosenescence, is well known and contributes to increased prevalence of infectious disease and vaccine failure in the elderly (8). A lower vaccine-induced immune response to influenza and hepatitis B viruses is well documented (9,10); however, such data are scarce for mRNA vaccines.

Of note, vaccination with 2 doses of BNT162b2 might not fully prevent SARS-CoV-2 outbreaks among elderly persons in congregate settings, such as long-term care facilities, possibly because of delayed and reduced immune response. However, vaccination protects against severe disease (11–13).

Conclusions

Although the immune response of elderly participants 4 weeks after the second dose of BNT162b2 nearly reached the level of younger participants, a small fraction of elderly participants did not demonstrate robust antibody and T-cell response. However, the immunologic correlates of protection remain unknown, and identification of persons with no or incomplete protection after vaccination remains challenging. Therefore, strategies focused solely on vaccinating high-risk groups might be insufficient to protect those at risk for severe disease. For the elderly, vaccinating caregivers and close contacts should be prioritized. Moreover, a booster vaccination, altered vaccine dose, or different COVID-19 vaccines should be considered for the elderly if further evidence demonstrates high rates of breakthrough infections despite 2-dose BNT162b2 vaccination.

These results are particularly relevant for vaccination strategies focused on broad administration of the first dose of a 2-dose vaccine while postponing the second vaccination. This practice might leave a relevant proportion of elderly with comparatively low levels of immunity for a prolonged period, emphasizing the need for nonpharmaceutical interventions, such as mask use and regular testing.

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V.M.C. is named together with Euroimmun GmbH on a patent application filed recently regarding SARS-CoV-2 diagnostics via antibody testing.

About the Author

Dr. Schwarz is a postdoctoral researcher in the Institute of Virology, Charité-Universitätsmedizin Berlin, Berlin, Germany. Her research interests include infectious disease surveillance and immunologic testing.

References

1. Brown AE, Heinsbroek E, Kall MM, Allen H, Beebejaun K, Blomquist P, et al.; PHE COVID-19 Mortality Working Group. Epidemiology of confirmed COVID-19 deaths in adults, England, March–December 2020. *Emerg Infect Dis.* 2021;27:1468–71. <https://doi.org/10.3201/eid2705.203524>
2. Walsh EE, Frenck RW Jr, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. *N Engl J Med.* 2020;383:2439–50. <https://doi.org/10.1056/NEJMoa2027906>
3. Dagan N, Barda N, Kepten E, Miron O, Perchik S, Katz MA, et al. BNT162b2 mRNA Covid-19 vaccine in a nationwide mass vaccination setting. *N Engl J Med.* 2021;384:1412–23. <https://doi.org/10.1056/NEJMoa2101765>
4. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al.; C4591001 Clinical Trial Group. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med.* 2020;383:2603–15. <https://doi.org/10.1056/NEJMoa2034577>
5. Van Praet JT, Vandecasteele S, De Roo A, De Vriese AS, Reynders M. Humoral and cellular immunogenicity of the BNT162b2 mRNA Covid-19 Vaccine in nursing home residents. *Clin Infect Dis.* 2021 Apr 7 [Epub ahead of print]. <https://doi.org/10.1093/cid/ciab300>
6. Tan CW, Chia WN, Qin X, Liu P, Chen MI-C, Tiu C, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. *Nat Biotechnol.* 2020;38:1073–8. <https://doi.org/10.1038/s41587-020-0631-z>
7. Müller L, André M, Moskorz W, Drexler I, Walotka L, Grothmann R, et al. Age-dependent immune response to the Biontech/Pfizer BNT162b2 COVID-19 vaccination. *Clin Infect Dis.* 2021 Apr 27 [Epub ahead of print]. <https://doi.org/10.1093/cid/ciab381>
8. Crooke SN, Ovsyannikova IG, Poland GA, Kennedy RB. Immunosenescence and human vaccine immune responses. *Immun Ageing.* 2019;16:25. <https://doi.org/10.1186/s12979-019-0164-9>
9. Fisman DN, Agrawal D, Leder K. The effect of age on immunologic response to recombinant hepatitis B vaccine: a meta-analysis. *Clin Infect Dis.* 2002;35:1368–75. <https://doi.org/10.1086/344271>
10. McElhaney JE, Verschoor CP, Andrew MK, Haynes L, Kuchel GA, Pawelec G. The immune response to influenza in older humans: beyond immune senescence. *Immun Ageing.* 2020;17:10. <https://doi.org/10.1186/s12979-020-00181-1>
11. Cavanaugh AM, Fortier S, Lewis P, Arora V, Johnson M, George K, et al. COVID-19 outbreak associated with a SARS-CoV-2 R.1 lineage variant in a skilled nursing facility after vaccination program—Kentucky, March 2021. *MMWR Morb Mortal Wkly Rep.* 2021;70:639–43. <https://doi.org/10.15585/mmwr.mm7017e2>

12. Teran RA, Walblay KA, Shane EL, Xydis S, Gretsches S, Gagner A, et al. Postvaccination SARS-CoV-2 infections among skilled nursing facility residents and staff members—Chicago, Illinois, December 2020–March 2021. *MMWR Morb Mortal Wkly Rep.* 2021;70:632–8. <https://doi.org/10.15585/mmwr.mm7017e1>
13. Tober-Lau P, Schwarz T, Hillus D, Spieckermann J, Helbig ET, Lippert LJ, et al. Outbreak of SARS-CoV-2 B.1.1.7 lineage after vaccination in long-term care facility, Germany,

February–March 2021. *Emerg Infect Dis.* 2021 Jun XX [Epub ahead of print]. <https://doi.org/10.3201/eid2708.210087>

Address for correspondence: Victor M. Corman, Leif Erik Sander, and Florian Kurth, Charité–Universitätsmedizin Berlin, Chariteplatz 1, D-10117, Berlin, Germany; email: victor.corman@charite.de, leif-erik.sander@charite.de, florian.kurth@charite.de

May 2021 COVID-19



- Coordinated Strategy for a Modeling-Based Decision Support Tool for COVID-19, Utah, USA
- Clinical Laboratory Perspective on Human Infections Caused by Unusual Nonhemolytic, Lancefield Group B *Streptococcus halichoeri*
- Case Series of Laboratory-Associated Zika Virus Disease, United States, 2016–2019
- Successful Control of an Onboard COVID-19 outbreak Using the Cruise Ship as a Quarantine Facility, Western Australia T
- Coccidioidomycosis and COVID-19 Co-Infection, United States, 2020
- Epidemiologic Findings From Case Investigations and Contact Tracing of the First 200 Cases of Coronavirus Disease 2019 (COVID-19) identified in Santa Clara County, California, USA
- SARS-CoV-2 in Nursing Homes after 3 Months of Serial, Facility-Wide Point Prevalence Testing, Connecticut, USA
- Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 during Border Quarantine and Air Travel, New Zealand (Aotearoa)
- Engineered NS1 Provides Sensitive and Specific Zika Diagnosis from Patient Serology
- Monitoring SARS-CoV-2 Circulation and Diversity through Community Wastewater Sequencing, the Netherlands and Belgium
- Characteristics and Clinical Implications of Carbapenemase-Producing *Klebsiella pneumoniae* Colonization and Infection, Italy
- Serologic Screening of Severe Acute Respiratory Syndrome Coronavirus 2 Infection in Cats and Dogs during First Coronavirus Disease Wave, the Netherlands
- Epidemiologic History and Genetic Diversity Origins of Chikungunya and Dengue Viruses, Paraguay
- Herd Immunity against Severe Acute Respiratory Syndrome Coronavirus 2 Infection in 10 Communities, Qatar
- Active Case Finding of Current Bornavirus Infections in Human Encephalitis Cases of Unknown Etiology, Germany, 2018–2020
- Susceptibility to SARS-CoV-2 of Cell Lines and Substrates Commonly used to Diagnose and Isolate Influenza and Other Viruses
- Symptom Diary–Based Analysis of COVID-19 Disease Course, Germany, 2020
- Use of Genomics to Track Coronavirus Disease Outbreaks, New Zealand
- Global Trends in Norovirus Genotype Distribution among Children with Acute Gastroenteritis
- Genetic Evidence and Host Immune Response in Persons Reinfected with SARS-CoV-2, Brazil
- Prevalence and Clinical Profile of SARS-CoV-2 Infection among Farmworkers, California, June–November 2020
- Epidemiology of Confirmed COVID-19 Deaths in Adults, England, March–December 2020
- Prescribing Antimicrobial Drugs for Acute Gastroenteritis, Primary Care, Australia, 2013–2018
- Evaluating Differences in Whole Blood, Serum, and Urine Screening Tests for Zika Virus, Puerto Rico 2016
- Longevity of Middle East Respiratory Syndrome Coronavirus Antibody Responses in Patients, Saudi Arabia
- Whole-Genome Sequencing of Shiga Toxin–Producing *Escherichia coli* OX18 from a Fatal Hemolytic Uremic Syndrome Case
- COVID-19–Associated Mold Infection in Critically ill Patients, Chile

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Appendix

Patients, Materials and Methods

Elderly Cohort

Patients were recruited within the COVIMMUNIZE study conducted under the auspices of Charité–Universitätsmedizin Berlin, a prospective observational cohort study on immunogenicity of coronavirus disease (COVID-19) vaccines in populations at risk. Written informed consent was obtained from patients or legal representatives according to regulations set by the ethics committee of Charité–Universitätsmedizin Berlin.

Subjects were eligible for inclusion if they were >70 years of age and scheduled for vaccination against COVID-19 with BNT162b2. To detect concurrent and asymptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, weekly reverse transcription PCR (RT-PCR) of oropharyngeal swab specimens was conducted for the first 8 weeks after enrollment. Blood sampling was performed immediately before the first (week 0) and second (week 3) dose of vaccine, and at week 7–8 (4 weeks after the second vaccination). Antibody and T cell data obtained from week 7 samples from 44 participants have been used as a control cohort in another study (E. Schrezenmeier et al., unpub. data, <http://medrxiv.org/lookup/doi/10.1101/2021.03.31.21254683>).

Healthcare Workers Cohort

Healthcare workers of the Charité–Universitätsmedizin Berlin were recruited within the EICOV study, a prospective observational cohort study on immunogenicity and reactogenicity of COVID-19 vaccines in healthcare workers. Subjects were eligible for inclusion if they were >18 years of age, employed at the Charité–Universitätsmedizin Berlin, and had no contraindication against a COVID-19 vaccination. To detect concurrent and asymptomatic infections with SARS-

CoV-2, weekly self-collected oropharyngeal swab specimens from participants were analyzed after enrollment. Blood sampling took place immediately before the first (week 0) and second (week 3) dose of vaccination with BNT162b2, as well as at week 7–8 (4 weeks after the second vaccination). Antibody and T cell data obtained from the 123 healthcare workers have been used as a control cohort in another study (D. Hillus et al., unpub. data, <https://www.medrxiv.org/content/10.1101/2021.05.19.21257334v2>).

Antibody Assessment

For detection of SARS-CoV-2–specific antibodies to the spike and nucleocapsid proteins, we used the SeraSpot Anti-SARS-CoV-2 IgG microarray-based multiparameter immunoassay according to manufacturer’s instructions (Seramun Diagnostica GmbH, <https://www.seramun.com>). In brief, this assay is based on the use of 4 recombinant SARS-CoV-2 proteins (complete spike, S1 domain, receptor-binding domain [RBD], and nucleocapsid) as capture antigens. These and test-specific controls are printed in an array arrangement on the bottom of each well. Bound antibodies from the patient serum samples are detected by horseradish peroxidase–labeled antibodies against human IgG. Color intensity at the site of formed immune complexes (pale blue to dark blue) correlates with antibody concentration. The SpotSight plate scanner was used for measurements. Results are calculated and normalized as signal-to-cutoff (S/CO) ratios by dividing the observed signal strength of a specific location by that of an internal cutoff control. Samples with an S/CO ratio ≥ 1.0 are defined as positive by the manufacturer.

Surrogate SARS-CoV-2 Neutralization Test

To detect neutralizing activity in serum samples 3 weeks after the first vaccination and 4 weeks after the second vaccination, we used the commercially available ELISA-based SARS-CoV-2 surrogate neutralization test cPass (medac GmbH, <https://international.medac.de>) according to the manufacturer’s instructions. Serum samples and positive and negative controls were diluted 1:10 in sample dilution buffer and preincubated 1:1 with RBD–horseradish peroxidase for 30 min at 37°C. Each reaction mixture was then added to the hACE2 precoated plate and incubated at 37°C for 15 min. After a washing step, 3',3',5,5-tetramethylbenzidine solution was added to each well and the plate was incubated at room temperature for 15 min. Following a stop solution step, the optical density (OD) at 450 nm was detected. Data were interpreted by the calculation of the relative inhibition using the following equation: inhibition

[%] = (1–OD value of sample/OD value of negative control) × 100. Samples were considered negative at an inhibition of <30% and considered positive otherwise.

IgG Avidity Assay

Maturation of IgG avidity was detected in younger (n = 30) and elderly (n = 16) participants at week 3 and week 7 by using an anti-SARS-CoV-2 S1 IgG ELISA Kit (Euroimmun, <https://www.euroimmun.com>), which showed a strong correlation with the SeraSpot Anti-SARS-CoV-2 IgG RBD (p < 0.001, Spearman's rank correlation coefficients = 0.662) used for general antibody testing (Appendix Figure 2). Serum samples were diluted 1:101 with sample buffer and incubated on plates precoated with recombinant SARS-CoV-2 spike proteins. After incubation for 1h at 37°C, wells were washed and 200 µL urea or 200 µL PBS were added to the plates and incubated for 10 min at 37°C. After a washing step, conjugate and substrate were added according to the manufacturer's instructions. OD was detected at 450 nm, and the relative avidity index was calculated by dividing the observed OD of the urea-treated sample by that of the PBS-treated sample, multiplied by 100. Values with an index of more than 60% were considered to be high avidity.

Interferon-γ Release of SARS-CoV-2-Specific T Cells

We applied a commercially available IGRA for assessment of interferon-γ (IFN-γ) release of SARS-CoV-2-specific T cells (Euroimmun). In parallel, 0.5 mL freshly collected Lithium-heparin blood was stimulated with a SARS-CoV-2 peptide pool from the spike S1 domain, 0.5 mL of blood was stimulated with mitogen as a positive control, and 0.5 mL of blood in a blank was used as a negative control. After 24 hours of incubation at 37°C, IFN-γ concentration in the plasma fraction of all 3 stimulation tubes was measured by ELISA. IFN-γ response in the blank served as a measure of patient-individual background IFN-γ activity and was subtracted from the IFN-γ response in the stimulation tubes. For analyses of IGRA outcome, we defined an arbitrary cutoff by using average IFN-γ activity (33.42 mIU/mL) determined in the 15 SARS-CoV-2 IgG-negative unvaccinated control multiplied by 5 as the threshold (167.1 mIU/mL) for borderline IGRA-reactive and multiplied by 10 for the threshold (334.2 mIU/mL) for IGRA-positive.

Statistical Analysis

Values are given as medians with interquartile range unless stated otherwise. GraphPad PRISM statistics version 27.0 (IBM Deutschland, <https://www.ibm.com/de-de>) was used for statistical analysis. Group differences were assessed in a univariate analysis by using Fisher exact test or nonparametric Mann Whitney U test. P values of <0.05 were considered statistically significant. All 95% CI for proportions were calculated by using the Wilson procedure with a correction for continuity (*I*).

References

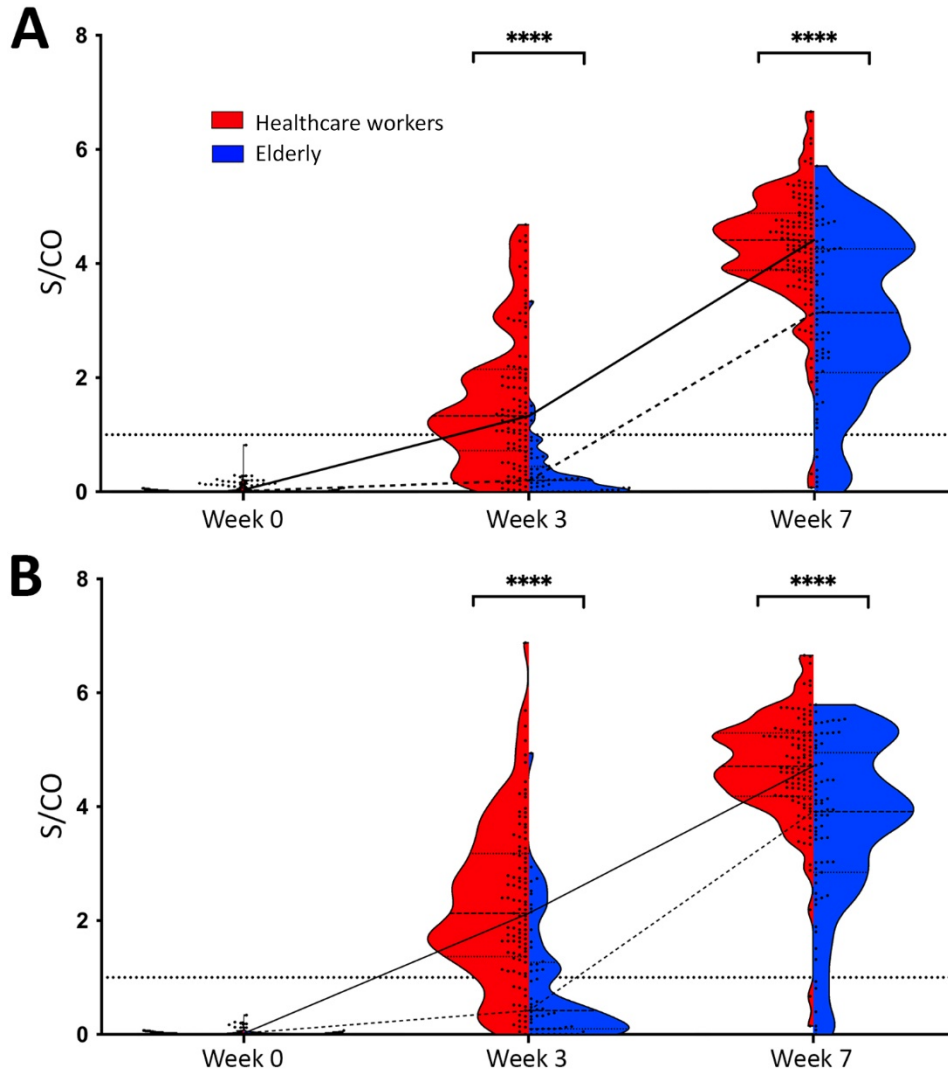
1. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med. 1998;17:857–72. PubMed [https://doi.org/10.1002/\(SICI\)1097-0258\(19980430\)17:8<857::AID-SIM777>3.0.CO;2-E](https://doi.org/10.1002/(SICI)1097-0258(19980430)17:8<857::AID-SIM777>3.0.CO;2-E)

Appendix Table. Proportion of positive outcome and outcome values in different test systems in study of delayed antibody and T-cell response to BNT162b2 vaccination in the elderly, Germany

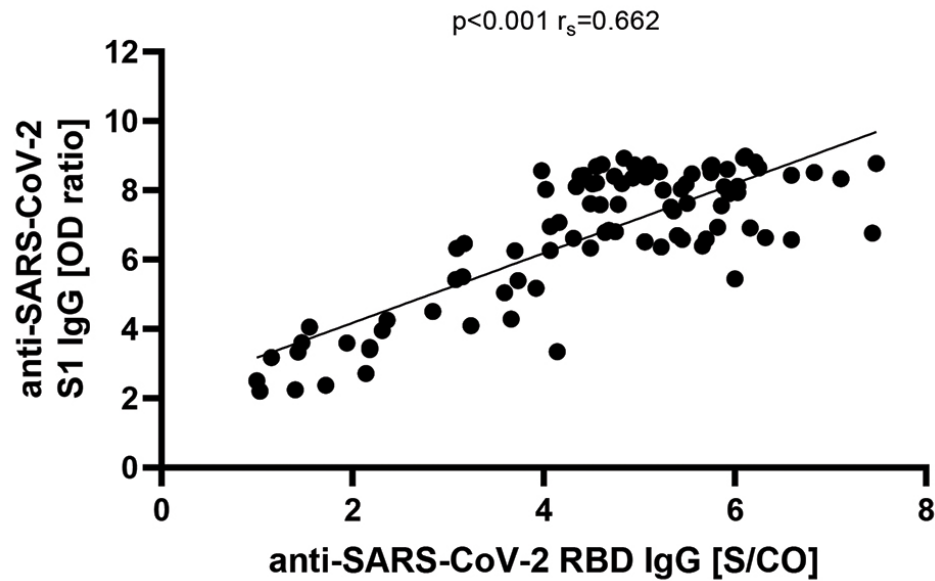
Time	Test system	Proportion of positive outcome (95% CI)			Outcome values median [IQR]		
		Healthcare workers	Elderly	p value*	Healthcare workers	Elderly	p value†
Week 7	NP spot array, S/CO	0.0 (0.0–3.3) [0/113]	0.0 (0.0–5.2) [0/70]		0.1 [0.1–0.2]	0.1 [0.0–0.1]	
	RBD spot array, S/CO	99.1 (95.2–100.0) [112/113]	91.4 (82.5–96.0) [64/70]	0.013	5.3 [4.6–5.8]	4.6 [3.2–5.5]	0.0003
	S1 spot array, S/CO	98.2 (93.8–99.7) [111/113]	90.0 (80.8–95.1) [63/70]	0.028	4.4 [3.9–4.89]	3.14 [2.07–4.26]	<0.0001
	Spike full spot array, S/CO	98.23 (93.78–99.69) [111/113]	91.43 (82.53–96.01) [64/70]	0.056	4.68 [4.19–5.3]	3.9 [2.8–5.0]	<0.0001
	ACE2/RBD inhibition, %	99.1 (95.2–100.0) [112/113]	90.0 (80.8–95.1) [63/70]	0.005	96.7 [95.6–97.2]	89.6 [70.9–95.2]	<0.0001
	IgG RAI, %	93.3 (78.7–98.8) [28/30]	50.0 (28.0–72.0) [8/16]	0.0015	76.2 [67.6–82.9]	59.3 [55.3–68.9]	0.0002
	IGRA S1 stimulation, mIU/mL	84.5 (74.4–91.1) [60/71]	51.2 (36.8–65.4) [22/43]	0.0002	2184 [1274–2484]	707.3 [215.5–1392]	<0.0001
	IGRA mitogen stimulation, mIU/mL	100.0 (94.9–100.0) [71/71]	100.0 (91.8–100.0) [43/43]		2483 [2438–2497]	2498 [2456–2500]	0.077
Week 3	NP spot array, S/CO	0.0 (0.0–3.5) [0/107]	0.0 (0.0–6.9) [0/52]		0.1 [0.1–0.2]	0.2 [0.1–0.2]	
	RBD spot array, S/CO	86.9 (79.2–92.0) [93/107]	30.8 (19.9–44.3) [16/52]	<0.0001	2.9 [1.8–4.1]	0.4 [0.1–1.3]	<0.0001
	S1 spot array, S/CO	66.4 (57.0–74.6) [71/107]	7.7 (3.0–18.2) [4/52]	<0.0001	1.3 [0.7–2.2]	0.2 [0.0–0.5]	<0.0001
	Spike full spot array, S/CO	84.1 (76.0–89.8) [90/107]	34.6 (23.2–48.2) [18/52]	<0.0001	2.1 [1.4–3.2]	0.4 [0.1–1.3]	<0.0001
	ACE2/RBD inhibition, %	90.7 (83.7–94.8) [97/107]	46.2 (33.3–59.5) [24/52]	<0.0001	60.2 [45.0–76.4]	26.4 [6.8–40.9]	<0.0001
Week 0	IgG RAI, %	0.0 (0.0–11.4) [0/30]	0.0 (0.0–19.4) [0/16]		23.6 [18.2–28.7]	13.6 [9.8–18.3]	0.0033
	NP spot array, S/CO	0.0 (0.0–3.7) [0/100]	0.0 [0.0–5.2] [0/70]		0.1 [0.1–0.1]	0.1 [0.1–0.2]	
	RBD spot array, S/CO	0.0 (0.0–3.7) [0/100]	0.0 [0.0–5.2] [0/70]		0.0 [0.0–0.0]	0.0 [0.0–0.0]	
	S1 spot array, S/CO	0.0 (0.0–3.7) [0/100]	0.0 [0.0–5.2] [0/70]		0.0 [0.0–0.0]	0.0 [0.0–0.0]	
	Spike full spot array, S/CO	0.0 (0.0–3.7) [0/100]	0.0 [0.0–5.2] [0/70]		0.0 [0.0–0.0]	0.0 [0.0–0.0]	

*p value was calculated by Fisher exact test. ACE2, angiotensin-converting enzyme 2; IGRA, interferon- γ release assay; IU, international units; NP, nucleocapsid protein; RAI, relative avidity index; RBD, receptor-binding domain; S1, spike subdomain 1; S/CO, signal-to-cutoff ratio.

†p value was calculated by the nonparametric Mann Whitney U test.



Appendix Figure 1. Anti-SARS-CoV-2 S1 and full spike IgG antibody response after BNT162b2 vaccination in the elderly, Germany. A) Anti-SARS-CoV-2 S1 and B) full spike IgG were measured in serum of BNT162b2-vaccinated healthcare workers (median age 34 years) (red) before the first vaccination (n = 100, week 0), 3 weeks after the first vaccination (n = 107, week 3), and 4 weeks after the second vaccination (n = 113, week 7) and in elderly participants (median age 81 years) (blue) at week 0 (n = 70), week 3 (n = 52), and week 7 (n = 70) using the SeraSpot Anti-SARS-CoV-2 IgG assay. Samples with an S/CO ratio of ≥ 1.0 are defined by the manufacturer as positive. P value was calculated by the nonparametric Mann Whitney U test and the median and interquartile range are depicted. S/CO, signal-to-cutoff ratio; ****, $p < 0.0001$.



Appendix Figure 2. Correlation between anti-S1 IgG and anti-RBD IgG in study of delayed antibody and T-cell response to BNT162b2 vaccination in the elderly, Germany. Anti-SARS-CoV-2 S1 IgG OD ratios of 92 samples were correlated with anti-SARS-CoV-2 RBD IgG S/CO measurements (p value, Spearman's rank correlation coefficients). OD, optical density; RBD, receptor-binding domain; S/CO, signal-to-cutoff ratio.