Increased Serum IgG Antibody, Avidity, and Surrogate Virus Neutralization Response after a Third Dose for the BNT162b2 mRNA Vaccine to SARS-CoV-2

TO THE EDITOR:

SARS-CoV-2 antibody concentrations decline over time following vaccination (1). On September 22, 2021, the Food and Drug Administration approved a booster dose of the Pfizer-BioNTech vaccine for SARS-CoV-2 under emergency use authorization. In a recent paper, Eliakim-Raz et al. showed that non-neutralizing IgG antibody concentrations increased dramatically over baseline (median 440 to 25 468 AU/mL) following a third dose in 97 individuals aged ≥60 years (2). No studies to date have compared antibody concentrations following the second and third dose in the same individuals and most have focused on the quantity rather than quality of the antibodies. Here we investigate the maturation of the humoral immune response in the same individuals, through measures of SARS-CoV-2 antibody concentration, avidity, and virus neutralization capacity, following the second and third vaccination.

We previously recruited and investigated antibody responses in 150 healthcare workers receiving the Pfizer-BioNTech vaccine (1). In the current study, we compared the antibody response in a subset n = 24, 75% female, median age 47 (9.6) at 3 timepoints: 1 month after the second dose (27 [8] days), baseline before the third dose (8.5 [0.6] months after the second dose), and 1 month after the third dose (32 ± 5 days). There was no statistical difference ($P > 0.05$) between the blood collection days after vaccination. Samples were tested for SARS-CoV-2 IgG antibody concentration using the Pylon assay as previously described (3), SARS-CoV-2 antibody avidity (4), and neutralizing capacity using surrogate virus neutralization tests (sVNT) from Bio-Rad Laboratories (5). Permission to collect serial blood samples, with signed informed consent, was granted by the University of California, San Francisco, Institutional Review Board.

As shown in Fig. 1, A, the IgG antibody responses measured in relative fluorescent units (RFU) averaged 2267 ± 1439 RFU 1 month after the second dose, 180 ± 111 RFU before the third dose ($P < 0.01$ vs second dose) and 3646 ± 1830 RFU 1 month after the third dose ($P < 0.01$ vs second dose and baseline for third dose). Only one individual had a significantly lower antibody response after the third dose (3545 RFU) compared to their response after the second dose (5419 RFU). In a subgroup analysis, we found no difference between RFU values for males (n = 6) vs females (n = 18; data not shown), recognizing that the number of samples tested is small.

Despite waning antibody concentrations from 1 to 8.5 months ("baseline") after the second dose, SARS-COV-2 IgG avidity continued to increase over time (Fig. 1, B). IgG avidity was 73.4 (9.1%) 1 month after the second dose and increased to 96.6 (9.4%) and 98.3 (3.2%), respectively, just before and 1 month after the third dose ($P < 0.001$ vs the second dose). Using the sVNT, percent inhibition at 1 month after the second dose was 88.5 (9.1%), which sharply declined to 39.7 (15.8%) just before the third dose ($P < 0.001$) and increased to 96.3 (4.0%) 1 month after the third dose ($P < 0.001$). The same pattern was observed when these samples were tested using the sVNT to evaluate antibody neutralization against SARS-CoV-2 variants (alpha, beta, gamma, delta, kappa, and epsilon, results not shown).

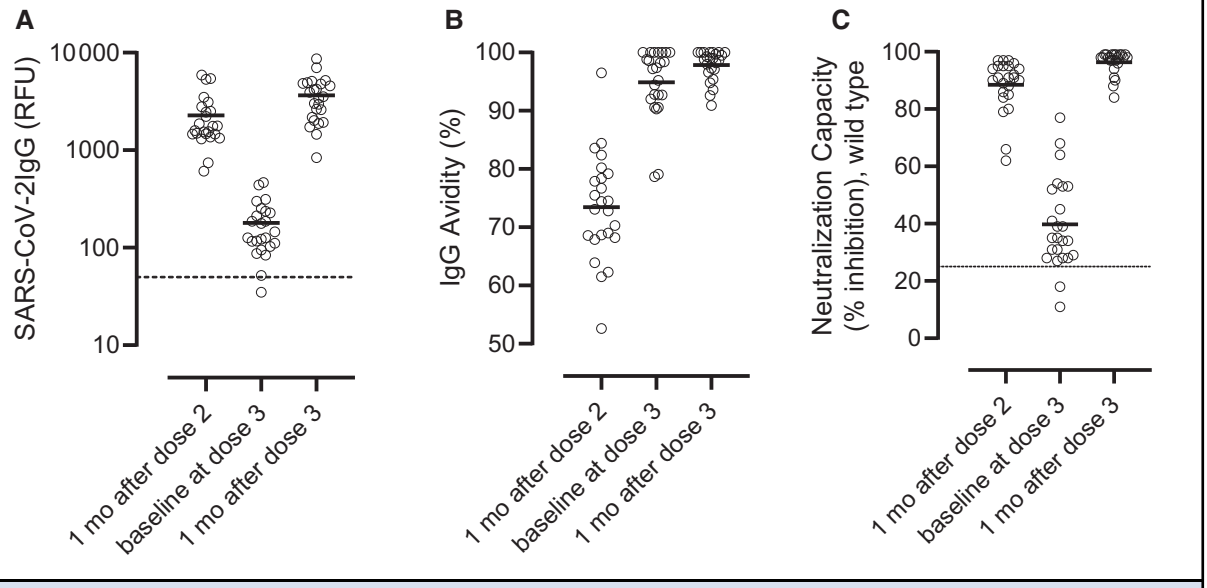

Fig. 1. SARS-CoV-2 IgG antibody response in serum after the second dose, before the third dose, and after the third dose of the Pfizer-BioNTech vaccine.
(A), SARS-CoV-2 IgG response (RFU); (B), SARS-CoV-2 avidity (%); (C), percent inhibition of neutralizing antibodies against wild-type SARS-CoV-2. mo = month.

An sVNT assay against the omicron variant was not available at the time of testing.

These data suggest that after the second dose of the Pfizer-BioNTech vaccine, the quantity of SARS-CoV-2 IgG antibodies declines over 9 months, while the remaining antibody pool continues to mature as measured by avidity. These antibodies are also still capable of producing some degree of virus neutralization at 9 months, however, in our study 2 individuals' sVNT results dropped below the cutoff prior to the third dose. The third dose not only restores but exceeds the antibody response relative to the second dose, in terms of concentration, avidity, and neutralizing capacity. Additional increases in avidity may be observed with a longer duration after vaccination, but no studies are being planned. Our data suggest that booster inoculations enhance the humoral immune response against SARS-CoV-2, however, it is still unknown what degree of neutralizing capacity of SARS-CoV-2 antibodies correlates with significant immunity or complete protection from COVID-19 infection. Similarly, more research is needed to determine what antibody measure or characteristic, if any, correlates with degree of severity in breakthrough infections.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest*: **Employment or Leadership:** None declared. **Consultant or Advisory Role:** None

declared. **Stock Ownership:** None declared. **Honoraria:** None declared. **Research Funding:** Funding for testing was provided by Bio-Rad Laboratories. **Expert Testimony:** None declared. **Patents:** None declared.

REFERENCES

1. Wu AHB, Nguyen ED, Ong CM, Yun C, Lynch KL. Rate of serum SARS-CoV-2 antibody decline for two mRNA vaccines. J Appl Lab Med 2022;7:625–7.
2. Eliakim-Raz N, Leibovici-Weisman Y, Stemmer A, Ness A, Awwad M, Ghantous N, Stemmer SM. Antibody titers before and after a third dose of the SARS-CoV-2 BNT162b2 vaccine in adults aged ≥60 years. JAMA 2021; 326:2203–4.
3. Lynch KL, Whitman JD, Lacanienta NP, Beckerdite EW, Kastner SA, Shy BR. Magnitude and kinetics of anti-SARS-CoV;2, antibody responses and their relationship to disease severity. Clin Infect Dis 2021;72:301–8.
4. Luo YR, Chakraborty I, Yun C, Wu AHB, Lynch KL. Kinetics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody avidity maturation and association with disease severity. Clin Infect Dis 2021;73:e3095-07.
5. Lynch KL, Zhou S, Kaul R, Wu AHB. Evaluation of neutralizing antibodies against SARS-CoV-2 variants after infection and vaccination using a multiplexed surrogate virus neutralization test. 2022;68:702–12.

Alan H.B. Wu*
Kara L. Lynch

Department of Laboratory Medicine, University of California, San Francisco, CA, USA

*Address correspondence to this author at: Department of Laboratory Medicine, Zuckerberg San Francisco General Hospital, 1001 Potrero Ave., San Francisco, CA 94110, USA. Fax 628-206-3045; E-mail alan.wu@ucsf.edu

https://doi.org/10.1093/jalm/jfac018

Persistent Elevation of Serum Human Chorionic Gonadotropin in a Nonpregnant Woman

TO THE EDITOR:

We read with interest the case report by Bidot et al. (1) describing an elevated serum human chorionic gonadotropin (hCG) concentration in a 39-year-old female with end-stage kidney disease (ESKD) on dialysis and an estimated glomerular filtration rate of 3 mL/min. The patient had a serum hCG of 85 IU/L. She had a serum hCG of 8 IU/L one year prior, and 6 weeks later, the concentration was again 8 IU/L. The authors state that this is an observation regularly encountered in women in ESKD.

Recently, we published a retrospective review of 4595 paired hCG and creatinine measurements for all serum hCG results from women that were performed at our hospital from June 9, 2016 to October 31, 2020 (2). We identified 246 samples with mildly elevated hCG. Our study demonstrated an association between mildly elevated hCG and degree of renal dysfunction in women > 40 years. In women ≤ 40 years of age and estimated glomerular filtration rate < 15 mL/min [like the patient in the Bidot et al. case (1)], 5.1% demonstrated low elevations in hCG. However, hCG concentrations >80 IU/L is rare in nonpregnant women of any age with or without ESKD (2–5). Synder et al. showed that the highest serum hCG value in a cohort of nonpregnant females ages 18 to 40 years was 4.6 IU/L (3). Haninger-Vacariu et al. found that an hCG cutoff of >14 IU/L had a sensitivity of 100% and a specificity of 86% for identifying pregnancy in a cohort of 71 female dialysis patients ages 18 to 50 years (4). And lastly, Soni et al. demonstrated that out of 62 females of any age with chronic kidney disease, the highest serum hCG concentration was 27 IU/L (5). All of the described studies used serum hCG assays designed to detect total (intact and free βhCG subunit) hCG.

In our retrospective cohort study, the highest mildly elevated hCG concentration in women > 40 years with decreased renal function was 22 IU/L. Among women ages 30 to 40 years with decreased renal function, 6 had hCG >22 IU/L (range 24–50 IU/L);