Supplementary Appendix

Supplement to: Rössler A, Knabl L, von Laer D, Kimpel J. Neutralization profile after recovery from SARS-CoV-2 omicron infection. N Engl J Med. DOI: 10.1056/NEJMc2201607

This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Appendix

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List of Investigators

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Methods

Ethics statement

The ethics committee (EC) of the Medical University of Innsbruck has approved the study with EC numbers 1064/2021 and 1191/2021.

Plasma samples

88 plasma samples from 59 individuals were collected 5 to 42 days after diagnosis of omicron BA.1 infection confirmed by qPCR. Patients with a confirmed omicron BA.1 infection were included in this retrospective study without further pre-selection. Of the unvaccinated cohort, 5 individuals had a previous infection with D614G (wild-type), 1 individual had a previous infection with B.1.1.7 (alpha), and 9 individuals had been tested positive for B.1.617.2 (delta) one to 24 months before their reinfection with omicron BA.1. In addition, 4 and 6 individuals in the vaccinated group had a confirmed prior infection with D614G (wild-type) or delta respectively one to 22 months before reinfection with omicron BA.1. Moreover, one participant had tested positive for alpha ten months before reinfection with omicron BA.1. For individuals, where more than one time point was analyzed, Figure 1 shows the time point with the maximal titer of neutralizing antibodies against omicron BA.1 (5-42 days after first positive PCR). Severity of infection was classified using self-reported symptoms using an adapted classification by the National Institutes of Health (NIH).[1] Infections were classified as asymptomatic, mild (symptoms of common cold like headache, cough, fever), mild/moderate (exertional dyspnea), moderate (breathing difficulties), or severe (SpO2 < 94).

Focus forming neutralization assay

An adapted protocol of a previously described [2] focus forming neutralization assays was performed using replication competent SARS-CoV-2 isolates (D614G (wild-type): Isolate B86.2, GISAID ID EPI_ISL_3305837; B.1.1.7 (alpha): isolate C69.1, GISAID ID EPI_ISL_3277382; B.1.351 (beta): isolate C24.1, GISAID ID EPI_ISL_1123262; P1.1 (gamma): isolate hCoV-19/Germany/BY-MVP-000005870/2021, GISAID ID EPI_ISL_2095177; B.1.617.2 (delta): isolate SARS-CoV-2-hCoV-19/USA/NY-MSHSPSP-PV29995/2021, GISAID ID EPI_ISL_2290769; BA.1 (omicron): isolate E16.1, GISAID ID EPI_ISL_6902053;). Briefly, plasma samples were heat inactivated for 30 minutes at 56 °C and clarified by centrifugation at 8,000 rpm for 5 minutes. Viruses were incubated in duplicates with serially four-fold diluted samples (1:16 to 1:16,384 dilutions in complete medium with 2% fetal calf serum) for 1 hour at 37 °C. Subsequently, confluent Vero cells stably expressing TMPRSS2 and ACE2 receptor were inoculated with serum/virus mixtures for 2h, resulting in approximately 100-200 infected cells in control wells without serum. Thereafter, serum/virus mixes were replaced by fresh medium and 8 hours later cells were fixed for 5 minutes using 96% ethanol. Infected cells were stained using serum of a convalescent patient and an Alexa Fluor Plus 488-conjugated goat anti-human IgG secondary antibody (Invitrogen, Thermo Fisher Scientific, Vienna, Austria). Infected cells were counted using an ImmunoSpot S6 Ultra-V reader and FluoroSpot software (CTL Europe GmbH, Bonn, Germany). Continuous 50% neutralization titers were calculated in GraphPad Prism 9.0.1 (GraphPad Software, Inc., La Jolla, CA, USA) using a non-linear regression. Titers <1:16 were considered negative as determined in a previous study and by analyzing pre-Corona samples.[2] Titers below 1 were set to 1 and titers >1:16,384 were set to 1:16,384.

Statistics

One-Way non-paired ANOVA (Kruskal-Wallis test) with Dunn's multiple comparisons was performed to determine statistical differences using GraphPad Prism 9.0.1 (GraphPad Software, Inc., La Jolla, CA, USA).

Acknowledgments
We thank Albert Falch, Eva Hochmuth, Evelyn Peer, Lisa-Maria Raschbichler, Bianca Neurauter, Lydia Riepler, David Bante, Lukas Perro, Stephan Amstler, Andreas Aufschnaiter, Luiza Hoch, Helena Schäfer for excellent technical and organizational support. We thank Prof. Florian Krammer and Prof. Viviana Simon for sharing their Delta isolate and Prof. Oliver T. Keppler and Dr. Marcel Stern for sharing their Gamma isolate with us.

Figure S1

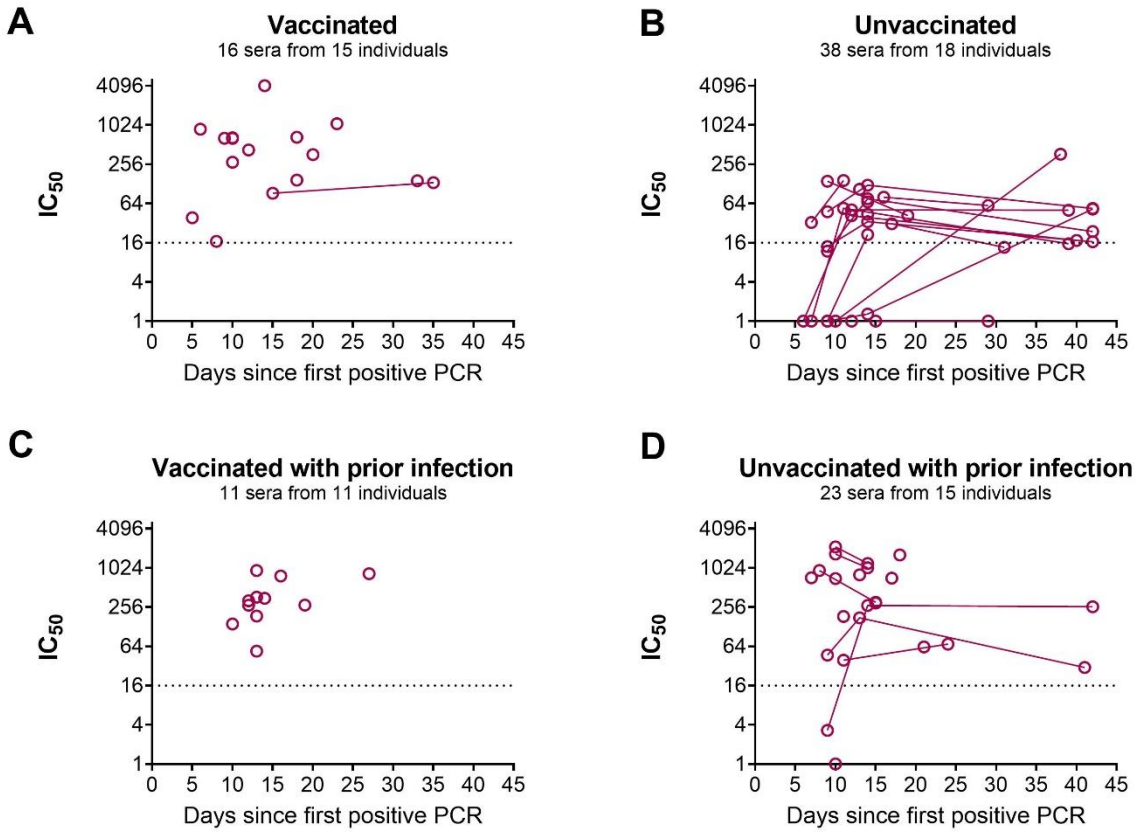

Figure S1. Titers of BA.1 neutralizing antibodies relative to time after positive PCR. Plasma samples from omicron BA.1 infected individuals were collected at indicated time points after first positive PCR of omicron BA.1 infection. 16 samples from 15 individuals were collected for vaccinated individuals with no prior infection (A), 38 samples from 18 individuals for unvaccinated individuals with no prior infection (B), 11 samples from 11 individuals for vaccinated individuals with a prior history of infection with a pre-omicron variant (C), and 23 samples from 15 individuals for unvaccinated individuals with a prior history of infection with a pre-omicron variant (D). 50 % neutralizing antibody titers (IC_{50}) against omicron BA.1 variant were determined and samples from the same patient were connected by lines. Titers below 1:16 are regarded as negative (dotted line).

Figure S2

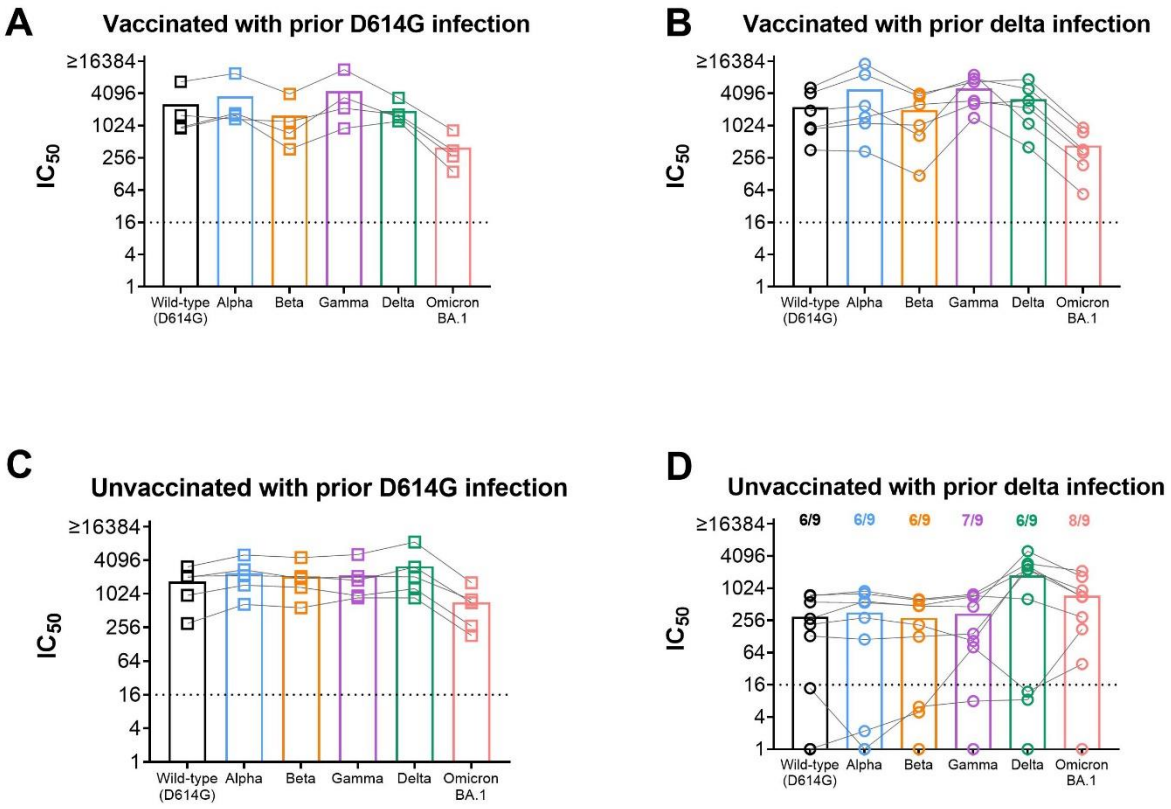

Figure S2. Titers of neutralizing antibodies for individuals with prior infection with a pre-omicron variant. Shown are neutralizing antibody titers after BA.1 infection for vaccinated individuals with a prior D614G (wild-type) (A) or B.1.617.2 (delta) variant infection (B) and for unvaccinated individuals with a prior wild-type (C) or delta variant infection (D). One individual among the vaccinated and one among the unvaccinated are not shown in the graph, both with a previous infection with the B.1.1.7 (alpha) variant. Samples were analyzed for 50 % neutralization titers (IC50) using live D614G (wild-type), alpha, B.1.351 (beta), P.1.1 (gamma), delta, or omicron BA.1 SARS-CoV-2 isolates. Individual values and mean titers (bars) are shown. Samples for each individual patient analyzed with the different virus variants are connected by lines. Titers below 1:16 are regarded as negative (dotted line).

Figure S3

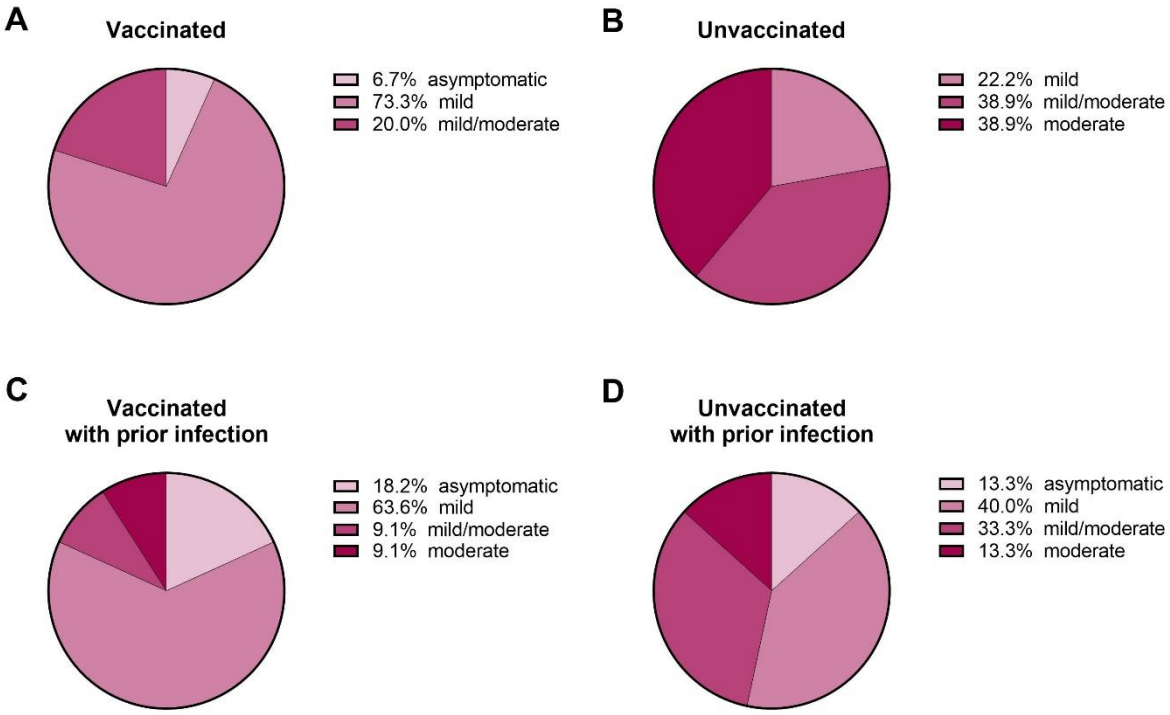

Figure S3. Clinical severity of infection. Severity of infection was defined using self-reported symptoms as asymptomatic, mild, mild/moderate, moderate, or severe using an adapted NIH classification for clinical severity of COVID-19. Shown is percentage of individuals with each severity of omicron BA.1 infection among vaccinated without prior infection (A, n=15), unvaccinated without prior infection (B, n=18), vaccinated with prior infection with a pre-Omicron variant (C, n=11), and unvaccinated with prior infection with a pre-Omicron variant (D, n=15).

Table S1. Characteristics of analyzed cohorts

	ALL PARTICIPANTS	VACCINATED	UNVACCINATED	VACCINATED WITH PRIOR INFECTION	UNVACCINATED WITH PRIOR INFECTION
NUMBER OF PARTICIPANTS (%)	**59 (100%)**	**15 (25.4%)**	**18 (30.5%)**	**11 (18.6%)**	**15 (25.4%)**
MEAN AGE IN YEARS (± SD)	38.7 (± 17.1)	34.9 (± 16.8)	46.3 (± 17.7)	36.4 (± 12.25)	35.5 (± 16.7)
ETNICITY WHITE	59 (100%)	15 (100%)	18 (100%)	11 (100%)	15 (100%)
MALE, N (% OF SUBGROUP)	26 (44.1%)	7 (46.7%)	7 (38.9%)	5 (45.5%)	7 (46.7%)
FEMALE, N (% OF SUBGROUP)	33 (55.9%)	8 (53.3%)	11 (61.1%)	6 (54.5%)	8 (53.3%)
MEAN DAYS SINCE INF.[*] (± SD)	14.3 (± 5.9)	15.4 (± 8.87)	17.0 (± 9.1)	14.73 (± 4.47)	13.4 (± 4.7)
VACCINATION, N (% OF ALL)	**26 (44.1%)**	**15 (25.4%)**	**-**	**11 (18.6%)**	**-**
N (% OF SUBGROUP)		**15 (100%)**	**-**	**11 (100%)**	**-**
BNT162B	**17 (28.8%)**	**10 (66.7%)**	-	**7 (63.6%)**	-
1 DOSE	4 (6.8)	1 (6.7%)	-	3 (27.3%)	-
2 DOSES	10 (16.9%)	6 (40%)	-	4 (36.4%)	-
3 DOSES	3 (5.1%)	3 (20%)	-	0 (0%)	-
CHADOX1	**3 (5.1%)**	**2 (13.3%)**	-	**1 (9.1%)**	-
1 DOSE	1 (1.7%)	1 (6.7%)	-	0 (0%)	-
2 DOSES	2 (3.4%)	1 (6.7%)	-	1 (9.1%)	-
3 DOSES	0 (0%)	0 (0%)	-	0 (0%)	-
MRNA-1273	**1 (1.7%)**	**1 (6.7%)**	-	**0 (0%)**	-
1 DOSE	0 (0%)	0 (0%)	-	0 (0%)	-
2 DOSES	0 (0%)	0 (0%)	-	0 (0%)	-
3 DOSES	1 (1.7%)	1 (6.7%)	-	0 (0%)	-
HETEROLOGOUS	**5 (8.5%)**	**2 (13.3%)**	-	**3 (27.3%)**	-
1 DOSE	0 (0%)	0 (0%)	-	0 (0%)	-
2 DOSES	3 (5.1%)	2 (13.3%)	-	1 (9.1%)	-
3 DOSES	2 (3.4%)	0 (0%)	-	2 (18.2%)	-
WEEKS SINCE VACC.[#] (± SD)	**15.9[$] (± 10.5)**	**12.1 (± 9.9)**	**-**	**20.4 (± 9.4)**	**-**
DISEASE SEVERITY, N (%)[§]					
ASYMPTOMATIC	5 (8.5%)	1 (6.7%)	0 (0%)	2 (18.2%)	2 (13.3%)
MILD	28 (47.5%)	11 (73.3%)	4 (22.2%)	7 (63.6%)	6 (40.0%)

MILD/MODERATE	16 (27.1%)	3 (20.0%)	7 (38.9%)	1 (9.1%)	5 (33.3%)
MODERATE	10 (16.9%)	0 (0%)	7 (38.9%)	1 (9.1%)	2 (13.3%)
SEVERE	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
PRIOR INF, N (% OF ALL)	**26 (44.1%)**	**-**	**-**	**11 (18.6%)**	**15 (25.4%)**
N (% OF SUBGROUP)		**-**	**-**	**11 (100%)**	**15 (100%)**
D614G (WILD-TYPE)	9 (15.3%)	-	-	4 (36.4%)	5 (33.3%)
B.1.1.7 (ALPHA)	2 (3.4%)	-	-	1 (9.1%)	1 (6.7%)
B.1.617.2 (DELTA)	15 (25.4%)	-	-	6 (54.5%)	9 (60.0%)
MONTHS[&] (± SD)	8.0 (± 7.3)	-	-	8.1 (± 6.7)	7.9 (± 7.7)
MEAN IC_{50}					
D614G (WILD-TYPE)	1591.9	3846.4	4.7	2239.5	768.7
B.1.1.7 (ALPHA)	2143.1	4186.4	4.3	4259.6	1114.4
B. 1.351 (BETA)	1067.2	1998.1	8.1	1773.2	889.5
P1 (GAMMA)	1711.2	2399.9	5.3	4607.1	946.0
B.1.617.2 (DELTA)	1809.0	3021.1	19.7	2595.7	2167.2
OMICRON BA.1	449.0	680.2	79.5	408.6	690.7

* Days since first positive PCR for omicron BA.1 infection; [#] Interval between last dose of vaccination and first positive PCR for omicron BA.1 infection; [§]Severity of COVID-19 was classified according to NIH guidelines of the NIH [1]; [$]for 4 of 26 individuals dates of vaccinations unknown; [&] Months since diagnosis of prior infection with wild-type or delta confirmed by qPCR

Table S2. Patient characteristics of vaccinated individuals without prior history of pre-Omicron infection

AGE (YEARS)	SEX[#]	DAYS SINCE BA.1 INFECTION*	VACCINATION	WEEKS SINCE LAST VACCINATION[$]	SEVERITY OF COVID-19
<20	f	10	BNT162b/BNT162b	16.3	mild
50-59	m	18	BNT162b/BNT162b	unknown	mild
50-59	f	18	BNT162b/BNT162b	unknown	mild
30-39	m	20	BNT162b/BNT162b	unknown	mild
30-39	m	8	BNT162b/BNT162b/BNT162b	3.1	mild/moderate
20-29	m	14	BNT162b/BNT162b/BNT162b[%]	1.6	mild/moderate
20-29	m	33	ChAdOx1	22.1	mild
20-29	f	35	BNT162b	6.0	mild
30-39	m	23	mRNA-1273/mRNA-1273/mRNA-1273[%]	0.6	mild
20-29	f	10	BNT162b/BNT162b	23.3	mild
30-39	f	6	mRNA-1273/mRNA-1273/BNT162b[%]	0.1	mild
20-29	f	10	BNT162b/BNT162b	23.0	mild/moderate
20-29	f	5	Ad26.COV2.S/BNT162b	7.6	asymptomatic
80-89	m	9	BNT162b/BNT162b/BNT162b	12.0	mild
40-49	f	12	ChAdOx1/ChAdOx1	29.3	mild

f = female; m = male; * Days since first positive PCR for omicron BA.1 infection; [%]Interval between last dose of vaccination and first positive PCR for omicron BA.1 infection less than 14 days; [$]Interval between last dose of vaccination and first positive PCR for omicron BA.1 infection

Table S3. Patient characteristics of unvaccinated individuals without prior history of pre-Omicron infection

AGE (YEARS)	SEX[#]	DAYS SINCE BA.1 INFECTION*	SEVERITY OF COVID-19
<20	m	13	mild
40-49	f	17	mild/moderate
40-49	m	14	mild/moderate
40-49	f	12	mild
60-69	m	14	mild
30-39	m	14	moderate
30-39	f	14	mild/moderate
30-39	f	42	moderate
50-59	f	14	moderate
50-59	m	14	moderate
20-29	f	11	mild/moderate
20-29	m	11	mild/moderate
60-69	m	38	moderate
60-69	f	12	moderate
80-89	f	12	mild
40-49	f	16	mild/moderate
30-39	f	9	moderate
70-79	f	29	mild/moderate

f = female; m = male; * Days since first positive PCR for omicron BA.1 infection

Table S4. Patient characteristics of vaccinated individuals with prior history of pre-Omicron infection

AGE (YEARS)	SEX[#]	DAYS SINCE BA.1 INFECTION*	VACCINATION	WEEKS SINCE LAST VACCINATION[$]	SEVERITY OF COVID-19	PRIOR INFECTION	
						MONTHS[§]	VARIANT
50-59	m	12	BNT162b/BNT162b	unknown	mild	7	delta
20-29	f	14	BNT162b/BNT162b	8.1	mild	14	wild-type
20-29	f	13	ChAdOx1/ChAdOx1	33.4	mild	2	delta
40-49	m	12	BNT162b	30.4	mild	13	D614G
20-29	m	13	BNT162b	30.3	mild	2	delta
40-49	m	13	ChAdOx1/ChAdOx1/BNT162b	8.6	mild/moderate	2	delta
50-59	f	16	ChAdOx1/ChAdOx1/BNT162b	9.0	asymptomatic	2	delta
30-39	f	13	BNT162b/BNT162b	23.3	mild	1	delta
20-29	f	10	BNT162b/BNT162b	24.6	mild	14	wild-type
20-29	m	19	BNT162b	23.0	asymptomatic	10	alpha
50-59	f	27	BNT162b/ChAdOx1	13.1	moderate	22	wild-type

f = female; m = male; * Days since first positive PCR for omicron BA.1 infection; [§] Months since confirmed diagnosis of prior D614G (wild-type), B.1.1.7 (alpha), or B.1.617.2 (delta) infection by qPCR; [$] Interval between last dose of vaccination and first positive PCR for omicron BA.1 infection

Table S5. Patient characteristics of unvaccinated individuals with prior history of pre-Omicron infection

AGE (YEARS)	SEX[#]	DAYS SINCE BA.1 INFECTION*	SEVERITY OF COVID-19	PRIOR INFECTION	
				MONTHS[§]	VARIANT
30-39	m	10	asymptomatic	1	delta
<20	f	13	mild	21	wild-type
50-59	m	17	mild	21	wild-type
<20	m	10	mild	2	delta
<20	f	8	mild	2	delta
<20	f	10	moderate	2	delta
20-29	m	15	mild/moderate	1	delta
50-59	f	14	mild	14	wild-type
20-29	f	7	mild/moderate	5	delta
20-29	m	10	mild/moderate	2	delta
60-69	m	13	moderate	2	delta
40-49	m	18	mild/moderate	14	wild-type
30-39	f	11	mild	20	wild-type
50-59	f	24	mild/moderate	2	delta
50-59	f	21	asymptomatic	10	alpha

f = female; m = male; * Days since first positive PCR for omicron BA.1 infection; [§] Months since confirmed diagnosis of prior D614G (wild-type), B.1.1.7 (alpha), or B.1.617.2 (delta) infection by qPCR

Table S6. Statistics Figure 1

	WILD-TYPE[#]	ALPHA[#]	BETA[#]	GAMMA[#]	DELTA[#]
VACCINATED	**	**	ns	ns	*
UNVACCINATED	**	****	**	****	***
VACCINATED WITH PRIOR INFECTION	ns	***	ns	****	**
UNVACCINATED WITH PRIOR INFECTION	ns	ns	ns	ns	ns

[#]Statistical difference of D614G (wild-type), B.1.1.7 (alpha), B.1.351 (beta), P1 (gamma), and B.1.617.2 (delta) to omicron BA.1 using unpaired, non-parametric ANOVA, followed by Kruskal-Wallis test with Dunn's multiple comparisons; * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001; ns = non-significant;

Table S7. Representativeness of study participants.

Disease, problem, or condition under investigation	Neutralizing antibody responses against SARS-CoV-2 variants after SARS-CoV-2 BA.1 (Omicron) infection
Special considerations related to	
Sex	Female study participants were slightly overrepresented. Seroprevalence through infection in Austria is similar among males and females.[3] A study found equal titers of neutralizing antibodies between male and female participants.[4]
Age	Severity of COVID-19 is age dependent with higher severity in older people. Mean age is similar in all 4 groups with a slight bias to older age in previously SARS-CoV-2 naïve individuals (unvaccinated without prior infection). This could influence clinical severity as presented in Supplementary Figure 3.
Ethnicity	Majority of population in Tyrol/Austria is white
Geography	Rates of vaccination and prior infection vary throughout the world. Austria has a vaccination rate of 72.2% (11.02.2022).[5] Seroprevalence in Tyrol has increased in a study among blood donors (healthy adults 18-70 years) from 3.4% in June 2020 to 82.7% in September 2021.[6]
Overall representativeness of this study	Small sample size and retrospective study design are major limitations of the current study. Larger prospective studies will be needed to decipher neutralizing antibody profiles after different constellations of prior immunity to SARS-CoV-2, e.g. different vaccination regiments.

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