Rate of Serum SARS-CoV-2 Antibody Decline for two mRNA Vaccines

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Nonstandard Abbreviations: COVID-19, Corona Virus Disease-2019; FDA, Food and Drug Administration; ZSFG, Zuckerberg San Francisco General Hospital; SARS-CoV-2, Severe Acute Respiratory Syndrome-Coronavirus 2; WHO, World Health Organization; RFU, relative fluorescence units; IRB, Institutional Review Board; UCSF, University of California, San Francisco.

To the Editor:

mRNA COVID-19 vaccines are effective in the prevention of infection. In the U.S., the Pfizer-BioNTech and Moderna vaccines were FDA cleared in December 2020. While initial studies showed similar antibody responses between the vaccines *(1)* there are no data extending beyond 6 months. The long-term antibody response to vaccines may be useful to assess long-term efficacy and the timing of a potential booster injection in adults.

We recruited 189 healthcare workers at ZSFG Hospital who were vaccinated in December 2020 or January 2021 (n=150 Pfizer-BioNTech, n=39 Moderna). We recorded the

age, sex, vaccine manufacturer, days from the vaccinations and date of blood collection. No one had a previous COVID-19 infection as determined by self-report and all were negative for antibodies to the SARS-CoV-2 nucleocapsid protein. Serum samples were collected via phlebotomy at least 1 week after the second vaccination. A second sample, separated by at least 1 month, was obtained from 87 subjects (n=56 Pfizer-BioNTech, n=31 Moderna). The protocol was approved by the UCSF IRB, with written informed consent.

The serum was tested for IgG antibodies to SARS-CoV-2 using Pylon (ET Healthcare, cutoff 50 relative fluorescence units, RFU) which preferentially targets the receptor binding domain of the spike protein *(2)*. During validation, a SARS-CoV-2 human IgG standard spiked into negative serum was measured at 6 concentrations ranging from 1-300 μg/mL and was linear to 300 μg/mL corresponding to 6976 RFU. The Pylon assay correlated to standards produced by the WHO (y=0.77x+18, r=0.986). IgG results were broken down into five bins representing 2-6 months since the initial vaccination. The rate of antibody change was determined for paired samples from the same individual, separated by >60 days. With two data points, we cannot determine if this rate is linear, therefore, we excluded pair samples that were separated by <2 months given that the rate of decline may be faster in the first month after the second dose We used the Student's *t* test to compare the IgG results and rate of change between the vaccines (ver. 19.6.4, MedCalc, Ostend, Belgium, a $p<0.05$ was considered statistically significant).

Fig. 1A shows all results plotted against the days since first vaccination. For all bins, the mean IgG concentration for the Moderna vaccine group was significantly higher than for the Pfizer-BioNTech vaccine group. For subjects with paired samples separated by >30 days, the antibody concentration was lower in the second sample. The normalized rate of decline was lower for the Moderna vaccine at -25.5%/month vs. the Pfizer-BioNTech vaccine at -

28.8%/month (p=0.025, Fig. 1B) on the subset of paired samples. Between the vaccines, there was no difference (p>0.05) in the distribution of sex (males 43% vs 27%, respectively), age (46 vs. 43), days from the first vaccine dose to first blood collection (51 vs. 45), days from the first dose to the second collection (133 vs. 122) and days between collections (85 vs. 80). It may be possible that the differences seen between the Pfizer-BioNTech and Moderna vaccines is a result of the different doses used (30 vs 100 μg, respectively) and recommended time interval between the first and second injections (3 vs. 4 weeks). One limitation is the smaller enrollments of subjects given the Moderna vs. Pfizer-BioNtech vaccines.

It cannot be concluded that higher SARS-CoV-2 antibody concentrations coupled with a slightly decreased rate of decline may indicate a higher degree of immunity for individuals receiving the Moderna relative to the Pfizer-BioNTech vaccine. Demonstration of higher protection requires a study on the rate of breakthrough infection, which are still uncommon *(3)*. A recent study of breakthrough infections in 1497 Pfizer-BioNTech vaccinated healthcare workers found neutralizing antibody titers to be lower in cases compared to matched uninfected controls *(4)*. It is unknown what serum antibody concentration is required for host protection; therefore the FDA has recommended against routine serological testing after vaccination *(5)*. Standardization of serological assays to demonstrate linearity provide quantitative results in a common unit of measurement and is a necessary step in determining an antibody concentration that infers immunity. Many commercially available methods have small analytical measurement ranges limiting comparison of results between methods. Recently there have been efforts to standardize assay results to a common unit (BAU/mL). The Pylon assay showed correlation of RFU vs BAU/ml using WHO standards however, significant biases still exist due to differences in the assay targets and design (7). A vaccinated individual is likely to have protection through

T-cell and B-memory cell immunity in the face of declining antibody levels, but further studies are necessary.

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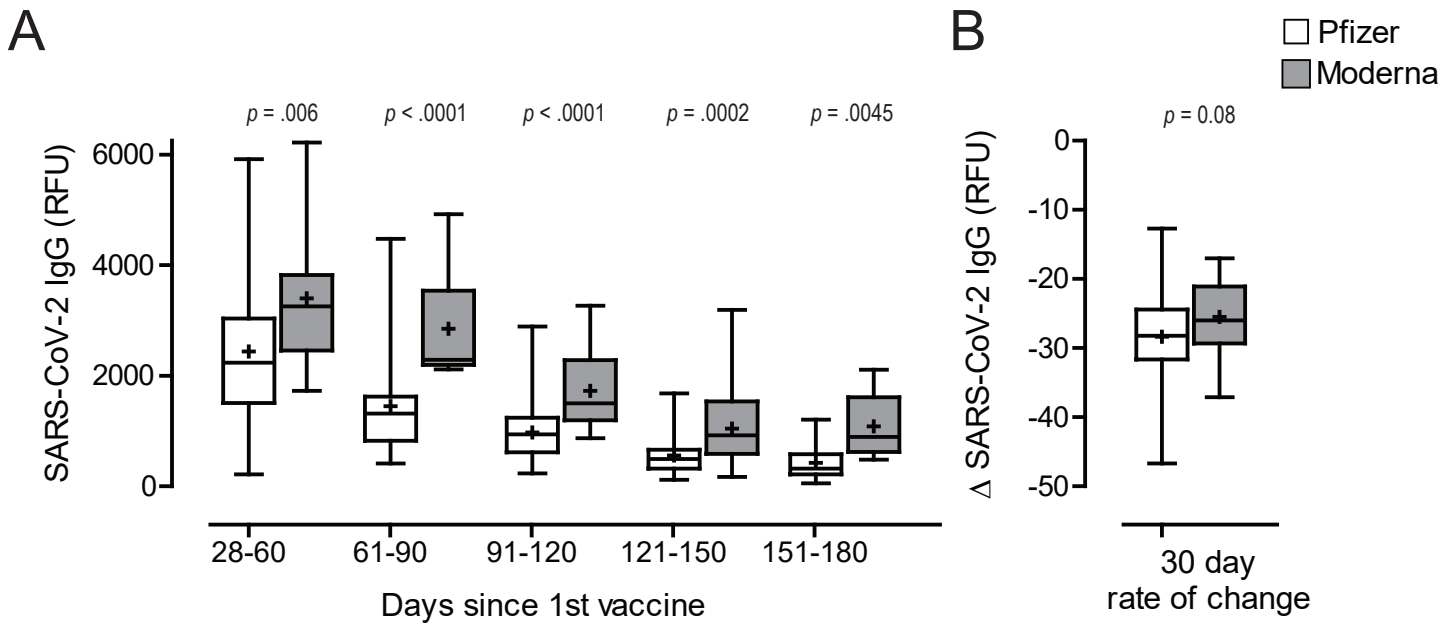
Figure caption

Figure 1. Serum antibody concentrations for healthcare workers after Pfizer-BioNTech and Moderna vaccine. A. Absolute antibody response versus days after the first vaccine injection. For each of the bins, n=52, 46, 54, 40, and 14 for the Pfizer-BioNTech vaccine and n=19, 10, 12, 23, and 5 for Moderna. B. Rate of decline in antibody response (in %/month) for paired samples separated by 2 months for Pfizer-BioNTech (n=34) and Moderna (n=24). + Arithmetic mean. – Median. The rate of antibody change was calculated as: $(IgG_{second} - IgG_{first})/(IgG_{first})/(Day_{second} - Day_{first.})$.


A
B
Pfizer
Moderna
p = .006
p < .0001
p < .0001
p = .0002
p = .0045
p = 0.08
SARS-CoV-2 IgG (RFU)
6000
4000
2000
0
Δ SARS-CoV-2 IgG (RFU)
0
-10
-20
-30
-40
-50
28-60
61-90
91-120
121-150
151-180
Days since 1st vaccine
30 day
rate of change