Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Ann Allergy Asthma Immunol 000 (2022) 1–3

Contents lists available at ScienceDirect

Letters

Split dosing of coronavirus disease 2019 vaccines provides noninferior antibody responsiveness to conventional vaccine dosing

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused an acute respiratory disease pandemic, termed coronavirus disease 2019 (COVID-19), that has resulted in considerable mortality and morbidity in the United States since early 2020.[1] Several vaccines, including the use of messenger RNA (mRNA) vaccines, have proven to be effective against the multiple variant strains of COVID-19,[2-5] and therefore, widespread vaccination of the public remains imperative. For patients with a history of allergic reaction to a previous COVID-19 vaccine, a graded administration protocol represents an option, with limited studies showing the safety or efficacy of this approach.[6-8] However, there are a few studies that evaluated whether split dosing of COVID-19 vaccines imparts similar immune responses to conventional dosing. The objective of this study was to quantitate pre- and post-COVID-19 vaccine antibody responses in subjects receiving vaccination in a 2-dose split manner compared with conventional (single or full) dosing, with the hypothesis that split dosing would be noninferior to conventional dosing.

Adult subjects between the ages of 19 and 65 years were enrolled from November 2021 to March 2022 at the allergy and immunology clinics and employee workforce of a tertiary care institution, approved by the center's institutional review board, with written informed consent. Subjects with medical reasons to have a poor antibody response to vaccination were excluded (eg, immunodeficiencies, immunocompromised, immunosuppressive medications including corticosteroids). Subjects were offered conventional or split dosing with an option of a prevaccine medication regimen of acetaminophen 500 mg and cetirizine 10 mg (to potentially reduce adverse effects of pain, itching, rash, headache, etc.). All subjects choosing split dosing were patients referred for vaccine allergy concerns. These concerns were heterogenous and did not meet the criteria for anaphylaxis to a previous COVID-19 vaccine, yet the subjects remained vaccine-hesitant. The subjects receiving the split dose first received 0.05 mL of vaccine, followed by administration of the remainder dose. The subjects completed a RedCap questionnaire regarding demographics, medical history, medications, and SARS-CoV-2 infections. Blood was collected at enrollment and at 6 weeks after vaccination.

To detect the presence of serum anti-immunoglobulin G antibodies against 3 different SARS-CoV-2 antigens, including receptor binding domain protein (RBD), spike protein 1 (S1), and nucleocapsid (N), the Luminex xMAP SARS-CoV-2 Multi-Antigen IgG assay (RUO; #30-00127) was used per the manufacturer's instructions. Paired samples (ie, before and after vaccination) were run simultaneously. The Luminex MAGPIX instrument measured the mean fluorescent intensity (MFI), and the cutoff for a positive specimen was greater than or equal to 700 MFI per the manufacturer's instructions. Clinical specimens positive for both N and RBD targets are considered positive for a natural infection or immunity (NI) with SARS-CoV-2. A positive RBD antibody result in the absence of nucleocapsid detection is consistent with a vaccine immune response. Paired pre- and postvaccination antibody responses as determined by MFI for RBD, S1, and N within the treatment group were compared using a Wilcoxon matched pairs rank test. Fold-change (post divided by pre-levels) differences between groups were assessed by a Mann-Whitney test. A P value $< .05$ was considered statistically significant. Statistical analyses were performed with GraphPad Prism software.

A total of 30 subjects, with 15 subjects in each group, completed pre- and postvaccine blood draws. Adult subjects were aged 43.4 years (mean), ranging from 21 to 63 years, White (83.3%), and female (86.7%). Twelve of them (40%) had a history of either a positive polymerase chain reaction (PCR) or antigen COVID-19 test result. The vaccines administered included Pfizer booster (73.3%), Pfizer 2-part series (10%), Moderna booster (3.3%), and Johnson & Johnson's Janssen (13.3%). All split-dosed subjects and 1 conventional-dosed subject received a predose regimen of antihistamine and acetaminophen.

For all subjects, there were significant increases in postvaccination MFI levels of RBD ($P < .001$) and S1 ($P < .001$), with no difference in nucleocapsid ($P = .93$) antibody levels. There were no differences in antibody responsiveness (fold-change) between conventional- and split-dosed groups for RBD and S1 (Fig 1A). However, there were 3 subjects who had positive nucleocapsid antibody seroconversion (Fig 1A), implying NI that could also account for increases in RBD and S1 antibody levels. After removal of these 3 subjects from analysis, there remained significant increases in postvaccination levels of RBD and S1 for all groups, with no differences in fold-change responsiveness between split- and conventional-dosed subjects (Fig 1B). There were also increases in RBD and S1 antibody expression after vaccination when assessed by type of vaccine administered (Fig 1C).

Seven subjects in the conventional-dosed and 3 subjects in the split-dosed group had positive nucleocapsid MFI levels > 700 at baseline. The nucleocapsid antibody levels of these subjects decreased over the course of the study, with mean (SEM) MFI levels

Dr Musa and Dr Wood are co-first authors.

Disclosures: Dr Poole has received funding from AstraZeneca and is a site investigator for human clinical research studies from GlaxoSmithKline, Takeda, and AstraZeneca, all unrelated to this project. The authors have no other conflicts of interest to report.

Funding: The authors report intramural research funding within the Division of Allergy and Immunology and Department of Pathology and Microbiology.

https://doi.org/10.1016/j.anai.2022.08.995

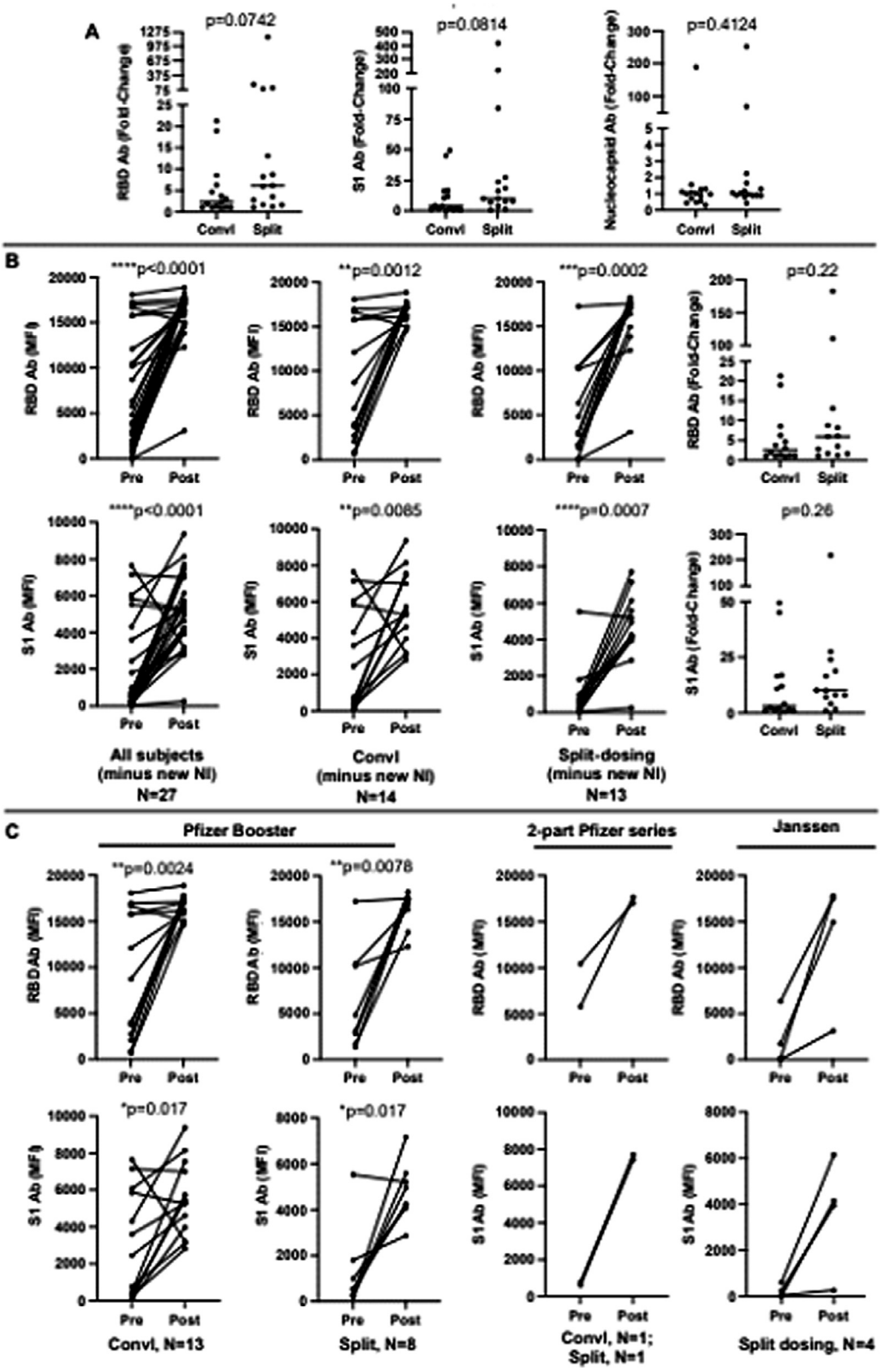

Figure 1. Split vaccine dosing-induced increase in receptor binding domain (RBD) and spike protein 1 (S1) antibody (Ab) expression is noninferior to conventional dosing. Scatter dot plots depict antibody fold-change (post-divided by pre-MFI levels), and line graphs depict mean fluorescent intensity (MFI). Panel A, Postvaccination fold-change antibody responses increased for RBD and S1 but not nucleocapsid, with no difference between conventional (Convl) (N = 15) and split-dosed (N = 15) groups. Note that 3 subjects showed positive test results for nucleocapsid antibody expression during the study interval. Panel B, Antibody expression and fold-change differences after removal of the 3 subjects with natural immunity (NI) conversion. Panel C, Antibody expression based on type of vaccine administration. Statistically significant values are denoted by asterisks (double asterisks denote $P < .01$, triple asterisks denote $P < 0.001$, four asterisks denote $P < 0.0001$).

prevaccination: 4167 (1320) and postvaccination: 2591 (983) (P = .01). Split dosing was well tolerated, and no significant adverse events occurred. Four subjects receiving split dosing reported headache, fatigue, numbness/tingling, facial flushing, and pruritus, with 3 subjects receiving additional acetaminophen and/or antihistamine.

Studies have now emerged on the safety of graded immunization for COVID-19 vaccines.[6–8] Almuhizi et al[8] reported that vaccine skin testing was not necessary, and that graded vaccine dosing was safely administered without any serious adverse reaction in patients referred for COVID-19 vaccine anaphylaxis risk. Moreover, the administration of acetaminophen and antihistamine premedications did not seem to affect antibody responses.

Most of our subjects (83.3%) had "positive" antibody levels to RBD and/or S1 (levels > 700 MFI) before administration of the COVID-19 vaccine, with very few subjects being vaccine or natural infection "naïve." Thus, providing a quantitative assessment of antibody responsiveness was a strength of this study; however, baseline antibody levels varied widely across individuals, representing a study limitation. The nucleocapsid antibody levels of the subjects with positive levels at baseline decreased over the 6-week study course. It is recognized that after natural infection, antinucleocapsid antibody responses wane the fastest, followed by RBD and S antibody responses, with wide variations among individuals.[9,10] Other considerations for future vaccine efficacy studies include assessing antibody responses at other time points, other immune measures (eg, T-cell responses), and inclusion of pediatric and elderly, immunosuppressed, and more diverse patient populations.

In conclusion, split dosing of COVID-19 vaccines remains an option that was shown to be as efficacious as conventional dosing in providing antibody responsiveness.

Acknowledgments

The authors thank Rhonda Walenz, BS, and Amy Nelson, BS, MT (ASP), for assistance with clinical study administration and coordination, Paul D. Fey, PhD, and Rick Starlin, MD, for study design assistance, and Lisa Chudomelka, MA, for assistance with manuscript submission. The authors also thank the subjects for participation in this study.

Amal Musa, MD*
Macy Wood, PhD†
Andrew Rorie, MD*
Sara M. May, MD†
Joel Van De Graaff, MD†
Jill A. Poole, MD*
* *Division of Allergy and Immunology*
Department of Internal Medicine, *University of Nebraska Medical Center*,
Omaha, Nebraska
† *Department of Pathology and Microbiology*
University of Nebraska Medical Center,
Omaha, Nebraska
japoole@unmc.edu

References

1. Centers for Disease Control and Prevention. COVID Data Tracker. 2022. Available at: https://covid.cdc.gov/covid-data-tracker. Accessed June 6, 2022.
2. Andrews N, Tessier E, Stowe J, Gower C, Kirsebom F, Simmons R, et al. Duration of protection against mild and severe disease by Covid-19 vaccines. *N Engl J Med*. 2022;386(4):340–350.
3. Rosenberg ES, Dorabawila V, Easton D, Bauer UE, Kumar J, Hoen R, et al. Covid-19 vaccine effectiveness in New York State. *N Engl J Med*. 2022;386(2):116–127.
4. Arbel R, Hammerman A, Sergienko R, Friger M, Peretz A, Netzer D, et al. BNT162b2 vaccine booster and mortality due to Covid-19. *N Engl J Med*. 2021;385(26):2413–2420.
5. Pilishvili T, Gierke R, Fleming-Dutra KE, Farrar JL, Mohr NM, Talan DA, et al. Effectiveness of mRNA Covid-19 vaccine among U.S. Health care personnel. *N Engl J Med*. 2021;385(25):e90.
6. Tuong LC, Capucilli P, Staicu M, Ramsey A, Walsh EE, Shahzad Mustafa S. Graded administration of second dose of Moderna and Pfizer-BioNTech COVID-19 mRNA vaccines in patients with hypersensitivity to first dose. *Open Forum Infect Dis*. 2021;8(12):ofab507.
7. Pitlick MM, Gonzalez-Estrada A, Park MA. Graded coronavirus disease 2019 vaccine administration: a safe alternative to vaccine avoidance. *Ann Allergy Asthma Immunol*. 2022;128(6):731–733.
8. Almuhizi F, Fein M, Gabrielli S, Gilbert L, Tsoukas C, Ben-Shoshan M, et al. Allergic reactions to the coronavirus disease 2019 vaccine (ARCOV) study: the McGill University Health Centre experience. *Ann Allergy Asthma Immunol*. 2022;129(2):182–188. e1.
9. Post N, Eddy D, Huntley C, van Schalkwyk MCI, Shrotri M, Leeman D, et al. Antibody response to SARS-CoV-2 infection in humans: a systematic review. *PLoS One*. 2020;15(12): e0244126.
10. Peluso MJ, Takahashi S, Hakim J, Kelly JD, Torres L, Iyer NS, et al. SARS-CoV-2 antibody magnitude and detectability are driven by disease severity, timing, and assay. *Sci Adv*. 2021;7(31): eabh3409. https://doi.org/10.1126/sciadv.abh3409.