Vaccination against SARS-CoV-2 is associated with a lower viral load and likelihood of systemic symptoms.

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Key Points:

Vaccination is associated with lower viral load and likelihood of having systemic symptoms following SARS-CoV-2 infection, but only for 6 months post-vaccination.

Potential Conflicts of Interest: No relevant. Disclosures: For the trial, the fluvoxamine placebo tablets were donated by Apotex. The ivermectin and ivermectin placebo tablets were donated by Edenbridge.

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Abstract

Background

Data conflict on whether vaccination decreases severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) viral load. The objective of this analysis was to compare baseline viral load and symptoms between vaccinated and unvaccinated adults enrolled in a randomized trial of outpatient COVID-19 treatment.

Methods

Baseline data from the first 433 sequential participants enrolling into the COVID-OUT trial were analyzed. Adults aged 30-85 with a body mass index (BMI) ≥ 25 kg/m² were eligible within 3 days of a positive SARS-CoV-2 test and <7 days of symptoms. Log₁₀ PCR viral loads were normalized to human RNase P by vaccination status, by time from vaccination, and by symptoms.

Results

274 participants with known vaccination status contributed optional nasal swabs for viral load measurement: median age 46 years; median BMI 31.2 kg/m² (IQR, 27.4, 36.4). Overall, 159 (58%) were women, and 217 (80%) were white. The mean relative log₁₀ viral load for those vaccinated <6 months from date of enrollment was 0.11 (95% CI, -0.48, 0.71), which was significantly lower than the unvaccinated group (p = 0.01). Those vaccinated ≥ 6 months prior to enrollment did not differ from the unvaccinated with respect to viral load (mean 0.99, 95% CI, -0.41 to 2.40; p = 0.85). The vaccinated group had fewer moderate/severe symptoms of subjective fever, chills, myalgias, nausea, and diarrhea (all P<0.05).

Conclusions

These data suggest that vaccination within 6 months of infection is associated with a lower viral load, and vaccination was associated with a lower likelihood of having systemic symptoms.
Introduction:

Breakthrough COVID-19 infections after vaccination do occur, and there are conflicting data on the influence of vaccination on the viral load of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). With the emergence of the delta variant of SARS-CoV-2, COVID-19 cases have dramatically increased worldwide, and breakthrough infections have occurred at increasing rates among those who were previously considered ‘fully vaccinated’ (i.e. having received 2 doses of the Moderna or Pfizer vaccines, or 1 dose of the Johnson and Johnson vaccine).

Several investigations of breakthrough infections have reported similar viral loads between vaccinated and unvaccinated individuals. In the Provincetown, Massachusetts outbreak, similar PCR cycle threshold (Ct) values were observed among 84 not fully vaccinated (median PCR Ct = 21.54) and 127 vaccinated individuals (median Ct = 22.77). Similarly, in a 719 person Wisconsin study during July 2021, when specimens were collected at a mode of 2 days post symptom onset, no difference in SARS-CoV-2 viral load was observed between vaccinated and unvaccinated individuals.

However, other investigations of breakthrough cases suggest that while initial viral loads are similar with the Delta variant, vaccinated persons have a more rapid decline in SARS-CoV-2 viral load. This was observed in Singapore among 71 vaccinated persons compared with 130 unvaccinated persons, and in a study in California of 171 fully vaccinated and 198 unvaccinated individuals.

The objective of this analysis was to assess for an association between prior vaccination on baseline viral load levels and symptoms among individuals enrolling in a nationwide randomized clinical trial testing early outpatient treatment for COVID-19. We hypothesized that the viral load from participants who had not been vaccinated would be higher compared to those who had been vaccinated, and those vaccinated within 6 months would have a lower viral load than those vaccinated more than 6 months ago. We further hypothesized that individuals who had been vaccinated would have less severe symptoms, and that symptom severity would correlate with viral load.
Methods:

Study Design

This is an analysis of baseline data collected from patients who enrolled in a phase 3, double-blind, factorial randomized, placebo-controlled COVID-OUT trial of early outpatient COVID-19 treatment (Clinicaltrials.gov NCT04510194). The trial is fully remote with no in-person visits. Patients received all study materials by FedEx or same-day courier, including a paper daily symptom log to fill out. Participants could opt into the collection of nasal swab samples at Days 1, 5, and 10. As such, this cross-sectional study was necessarily limited to those participating in voluntary biospecimen collection.

Study Sample

The trial enrolled adults aged 30 to 85 years throughout the US and is ongoing. Inclusion criteria included: a positive test for SARS-CoV-2 within the past 3 days; no known history of confirmed SARS-CoV-2 infection. Participants for the trial were limited to those with a BMI ≥ 25 kg/m² (or ≥ 23 kg/m² for participants of Latinx or Asian race/ethnicity) by self-reported height and weight. Exclusion criteria included: currently hospitalized; symptom onset ≥7 days (though asymptomatic individuals may enroll); immune compromised state; history of unstable heart, liver, or kidney disease; use of insulin or sulfonylureas; other medication exclusions.

Laboratory procedures

Participants opted into self-collection of nasal swabs then received the supplies for collection of anterior-nares nasal swabs. They received written instructions with pictures on how to collect the anterior mid-turbinate nasal swabs, as well as instructions over the phone from research coordinators. Once collected, the samples were placed in the participant’s refrigerators, and the research coordinators arranged for the samples to be returned to the lab via overnight FedEx. Just before being placed outside for FedEx pick-up, participants placed the samples in a small Styrofoam cooler with activated instant cold packs, and then the Styrofoam was placed in a rigid cardboard box.

The nasal samples were placed into tubes that contained Smart Transport Medium. They were received, processed, and tested at the Advanced Research and Diagnostic Laboratory (ARDL), a CLIA-certified lab.
at the University of Minnesota. Testing was performed with the HDPCR™ SARS-CoV-2 assay (ChromaCode) on the QuantStudio 7 (Applied Biosystems), using a validated extractionless protocol, and results were captured with the StarLims Laboratory Information Management System (Abbott). The final result is derived from cycle threshold (CT) values from three targets, N1, N2 and RNAse P. N1 and N2 are targets in the nucleocapsid protein, and RNAse P is used as the internal control target.

The viral load is calculated relative to the human RNAse P (RP): $2^{\frac{CT_{RP} - (N1+N2)}{2}}$. This calculation normalizes the raw Ct value for the SARS-CoV-2 viral targets (N1 and N2) to the Ct value of the human RP internal sample control. This provides a normalized relative value of the amount of viral RNA compared to total human nucleic acid to compensate for sampling and extraction quality.\(^5,6\) When N1 or N2 were undetected they were replaced with a value of 45, the maximum number of cycles.

**Statistical Methods**

We utilized standard descriptive statistics to summarize the distribution of baseline covariates by COVID-19 vaccination status. Normally distributed continuous variables were compared using Student’s t-test with unequal variances whereas skewed variables were compared using the Wilcoxon rank sum test. Categorical variables were compared using Fisher’s exact test and a quasi-Poisson regression model was used to compare counts while accounting for overdispersion. We computed the standardized mean difference to assess covariate balance between COVID-19 vaccination status groups. Covariates with a standardized mean difference >0.10 were adjusted for in subsequent analyses to reduce the chance of a biased association due to potential confounding.

We dichotomized symptom severity as moderate or severe versus absent or mild for a 10 symptom daily log.\(^7\) Participants were defined as having a loss of smell or taste if their sense of smell or taste was less than usual or completely absent. Participants were defined as having vomited or had diarrhea in the last 24 hours if they vomited at least once or had diarrhea at least 3 times in the last 24 hours, respectively. We compared the proportion of participants with COVID-19 symptoms at baseline by vaccination status and log\(_{10}\) viral load tertiles using Fisher’s exact test. The latter was achieved by grouping participants into log\(_{10}\) viral load tertiles using baseline measurements for all study participants, regardless of whether or not they reported symptom data.

To assess the association between log\(_{10}\) viral load and vaccination status, we fit a linear regression model initially treating vaccination status as binary. We then dichotomized the vaccinated subgroup based on date of vaccination to create a trichotomous variable with the following categories: unvaccinated, vaccinated within 6 months of enrollment, and vaccinated at least 6 months prior to enrollment. We assumed that all vaccinated individuals randomized prior to July 15\(^{th}\), 2021 were vaccinated within 6 months of enrollment due to when the coronavirus vaccines became available. A linear regression model regressing log\(_{10}\) viral load against the trichotomous predictor was used to assess
the impact of the duration of antecedent vaccination on viral load. A sensitivity analysis looking at those vaccinated within 4 months versus >= 4 months was also completed.

Of further interest was whether the relationship between log_{10} viral load and vaccination status was impacted by the emergence of the delta variant. To this end, we fit two additional linear regression models regressing log_{10} viral load against vaccination status after stratifying by the emergence of the delta variant. Participants were considered to have joined the study prior to the emergence of the delta variant if their randomization date was before June 19, 2021, and after the emergence of the delta variant if their randomization date was June 19, 2021 or later. This date has been used by the CDC to indicate pre-delta versus post-delta times. Between June 19 and July, 15, 2021 only 3 trial participants were enrolled, thus the assumption of dates for vaccination and delta variant are relatively distinct.

All analyses were conducted using R version 4.1.1 and we used two-sided p-values <0.05 for statistical significance.

**Patient Consent Statement**

This protocol was reviewed by the Food and Drug Association, investigational new drug license #152439. Institutional Review Board (IRB) approval for this protocol was obtained from the Advarra Central IRB (protocol MET29324) in compliance with the International Conference of Harmonization – Good Clinical Practices. Written consent was obtained from all participants. The independent data safety monitoring board approved the release of baseline data.

**Results:**

Among 434 sequential consented participants who had enrolled into the COVID-OUT trial through September 12, 2021, 274 agreed to provide optional self-collect nasal swabs for SARS-CoV-2 PCR and returned a specimen. Of these 274 participants, 272 provided vaccination status and were considered for analysis in this study. Of the 272, 112 (41%) were fully vaccinated of whom 94 were vaccinated within 6 months of enrollment. Date of vaccination was available for 17 of the remaining 18 vaccinated patients, all of whom were vaccinated at least 6 months prior to enrollment. See Figure 1.

Supplemental Table 1 compares those who submitted nasal swabs to those who did not. Most notably, those who submitted swabs had more chronic medical conditions, had a higher mean BMI, and were more likely to have a loss of smell.

The demographics for the overall study population and by vaccination status are provided in Table 1. The median age was 46 years (IQR: 38-53) and the median body mass index (BMI) was 31.2 kg/m^2 (IQR: 27.4-36.4). Overall, 159 (58%) were women and 217 (80%) were white. The median PCR cycle threshold (Ct) at baseline was 24.3 (IQR: 19.8-30.6). Vaccinated participants had a higher median PCR Ct value of 25.8 (IQR, 20.6-31.2) reflective of a lower viral load than unvaccinated participants who had a median PCR Ct value of 23.1 (IQR, 19.4-29.0). The mean log_{10} viral load for the overall study population was 0.78 (SD ±3.0), log_{10} viral load was lower in the vaccinated group, 0.26 (SD ±2.6), compared to the unvaccinated group to 1.1 (SD ±3.2). The missingness for baseline symptoms was 24% as it was a
secondary outcome. Among those for whom baseline COVID-19 symptoms were reported, the vaccinated group had fewer moderate or severe symptoms (mean 2.8 (SD ±2.3) symptoms) than those unvaccinated (mean 3.4 (SD ±2.7) symptoms).

The cycle threshold mean and median were lower for the participants who were unvaccinated, Table 2. A higher percentage of unvaccinated individuals were in the lowest PCR cycle threshold group compared to vaccinated individuals (31% vs. 21%).

A larger proportion of unvaccinated vs. vaccinated participants reported chills or shivering: 0.19 (24/127) vs. 0.05 (4/81), p<0.01; diarrhea in the last 24h: 0.18 (23/127) vs. 0.04 (3/81), p<0.01; feeling hot or feverish: 0.29 (37/127) vs. 0.10 (8/81); p<0.01; muscle or body aches: 0.54 (69/127) vs. 0.31 (25/81), p<0.01; and nausea: 0.19 (24/127) vs. 0.07 (6/81), p=0.03. Conversely, a larger proportion of vaccinated vs. unvaccinated participants had a stuffy or runny nose, 0.58 (47/81) vs. 0.35 (45/127), p<0.01. The vaccinated and unvaccinated subgroups did not significantly differ with respect to the frequency of cough, headache, loss of smell, loss of taste, fatigue, shortness of breath or difficulty breathing, sore throat, and vomiting (Figure 2).

Figure 3 displays the proportion of participants with moderate or severe COVID-19 symptoms at baseline and associated 95% Wilson-score CIs after dividing participants into the low viral load tertile [-10.3, -0.32 viral load], medium tertile (-0.32, 1.95), and high tertile (1.95, 8.82). The proportion of participants with a given symptom significantly varied by viral load tertiles for subjective fever (p<0.01), loss of smell (p<0.01), loss of taste (p=0.01), fatigue (p<0.01), myalgia (p=0.02), and stuffy or runny nose (p=0.02), with the middle tertile containing the largest proportion of sick participants for each of these symptoms besides stuffy or runny nose. There were no significant differences between viral load tertile groups at baseline for the following symptoms: chills, cough, diarrhea, headache, nausea, dyspnea, sore throat, and vomiting.

Log_{10} viral load values by COVID-19 vaccination status are presented in Figure 4. Each beige dot represents a unique observation whereas error bars reflect average log_{10} viral load values and associated 95% CIs. The expected log_{10} viral load value for unvaccinated participants was 1.14 (95% CI, 0.68, 1.60), which was significantly larger than the mean value of 0.26 (95% CI, -0.29, 0.81) observed for those who received a vaccine (p = 0.02). The log_{10} viral load values also appear to be more dispersed for unvaccinated patients. In this study sample, log_{10} viral load values ranged from [-6.14, 7.48] for vaccinated and [-10.3, 8.82] for unvaccinated participants.

Figure 5 displays a similar plot for the trichotomous variable that considers vaccination duration prior to enrollment. Notably, the significant difference in average log_{10} viral load values between vaccinated and unvaccinated individuals found previously appears to be driven by those who were vaccinated within 6
months of enrollment. Whereas log_{10} viral load values were, on average, 1.03 units less for participants who were vaccinated within 6 months (mean: 0.11; 95% CI, -0.48 to 0.71; p=0.01) compared to the unvaccinated, this difference attenuated to -0.15 for participants who were vaccinated ≥6 months prior to enrollment (mean: 0.99; 95% CI, -0.41 to 2.4; p=0.85). It is worth noting that the small sample size for the group of individuals vaccinated ≥6 months limited the power of the latter comparison. There was no difference in RNase between groups (Supplemental Figure 1). The sensitivity analysis using a cut-off of 4 months also found that those vaccinated within 4 months prior to enrollment (n=39) had a lower viral load than those who were unvaccinated, but not those vaccinated ≥4 months prior to enrollment (n=58), Supplemental Figure 2.

The relationship between log_{10} viral load and vaccination status after stratifying by June 19, 2021 (an assumed relation to the emergence of the delta variant) are presented in Supplemental Figure 3. 119 study participants were randomized prior to June 19, 2021, of whom 30 (25%) were vaccinated. Of the remaining 153 participants, 82 (54%) were vaccinated. Prior to the emergence of the delta variant, vaccinated individuals had significantly lower log_{10} viral load values than unvaccinated individuals (mean: -0.44; 95% CI, -1.46 to 0.58 vaccinated vs. mean: 1.22; 95% CI, 0.62 to 1.81 unvaccinated; p<0.01).

However, this association between viral load and vaccination status was no longer significant after the delta variant emerged (p=0.28).

As a sensitivity analysis, we adjusted linear regression models for age, BMI, sex, and self-identified white vs. non-white race. The results for the adjusted analyses are provided in the Supplemental Tables 2 and 3 and do not meaningfully differ from the unadjusted analyses.

Discussion

This primary data collection adds to growing data about viral load after vaccination against SARS-CoV-2, and about the timing of vaccination. In this sample of participants diagnosed with SARS-CoV-2 infection within 3 days, vaccination was associated with a lower viral load. This association with lower viral load is supported by the epidemiologic data that transmission rates are less after vaccination.\textsuperscript{9} This is a significant public health message for individuals who may be forgoing the vaccine because they feel they are not at risk for serious COVID-19 disease.\textsuperscript{10}

However, this beneficial reduction on viral load appears to no longer exist for individuals vaccinated ≥6 months before enrolling in the trial, though the small number vaccinated more than 6 months prior to enrollment limits interpretation, as does the fact that 6 months aligns with the emergence of the delta variant. The sensitivity analysis showing that the association with lower viral load decreases with a cut-off of 4 months suggests that waning immunity is not only due to the delta variant. Previous work also suggested that vaccine protection against infection waned over time after 12 weeks.\textsuperscript{11} It may be appropriate to revisit what should be considered fully vaccinated, especially after recent data suggest that protection against infection and severe disease also decrease after 6 months.\textsuperscript{11,12}
It is also notable that persons who had been vaccinated were less likely to have systemic symptoms (i.e. chills, fever, body aches, or diarrhea). This association with a lower likelihood of having symptoms is similar to what has been found previously when looking at effectiveness of mRNA vaccines against the delta variant.\textsuperscript{3,13} In our sample, vaccinated individuals were more likely to have a runny nose, perhaps indicating that the virus is less likely to invade beyond the upper respiratory tract in vaccinated individuals, though this is speculative. There was also no difference in cough between those who were vaccinated or unvaccinated. Viral load correlated with the frequency of the presence of moderate/severe symptoms. While the numbers in each tertile are small, the middle tertile of viral load were more likely to have symptoms of neurologic involvement (e.g. lack of taste and smell). Those in the lowest tertile were generally least likely to have symptoms. That the highest viral loads had fewer neurologic symptoms may reflect the lack of an immune response in this group.

Previous data suggesting that there was no difference in viral load between vaccinated and unvaccinated persons were from nasal swabs taken after 2 days of symptoms.\textsuperscript{2} The mean days since symptom onset in our sample was 5 days. The fact that we observed a difference between vaccinated and unvaccinated may be consistent with other analyses showing that viral load degraded more quickly in vaccinated individuals.\textsuperscript{3,4} The effect of vaccination on viral load in our sample was less significant after June 19\textsuperscript{th}; more data will be needed to understand whether vaccines lower viral load in persons with the delta variant. Other work does suggest that while vaccinated individuals can transmit the delta variant to others, the delta viral load does clear faster in persons who have been vaccinated.\textsuperscript{14} Additionally, it is possible that viral particles in vaccinated individuals may be less able to replicate than those from unvaccinated individuals.\textsuperscript{15}

**Limitations**

There is likely a selection bias in who enters the study, such that individuals with more symptoms are more likely to enter the study. Thus, the vaccinated individuals may be those with a suboptimal vaccine response. Therefore, the difference in symptoms between vaccinated and unvaccinated individuals may be biased in this analysis. This comparison was among persons who submitted optional self-collected nasal swabs. There may be an unknown selection bias as to who opted into this substudy of the main trial. It could be possible that participants who had a higher degree of symptomatology were less likely to complete the steps for baseline nasal swab collection and submission to the study team. Subjects in this analysis were not randomized to being in the vaccinated versus unvaccinated group, and there may be unobserved confounding in comparing these groups. This dataset was taken from individuals who enrolled before mid-September, 2021. Thus, those who were vaccinated more than 6 months prior to their enrollment in the study were receiving the vaccine before it was widely available. This may be
because they were higher risk individuals; or because they were more likely to be in occupations that put them at risk of exposure to a higher viral load; may be more likely to have comorbidities. Alternatively, they valued preventive measures and thus received the vaccine as early as they could and may continue to avoid high exposure to the virus. These unobserved potential influences on the viral load at baseline may bias both towards and away from the null hypothesis. Lastly, while efforts were made to standardize how the samples were collected, transport time via overnight FedEx, and the storage temperature during transport, it is possible that there is variation between participants in any or all of these variables. We are not aware of a reason why that variation would be systematically different between vaccinated and unvaccinated individuals.

Conclusions

In this cross-sectional analysis of baseline data collected from patients who enrolled in a phase 3 randomized trial of early outpatient treatment of SARS-CoV-2 infection, vaccinated individuals had a lower viral load and lower prevalence of systemic symptoms than those who had not been vaccinated. The effect on viral load was no longer present for persons vaccinated more than 6 months prior. The primary goal of being “fully vaccinated” is to prevent serious disease, which may still occur after 6 months. Future research should look at viral load beyond 6 months to understand whether the term “fully vaccinated” for viral load and transmission should define only those vaccinated within the previous 6 months. Vaccine- and booster-induced reduction in viral load may be an important component for achieving reduced coronavirus spread.
| Table 1. Demographic characteristics, comorbidities, and baseline symptoms. | Overall (n=272) | Unvaccinated (n=160) | Vaccinated (n=112) | p-value |
|---|---|---|---|---|
| **Women, n (%)** | 159 (58%) | 99 (62%) | 60 (54%) | 0.17 |
| **Age, median [IQR], years** | 46 [38, 53] | 44 [38, 51] | 47 [39, 56] | 0.02 |
| **BMI, median [IQR], kg/m²** | 31.2 [27.4, 36.4] | 31.5 [27.8, 36.0] | 31.0 [27.3, 37.0] | 0.27 |
| **Race, n (%)** | | | | |
| Native American | 6 (2.2%) | 2 (1.2%) | 4 (3.6%) | 0.39 |
| Asian | 6 (2.2%) | 1 (0.6%) | 5 (4.5%) | 0.09 |
| Native Hawaiian or Pacific Islander | 2 (0.7%) | 0 (0%) | 2 (1.8%) | 0.33 |
| Black or African American | 16 (5.9%) | 11 (6.9%) | 5 (4.5%) | 0.57 |
| White | 217 (80%) | 133 (83%) | 84 (75%) | 0.14 |
| Other / Declined | 17 (6.2%) | 8 (5.0%) | 9 (8.0%) | 0.45 |
| **Insurance status, n(%)** | | | | |
| Private | 170 (66%) | 97 (63%) | 73 (72%) | <0.01 |
| Public | 29 (11%) | 23 (15%) | 6 (5.9%) | |
| Medicare | 24 (9.4%) | 10 (6.5%) | 14 (14%) | |
| None | 33 (13%) | 25 (16%) | 8 (7.9%) | |
| **Number of high-risk comorbidities** | 0.45 (±0.55) | 0.44 (±0.56) | 0.46 (±0.55) | 0.70 |
Days from symptom onset to sample collection, median [IQR]  
5 [4, 6]  
5 [4, 6]  
5 [4, 6]  
0.68

|                         | Group 1 | Group 2 | Group 3 | p-value |
|-------------------------|---------|---------|---------|---------|
| Number of symptoms², mean (+sd) | 3.2 (+2.5) | 3.4 (+2.7) | 2.8 (+2.3) | 0.08 |
| Vomited in the last 24h, n (%) | 21 (10%) | 13 (10%) | 8 (9.9%) | 1.0 |
| Diarrhea in the last 24h, n (%) | 26 (13%) | 23 (19%) | 3 (3.7%) | <0.01 |
| Loss of taste, n (%) | 124 (60%) | 71 (56%) | 53 (65%) | 0.19 |
| Loss of smell, n (%) | 122 (59%) | 71 (56%) | 51 (63%) | 0.39 |
| Number of Asymptomatic, n (%) | 24 (12%) | 13 (10%) | 11 (14%) | 0.51 |

¹ Mean, (+sd) of high risk comorbidities include diabetes mellitus, coronary artery disease, congestive heart failure, or obesity.

² Moderate or severe value for symptoms: chills or shivering, cough, feeling hot or feverish, headache, fatigue, muscle or body ache, nausea, shortness of breath or difficulty breathing, sore throat, or stuffy or runny nose.
| Table 2. PCR Cycle Threshold (Ct) | Overall | Unvaccinated | Vaccinated | P-value |
|----------------------------------|---------|--------------|------------|---------|
| Overall, median [IQR]            | 24.3[19.8, 30.6] | 23.1[19.4, 29.0] | 25.8 [20.6, 31.2] | 0.02 |
| Overall, mean (sd)               | 25.8 (+7.6) | 25.0 (+7.5) | 27.1 (+7.6) | 0.03 |
| <20, n(%)                        | 72 (26%) | 49 (31%) | 23 (21%) | 0.25 |
| 20,<25, n(%)                     | 76 (28%) | 46 (29%) | 30 (27%) |         |
| 25,<30, n(%)                     | 50 (18.4%) | 27 (17%) | 23 (20.5%) |         |
| 30,<35, n(%)                     | 34 (12.5%) | 19 (12%) | 15 (13%) |         |
| ≥35, n(%)                        | 40 (15%) | 19 (12%) | 21 (19%) |         |
| Log₁₀ Viral Load, mean (±sd)     | 0.78 (+3.0) | 1.14 (+3.2) | 0.26 (+2.6) | 0.01 |
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Unrelated Disclosures:

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Figure Legends:

Figure 1. Overview of the participants whose nasal swabs were included in the analysis.

Figure 2: COVID-19 symptoms by COVID-19 vaccination status. Proportions reflect the number of eligible patients with a moderate or severe symptom at baseline, as well as presence of symptoms for diarrhea in the last 24h, vomited in the last 24h, loss of smell, or loss of taste. 95% Wilson-score confidence intervals and p-values for the differences in proportions are provided. Vaccinated participants had less frequent chills, diarrhea, subjective fever, myalgias, and nausea than unvaccinated participants.

Figure 3: COVID-19 symptoms by log_{10} viral load tertile. Proportions reflect the number of eligible patients with a moderate or severe symptom at baseline, as well as presence of symptoms for diarrhea in the last 24h, vomited in the last 24h, loss of smell, or loss of taste. 95% Wilson-score confidence intervals and p-values for the differences in proportions are provided.

Figure 4: SARS-CoV-2 log_{10} viral load value by COVID-19 vaccination status. The beige dots reflect each observation in the study sample whereas error bars reflect average log_{10} viral load values and associated 95% confidence intervals. Random jittering was applied along the horizontal axis for visual clarity.

Figure 5: SARS-CoV-2 log_{10} viral load values by COVID-19 vaccination status and duration of antecedent vaccination. The beige dots reflect each observation in the study sample whereas error bars reflect average log_{10} viral load values and associated 95% confidence intervals. Random jittering was applied along the horizontal axis for visual clarity.
1. Brown CM, Vostok J, Johnson H, et al. Outbreak of SARS-CoV-2 Infections, Including COVID-19 Vaccine Breakthrough Infections, Associated with Large Public Gatherings - Barnsy, Massachusetts, July 2021. MMWR Morb Mortal Wkly Rep. 2021;70(31):1059-1062.

2. Riemersma KK, Grogan BE, Kita-Yarbro A, et al. Shedding of Infectious SARS-CoV-2 Despite Vaccination. medRxiv. 2021:2021.2007.2031.21261387.

3. Chia PY, Xiang Ong SW, Chiew CJ, et al. Virological and serological kinetics of SARS-CoV-2 Delta variant vaccine-breakthrough infections: a multi-center cohort study. medRxiv. 2021:2021.2007.2028.21261295.

4. Acharya CB, Schrom J, Mitchell AM, et al. No Significant Difference in Viral Load Between Vaccinated and Unvaccinated, Asymptomatic and Symptomatic Groups When Infected with SARS-CoV-2 Delta Variant. medRxiv. 2021:2021.2009.2028.21264262.

5. Puskarich MA, Cummins NW, Ingraham NE, et al. A multi-center phase II randomized clinical trial of losartan on symptomatic outpatients with COVID-19. EClinicalMedicine. 2021;37.

6. Nelson AC, Auch B, Schomaker M, et al. Analytical Validation of a COVID-19 qRT-PCR Detection Assay Using a 384-well Format and Three Extraction Methods. bioRxiv. 2020:2020.2004.2002.022186.

7. FDA. Guidance for Industry. .

8. MJ D. Hospitalizations Associated with COVID-19 Among Children and Adolescents — COVID-NET, 14 States, March 1, 2020 - Aug 14, 2021. 2021;70(36):1255-1260.

9. de Gier B, Andeweg S, Joosten R, et al. Vaccine effectiveness against SARS-CoV-2 transmission and infections among household and other close contacts of confirmed cases, the Netherlands, February to May 2021. Eurosurveillance. 2021;26(31):2100640.

10. Hopkins J. https://publichealth.jhu.edu/2021/im-a-healthy-young-person-why-should-i-get-a-covid-vaccine. Accessed 11/19/21, 2021.

11. Chemaitelly H, Tang P, Hasan MR, et al. Waning of BNT162b2 Vaccine Protection against SARS-CoV-2 Infection in Qatar. New England Journal of Medicine. 2021.

12. Goldberg Y, Mandel M, Bar-On YM, et al. Waning Immunity after the BNT162b2 Vaccine in Israel. New England Journal of Medicine. 2021.

13. Pouwels KB, Pritchard E, Matthews PC, et al. Effect of Delta variant on viral burden and vaccine effectiveness against new SARS-CoV-2 infections in the UK. Nature Medicine. 2021;27(12):2127-2135.

14. Singanayagam A, Hakki S, Dunning J, et al. Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. The Lancet Infectious Diseases.

15. Shamier MC, Tostmann A, Bogers S, et al. Virological characteristics of SARS-CoV-2 vaccine breakthrough infections in health care workers. medRxiv. 2021:2021.2008.2020.21262158.
Figure 1

Adults with SARS-CoV-2 positive, age 30-85, screened between December 30, 2020 and September 12, 2021 (n=3,209)

Abbreviations: BMI=body mass index (kg/m²); CKD=chronic kidney disease; GFR=glomerular filtration rate (mL/min/1.73 m²)
**Symptoms not required for participation
**Medication exclusion list: metformin, insulin, cimetidine, hydroxychloroquine, sulfonurea, dolutegravir, atazanavir, ramelteon, lopinavir, efavirenz, sodium bicarbonate, lithium, valproate, fluvoxamine, rasagiline, selegiline, MAOIs, linezolid, duloxetine, methylene blue, tizanidine, ramelteon, alogliptin, agomelatine, bromopride, dapoxetine, temazepam, thioridazine, urokinase, pimozone.
Dose-dependent: SSRI, SNRI, tricyclic antidepressant, amlodipine, diazepam, theophylline, clozapine, olanzapine, NSAIDS, aspirin, warfarin, phenytoin, clopidogrel, St. John’s wort, high dose antipsychotic

Total excluded (n=2,775)
- BMI < 25kg/m² (n=402)
- Previously tested positive for SARS-CoV-2 (n=303)
- Currently admitted to hospital (n=300)
- More than 3 days since positive SARS-CoV-2 test (n=288)
- **Symptoms started > 7 days ago (n=273)
- **Currently taking a medication exclusion (n=273)
- Spoke language not available in translated materials (n=180)
- Immunocompromised (n=110)
- Incarcerated (n=41)
- GFR < 45mL/min (n=40)
- Multiple reasons or all other reasons (n=565)

Participants enrolled in trial: (n=434)
Did not submit nasal swabs (n=160)
Submitted nasal swabs (n=272)
Vaccination status unknown (n=2)
Vaccinated (n=112)
Unvaccinated (n=160)
Figure 2

Symptoms at Baseline

| Symptom                              | Vaccinated | Unvaccinated | p-value |
|--------------------------------------|------------|--------------|---------|
| Chills or Shivering                  |            |              | < 0.01  |
| Cough                                |            |              | 0.88    |
| Diarrhea in Last 24h                 |            |              | < 0.01  |
| Fatigue                              |            |              | 0.30    |
| Feeling Hot or Feverish              |            |              | < 0.01  |
| Headache                             |            |              | 0.24    |
| Loss of Smell                        |            |              | 0.39    |
| Loss of Taste                        |            |              | 0.19    |
| Muscle or Body ache                  |            |              | < 0.01  |
| Nausea                               |            |              | 0.03    |
| Shortness of Breath or Difficulty Breathing |      |              | 0.30    |
| Sore Throat                          |            |              | 0.56    |
| Stuffy or Runny Nose                 |            |              | 0.01    |
| Vomited in Last 24h                  |            |              | 1.00    |

Proportion (95% CI)
Figure 3

The figure shows the proportion of patients experiencing various symptoms at baseline. The symptoms are listed on the y-axis, and the proportion is indicated on the x-axis with 95% confidence intervals (CI). The p-values are listed for each symptom, indicating the statistical significance of the observed proportions. Symptoms are categorized into High, Medium, and Low severity levels.
Figure 5

- No vs. Yes (<6 mo.): $p = 0.01$
- No vs. Yes (≥6 mo.): $p = 0.85$
- Yes (<6 mo.) vs. Yes (≥6 mo.): $p = 0.20$

Average Log10 Relative Viral Load

COVID-19 Vaccine