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**Graphical abstract**

BNT162b2 Vaccination Timing

COVID-19 Infection Risk

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Biological rhythms in COVID-19 vaccine effectiveness in an observational cohort study of 1.5 million patients

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BACKGROUND. Circadian rhythms are evident in basic immune processes, but it is unclear if rhythms exist in clinical endpoints like vaccine protection. Here, we examined associations between COVID-19 vaccination timing and effectiveness.

METHODS. We retrospectively analyzed a large Israeli cohort with timestamped COVID-19 vaccinations (n = 1,515,754 patients over 12 years old, 99.2% receiving BNT162b2). Endpoints included COVID-19 breakthrough infection and COVID-19–associated emergency department visits and hospitalizations. Our main comparison was among patients vaccinated during morning (800–1159 hours), afternoon (1200–1559 hours), or evening hours (1600–1959 hours). We employed Cox regression to adjust for differences in age, sex, and comorbidities.

RESULTS. Breakthrough infections differed based on vaccination time, with lowest the rates associated with late morning to early afternoon and highest rates associated with evening vaccination. Vaccination timing remained significant after adjustment for patient age, sex, and comorbidities. Results were consistent in patients who received the basic 2-dose series and who received booster doses. The relationship between COVID-19 immunization time and breakthrough infections was sinusoidal, consistent with a biological rhythm that modifies vaccine effectiveness by 8.6%–25%. The benefits of daytime vaccination were concentrated in younger (<20 years old) and older patients (>50 years old). COVID-19–related hospitalizations varied significantly with the timing of the second booster dose, an intervention reserved for older and immunosuppressed patients (HR = 0.64, morning vs. evening; 95% CI, 0.43–0.97; P = 0.038).

CONCLUSION. We report a significant association between the time of COVID-19 vaccination and its effectiveness. This has implications for mass vaccination programs.

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2-dose immunization series, we based our analysis on the timing of dose 2, which completes the intervention. As a group, patients vaccinated in the morning were older and had more comorbidities than at other times, although the magnitude of these differences were small (Table 1). Over the study period, the cohort incurred 278,488 COVID-19–positive tests. There were also 4,501 COVID-19–associated emergency department (ED) visits and 3,824 COVID-19–associated hospitalizations, defined as occurring within –7 to +7 days of a COVID-19–positive test (see Methods).

Patients immunized against COVID-19 in the morning and afternoon had less frequent breakthrough infections than those vaccinated in the evening, with the groups diverging prior to the wave of infections caused by the Delta variant (Figure 2A). The difference remained significant after stratification by sex, age, and total number of comorbidities (Figure 2, B–D). A subgroup analysis of patients who received both doses 1 and 2 exclusively in the morning, midday, or evening yielded similar results (Sup-

Of approximately 2.6 million MHS participants, 1,515,574 had timestamps recorded for at least 1 immunization (Figure 1A and Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/JCI167339DS1). Our study population almost exclusively received the Pfizer-produced BNT162b2 vaccine (99.2%, n = 1,503,599), with a minority receiving the Moderna mRNA-1273 product (0.74%, n = 11,220).

Most patients received immunizations within a 12-hour time span stretching from 800 to 2000 hours (Figure 1B). Based on this distribution, we began by comparing COVID-19 breakthrough infections in patients who received their immunization in the morning (800–1159 hours, n = 552,423), afternoon (1200–1359 hours, n = 418,516), or evening (1600–1959 hours, n = 374,786). For the initial

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Table 1. Patient demographics based on time of COVID-19 vaccinations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Evening, n = 374,786</th>
<th>Morning, n = 351,857</th>
<th>Dose 2</th>
<th>Dose 3</th>
<th>Dose 4</th>
<th>Dose 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in yr, median (IQR)</td>
<td>40 (24–55)</td>
<td>46 (30–60)</td>
<td>44 (27–57)</td>
<td>47 (30–60)</td>
<td>46 (32–60)</td>
<td>46 (39–60)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male (61%)</td>
<td>181,481 (48)</td>
<td>130,592 (41)</td>
<td>165,086 (49)</td>
<td>185,319 (47)</td>
<td>189,793 (48)</td>
</tr>
<tr>
<td>Sex</td>
<td>Female (39%)</td>
<td>193,305 (52)</td>
<td>120,993 (39)</td>
<td>183,797 (52)</td>
<td>205,816 (53)</td>
<td>211,664 (54)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>0.001</td>
<td>60,049 (16)</td>
<td>116,686 (21)</td>
<td>75,120 (21)</td>
<td>82,732 (21)</td>
<td>33,129 (50)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>0.001</td>
<td>27,922 (7.5)</td>
<td>34,693 (9.3)</td>
<td>37,803 (9.7)</td>
<td>16,252 (24)</td>
<td>19,474 (26)</td>
</tr>
<tr>
<td>Chronic kidney disease, n (%)</td>
<td>0.001</td>
<td>14,250 (3.8)</td>
<td>21,967 (5.6)</td>
<td>9,736 (15)</td>
<td>14,203 (19)</td>
<td>11,994 (18)</td>
</tr>
<tr>
<td>Neurological disease, n (%)</td>
<td>0.001</td>
<td>32,950 (8.8)</td>
<td>44,828 (11)</td>
<td>39,046 (11)</td>
<td>42,172 (11)</td>
<td>17,983 (24)</td>
</tr>
<tr>
<td>Dialysis, n (%)</td>
<td>0.001</td>
<td>155 (&lt;0.1)</td>
<td>345 (&lt;0.1)</td>
<td>249 (&lt;0.1)</td>
<td>283 (&lt;0.1)</td>
<td>118 (0.2)</td>
</tr>
<tr>
<td>Asthma, n (%)</td>
<td>0.001</td>
<td>69,068 (18)</td>
<td>75,327 (18)</td>
<td>61,236 (17)</td>
<td>65,317 (17)</td>
<td>11,400 (15)</td>
</tr>
<tr>
<td>Immunosuppression, n (%)</td>
<td>0.001</td>
<td>7,039 (1.9)</td>
<td>9,193 (2.2)</td>
<td>8,587 (2.4)</td>
<td>9,169 (2.3)</td>
<td>3,855 (5.2)</td>
</tr>
<tr>
<td>Diagnosis of cancer in the past 5 yr, n (%)</td>
<td>0.001</td>
<td>16,719 (4.5)</td>
<td>24,076 (5.8)</td>
<td>16,697 (5.1)</td>
<td>22,127 (5.5)</td>
<td>23,681 (6.1)</td>
</tr>
<tr>
<td>Obesity, n (%)</td>
<td>0.001</td>
<td>68,386 (18)</td>
<td>71,408 (19)</td>
<td>63,978 (17)</td>
<td>65,317 (17)</td>
<td>11,400 (15)</td>
</tr>
<tr>
<td>Heart disease, n (%)</td>
<td>0.001</td>
<td>26,548 (7.1)</td>
<td>37,670 (9.0)</td>
<td>33,114 (9.4)</td>
<td>36,730 (9.4)</td>
<td>15,431 (26)</td>
</tr>
<tr>
<td>Days between vaccinations 2 and 3, median (IQR)</td>
<td>0.001</td>
<td>197 (187–208)</td>
<td>197 (187–208)</td>
<td>196 (187–206)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

aP < 0.001, Wilcoxon rank-sum test. bP < 0.001, Pearson’s χ² test.
HR for breakthrough infection was sinusoidal, with period durations ranging from 7.4 to 16.7 hours, depending on the vaccine dose and the method used to assess rhythm parameters (Figure 3, A–C, and Supplemental Table 14). We estimated maximum peak-to-trough change in the HR for breakthrough infection as 0.13 for doses 1 and 2, 0.086 for dose 3, and 0.25 for dose 4 (Figure 3, A–C). In our cohort, this translates to a number needed to treat (NNT) range of 18.7 to 54.5 by study end if patients were moved from the least favorable to most favorable COVID-19 vaccination times. It also corresponds to a relative change of vaccine effectiveness of 8.6%–25%. For doses 2 and 3, time of vaccination did not significantly associate with COVID-19–related ED visits or hospitalizations, nor was there a sinusoidal trend in HRs across the day (Table 2 and Supplemental Figure 6, A and B). However, for dose 4 (the second booster dose), vaccination timing did significantly associate with COVID-19 hospitalizations, favoring morning immunization (HR = 0.64; morning vs. evening, 0.43–0.97 95% CI; \( P = 0.038 \)), and there was a nonsignificant trend for ED visits (Table 2). In both cases, the relationship between dose 4 timing and outcomes was sinusoidal (Figure 3D, Supplemental Figure 6C, and Supplemental Table 12) and resembled that of breakthrough infection risk (Figure 3C). Thus, COVID-19 vaccination timing had a significant association with effectiveness, as ita, respectively) and a similar distribution of testing times around the clock (Supplemental Figure 3A). We did observe reduced positivity rates in COVID-19 tests obtained in the early morning, similar to one recent report (40), but this pattern was independent of vaccination timing (Supplemental Figure 3B). Overall, univariate analysis suggested a clinical advantage to dosing COVID-19 vaccines in the morning or afternoon in terms of fewer breakthrough infections. These results are unlikely to reflect differences in the baseline velocity of COVID-19 infection, diagnostic testing patterns, or diurnal variations in COVID-19 detection.

Using Cox regression to adjust for age, sex, comorbidities, and the protection afforded by COVID-19 boosters, we mapped the relationship between vaccination time and breakthrough infections (Figure 3). Across the time span when most COVID-19 vaccinations were given, the HR for breakthrough infections was lowest between late morning and early afternoon and highest for evening vaccination times (Figure 3, A–C). Stratifying participants into morning, midday, and evening vaccination groups produced similar conclusions, with optimal vaccine timing straddling the morning and midday bins (Table 2). Two alternative statistical approaches to Cox modeling, logistic regression and bootstrap analysis, also produced similar conclusions (Supplemental Tables 4 and 5). The relationship between vaccination time and HR for breakthrough infection was sinusoidal, with period durations ranging from 7.4 to 16.7 hours, depending on the vaccine dose and the method used to assess rhythm parameters (Figure 3, A–C, and Supplemental Table 14). We estimated maximum peak-to-trough change in the HR for breakthrough infection as 0.13 for doses 1 and 2, 0.086 for dose 3, and 0.25 for dose 4 (Figure 3, A–C). In our cohort, this translates to a number needed to treat (NNT) range of 18.7 to 54.5 by study end if patients were moved from the least favorable to most favorable COVID-19 vaccination times. It also corresponds to a relative change of vaccine effectiveness of 8.6%–25%. For doses 2 and 3, time of vaccination did not significantly associate with COVID-19–related ED visits or hospitalizations, nor was there a sinusoidal trend in HRs across the day (Table 2 and Supplemental Figure 6, A and B). However, for dose 4 (the second booster dose), vaccination timing did significantly associate with COVID-19 hospitalizations, favoring morning immunization (HR = 0.64; morning vs. evening, 0.43–0.97 95% CI; \( P = 0.038 \)), and there was a nonsignificant trend for ED visits (Table 2). In both cases, the relationship between dose 4 timing and outcomes was sinusoidal (Figure 3D, Supplemental Figure 6C, and Supplemental Table 12) and resembled that of breakthrough infection risk (Figure 3C). Thus, COVID-19 vaccination timing had a significant association with effectiveness, as
with a shift in the peak HR to later hours (Figure 3C and Figure 4, B–D). These data suggest a strategy for leveraging biological rhythms to optimize COVID-19 vaccine effectiveness in a population, where younger patients and the elderly would be prioritized for immunizations in the late morning to early afternoon.

Discussion
Our data indicate a significant association between the time of COVID-19 vaccination and its clinical effectiveness in terms of breakthrough infection and hospitalization. While the effect of vaccination timing is additive on top of an effective vaccine, it is defined by breakthrough infection and, in the case of the second booster shot, hospitalization. The sinusoidal trend suggests a biological rhythm in COVID-19 vaccine effectiveness based on time of administration.

During the study period, the second booster was restricted to patients over 60 and those with a history of immunosuppression. As such we examined how chronological age interacts with immunization timing with regards to vaccine effectiveness (Figure 4). We found that the benefits of morning vaccination were concentrated in younger (<20 years old) and older (>50 years old) individuals (Figure 4A and Supplemental Figure 7). Older age correlated with a shift in the peak HR to later hours (Figure 3C and Figure 4, B–D). These data suggest a strategy for leveraging biological rhythms to optimize COVID-19 vaccine effectiveness in a population, where younger patients and the elderly would be prioritized for immunizations in the late morning to early afternoon.

### Table 2. Cox-adjusted HRs for BNT162b2 effectiveness based on morning, afternoon, or evening vaccination times

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Dose 2</th>
<th>Dose 3</th>
<th>Dose 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PM</td>
<td>Mid</td>
<td>AM</td>
</tr>
<tr>
<td>Infection, HR</td>
<td>– 0.92</td>
<td>0.95</td>
<td>– 0.93</td>
</tr>
<tr>
<td>95% CI range</td>
<td>&lt;0.001 &lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ED visit, HR</td>
<td>– 0.98</td>
<td>0.92</td>
<td>– 1.02</td>
</tr>
<tr>
<td>95% CI range</td>
<td>– 0.87–1.11</td>
<td>0.83–1.03</td>
<td>– 0.87–1.20</td>
</tr>
<tr>
<td>P value</td>
<td>0.8</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Hospitalization, HR</td>
<td>– 0.99</td>
<td>0.90</td>
<td>– 0.96</td>
</tr>
<tr>
<td>95% CI range</td>
<td>– 0.86–1.14</td>
<td>0.78–1.04</td>
<td>– 0.78–1.12</td>
</tr>
<tr>
<td>P value</td>
<td>0.9</td>
<td>0.15</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Cox-adjusted HRs for BNT162b2 effectiveness based on morning (800–1159 hours [AM]), afternoon (1200–1559 hours [Mid]), or evening (1600–1959 hours [PM]) vaccination times. Significant associations (P < 0.05) are in bold. See Supplemental Tables 7–9 for HRs of all model covariates.
an easily modifiable factor that when extended over millions of immunizations can amount to a large aggregate benefit. The relationship between vaccination timing and infection risk is sinusoidal, suggesting a biological rhythm in vaccine effectiveness consistent with circadian regulation of underlying immune processes. Studies indicate that fundamental immune processes exhibit circadian and ultradian rhythms in controlled model systems (6–12, 17, 18). Our study contributes for the first time to our knowledge a “bottom line,” real-world estimate of clinical protection afforded by optimal vaccination timing.

While breakthrough COVID-19 infections varied with vaccination timing for all doses studied, ED presentations did not achieve statistical significance, and hospitalizations showed a time effect for only the second booster (dose 4). Of note, our statistical power for detecting associations with ED presentations or hospitalizations was much lower, as they were infrequent in our cohort (60.8 and 71.5 positive COVID tests per ED visit or hospitalization, respectively). This fact accentuates the value of analyzing large cohorts for detecting diurnal effects on infrequent but clinically important outcomes. Another explanation is that patients presenting with pneumonia severe enough to merit emergency care are more likely to have disrupted circadian rhythms at baseline (41), and thus time of vaccination might be less biologically pertinent to them. Regardless, minimizing COVID-19 infections of any severity is desirable. Asymptomatic patients can still infect others, and even mild symptoms can complicate the management of chronic conditions. Moreover, reducing hospitalizations in older patients is important, as they are at increased risk for poor outcomes.

We observed that the time of vaccination was most salient for patients at the extremes of age (<20 and >50 years old). Potentially this reflects changes to the circadian system and immune experience with aging (Figure 4, B–D) (41, 42). Alternatively, working age adults may be more likely to engage in night shift work or other nighttime activities that could mask circadian effects at a population level. Regardless, the observation is useful because it suggests that prioritizing young patients and the elderly for morning-to-midday immunization is enough to improve the effectiveness of mass vaccinations against COVID-19. Such a prioritization might extend to other vaccinations like influenza, given studies suggesting that morning influenza immunizations are beneficial specifically in the elderly (30, 33). We suggest that a timed vaccination approach for specific age ranges is practical and warrants prospective study. Such a prospective trial could combine actigraphy and longitudinal viral surveillance with immunogenicity biomarkers, including neutral-
izing antibody titers, pathogen-specific memory B and T cells, and innate immune memory responses in peripheral blood mononuclear cells. That way, the trial would deliver a clinical conclusion on efficacy while helping to elucidate potential mechanisms. It would also help to resolve conflicting studies about time-of-day variations in SARS-CoV-2 antibody titers (28, 31, 32).

Human populations have less consistent behavioral patterns than other organisms, making it challenging to observe how circadian rhythms affect medical interventions in real-world settings. This is because individuals lead diverse lifestyles, are routinely exposed to light at night, and engage in activities like night shift work that alter the phase of the circadian clock (19, 42). Our ability to detect rhythms in COVID-19 vaccine effectiveness in this study is due to the unique circumstances of the COVID-19 pandemic and the Israeli response to the pandemic. This includes a population-level sample of participants that is two orders of magnitude larger than prior cohorts, a concerted intervention largely consisting of a single vaccine product (BNT162b2), a population highly motivated to report COVID-19 infection, substantial clinical follow-up (>1 year), and, finally, a high level of data integration within the Israeli healthcare system. Our data do not directly address whether rhythms in BNT162b2 effectiveness extend to other COVID-19 vaccine products or to vaccinations against other pathogens. However, it is worth noting that the plurality of studies that do report diurnal rhythms in vaccine immunogenicity found morning-to-afternoon dosing to be optimal (25, 29, 30). Therefore, our data may have implications for diseases beyond COVID-19.

Our study has several strengths. Biological rhythms in vaccination are an understudied topic, with only 12 published studies in patients, including 6 focused on COVID-19 (24–35). These prior studies differ on whether optimal vaccination timing exists, what times of day produce optimal vaccine responses, and whether the effect is specific to certain patient subsets. While important, earlier studies had relatively low sample sizes, variable design, short-term follow-up, differences in vaccine type, and, most notably, a focus on markers of vaccine immunogenicity like antibody titers as endpoints rather than clinical protection. For BNT162b2, antibody titers vary by up to two orders of magnitude in healthy individuals, making this a noisy readout for observing circadian effects (43). Moreover, the dose-response relationship between spike antibody titers and COVID-19 vaccine efficacy appears weak (44). Our study avoids this pitfall by directly examining how immunization time of day relates to real-world BNT162b2 vaccine protection. Other strengths include a cohort an order of magnitude larger than all prior studies combined, longer clinical follow-up (>16 months), and orthogonal validation of conclusions using independent statistical approaches. Recent studies reveal time of day to be a significant factor in natural coronavirus infection beyond vaccine responses, including SARS-CoV-2 viral entry (45), mucosal barrier function (46), and, as we confirm here, SARS-CoV-2 positivity rates (Supplemental Figure 3B) (40). Thus, biological rhythms are evident at multiple levels of COVID-19 infection, and our data demonstrate that they extend to a key medical intervention.

Our analysis also has limitations. As with any observational study, patients were not randomly assigned to specific vaccination times, and demographic differences between groups can bias results. We attempted to compensate for this by adjusting for variables known to affect COVID-19 infection rates and complications (39, 47, 48), assessing the role of unmeasured variables, and using independent statistical models. Nevertheless, there may be sources of bias missed by our methods. There were also a limited number of ED and hospitalization events in the study population to support conclusions related to this specific outcome.

Our data set lacks comprehensive viral genotyping, and so we cannot determine whether time of vaccination influences breakthrough infections to different extents across variants. However, given rhythms were evident with vaccine boosters given while different viral variants were dominant our findings are likely to be broadly applicable.

Because vaccinations were not provided around the clock in our population, we cannot be certain whether the observed rhythms in vaccine effectiveness are diurnal (i.e., 24-hour cycles) or ultradian in nature (<24-hour cycles) as suggested by periodicity analysis (Figures 3 and 4 and Supplemental Table 14). Regardless, both diurnal and ultradian rhythms are compatible with upstream circadian clock influence (18, 49).

Israel is located at 31 degrees latitude and experiences warmer winters and less seasonal variation in photoperiod than more northerly or southerly latitudes. It is conceivable that viral transmission might be faster in areas with colder air temperature (50), thereby obscuring circadian influences in vaccine effectiveness in those regions. However, COVID-19 incidence did not vary by latitude within the US, which lies between the 20th and 66th parallels (51). Moreover, we observed a similar diurnal pattern in SARS-CoV-2 positivity rates as a cohort from the US (40). Thus, it is likely that rhythms in BNT162b2 vaccine effectiveness extend to other countries.

Our cohort is relatively ethnically homogenous (91% identifying as Israeli Jewish), and validation in more diverse populations would be valuable. That said, data indicate that BNT162b2 exhibits similar efficacy across ethnic boundaries (52), suggesting that demographics are likely not a limitation to generalizability.

Our results reflect the cumulative behavior of our cohort, and individuals with acutely disrupted circadian rhythms due to shift work, jet lag, or sleep deprivation might react differently to immunization timing. Research suggests these patients are more vulnerable to COVID-19 infection, and future research should focus on vaccination strategies specific to this subgroup (53).

Finally, our data cannot determine by themselves the mechanism by which time of day regulates COVID-19 vaccine protection. Based on observations in model systems, it is tempting to speculate that circadian clock regulation of innate inflammation and T cell activation might explain the oscillations in vaccine effectiveness reported here (12, 17, 18). Further research should explore these possibilities.

In summary, circadian rhythms represent a fundamental property of living things that regulate the immune system at a basic biological level. This study provides the first estimate to our knowledge of how far this core process extends into an important clinical realization of immune function: vaccine effectiveness. Our data suggest a way of leveraging biological rhythms to optimize immunizations against SARS-CoV-2 variants using reformulated vaccines and potentially vaccines against other pathogens.
Methods

Study design. This retrospective cohort study analyzes database records from MHS in Israel. MHS is the second largest state-managed, not-for-profit, HMO in Israel with over 2.6 million members, constituting one-quarter of the country’s population. MHS maintains extensive medical, demographic, and anthropometric information linked to a nation-wide electronic medical record servicing the entire Israeli civilian population. The MHS database captures all clinical encounters, diagnoses, medications, and laboratory data for its participants anywhere within the country regardless of setting, including all clinical activity and diagnostic testing related to COVID-19.

Our analysis used the target-trial framework (54) and focused on the time of day vaccines were given for the initial series (doses 1 and 2), the first booster dose (dose 3), or the second booster (dose 4). We conceptualized each vaccine encounter as a separate clinical question as to the optimal time of vaccine effectiveness.

Setting. In December 2020, Israel launched a national immunization campaign against COVID-19 consisting almost exclusively of the Pfizer BNT162b2 vaccine series delivered in 2 doses 3–4 weeks apart (Supplemental Figure 1). In July and September 2021, the Israeli Ministry of Health initiated third (first booster) and fourth (second booster) dose campaigns, respectively. Vaccines and point-of-care COVID-19 testing were provided free of charge to all citizens. During the study period, 96.2% of point-of-care tests were PCR based, and antigen-based point-of-care testing was only briefly significant during the wave of Omicron variant (B.1.1.529) infections (maximum monthly prevalence 10.8% of all tests). A monthly breakdown of point-of-care testing by detection modality is provided in Supplemental Figure 4. At-home antigen test kits were available for purchase (retail cost range, NIS 32–100, or $11–$32 per kit), and in fall 2021 20 home kits were provided free to families with school-aged children to support the back-to-school effort (Supplemental Figure 4, arrow). However, the Israeli Ministry of Health required a documented positive test prior to quarantine and a negative test after quarantine before issuing a certificate of recovery to patients throughout the study period (https://corona.health.gov.il/en/confirmed-cases-and-patients/cases-recovered/). Thus, censoring of COVID-19 infection is unlikely in our cohort as there was strong incentive to obtain a laboratory diagnosis even if initial testing occurred at home.

Participants. Among 1,624,601 MHS participants over 12 years of age 1,594,180 (98.13%) received the Pfizer BNT162b2 vaccine, 29,253 (1.8%) received the Moderna mRNA-1273 vaccine, 437 (0.03%) received the AstraZeneca ChAdOx1-S product, 400 (0.02%) received Sputnik V, 232 (0.01%) received the Jansen Jcovden vaccine, and 46 (0.003%) received the Sinovac CoronaVac product; for 53 patients (0.003%) the vaccine identity was unspecified.

We analyzed data from December 19, 2020 (the first day of vaccine administration in the study population), to April 25, 2022 (the last day of data extraction). This time span encompasses two spikes in COVID infection dominated by the Delta (B.1.617.2) and Omicron (B.1.1.529) SARS-CoV-2 variants (Supplemental Figure 1, black and blue bars).

We extracted data from all MHS members aged 12 years and older who received at least 1 dose of SARS-CoV-2 vaccine and had joined MHS prior to February 2020 and, therefore, had a complete medical history on file. For analysis of each vaccine dose, we excluded patients who had a documented SARS-CoV-2–positive test prior to the date of vaccination (Figure 1A). We further excluded patients with missing timestamps (Figure 1A). 81.1% of MHS members identified as Jewish, 6% identified as orthodox Jews, 5.8% as Arabs, and 6.1% as former Soviet Union residents.

Variables. We analyzed deidentified patient-level data extracted from MHS electronic records. Continuous variables included age at immunization, time and date of vaccine administration, and body mass index. Dichotomous variables included sex and comorbidities linked to COVID-19 severity (47, 48). For variable definitions, see Supplemental Tables 1 and 2.

Data sources/measurements. All data used for analysis was extracted from the MHS database. The primary outcome was COVID-19 infection as defined by positive SARS-CoV-2 PCR or antigen test performed at any official site. Infections in the first 7 days following vaccination were excluded from the analysis, since patients during this interval are not considered to have a complete immunologic response to vaccination. Secondary outcomes were ED visits and hospitalizations associated with COVID-19 infection, defined as encounters within a period –7 to +7 days after a documented COVID-19–positive test.

Bias. Typically, a Schoenfeld’s global test is used to validate the assumption of Cox proportional hazards. However, the large sample sizes of our study present a challenge to this approach because this leads to overpowered (55). We found evidence of this in our data by applying the Schoenfeld’s global test on stratified data with smaller sample sizes (Supplemental Table 3), demonstrating reduction in test significance compared with the complete data set. Therefore, we used two alternate approaches to orthogonally validate results from our Cox model as recommended (55): multivariate logistic regression (Supplemental Table 4) and bootstrap multivariate analysis (Supplemental Table 5). For logistic regression, we applied the same multivariate Cox regression model, with the exception that follow-up time was not included. The reason for this modification was that our goal with logistic regression was to validate the trend rather than the effect size estimated by the Cox model and that follow-up times were not materially different between vaccination groups (Table 1). For bootstrap analysis, we sampled the entire cohort for 2,000 iterations with replacement calculating HRs and CIs for the same multivariate regression model. Results from both approaches showed similar trends as our multiple Cox regression model, supporting this approach for analyzing the data. As additional support, a plot of scaled Schoenfeld residuals for the variable time of vaccination demonstrated stable proportional HRs across the study period (Supplemental Figure 5) (56).

Study size. We used expansive inclusion criteria covering all vaccinated patients without prior documented COVID-19 infection in the MHS database that had demographic information and timestamped vaccinations.

Data availability. Deidentified data will be made available with a transfer agreement.

Statistics. An initial descriptive analysis included calculations of single variable distribution, central tendency, and dispersion for all data in Table 1. We stratified vaccine timing in 4-hour bins for comparison: 800–1159 hours (morning), 1200–1559 hours (afternoon), and 1600–1959 hours (evening) (Figure 1B). For analysis of the initial vaccine series (dose 1 and 2), day 0 in our analysis was individually defined for each patient as 7 days after the receipt of vaccine dose 2 as described previously (36). Given that the first 2 doses of BNT162b2 constitute a single intervention, we combined these doses into one
analytic model. Our main comparison for the initial vaccine series (doses 1 and 2) was based on the timing of dose 2 only. In support of this, a sensitivity analysis demonstrated that dose 2 timing was more important than dose 1 in modifying breakthrough infection risk for the initial vaccine series (Supplemental Table 6). For analysis of the booster doses (doses 3 and 4), day 0 was defined as 7 days after the immunization was given. Note that only the time of booster administration was considered, as we regarded boosters as separate medical interventions to augment waning COVID-19 immunity.

Because one independent variable (booster status) differed over time, univariate and multivariate survival analyses were performed with time-dependent covariates (57, 58). Kaplan–Meier analysis with a log-rank test was used for the univariate analysis. For analyzing the initial vaccine series based on the timing of dose 2, we employed a Cox proportional-hazards regression model with random mixed effects to estimate the association between vaccine administration time of day and study endpoints. For analyzing the first booster dose (dose 3), we used a Cox proportional-hazards regression model and adjusted for dose 4 as a time-dependent covariant. For analyzing time of vaccination of the second booster (dose 4), we applied a Cox proportional-hazards regression model without time-dependent covariates. Cox modeling was previously applied to Israeli HMO data for analyzing associations between COVID-19 immunization and clinical endpoints over extended periods (39). Our choice of variables to include in the Cox model was based on previously published associations between age, comorbidities, and COVID-19 endpoints of infection or disease severity (39, 47, 48). Then, we interrogated violation of Cox assumptions for the variables of interest by computing a Schoenfeld global test (see Bias above). Variables that were associated with outcomes measured (breakthrough COVID-19 infection or COVID-19-associated ED visits) with a \( P < 0.1 \) in the univariate comparison were included in the multivariate model. For the multivariate analysis, we used goodness-of-fit parameters (Akaike Information Criterion [AIC] = –2 log likelihood + 2 × number of estimated parameters in this equation with a backward elimination approach). To simplify the modeling of breakthrough events after the initial immunization series (doses 1 and 2), we censored events in the subset of patients receiving the second booster (dose 4) after the date it was administered. For modeling events after doses 3 and 4, there was no censoring. We treated the first COVID-19 infection as a terminal event. As such, recurrent infections did not factor into our analysis.

We calculated NNT as described previously (59): \( \text{NNT} = \frac{\text{Sc}_{\text{HR}}}{\text{Sc}} - 1 \), where \( \text{Sc} \) is event-free survival in the reference group, and \( \text{HR} \) is the HR at the most preferable minus the least preferable vaccination time. We estimated \( \text{Sc} \) as 0.75 at study end based on the survival curve generated for univariate analysis (Figure 2A) and \( \text{HR} \) as the peak-to-trough difference in HR for breakthrough infection across the day (Figures 3 and 4). We estimated the change in vaccine effectiveness based on moving vaccinations from the best to worst time of day as 1-HR as described previously (39).

**Time bin sensitivity analysis.** To examine how the choice of time bins affected conclusions from our Cox regression model, we generated HRs comparing the 800–959-hour time bin to successive 2-hour intervals across the day, incrementing by 1 hour with each iteration. Note that to avoid overlap with the index bin, HRs were generated from 1000 hours in this analysis. Our hypothesis was that a biological rhythm in vaccine effectiveness should produce a sinusoidal trend in HRs as a function of vaccination time. To statistically evaluate the trend in HRs across the day for periodicity we used METACYCLE, an algorithm commonly used for circadian rhythm detection in gene expression data (60). The COSOPT algorithm (61) was used to generate best-fit sinusoidal trendlines for data display. We observed very high goodness of fit with this approach (\( R^2 \) range, 0.77–0.97, Figure 3), supporting the use of a sinusoidal model. To avoid forced fitting these data, we programmed COSOPT and METACYCLE to select the best fitting periodic function across a range of cycle lengths (4–24 hours) as described previously (61).

We performed all statistical analyses using R version 3.5.0. Code for all analyses can be found in the Supplemental Methods. A \( P \) value of less than 0.05 was used to indicate significance in all analyses.

**Study approval.** The study was approved by the MHS Ethical Committee. All data were deidentified prior to analysis. Because this was a retrospective study, patient informed consent was not required.

**Author contributions**

GH and JAH conceived the study. GH, OAD, HM, AG, EO, CG, DG, and JH contributed to the study design. Data were extracted by HA, GH, and OAD. GC oversaw the data gathering. All authors contributed to the data analysis. GH and JAH wrote the initial manuscript. All authors vouch for the data and analysis. All authors participated in the editing and critical review of the manuscript and decided to submit it for publication.

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