greater disease severity among hospitalised women but not men: multicentre cohort study. BMJ Open Respir Res. 2021;8:e001029. https://doi.org/10.1136/bmjresp-2021-001029
7. Seasely AR, Blanchard CT, Arora N, Battarbee AN, Casey BM, Dionne-Odom J, et al.; CWRH COVID-19 Working Group. Maternal and perinatal outcomes associated with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Delta (B.1.617.2) variant. Obstet Gynecol. 2021;138:842–4. https://doi.org/10.1097/AOG.0000000000004607
8. Wang AM, Berry M, Moutos CP, Omere C, Clark SM, Harirah HM, et al. Association of the Delta (B.1.617.2) variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with pregnancy outcomes. Obstet Gynecol. 2021; 138:838–41. https://doi.org/10.1097/AOG.0000000000004595
9. Klopfenstein T, Zayet S, Lohse A, Selles P, Zahra H, Kadiane-Oussou NJ, et al.; HNF Hospital Tocilizumab multidisciplinary team. Impact of tocilizumab on mortality and/or invasive mechanical ventilation requirement in a cohort of 206 COVID-19 patients. Int J Infect Dis. 2020; 99:491–5. https://doi.org/10.1016/j.ijid.2020.08.024

Address for correspondence: Timothee Klopfenstein, Department of Infectious Disease, Nord Franche-Comte Hospital, 90400 Trevenans, France; email: timothee.klopfenstein@hnfc.fr

Cross-Variant Neutralizing Serum Activity after SARS-CoV-2 Breakthrough Infections

Pinkus Tober-Lau,[1] Henning Gruell,[1] Kanika Vanshylla,[1] Willi M. Koch, David Hillus, Philipp Schommers, Isabelle Suárez, Norbert Suttorp, Leif Erik Sander,[2] Florian Klein,[2] Florian Kurth[2]

Author affiliations: Charité–Universitätsmedizin Berlin, Berlin, Germany (P. Tober-Lau, W.M. Koch, D. Hillus, N. Suttorp, L.E. Sander, F. Kurth); University of Cologne, Cologne, Germany (H. Gruell, K. Vanshylla, P. Schommers, I. Suárez, F. Klein); German Center for Infection Research, Bonn-Cologne, Germany (F. Klein); Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany (F. Kurth); University Medical Centre Hamburg-Eppendorf, 20359, Hamburg (F. Kurth)

DOI: https://doi.org/10.3201/eid2805.220271

[1]These authors contributed equally to this article.

[2]These authors co-led this study.

To determine neutralizing activity against the severe acute respiratory syndrome coronavirus 2 ancestral strain and 4 variants of concern, we tested serum from 30 persons with breakthrough infection after 2-dose vaccination. Cross-variant neutralizing activity was comparable to that after 3-dose vaccination. Shorter intervals between vaccination and breakthrough infection correlated with lower neutralizing titers.

The B.1.1.529 (Omicron) variant of concern of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) carries a high number of nonsynonymous mutations in the spike glycoprotein, relative to that of the ancestral (wild-type) strain (Wu01). Those mutations result in a strong immune evasion phenotype, as demonstrated by severely reduced serum neutralization after vaccination or previous infection with ancestral variants in most persons (*1*–*3*), lower vaccine effectiveness, and increased rates of reinfection (N. Andrews et al., unpub. data, https://www.medrxiv.org/content/10.1101/2021.12.14.21267615v1). However, booster vaccinations with 1 dose of mRNA vaccine after priming with an initial 2 doses induce high levels of serum neutralizing activity against Omicron (*1*,*4*). Substantial efforts have therefore been made to speed up booster vaccination campaigns in light of the rapid spread of Omicron and the recent surge of infections worldwide. Breakthrough infections after 2-dose mRNA vaccination can result in a natural boost to humoral immunity against SARS-CoV-2 (*5*; L.J. Abu-Raddad et al., unpub. data, https://www.medrxiv.org/content/10.1101/2022.01.18.222694502v2), and emerging evidence suggests that breakthrough infections with non-Omicron SARS-CoV-2 variants also elicit cross-neutralizing serum activity against Omicron (*6*).

We determined serum neutralizing activity against the spike pseudotypes of SARS-CoV-2 Wu01 strain and 4 variants of concern (Alpha, Beta, Delta, Omicron [BA.1]) in 20 persons with non-Omicron (Alpha, Delta) SARS-CoV-2 infection after 2-dose mRNA vaccination with BNT162b2 (Comirnaty; Pfizer-BioNTech, https://www.comirnaty.com) or heterologous vaccination with ChAdOx1 (Vaxzevria; AstraZeneca, https://www.astrazeneca.com) and BNT162b2 (Appendix, https://wwwnc.cdc.gov/EID/article/28/5/22-0271-App1.pdf). We compared serum neutralization activity for this cohort with that of 2 age-matched cohorts, 1 consisting of 20 persons who received 2 or 3 doses of mRNA vaccine (*1*) and did not experience breakthrough infection and another cohort of 10 persons who experienced

Omicron breakthrough infection after 2-dose vaccination (Figure, panel A; Appendix Table).

We detected significantly higher serum neutralizing activity against all investigated variants in serum from vaccinated persons with subsequent non-Omicron SARS-CoV-2 infection (Figure, panel B) than in serum from persons who received the regular 2 doses of vaccine and experienced no subsequent infection. The geometric mean 50% inhibitory serum dilution (ID_{50}) against Wu01 was 6.3-fold higher after breakthrough infection (640 [95% CI 409–1,003] vs. 4,056 [95% CI 2,174–7,568]). This difference in serum neutralizing activity was particularly pronounced against the Beta (23.5-fold higher ID_{50}, 49 [95% CI 28–85] vs. 1,148 [95% CI 524–2,514]) and Omicron (23.8-fold higher ID_{50}, 9 [95% CI 5–13] vs. 202 [95% CI 79–515]) variants, each of which exhibits substantial immune escape. The boosting effect of non-Omicron breakthrough infections was highly variable (Figure, panel B) because serum

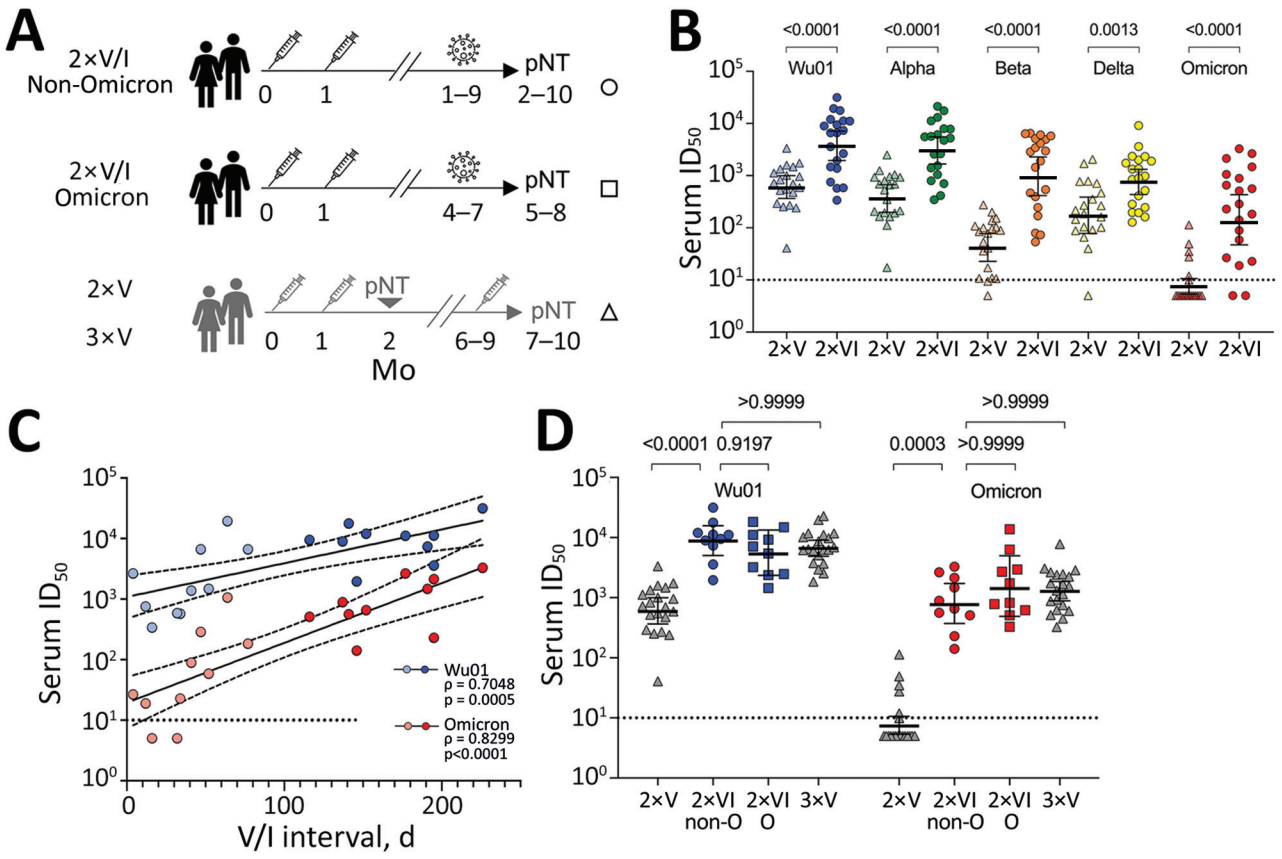


Figure. SARS-CoV-2 serum neutralizing titers across variants after postvaccination breakthrough infection. A) Schematic of the study cohort of 2×VI patients and age-matched reference cohorts (1). B) Serum neutralizing activity against Wu01 and SARS-CoV-2 variants in 2×V persons (triangles) and 2×V/I persons (circles). Horizontal lines indicate geometric mean ID50s; error bars, 95% CIs. Groups were compared by using the Mann-Whitney test. p values are shown at top. C) Correlation of serum neutralizing activity against SARS-CoV-2 Wu01 (blue) or Omicron (red) and interval between second vaccination and non-Omicron breakthrough infection (Spearman ρ and p values). Breakthrough infections within 3 months (90 days) from vaccination are indicated by light shaded symbols. Solid lines indicate linear regression, and dashed lines indicate 95% CIs. Correlation was determined by Spearman ρ. D) Serum neutralizing activity against SARS-CoV-2 Wu01 (blue) and Omicron (red) in 2×V or 3×V persons (triangles) compared with 2×V/I non-Omicron (circles) or Omicron (triangles) persons after 2 and 3 doses of mRNA vaccine. Only persons with vaccine-to-infection intervals >3 months are shown. Groups were compared by using the Kruskal-Wallis test with the Dunn multiple testing correction. Horizontal lines indicate geometric mean ID50s; error bars, 95% CIs. p values are shown at top. Black dotted lines in panels B, C, and D indicate the lower limit of quantification (ID50 = 10); ID50s <10 were imputed to half the lower limit of quantification (ID50 = 5). ID50, 50% inhibitory serum dilution; O, Omicron; pNT, pseudovirus neutralization test; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; V/I, vaccination with subsequent breakthrough infection; Wu01, ancestral (wild-type) SARS-CoV-2 strain; 2xV/I non-Omicron, vaccinated persons with non-Omicron breakthrough infection that occurred 1–8 months after vaccination (circles); 2xV/I Omicron, vaccinated persons with Omicron breakthrough infection that occurred 4–7 months after vaccination (squares); 2xV, vaccinated persons after 2 doses of mRNA vaccine; 3xV, vaccinated persons after 3 doses of mRNA vaccine (triangles).

neutralizing titers (ID_{50}) showed a strong correlation with the interval between second vaccination and diagnosis of breakthrough infection (Omicron, Spearman ρ = 0.8299, p<0.0001; Wu01, ρ = 0.7048, p = 0.0005) (Figure, panel C; Appendix Figure, panels A–C). Breakthrough infections acquired >3 months after the second vaccination resulted in serum neutralizing capacity against both Wu01 and Omicron, which was comparable to that after 3-dose vaccination. This effect was observed after both non-Omicron and Omicron breakthrough infections (Figure, panel D). Similarly, neutralizing capacity against the Delta variant was increased after Omicron breakthrough infections (Appendix Figure, panel D). Limitations of this study include limited sample size and application of a pseudovirus-based neutralization assay.

In summary, we found that Omicron and non-Omicron SARS-CoV-2 breakthrough infections elicit cross-variant neutralizing antibodies. Our results suggest that short vaccination-to-infection intervals correlate with lower neutralizing titers, which may be relevant for recommendations concerning additional booster vaccination of persons who experience early breakthrough infections after initial immunization.

Acknowledgments

We are grateful to all study participants. We also thank Florian Behr, Julia Blum, Annelene Kossow, Göksu Oral, Elham Rezaei, Maike Schlotz, and the members of the EICOV/COVIM Study Group for sample acquisition and processing as well as logistical support: Yvonne Ahlgrimm, Ben Al-Rim, Kerstin Behn, Norma Bethke, Harald Bias, Tobias Bleicker, Dana Briesemeister, Claudia Conrad, Victor Max Corman, Chantip Dang-Heine, Doris Frey, Julie-Anne Gabelich, Janine Gerdes, Ute Gläser, Andreas Hetey, Lisbeth Hasler, Anja Heiduk, Elisa-Theresa Helbig, Alexandra Horn, Claudia Hülso, Stefanie Jentzsch, Luisa Kegel, Paolo Kroneberg, Sebastian Kühn, Irmgard Landgraf, Ngoc Han Le, Michelle Lisy, Lena Johanna Lippert, Constanze Dorothea Lüttke, Pedro de Macedo Gomes, Birgit Maeß, Janine Michel, Friederike Münn, Andreas Nitsche, Anne-Maria Ollech, Christina Pley, Anita Pioch, Annelie Richter, Mira Risham, Carolin Rubisch, Angela Sanchez Rezza, Lisa Ruby, Isabelle Schellenberger, Jenny Schlesinger, Angelika Schliephake, Georg Schwanitz, Tatjana Schwarz, Sevda Senaydin, Alexandra Stege, Sarah Steinbrecher, Paula Stubbemann, Charlotte Thibeault, Denise Treue, and Saskia Zvorc.

This work was supported by grants from COVIM: "NaFoUniMedCovid19" (FKZ: 01KX2021 to L.E.S. and F.K.L.), the Federal Institute for Drugs and Medical Devices (V-2021.3 / 1503_68403 / 2021-2022 to L.E.S. and F.K.U.), the German Center for Infection Research (DZIF) (to F.K.L.), and the Deutsche Forschungsgemeinschaft (DFG) (CRC1310 to F.K.L. and SFB-TR84 to N.S. and L.E.S.). The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

H.G., K.V., and F.K.L. are listed as inventors on pending patent application(s) on SARS-CoV-2-neutralizing antibodies filed by the University of Cologne.

About the Author

Dr. Tober-Lau is a physician and researcher at the Department of Infectious Diseases and Respiratory Medicine at Charité–Universitätsmedizin Berlin, Germany. His research interests focus on infectious diseases and global health.

References

1. Gruell H, Vanshylla K, Tober-Lau P, Hillus D, Schommers P, Lehmann C, et al. mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant. Nat Med. 2022 Jan 19 [Epub ahead of print]. https://doi.org/10.1038/s41591-021-01676-0
2. Schmidt F, Muecksch F, Weisblum Y, Da Silva J, Bednarski E, Cho A, et al. Plasma neutralization of the SARS-CoV-2 Omicron variant. N Engl J Med. 2021;386:599–601. https://doi.org/10.1056/NEJMc2119641
3. Carreño JM, Alshammary H, Tcheou J, Singh G, Raskin AJ, Kawabata H, et al.; PSP-PARIS Study Group. Activity of convalescent and vaccine serum against SARS-CoV-2 Omicron. Nature. 2021;602:682–8.
4. Nemet I, Kliker L, Lustig Y, Zuckerman N, Erster O, Cohen C, et al. Third BNT162b2 vaccination neutralization of SARS-CoV-2 Omicron infection. N Engl J Med. 2021;386:492–4. https://doi.org/10.1056/NEJMc2119358 386(5):492-49.
5. Bates TA, McBride SK, Winders B, Schoen D, Trautmann L, Curlin ME, et al. Antibody response and variant cross-neutralization after SARS-CoV-2 breakthrough infection. JAMA. 2022;327:179–81. https://doi.org/10.1001/jama.2021.22898
6. Rössler A, Riepler L, Bante D, von Laer D, Kimpel J. SARS-CoV-2 Omicron variant neutralization in serum from vaccinated and convalescent persons. N Engl J Med. 2022;386:698–700. https://doi.org/10.1056/NEJMc2119236

Address for correspondence: Florian Kurth, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Infectious Diseases and Respiratory Medicine, Augustenburger Platz 1, D-13353 Berlin, Germany; email: florian.kurth@charite.de

Article DOI: https://doi.org/10.3201/eid2805.220271

Cross-Variant Neutralizing Serum Activity after SARS-CoV-2 Breakthrough Infections

Appendix

Patients, Materials and Methods

Ethics Statement

Subjects were recruited under protocols approved by the ethics committee (EC) of Charité-Universitätsmedizin Berlin (PaCOVID-19 (*1*) Study, EA2/066/20, DRKS00021688) or the EC of the Medical Faculty of Cologne (20–1187), conducted in accordance with the Declaration of Helsinki and Good Clinical Practice principles (ICH 1996). Written informed consent was obtained from all patients or legal representatives according to regulations set by the ethics committees of Charité - Universitätsmedizin Berlin and the Medical Faculty of Cologne, respectively.

Clinical data

Study participants were eligible for inclusion in case of PCR-confirmed SARS-CoV-2 infection following vaccination with 2 doses of BNT162b2, 2 doses of mRNA-1273, or 1 dose of ChAdOx followed by 1 dose of BNT162b2 ("breakthrough cases"). Individuals with non-Omicron breakthrough infections were diagnosed between February and November 2021, before the emergence of SARS-CoV-2 Omicron variant (B.1.1.529). Out of 20 individuals, seven were diagnosed with Alpha (B.1.1.7), nine with Delta (B.1.617.2). In four cases sequencing data was not available. Individuals with Omicron breakthrough infections were diagnosed in December 2021. Two out of 20 (10%) patients with non-Omicron and 1 out of 10 (10%) with Omicron SARS-CoV-2 infection were asymptomatic, the remaining patients exhibiting mild symptoms. Non-infected vaccinated individuals were recruited from the previously published prospective observational cohort studies EICOV, COVIMMUNIZE, and COVIM, approved by the EC of Charité - Universitätsmedizin Berlin (EA4/245/20 and EA4/244/20), by the Federal Institute for Vaccines and Biomedicines (Paul Ehrlich Institute), and the EC of the state of Berlin (*2–4*). To

detect concurrent SARS-CoV-2 infection, all participants received nucleic acid amplification tests at time of sampling, and all samples were tested for anti-nucleocapsid antibodies using the SeraSpot Anti-SARS-CoV-2 IgG microarray-based immunoassay (Seramun Diagnostica). Participants with history of infection, determined by self-reporting, positive nucleic acid amplification test, or presence of nucleocapsid antibodies according to the manufacturer's specifications were excluded from analysis. Further cohort details are described in the Appendix Table. Serum samples were stored at −80°C. All study participants provided written informed consent.

Pseudovirus neutralisation assays

Serum neutralising activity was determined using a single-round infection lentivirus-based assay (*5*,*6*). Pseudoviruses were generated in HEK293T cells by co-transfecting plasmids encoding the SARS-CoV-2 spike protein, HIV-1 Tat, HIV-1 Gag/Pol, HIV-1 Rev, and luciferase using the FuGENE 6 Transfection Reagent (Promega). Culture supernatant was replaced after 24 hours, and pseudovirus-containing supernatants were harvested at 48 h to 72 h after transfection. After centrifugation and filtration (0.45 μm), pseudoviruses were stored at −80°C until use. Pseudovirus titers were determined by infecting 293T-ACE2 cells and luciferase activity was measured in relative light units (RLUs) following a 48-hour incubation period at 37°C and 5% CO2 using a microplate reader (Berthold), by adding 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). Serum was heat-inactivated at 56°C for 45 min before use. Serial serum dilutions (1:3 dilution series starting at 1:10) were co-incubated with pseudovirus supernatants for 1 hour at 37°C before addition of 293T-ACE2 cells. After 48-hours at 37°C and 5% CO2, luciferase activity was measured as described above. After subtracting background RLUs of non-infected cells, serum ID_{50}s were determined as the serum dilution resulting in a 50% RLU reduction compared to virus-infected untreated controls cells by plotting a nonlinear fit-based agonist vs normalized dose response curve with variable slope and least squares fit in GraphPad Prism 7.0 (GraphPad).

Statistical methods

Statistics were conducted in GraphPad Prism 9.0 (GraphPad). Serum samples that did not show 50% inhibition (ID_{50}) at the lowest tested dilution of 10 (lower limit of quantification, LLOQ) were assigned a value 1/2 of the LLOQ (ID_{50} = 5) for plotting graphs and for statistical

analysis. Group comparisons were done by Mann-Whitney test or Kruskal-Wallis test with Dunn's multiple testing correction, as indicated. Correlation between ID_{50}s and vaccine - infection interval was done by Spearman r.

References

1. Kurth F, Roennefarth M, Thibeault C, Corman VM, Müller-Redetzky H, Mittermaier M, et al. Studying the pathophysiology of coronavirus disease 2019: a protocol for the Berlin prospective COVID-19 patient cohort (Pa-COVID-19). Infection. 2020;48:619–26.

2. Gruell H, Vanshylla K, Tober-Lau P, Hillus D, Schommers P, Lehmann C, et al. mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant. [Internet]. Nat Med. 2022;•••. 10.1038/s41591-021-01676-0

3. Hillus D, Schwarz T, Tober-Lau P, Vanshylla K, Hastor H, Thibeault C, et al.; EICOV/COVIM Study Group. Safety, reactogenicity, and immunogenicity of homologous and heterologous prime-boost immunisation with ChAdOx1 nCoV-19 and BNT162b2: a prospective cohort study. [Internet]. Lancet Respir Med. 2021;9:1255–65. 10.1016/S2213-2600(21)00357-X

4. Tober-Lau P, Schwarz T, Vanshylla K, Hillus D, Gruell H, Suttorp N, et al.; EICOV/COVIM Study Group. Long-term immunogenicity of BNT162b2 vaccination in older people and younger health-care workers. Lancet Respir Med. 2021;9:e104–5.

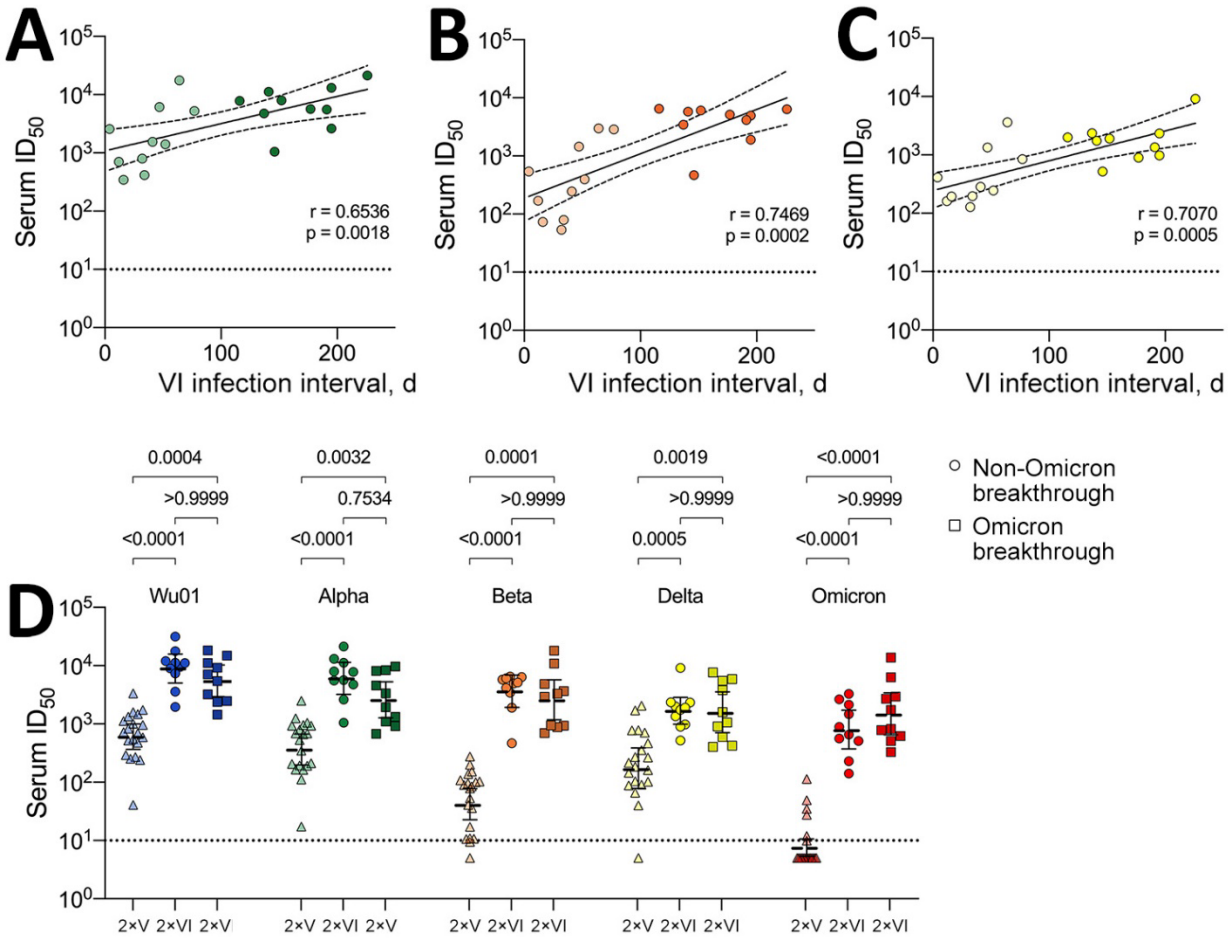
5. Vanshylla K, Di Cristanziano V, Kleipass F, Dewald F, Schommers P, Gieselmann L, et al. Kinetics and correlates of the neutralizing antibody response to SARS-CoV-2 infection in humans. Cell Host Microbe. 2021;29:917–929.e4.

6. Crawford KHD, Eguia R, Dingens AS, Loes AN, Malone KD, Wolf CR, et al. Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 Spike Protein for Neutralization Assays. [Internet]. Viruses. 2020;12:E513. 10.3390/v12050513

Appendix Table. Characteristics of participants in study of cross-variant neutralizing serum activity after SARS-CoV-2 breakthrough infections

Non-Omicron breakthrough infection cohort, n = 20	Value
Gender, no. (%)	
Male	7 (35)
Female	13 (65)
Age, median years (IQR; range)	39 (28–52; 21–93)
Reported comorbidities, no. (%)	
Arterial hypertension	4 (20)
Asthma	3 (15)
Arrhythmia	3 (15)
Tumor	1 (5)
Diabetes	1 (5)
Sarcoidosis	1 (5)
Time period of SARS-CoV-2 infection	February - November 2021
COVID-19 severity, no. (%)	
Asymptomatic	2 (10)
Mild symptoms	18 (90)
Vaccination received, no. (%)- n (%)	
2 doses BNT162b2	18 (90)
1 dose ChAdOx1 nCoV-19, 1 dose BNT162b2	2 (10)
Sampling time point, median weeks from positive PCR (IQR; range)	6.3 (5.2–7.3; 4.4–9.9)
Time between first and second dose, median weeks (IQR; range)	3.3 (3.0–5.8; 3.0–12.9)
Time between second dose and infection, median weeks (IQR; range)	13.8 (5.1–24.4; 0.6–32.3)
Omicron Breakthrough infection cohort, n = 10	
Gender, no. (%)	
Male	4 (40)
Female	6 (60)
Age, median years (IQR; range)	38 (30-47; 26-56)
Reported comorbidities, no. (%)	
Thyroidectomy	1 (10)
Time period of SARS-CoV-2 infection	December 2021
COVID-19 severity, no. (%)	
Asymptomatic	1 (10)
Mild symptoms	9 (90)
Vaccination received, no. (%)	
2 doses BNT162b2	7 (70)
1 dose ChAdOx1 nCoV-19, 1 dose BNT162b2	2 (20)
2 doses mRNA-1273	1 (10)
Sampling time point, median weeks from positive PCR (IQR; range)	2.1 (2.0-2.3; 2.0-5.7)
Time between first and second dose, median weeks (IQR; range)	6.0 (4.8-6.8; 4.0-10.4)
Time between second dose and infection, median weeks (IQR; range)	22.2 (17.7-24.9; 17.6-30.7)
Vaccinated cohort (*2*), n = 20	
Gender, no. (%)	
Male	7 (35)
Female	13 (65)
Age, median years (IQR, range)	40 (30-54; 27-78)
Reported comorbidities, no. (%)	
Cardiovascular disease	7 (35)
Respiratory disease	3 (15)
Rheumatoid arthritis	1 (5)
Polymyalgia rheumatica	1 (5)
Body mass index, median (IQR; range)	25.6 (22.6-29.8; 18.8-37.0)
Vaccination received	BNT162b2
Sampling time point, median weeks (IQR; range)	
After second dose	3.9 (3.7-4.3; 3.6-6.0)
After third dose	3.3 (3.1-4.1; 2.9-5)
Vaccination intervals, median weeks (IQR; range)	
Time between first and second dose	3.0 (3.0-3.0; 3.0-4.0)
Time between second and third dose	36.7 (33.9-38.8; 26.9-40.9)

*IQR, interquartile range; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.



Appendix Figure. Influence of time between vaccination and infection, and infecting SARS-CoV-2 variant on serum neutralizing capacity. A, B, C) Correlation between serum neutralizing activity and interval between second vaccination and non-Omicron breakthrough infection against SARS-CoV-2 Alpha (B, green), Beta (C, orange), and Delta (D, yellow) variants. Breakthrough infections within 3 months (90 days) from vaccination are indicated by light shaded symbols. Lines indicate linear regression with 95% CIs. Correlation was determined by Spearman r. D) Serum neutralizing activity against the indicated SARS-CoV-2 variants in individuals after 2-dose vaccination (triangles), 2-dose vaccination with subsequent non-Omicron (circles) or Omicron (squares) breakthrough infection. Breakthrough infections with vaccine-infection intervals ≥three months are shown. Bars indicate geometric mean ID_{50}s and 95% confidence intervals. Group comparisons by Kruskal-Wallis test. ID_{50}: 50% inhibitory serum dilution; 2x/3x vac: double/triple vaccination; vac-inf: double vaccination with subsequent breakthrough infection; Wu01: SARS-CoV-2 wildtype.