

**Determining the Incidence of Asymptomatic SARS-CoV-2 among Early Recipients of COVID-19 Vaccines: A Prospective Cohort Study of Healthcare Workers before, during and after Vaccination [DISCOVER-COVID-19]**

Crystal M. North, MD, MPH<sup>1,2\*</sup>, Amy Barczak, MD<sup>1,2,5\*</sup>, Robert H. Goldstein, MD, PhD<sup>1,2</sup>, Brian C. Healy, PhD<sup>2,4</sup>, Dianne M. Finkelstein, PhD<sup>4</sup>, Delaney D. Ding<sup>1</sup>, Andy Kim<sup>3</sup>, Julie Boucau, PhD<sup>5</sup>, Bennett Shaw<sup>1</sup>, Rebecca F. Gilbert<sup>1</sup>, Tammy Vyas, Zahra Reynolds<sup>1</sup>, Katherine J. Siddle, PhD<sup>6</sup>, Bronwyn L. MacInnis, PhD<sup>6</sup>, James Regan<sup>3</sup>, James P. Flynn<sup>3</sup>, Manish C. Choudhary, PhD<sup>2,3</sup>, Jatin M. Vyas, MD, PhD<sup>1,2</sup>, Karl Laskowski, MD<sup>3</sup>, Anand S. Dighe, MD, PhD<sup>2,7</sup>, Jacob E. Lemieux, MD, D.Phil<sup>1,2</sup>, Jonathan Z. Li, MD, MMSc<sup>2,3</sup>, Lindsey R. Baden, MD<sup>2,3</sup>, Mark J. Siedner, MD MPH<sup>1,2</sup>, Ann E. Woolley, MD, MPH<sup>2,3</sup>, Chana A. Sacks, MD, MPH<sup>1,2</sup>

[1] Department of Medicine, Massachusetts General Hospital, Boston, MA, USA

[2] Harvard Medical School, Boston, MA, USA

[3] Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA

[4] Department of Biostatistics, Massachusetts General Hospital, Boston, MA, USA

[5] The Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA, USA

[6] The Broad Institute of MIT and Harvard, Cambridge, MA, USA

[7] Department of Pathology, Massachusetts General Hospital, Boston, MA, USA

*\*Authors contributed equally to this manuscript*

Corresponding Author: Dr. Sacks

Division of General Internal Medicine

100 Cambridge St, Suite 1600

Boston, MA, USA 02114

Phone: 617-643-7102

Email: csacks@partners.org

Alternate Corresponding Author:

Dr. North

Division of Pulmonary and Critical Care Medicine

55 Fruit Street, BUL-148

Boston, MA, USA 02114

Phone: 617-724-9674

Email: cnorth@mgh.harvard.edu

## **Abstract**

The impact of COVID19 vaccination on viral characteristics of breakthrough infections is unknown. In this prospective cohort study, incidence of SARS-CoV-2 infection decreased following vaccination. Although asymptomatic positive tests were observed following vaccination, higher cycle thresholds, repeat negative tests and inability to culture virus raises questions about their clinical significance.

**Keywords:** COVID, SARS-CoV2, pandemic, incidence, vaccine

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## Introduction

Vaccination substantially reduces COVID-19-related symptomatic infection, severe disease, hospitalization, and death in both clinical trial<sup>1-3</sup> and real-world settings.<sup>4,5</sup> Less is known about the effect of vaccination on asymptomatic infection. Early reports from observational studies suggest reduced rates of asymptomatic infection among vaccinated individuals,<sup>6,7</sup> but studies so far have provided no information on virologic characteristics of breakthrough infections. As asymptomatic infections have been important drivers of SARS-CoV-2 transmission prior to vaccination,<sup>8,9</sup> improved understanding of post-vaccination asymptomatic infections, including viral load and duration of shedding, is a public health priority.

## Methods

### *Study design*

We conducted a prospective cohort study of healthcare workers before, during and after COVID-19 vaccination in a large healthcare system (Mass General Brigham, MGB) in Boston, Massachusetts from December 30, 2020 through April 2, 2021. Employees were eligible for participation if they were eligible for vaccination through MGB's vaccination program, were planning to or had recently received the first dose of a COVID-19 vaccine through MGB and were able to drop off weekly self-swab testing kits. We recruited healthcare workers through email announcements and posted advertisements in English and Spanish.

### *Study procedures and data collection*

Participants completed weekly self-administered anterior nasal swabs and online symptom surveys. Participants who developed symptoms suggestive of COVID-19 accessed additional testing through Occupational Health protocols. Weekly testing continued from study enrollment through eight weeks after the first vaccine dose; if a participant enrolled more than 4 weeks after the first vaccine dose, testing continued for 4 weeks. Those who tested positive underwent independent symptom

evaluation through Occupational Health. Nasal swabs underwent real-time reverse transcriptase PCR testing at the Broad Institute of MIT and Harvard (Cambridge, Massachusetts), with cycle thresholds reported for positive tests (three cycles is about one log difference in viral load).<sup>10</sup> Testing through Occupational Health was completed at either the Broad Institute or a CLIA-approved, hospital-affiliated laboratory. Study investigators had access to all participants' SARS-CoV-2 test results in the MGB system and Occupational Health symptom assessments during the study period.

#### *Virologic characteristics sub-study*

On January 29, 2021, we initiated a sub-study to elucidate virologic characteristics of post-vaccination infection. For study participants who tested positive after their first vaccine dose, we conducted home visits approximately every other day for up to 14 days to collect self-administered nasal swabs for SARS-CoV-2 PCR testing, viral culture, and sequencing (see Supplementary Material).

#### *Statistical Analysis*

Our primary outcome was infection incidence rates in the pre-vaccination, partially vaccinated, and fully vaccinated intervals. Our primary exposure was vaccination period, classified based on time since the first and second vaccine doses, as pre-vaccination, partially vaccinated, and fully vaccinated intervals. For the two-dose mRNA vaccine regimens, we defined the pre-vaccination period as observation time up to 5 days after the first vaccine dose; the partially vaccinated period as 6 days after the first vaccine dose through 13 days after the second vaccine dose; and the fully vaccinated period as 14 days after the second vaccine dose until the end of the follow-up interval. We completed sensitivity analyses varying the delineation between the pre and partial vaccination periods.

Participants contributed observation time between their first and last swab. Observation time for participants who tested positive ended at the time of the positive test. We calculated incidence rates as the number of positive swabs divided by the total observation time in each vaccination period and estimated incidence rate ratios comparing the time periods using generalized estimating equations with a log link, Poisson distribution, and robust standard errors. Among those who tested positive, we compared cycle thresholds by symptom and vaccination status, compared the ratio of symptomatic to asymptomatic infections by vaccination status, and summarized the duration of culture positivity. Analyses were completed using Stata (Version 16, StataCorp, College Station, TX) and R ([www.r-project.org](http://www.r-project.org)). The study was approved by the MGB Human Subjects Review Committee.

## Results

In total, 2,247 healthcare workers were included in the analytic cohort (eTable 1 & 2). More women enrolled (n=1,760; 78%); median age was 37 years (IQR 30, 50); the most common healthcare role was physician/nurse practitioner/physician assistant (n=873; 39%), and 84% (n=1,879) identified as White or Caucasian. Nearly half (n=1,015; 47%) cared for COVID-19 patients during the study, and nearly all participants (n=2,243; >99%) were fully vaccinated by the end of the study period. The most common vaccine manufacturers were Moderna (n=1,428; 64%) and Pfizer-BioNTech (n=814; 36%).

Participants completed 13,359 swabs (median 6 swabs per participant [IQR 3,8]) over 85,109 person-days (median 42 person days [IQR 26, 50]). Nineteen (0.8%) participants had a positive test during the study period (Figure 1a), an incidence rate of 81.5 infections per 1000 person-years (95% CI 49.1, 127.3). No participants were hospitalized for COVID19-associated illness. Of these 19 infections, 6

(31.6%) occurred in the pre-vaccination period (median 1.5 days after dose 1), 10 (52.6%) occurred during the partial vaccination period (median 21.5 days after dose 1), and 3 (15.8%) occurred after full vaccination (median 52 days after dose 1). Incidence was lower among those who were fully vaccinated (34.6 per 1000 person-years (95% CI 7.1–101.1); rate ratio 0.052 (95% CI 0.013–0.21);  $p < 0.001$ ) and those who were partially vaccinated (72.8 per 1000 person-years (95% CI 34.9–133.9); rate ratio 0.11 (95% CI 0.040–0.30);  $p < 0.001$ ), compared to those who were unvaccinated (666.3 per 1000 person-years (95% CI 244.5–1450.3)) at the time of the positive test (eTable 3). Results were similar after varying the vaccine interval period definitions (eTable 4).

Cycle thresholds were available for 17 (89%) of the positive tests and were lower (indicating higher viral loads) in participants with symptomatic compared with asymptomatic infections (21.1 [IQR 15.6–33.5] versus 36.0 [IQR 26.8–37.4],  $p = 0.01$ ). Differences in cycle thresholds among those who tested positive before vaccination versus those with partial or full vaccination were not statistically significant (21.0 (IQR 15.2–33.5) versus 31.0 (IQR 21.3–36.2) or 31.4 (IQR 26.8–36.0), respectively;  $p = 0.50$ ). Five of 6 (83%) infections that occurred before vaccination were symptomatic, compared with 6 of 10 (60%) infections that occurred in the partial vaccination period and 1 of 3 (33%) infections that occurred after full vaccination ( $p = 0.39$ ).

Seven participants with positive tests after the virologic sub-study began (4 asymptomatic, 3 symptomatic individuals; all in the partially or fully vaccinated period) underwent additional characterization (eTable 5). All four individuals with asymptomatic infections had undetectable viral loads a median of 2 days (IQR 2,2) after their positive test and none had culturable virus at that time. One individual with symptomatic infection had an undetectable nasal swab viral load and negative culture 2 days after their positive test. Two of the three participants with symptomatic infections had a detectable viral load for 10 and 11 days, and culturable virus for 4 and 7 days, respectively (Figure 1b). Both were sequenced and determined to be the alpha variant.

## Discussion

By coordinating a systematic testing infrastructure amid a large health system's rapid COVID-19 vaccination rollout, we found that SARS-CoV-2 infection incidence decreased in a stepwise fashion following vaccination. We did identify asymptomatic infections in the partial and fully vaccinated periods; however, all asymptomatic individuals who underwent serial retesting had negative repeat PCR tests within two days and none had culturable live virus. This suggests a shorter duration of viral shedding in these asymptomatic individuals than has been previously reported for pre-vaccination symptomatic infections.<sup>11,12</sup> These findings raise important questions about whether post-vaccination asymptomatic, high cycle threshold positive tests represent transient infections or, alternatively, false positive tests.

By contrast, 2 of 3 participants with symptomatic infection in the partial or fully vaccinated periods had prolonged viral shedding and culturable virus up to 7 days after the initial positive test. Future work to elucidate viral and immunologic contributors to post-vaccination infection will be critical to understanding why some individuals are not protected from infection.

Strengths of this study are the prospective design, systematic weekly sampling regardless of symptoms, and in-depth virologic characterization among a subset of participants who tested positive. This study also has limitations. First, participants completed nasal swabs weekly, which may have been longer than the interval in which an asymptomatic participant could be infected and have stopped shedding, thereby underestimating asymptomatic infection incidence. Second, we were unable to enroll an unvaccinated control group in the healthcare setting, limiting our ability to definitively separate incidence trends in our study from regional trends during the study period. However, the incidence rate in our study cohort continued to decline while local test positivity began to increase (eFigure 1), suggesting that incidence rates in our cohort are not simply a mirror of community trends. Lastly, our cohort is comprised of healthcare workers that are predominantly

young, female, and of Caucasian/non-Hispanic race and ethnicity, and may not reflect the general population, including older adults and those who are immunosuppressed.

### **Conclusion**

COVID-19 incidence decreased from pre- to post-vaccination. Although positive tests were observed in asymptomatic individuals following vaccination, higher cycle thresholds, subsequent negative repeat PCR testing, and inability to culture the virus in those cases calls into question the clinical significance of these asymptomatic infections.

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## NOTES:

### Acknowledgements

We thank Giancarlo Buonomo, Grace Chamberlin, Alan Chen, Brandon Duffy, Gina Endres, Eliana Epstein, Harmanjot Kaur, Hope Koene, Taryn Lipiner, Dan Morash, Jacklyn Pagliaro, Nicholas Rahim, Dakarai Saunders, Gary Smith, Tatiana Sultzbach and Ryan Vyas for their effort in collecting samples, making this study possible.

### Funding

This work was supported by Mass General Brigham and the Massachusetts General Hospital (COVID Clinical Trials Pilot Grant, Executive Committee on Research, and Department of Medicine) and the Broad Institute of MIT and Harvard, with philanthropic support from F/Prime Capital. The BSL3 portion of this work was supported by the Massachusetts Consortium on Pathogen Readiness and the Harvard Center for AIDS Research, a National Institutes of Health funded program [P30AI060354]. The viral genome sequencing portion of this work was supported by the Broad Institute Viral Genomics Group and funding from the Centers for Disease Control and Prevention [BAA 75D30120C09605]. JZL is supported by P30AI060354 and a gift from Mark and Lisa Schwartz. CMN is supported by K23HL154863. The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the views of Mass General Brigham, the National Institutes of Health, or any of the other funders.

## Conflicts of Interest

CMN receives consulting fees from Axle Informatics for safety monitoring related to COVID-19 vaccine clinical trials. DMF receives consulting fees from Janssen Pharmaceuticals for serving on Data Safety and Monitoring Boards. JEL received consulting fees from Sherlock Biosciences. BCH has received grant support from Analysis Group, Celgene (Bristol Myers Squibb), Verily Life Sciences, Novartis, Merck Serono, and Genzyme. AEW received personal fees from COVAXX outside the submitted work. LRB is involved in HIV and COVID vaccine clinical trials conducted in collaboration with the NIH, HIV Vaccine Trials, Network (HVTN), COVID Vaccine Prevention Network (CoVPN), International AIDS Vaccine Initiative (IAVI), Crucell/Janssen, Moderna, Military HIV Research Program (MHRP), Gates Foundation, and the Ragon Institute. LRB reports grants from Ragon Institute, grants from NIH/NIAID, grants from Gates Foundation, grants from Wellcome Trust outside the submitted work. All other authors report nothing to disclose.

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### Figure Legend

**Figure 1.** Cycle thresholds (Panel A), duration of detectable SARS-CoV-2 virus, and culture positivity (Panel B) among participants with detectable SARS-CoV2 in relation to time since first vaccine dose. In figure 1b, each line represents a single participant over time. Samples collected after Day 0 were tested with a quantitative viral load assay.

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Figure 1

