informational supplement (M100-S33). Wayne (PA): The Institute; 2023.
7. Public Health Ontario. CPE – carbapenemase producing Enterobacteriaceae – Confirmation [cited 2024 Feb 26]. https://www.publichealthontario.ca/en/Laboratory-Services/Test-Information-Index/CPE-confirmation
8. Fattouh R, Tijet N, McGeer A, Poutanen SM, Melano RG, Patel SN. What is the appropriate meropenem MIC for screening of carbapenemase-producing Enterobacteriaceae in low-prevalence settings? Antimicrob Agents Chemother. 2015;60:1556–9. https://doi.org/10.1128/AAC.02304-15
9. European Committee on Antimicrobial Susceptibility Testing. EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.0. 2017 Jul [cited 2024 Feb 26]. https://www.eucast.org/resistance_mechanisms
10. Baker S, Scott TA. Antimicrobial-resistant *Shigella*: where do we go next? Nat Rev Microbiol. 2023;21:409–10. https://doi.org/10.1038/s41579-023-00906-1

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SARS-CoV-2 IgG Levels as Predictors of XBB Variant Neutralization, Israel, 2022 and 2023

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DOI: https://doi.org/10.3201/eid3005.231739

Although a vaccine against SARS-CoV-2 Omicron-XBB.1.5 variant is available worldwide and recent infection is protective, the lack of recorded infection data highlights the need to assess variant-specific antibody neutralization levels. We analyzed IgG levels against receptor-binding domain–specific SARS-CoV-2 ancestral strain as a correlate for high neutralizing titers against XBB variants.

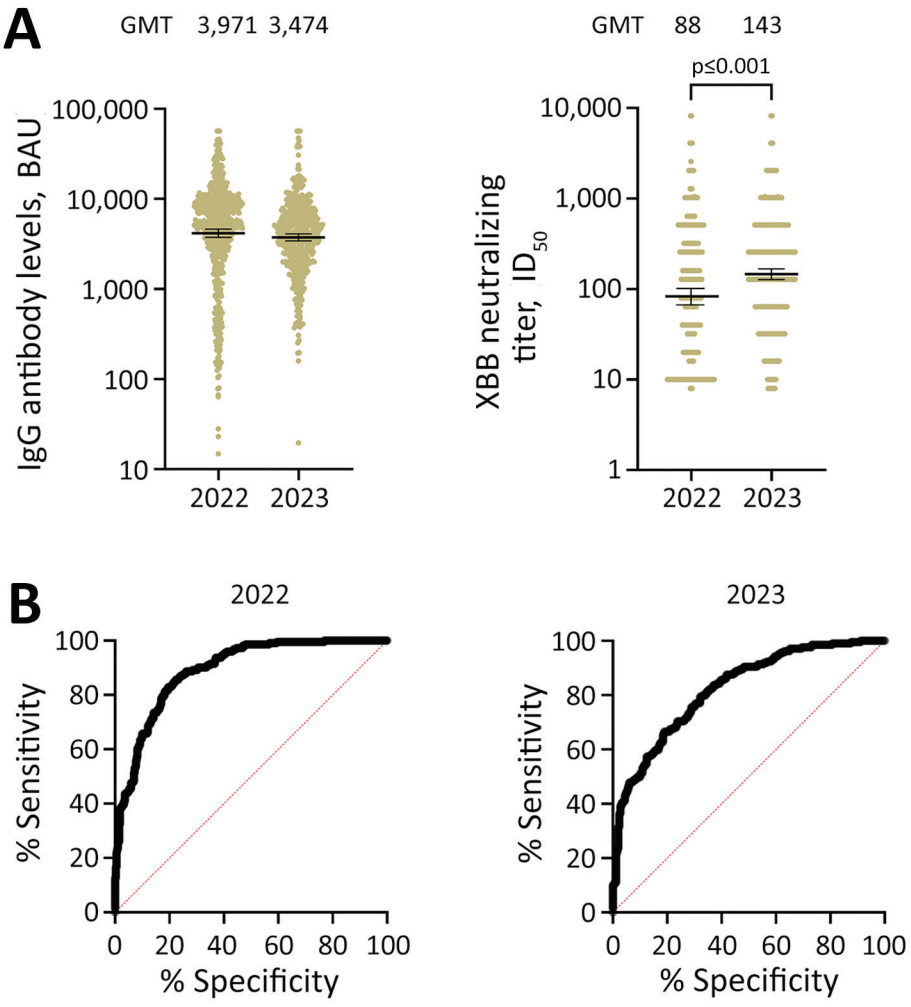
Since the beginning of 2023, SARS-CoV-2 Omicron XBB variants have led as the cause of global SARS-CoV-2 infections (*1*,*2*). SARS-CoV-2 mRNA vaccines based on the ancestral variant were shown to be less effective against Omicron variants, with reduced neutralization efficiency (*3*,*4*). Because of this reduced neutralization efficiency, updated mRNA vaccines, like the monovalent XBB1.15 vaccine, were developed and distributed (*5*). High levels of neutralizing and receptor-binding domain (RBD) binding IgG levels are known to be correlated with protection from infection or severe disease (*6*,*7*). The evasiveness of Omicron variants against neutralizing antibodies induced by vaccination or infection with previous variants demonstrated the importance of determining variant-specific neutralizing antibodies (*4*). In this study, we investigated the utility of measuring RBD IgG levels against the SARS-CoV-2 ancestral (wild-type [WT]) strain to predict titers of XBB-specific neutralizing antibodies.

During February 2022–August 2023, we obtained 1,070 samples from 373 study participants at Sheba Medical Center in Ramat Gan, Israel, and tested the samples for levels of IgG against WT-RBD and XBB-specific neutralizing antibody levels (Appendix, https://wwwnc.cdc.gov/EID/article/30/5/23-1739-

Table. Sex, age range, and COVID-19 history of patient participants who provided samples for testing IgG against SARS-CoV-2 ancestral strain and Omicron XBB-specific neutralizing antibody levels in 2022 and 2023, Israel*

Variable	Value
Sex	
F	251 (67)
M	122 (33)
No. COVID-19 vaccinations received	
0	1 (0.3)
1	13 (3.5)
2	5 (1.3)
3	102 (27)
4	215 (58)
5	36 (9.7)
6	1 (0.3)
No. COVID-19 infections	
0	227 (61)
1	120 (32)
2	22 (5.9)
3	3 (0.8)
4	1 (0.3)

*Values are no. (%) except as indicated.



Year	Cutoff (BAU)	Sensitivity (95% CI)	Specificity (95% CI)
2022	>8,712	66% (59–72)	90% (87–93)
2023	>6,278	51% (44–58)	90% (85–93)

Figure. Binding IgG and neutralizing titer levels from samples collected in 2022 and 2023 from patient participants at the Sheba Medical Center, Israel, and the prediction of SARS-CoV-2 Omicron XBB neutralization by RBD-WT IgG levels from those samples. A) Scatter plot analyses of 1,071 WT IgG and XBB-specific neutralizing titers in samples obtained from healthcare workers during 2022 and 2023. Horizontal lines indicate GMTs; error bars indicate 95% CIs. GMT of each timepoint is indicated. B) ROC curves showing the diagnostic value of WT IgG levels for high (titer ≥250) XBB-specific neutralization levels. Sensitivity and specificity determinants for specific cut off levels are shown. BAU, binding antibody unit; GMT, geometric mean titer; ID_{50}, 50% inhibitory dilution; RBD, receptor-binding domain; ROC, receiver operating characteristic; WT, SARS-CoV-2 ancestral (wild-type) strain.

App1.pdf). Most of the study participants were vaccinated ≥3 times with the BNT162b2 (Pfizer-BioNTech, https://www.pfizer.com) or mRNA1273 (Moderna, https://www.modernatx.com) vaccines, and 39% were previously infected (Table; Appendix Table). Because XBB variants were only marginally circulating in Israel during 2022 but were the dominant variants during 2023 (Appendix Figure 1), we examined antibody levels separately for 2022 and 2023. Although IgG levels against WT virus were lower in 2023 (geometric mean titer of 3,474 binding antibody units [BAU] [95% CI 3,093–3,902] in 2022 vs. 3,971 BAU [95% CI 3,496–4,511] in 2023), 50% inhibitory dilution neutralizing antibody titers against XBB were significantly higher (geometric mean titer of 88 [95% CI 75–1,040] in 2022 vs. 143 [95% CI 121–168] in 2023) 2 (Figure 1, panel A).

We assessed the correlation between WT IgG and XBB neutralizing antibody levels. Although a strong correlation between RBD IgG and neutralizing antibody titers was maintained in both years, a stronger correlation was detected in 2022 (repeated measures correlation of 0.54 [95% CI 0.46–0.60]) compared with 2023 (repeated measures correlation of 0.31 [95% CI 0.17–0.44]). The regression co-efficient between IgG and neutralizing antibody levels was different for 2022 and 2023 (Appendix Figure 2). We found the expected

value of XBB specific neutralizing antibody titers for IgG of 7,000 BAU was 156 in 2022 and 276 in 2023.

We investigated if the correlation between WT IgG and XBB neutralization levels could be applied to predict persons with high XBB neutralization titers. A titer of 50% inhibitory dilution ≥1:250 was considered to be high neutralizing. US Food and Drug Administration guidelines consider titers of 50% inhibitory dilution ≥1:250 as eligible for transfusion as COVID-19 convalescent plasma (*8*,*9*). We found 36% of samples in 2022 and 46% of samples in 2023 had 50% inhibitory dilution ≥1:250. The area under the receiver operating characteristic curve was 0.89 (95% CI 0.87–0.92) for 2022 and 0.82 (95% CI 0.79–0.86) for 2023, suggesting a good discrimination between high and low titers based on WT IgG levels. Requiring a specificity of 90%, the receiver operating characteristic analysis showed a sensitivity of 66% (95% CI 59%–72%) for WT IgG levels >8,712 BAU in 2022 and a sensitivity of 51% (95% CI 44%–58%) for WT IgG levels >6,278 BAU in 2023 (Figure 1, panel B).

The results of our study show that measuring IgG against the SARS-CoV-2 ancestral strain (WT-RDB) can predict the presence of high neutralizing antibody levels against current circulating variants. We focused on the prediction of neutralizing antibodies against XBB variants because it was the immune antigen present in the vaccines available during the study period. We found that significantly higher XBB neutralizing antibody titers, but lower WT-RBD IgG levels were detected in samples obtained during 2023 compared with 2022. One explanation is that increased exposure to XBB-related variants in 2023 led to the development of XBB-specific antibodies paired with waning WT IgG levels. Our regression co-efficient analysis showed that samples obtained in 2022 had higher mean WT IgG levels than in 2023, despite having similar XBB neutralizing levels. The WT IgG level cutoff that can predict XBB-specific high neutralizing antibodies with 90% specificity was lower in 2023 compared with 2022.

The continued waves of COVID-19 infections together with SARS-CoV-2 vaccinations have diversified the immune protection of humans worldwide. Vital public health actions to prevent COVID-19 infections include prioritizing vaccination on the basis of known immunity, estimating the immune status of the population, ensuring COVID-19 convalescent plasma has high neutralizing antibodies, and investigating the effects of updated vaccines in persons with varying levels of neutralizing antibodies. Our results show that, regardless of any knowledge of previous SARS-CoV-2 infections, WT IgG levels are correlated and can predict XBB-specific neutralizing antibody titers.

About the Author

Dr. Lustig is the director of the Israeli Central Virology Laboratory of the Ministry of Health at Sheba Medical Center. His primary research interest is characterization of the immune response against emerging viral pathogens.

References

1. Chatterjee S, Bhattacharya M, Nag S, Dhama K, Chakraborty C. A detailed overview of SARS-CoV-2 omicron: its sub-variants, mutations and pathophysiology, clinical characteristics, immunological landscape, immune escape, and therapies. Viruses. 2023;15:167. https://doi.org/10.3390/v15010167
2. Miller J, Hachmann NP, Collier AY, Lasrado N, Mazurek CR, Patio RC, et al. Substantial neutralization escape by SARS-CoV-2 omicron variants BQ.1.1 and XBB.1. N Engl J Med. 2023;388:662–4. https://doi.org/10.1056/NEJMc2214314
3. Accorsi EK, Britton A, Fleming-Dutra KE, Smith ZR, Shang N, Derado G, et al. Association between 3 doses of mRNA COVID-19 vaccine and symptomatic infection caused by the SARS-CoV-2 omicron and delta variants. JAMA. 2022;327:639–51. https://doi.org/10.1001/jama.2022.0470
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. 2022;386:492–4. https://doi.org/10.1056/NEJMc2119358
5. Regan JJ, Moulia DL, Link-Gelles R, Godfrey M, Mak J, Najdowski M, et al. Use of updated COVID-19 vaccines 2023–2024 formula for persons aged ≥6 months: recommendations of the advisory committee on immunization practices—United States, September 2023. MMWR Morb Mortal Wkly Rep. 2023;72:1140–6. https://doi.org/10.15585/mmwr.mm7242e1
6. Bergwerk M, Gonen T, Lustig Y, Amit S, Lipsitch M, Cohen C, et al. Covid-19 breakthrough infections in vaccinated health care workers. N Engl J Med. 2021;385:1474–84. https://doi.org/10.1056/NEJMoa2109072
7. Garcia-Beltran WF, Lam EC, Astudillo MG, Yang D, Miller TE, Feldman J, et al. COVID-19-neutralizing antibodies predict disease severity and survival. Cell. 2021;184:476–488.e11. https://doi.org/10.1016/j.cell.2020.12.015
8. McDyer JF, Azimpouran M, Durkalski-Mauldin VL, Clevenger RG, Yeatts SD, Deng X, et al. COVID-19 convalescent plasma boosts early antibody titer and does not influence the adaptive immune response. JCI Insight. 2023;8:e167890. https://doi.org/10.1172/jci.insight.167890
9. US Food and Drug Administration. 2021. COVID-19 convalescent plasma EUA decision memo 02052021 [cited 2021 Dec 27]. https://www.fda.gov/media/141480/download

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Article DOI: https://doi.org/10.3201/eid3005.231739

SARS-CoV-2 IgG Levels as Predictors of XBB Variant Neutralization, 2022 and 2023

Appendix

Materials and Methods

Study Samples

All of the study participants were part of the Sheba COVID-19 study cohorts. These include health care workers (HCW) from the Sheba serology study (*1*,*2*), which was initiated before the rollout of the first COVID-19 vaccine dose and volunteers from clinical studies initiated by Sheba (*3*,*4*). All of Sheba COVID-19 study cohorts participants (SSP) were encouraged to undergo antigen rapid diagnostic testing (Ag-RDT) or quantitative real-time polymerase chain reaction (qRT-PCR) for SARS-CoV-2 detection in the event of exposure to an infected person or if they exhibited any COVID-19–related symptoms. Additionally, SSP were encouraged to test weekly and received reminders through emails, text messages, or phone calls. All cohort members were asked to perform serology testing monthly.

The 1070 samples included in this study were randomly obtained from 373 Sheba Medical Center Study Participants from February 23rd 2022 until August 16th 2023. Since each SSP donated several samples to this study, Table 1 shows the demographic and SARS-CoV-2 exposure (COVID-19 vaccination and infection) history of SSP and appendix Table 1, the SARS-CoV-2 exposure history of each sample.

Statistical Analysis

Geometric Mean Titers (GMTs) with 95% confidence intervals were estimated for each antibody each year by fitting an intercept-only linear regression model with the logarithm of the antibody measurement as the outcome, with a random effect per person to account for repeated

measurements. A direct comparison between years was performed by fitting the same model with the year as the sole predictor.

Correlation between the two antibody types was estimated for each year using repeated measures correlation [https://www.frontiersin.org/articles/10.3389/fpsyg.2017.00456/full]. A linear regression with XBB neutralizing antibodies as the outcome, WT IgG antibodies as the sole predictor, and a random effect per person, was used to estimate and plot a regression line, and to estimate the expected value of XBB antibodies for a person with IgG antibody levels of 7000. A confidence interval for this prediction was estimated using the non-parametric percentile bootstrap with 1000 repetitions.

ROC curves were estimated and plotted in the standard manner, with the sensitivity level at 90% specificity noted. A 95% confidence interval for this level was estimated using the exact binomial distribution.

Serology Assays

Samples were tested using the SARS-CoV-2 IgG II Quant (6S60, Abbott) test. These commercial tests were performed according to the manufacturer's instructions.

A SARS-CoV-2 lentivirus-based neutralization assay was performed to assess XBB-specific (either XBB1.9 or XBB1.16) neutralizing antibody levels measured in 50% inhibitory dilution (ID50). Neutralization assay was adapted from (*5*) with minor modifications.

Lentiviral particles were produced by co-transfecting HEK293T/17 cells with an expression vector encoding variant specific SARS-CoV-2 spike alongside packaging vector pCMVDR8.2, luciferase reporter vector pHR′CMV-Luc and a TMPRSS2 expression vector (a gift from Dr. Daniel Douek, Vaccine Research Institute (VRC), National Institute of Health (NIH). MD, USA). Supernatant was collected from cells 48-hour post transfection and used for subsequent neutralization. Transfection was done using Lipofectamine 3000 (Thermo Scientific, cat# L3000001) as specified by the manufacture.

For neutralization, serum samples were heat inactivated in 56°C for 30 minutes. Serum samples were 2-fold diluted in a 96-well plate in dilution medium (MEM 5% FBS), overlaid with pseud-typed Lentivirus solution and incubated in 37°C for one hour. Pseudovirus-serum complexes were than overlaid with HEK293 TMPRSS2-ACE2 cells suspended in dilution

medium. Cells were incubated in 37°C for 72 hours. Following incubation, luminesces was quantified by lysing the cells with tissue culture lysis reagent (Promega, cat# E1531) and adding luciferase assay substrate (Promega, cat# E1501). Luminescence was read using a Varioskan LUX Multimode Microplate Reader (Thermo Scientific).

References

1. Gilboa M, Gonen T, Barda N, Cohn S, Indenbaum V, Weiss-Ottolenghi Y, et al. Factors associated with protection from SARS-CoV-2 omicron variant infection and disease among vaccinated health care workers in Israel. JAMA Netw Open. 2023;6:e2314757. PubMed https://doi.org/10.1001/jamanetworkopen.2023.14757

2. Lustig Y, Sapir E, Regev-Yochay G, Cohen C, Fluss R, Olmer L, et al. BNT162b2 COVID-19 vaccine and correlates of humoral immune responses and dynamics: a prospective, single-centre, longitudinal cohort study in health-care workers. Lancet Respir Med. 2021;9:999–1009. PubMed https://doi.org/10.1016/S2213-2600(21)00220-4

3. Barda N, Lustig Y, Indenbaum V, Zibly D, Joseph G, Asraf K, et al. Immunogenicity of Omicron BA.1-adapted BNT162b2 vaccines: randomized trial, 3-month follow-up. Clin Microbiol Infect. 2023;29:918–923.e7. PubMed https://doi.org/10.1016/j.cmi.2023.03.007

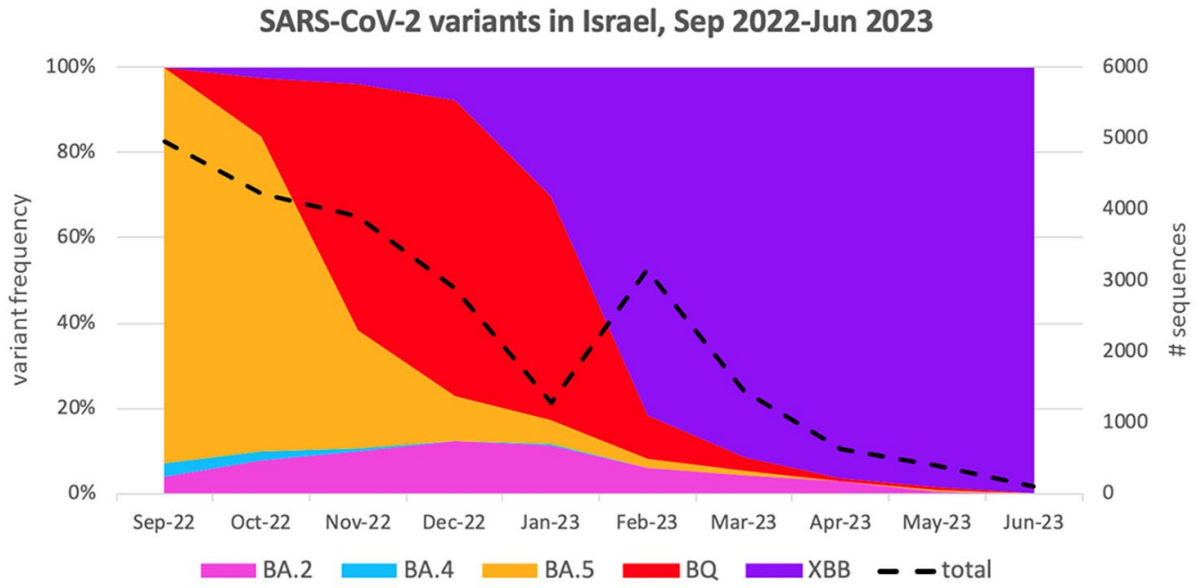
4. Canetti M, Barda N, Gilboa M, Indenbaum V, Mandelboim M, Gonen T, et al. Immunogenicity and efficacy of fourth BNT162b2 and mRNA1273 COVID-19 vaccine doses; three months follow-up. Nat Commun. 2022;13:7711. PubMed https://doi.org/10.1038/s41467-022-35480-2

5. Pegu A, O'Connell SE, Schmidt SD, O'Dell S, Talana CA, Lai L, et al. Durability of mRNA-1273 vaccine-induced antibodies against SARS-CoV-2 variants. Science. 2021;373:1372–7. PubMed https://doi.org/10.1126/science.abj4176

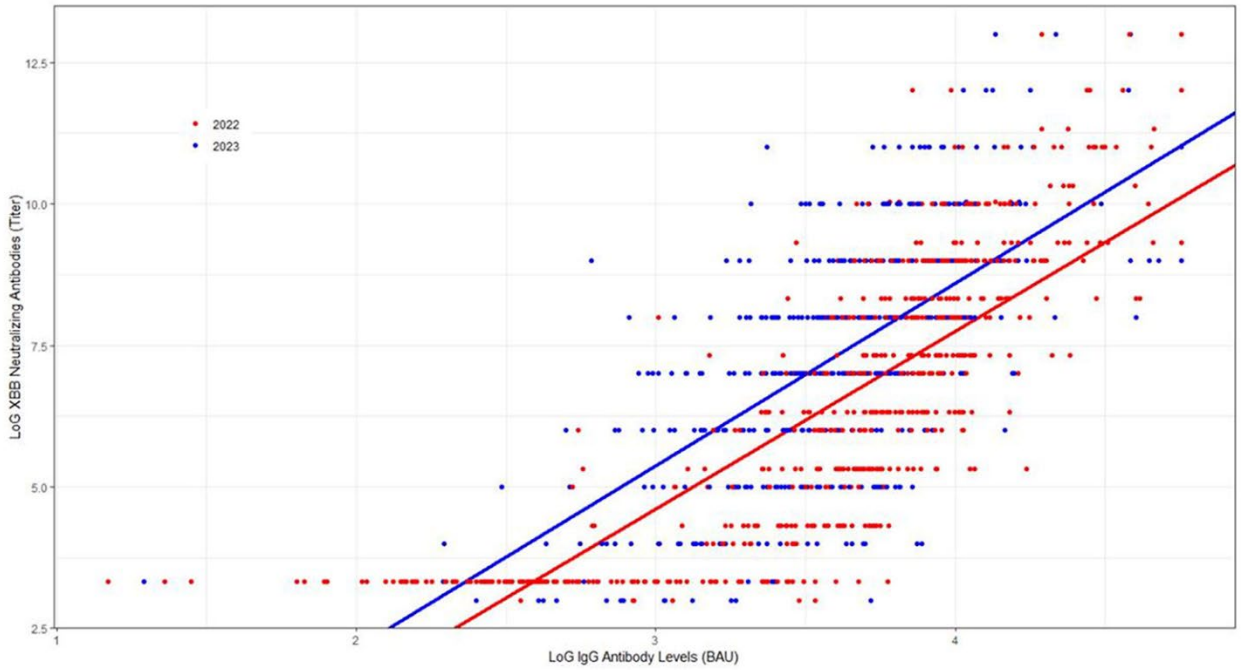
Appendix Table. Previous exposure of the samples used in this study in 2022 and 2023.

Variable	*2022, N = 650*[1]	*2023, N = 420*[1]
Number of COVID-19 Vaccines		
0	0 (0%)	2 (0.5%)
1	20 (3.1%)	18 (4.3%)
2	3 (0.5%)	4 (1%)
3	38 (5.8%)	112 (27%)
4	520 (80%)	250 (60%)
5	69 (11%)	33 (7.9%)
6	0 (0%)	1 (0.2%)
Number of COVID-19 Events Documented		
0	519 (80%)	250 (60%)
1	111 (17%)	138 (33%)
2	19 (2.9%)	28 (6.7%)
3	1 (0.2%)	3 (0.7%)
4	0 (0%)	1 (0.2%)

[1]n(%)



Appendix Figure 1. SARS-CoV-2 variants circulating in Israel between September 2022 and June 2023. Variant frequency of sequenced SARS-CoV-2 positive samples in Israel, depicted over time (left y-axis). The dashed line (right y-axis) represents the number of total sequenced SARS-CoV-2 positive samples over time.



Appendix Figure 2. Correlation of XBB neutralization by RBD-WT IgG antibody levels. Correlation of anti RBD IgG antibody levels with XBB specific neutralizing titers in 2022 (red) and 2023 (blue). Each line was obtained by linear regression and represents the association between binding and neutralizing antibodies in 2022 and 2023.