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Superior immunogenicity and effectiveness of the third compared to the second BNT162b2 vaccine dose

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As the effectiveness of a two-dose messenger RNA (mRNA) severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine regimen decreases with time, a third dose has been recommended. Here, we assessed immunogenicity, vaccine effectiveness and safety of the third BNT162b2 vaccine dose in a prospective cohort study of 12,413 healthcare workers (HCWs). Anti-RBD immunoglobulin G (IgG) levels were increased 1.7-fold after a third dose compared with following the second dose. Increased avidity from 61.1% (95% confidence interval (CI), 56.1–66.7) to 96.3% (95% CI, 94.2–98.5) resulted in a 6.1-fold increase in neutralization titer. Peri-infection humoral markers of 13 third-dose Delta variant of concern (VOC) breakthrough cases were lower compared with 52 matched controls. Vaccine effectiveness of the third dose relative to two doses was 85.6% (95% CI, 79.2–90.1). No serious adverse effects were reported. These results suggest that the third dose is superior to the second dose in both quantity and quality of IgG antibodies and safely boosts protection from infection.

The vaccination campaign against coronavirus disease 2019 (COVID-19) is expanding worldwide, and studies demonstrate that all Food and Drug Administration (FDA)-approved vaccines, in particular mRNA-based BNT162b2 and mRNA-1273, have high vaccine efficacy and effectiveness in preventing symptomatic COVID-19 (refs. [1–6]).

Disturbingly, COVID-19 breakthrough infections are substantially increasing, especially in countries that were the first ones to initiate vaccine rollout. Accumulating evidence suggests that vaccine effectiveness is declining in all age groups a few months after receipt of the second dose of vaccine[3,7,8] and that humoral immunity follows a similar path[9]. These findings, along with evidence that SARS-CoV-2 breakthrough infections correlate with lower IgG and neutralizing antibody levels[10], prompted Israeli authorities to approve the administration of a third vaccine dose. Early reports from Israel on the effectiveness of the third dose have been published[11–16] and demonstrate a marked decrease in new infections, specifically in severe cases. Following these reports, other countries recommended a third dose of the vaccine. Although early data demonstrated that a third BNT162b2 vaccine dose increased vaccine effectiveness to approximately 93–95%[11–14], only limited real-world data are currently available for the BNT162b2 third vaccine dose safety or its effect on immunogenicity.

Here, we present real-world data on vaccine effectiveness, safety and immunogenicity within a large-scale cohort of HCWs in a large tertiary center in Israel, the Sheba HCW COVID cohort.

Results

The study included four arms (Fig. 1): (1) the immunogenicity arm, in which serological tests of samples from vaccinated HCWs at three time points were assessed and compared (after receiving second dose, before receiving the third dose and after receiving the third dose); (2) the correlates of risk arm, in which antibody levels of breakthrough cases following the third vaccine dose were compared to matched noninfected controls; (3) the vaccine effectiveness arm, in which incidence of SARS-CoV-2 infections regardless of symptoms among HCWs with two vaccine doses given at least 5 months previously was compared to incidence among HCWs with three doses; and (4) the safety arm, in which adverse events among those vaccinated with the third dose by 2 September 2021 were assessed via an electronic questionnaire.

Immunogenicity data from the third BNT162b2 vaccine dose. Of 4,526 HCWs eligible for the study, 1,047 had serum samples from both pre- and post-third dose time points (up to 45 days before, as well as 14–45 days after the third dose). IgG and neutralizing antibody levels at these two time points were tested for 1,047 and 512 HCWs, respectively. A 31-fold (95% CI, 28–34) and 41-fold (95% CI, 39–42) increase in IgG and neutralizing levels, respectively, was observed after the third vaccine dose (Fig. 2a,b). Administration of the third dose also resulted in a minor but significant increase ($P = 0.025$, Wilcoxon signed rank test) in T cell activation, which was tested in 16 HCWs (Fig. 2c).

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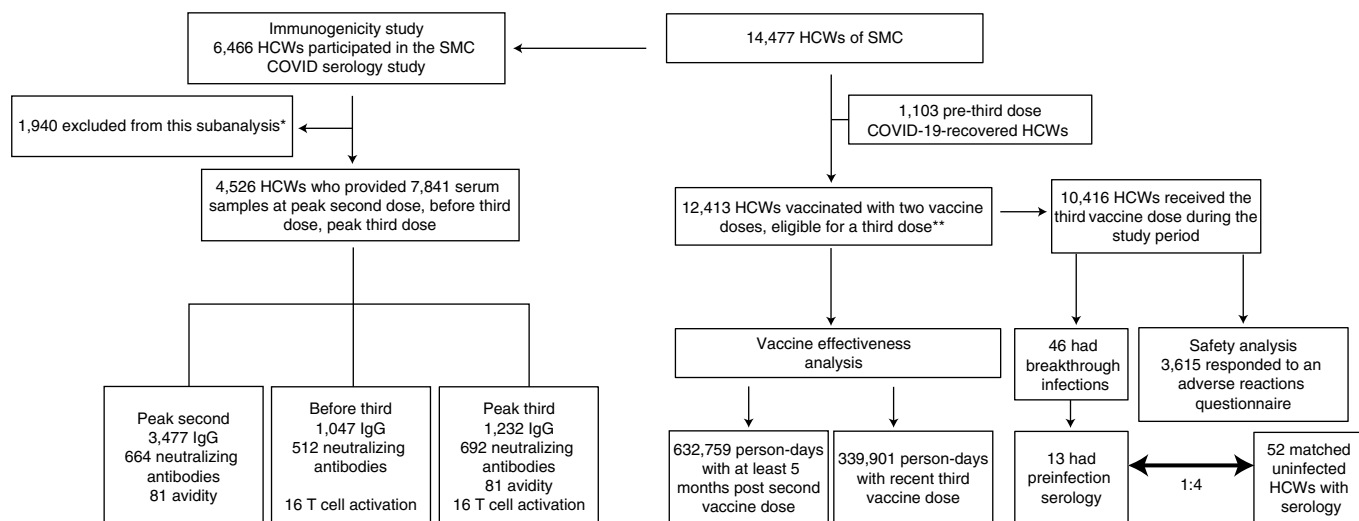
Fig. 1 | Study profile. The BNT162b2-vaccinated Sheba HCW cohort used for the vaccine effectiveness, safety and serology analyses following the second and third vaccination. SMC, Sheba Medical Center. *HCW without serum samples before and after the second vaccine dose and after the third vaccine dose were excluded. **HCW that have not been previously infected with SARS-CoV-2 and were vaccinated with the second vaccine dose at least 5 months prior, were eligible for a third dose.
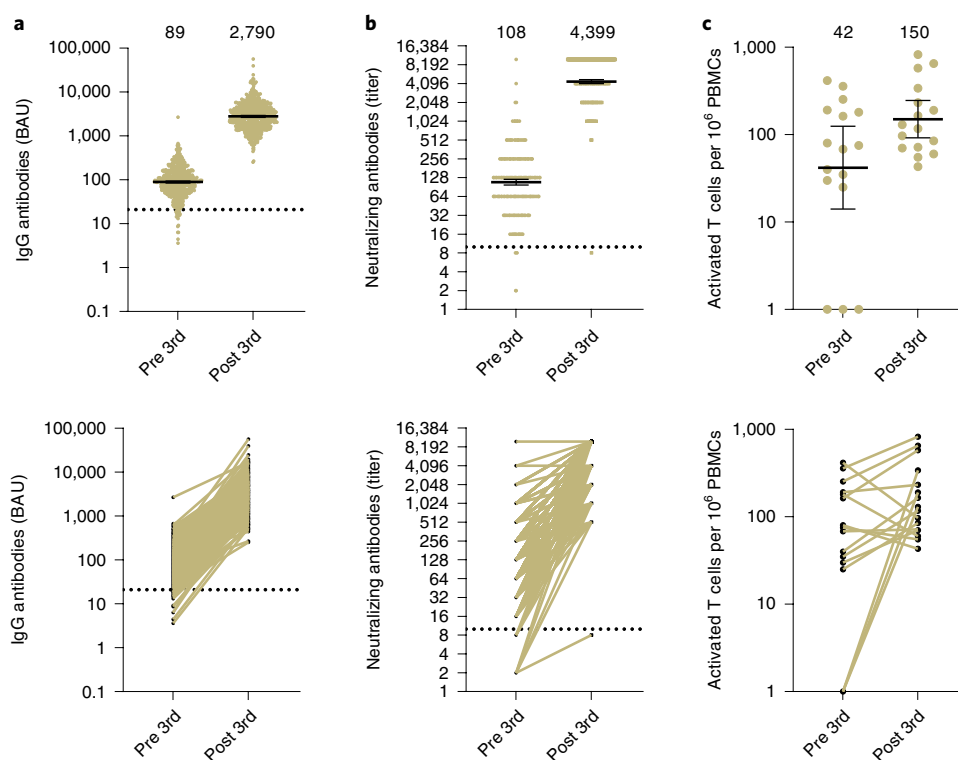

Fig. 2 | Immunogenicity before and after the third vaccine dose. a–c, Scatter plot and before/after analysis of IgG antibodies ($n = 940$ biologically independent samples) (a), neutralizing titers ($n = 513$ biologically independent samples) (b) and number of activated T cells ($n = 16$ biologically independent samples) (c) in HCWs ≤45 days before (Pre 3rd) or 14–45 days after (Post 3rd) the third vaccine dose. The dotted black line indicates the cutoff level of positive antibodies and neutralizing concentrations. The black bars indicate GMT ± 95% CI. GMT of each time point is indicated. BAU, binding antibody unit; GMT, geometric mean titer; PBMCs, peripheral blood mononuclear cells.

To investigate any added effect of the third vaccine dose on humoral response, we compared IgG and neutralizing antibodies at their peak levels following the second vaccine dose (post-second) to that of the third dose (post-third). IgG and neutralizing antibody results were available for 3,477 and 664 HCWs, respectively, after their second vaccine dose and for 1,232 and 692 HCWs after their third vaccine dose. A linear mixed model was used to examine the differences in immunogenicity across age, sex and number of comorbidities, comparing post-second dose IgG levels and neutralizing antibody titers. The estimated geometric mean titer (GMT) for IgG
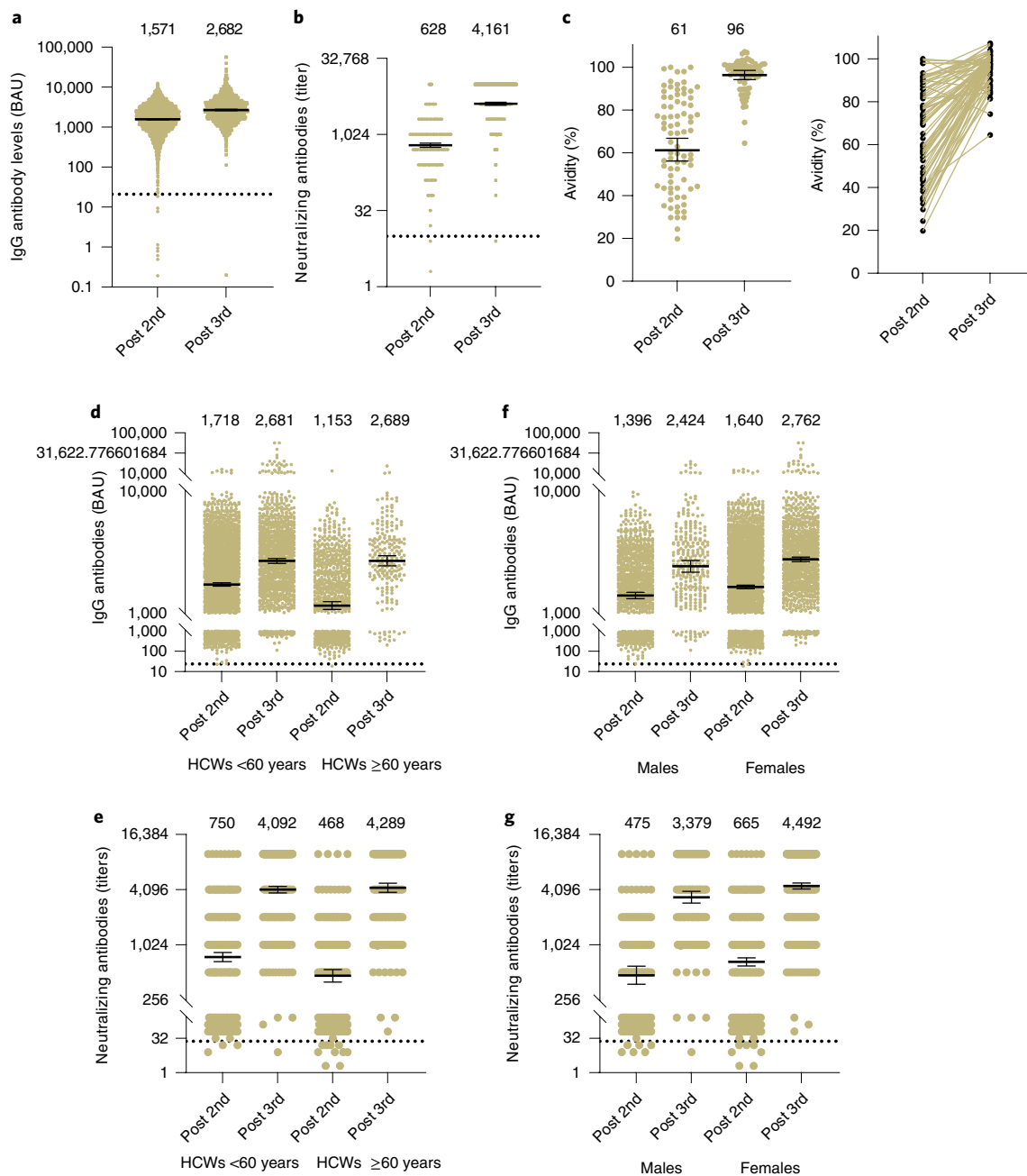
Fig. 3 | Humoral response 14–45 days after the second and third vaccine doses. a–c, Scatter plot analysis of IgG antibodies (*n* = 3,478 after the second dose and *n* = 1,098 after the third dose) (a) and neutralizing titers (*n* = 665 after the second dose and *n* = 693 after the third dose) (b) and avidity scatter plot and before/after analysis (*n* = 81) (c) in HCWs after the second (Post 2nd) and third (Post 3rd) vaccine doses. The dotted black line indicates the cutoff level of positive antibodies and neutralizing concentrations. The black bars indicate GMT ± 95% CI. All numbers (*n*) reflect biologically independent samples. GMT of each time point is indicated. **d–g**, IgG (d,f) and neutralizing antibodies (e,g) after the second and third doses according to age group (*n* = 2,697 and *n* = 780 after the second dose and *n* = 1,028 and *n* = 204 after the third dose in HCWs <60 years and ≥60 years, respectively, for IgG (d) and *n* = 383 and *n* = 281 after the second dose and *n* = 444 and *n* = 248 after third dose in HCWs <60 years and ≥60 years, respectively, for neutralizing antibodies (e)) and sex (*n* = 932 and *n* = 2,545 after the second dose and *n* = 276 and *n* = 956 after the third dose in male and female HCWs, respectively, for IgG (f) and *n* = 154 and *n* = 510 after the second dose and *n* = 186 and *n* = 506 after the third dose in male and female HCWs, respectively, for neutralizing antibodies (g)). Expected GMT is indicated for each group. Dots represent individual observed serum samples. The black bars indicate GMT ± 95%CI.. All numbers (*n*) reflect biologically independent samples.

following the second dose, in binding antibody units (BAUs), was 1,586 (95% CI, 1,458–1,709), and for IgG following the third dose, it was 2,745 (95% CI, 2,641–2,853). Thus, a 1.7-fold (95% CI, 1.6–1.9) increase in IgG levels occurred after the third dose in comparison to after the second dose. Neutralizing antibody levels after the second and third vaccine doses were 646 (95% CI, 589–709) and 3,948

(95% CI, 3,735–4,191), respectively (Fig. 3a,b). The neutralizing titer after the third dose was thus 6.1-fold (95% CI, 5.5–6.8) greater than that of the second dose. Because both quantity and the strength of interaction of antibodies are important for neutralization, we tested IgG avidity after the second and third doses in 81 randomly selected HCWs. Although a 61.1% (95% CI, 56.1–66.7)

Table 1 | Variables associated with IgG and neutralizing antibody titers

Variable	IgG (after[a] V2)	IgG (after[a] V3)	IgG ratio V2/V3	Neutralizing antibodies (after[a] V2)	Neutralizing antibodies (after[a] V3)	Neutralizing antibodies ratio V2/V3
Total	3,477 ratio of mean (95% CI)	1,232 ratio of mean (95% CI)	Ratio of mean (95% CI)	644 ratio of mean (95% CI)	692 ratio of mean (95% CI)	Ratio of mean (95% CI)
Age (years)						
<60	1	1	n/a	1	1	n/a
≥60	0.70 (0.65–0.75)	1.03 (0.93–1.13)	1.47 (1.32–1.62)	0.61 (0.52–0.72)	1.13 (0.96–1.33)	1.86 (1.50–2.30)
Sex						
Female	1	1	n/a	1	1	n/a
Male	0.87 (0.82–0.93)	0.95 (0.85–1.05)	1.08 (0.98–1.19)	0.79 (0.66–0.96)	0.77 (0.65–0.92)	0.97 (0.77–1.23)
Comorbidities						
BMI < 30	1	1	n/a	1	1	n/a
BMI ≥ 30	1.10 (1.02–1.18)	1.31 (1.17–1.47)	1.19 (1.07–1.34)	0.99 (0.79–1.25)	1.12 (0.89–1.42)	1.13 (0.84–1.53)
No specific comorbidity[b]	1	1	n/a	1	1	n/a
One specific comorbidity[b]	0.88 (0.81–0.95)	0.93 (0.83–1.04)	1.05 (0.94–1.17)	0.98 (0.81–1.20)	0.98 (0.78–1.22)	0.99 (0.75–1.31)
Two or more specific comorbidities[b]	0.73 (0.61–0.87)	0.86 (0.69–1.10)	1.18 (0.92–1.53)	0.73 (0.54–0.98)	0.96 (0.69–1.32)	1.31 (0.88–1.94)

[a]The post-vaccine period was defined as days 14–45 after the second or third vaccine doses. [b]Specific comorbidities included the following: hypertension, diabetes, dyslipidemia, heart disease, lung disease, kidney disease and liver disease. n/a, not applicable; V2, second dose; V3, third dose.

avidity was observed after the second dose, a substantially higher avidity of 96.3% (95% CI, 94.2–98.5) was found after the third dose (Fig. 3c). No substantial differences in avidity after the second and third doses were observed between HCWs 60 years or older and those younger than 60 years (Extended Data Fig. 1).

Following the second dose, lower IgG and neutralizing titers were associated with older age, male sex and the presence of at least one (for IgG) or two (for neutralizing antibodies) coexisting conditions, whereas higher IgG titers were associated with a body mass index (BMI) of 30 or higher (obesity) as compared with a BMI of less than 30 (Table 1 and Supplementary Data, Section 4.3). The third dose elicited a 1.41-fold (95% CI, 1.27–1.58) and 1.19-fold (95% CI, 1.06–1.34) increased expression of IgG antibodies and 1.66-fold (95% CI, 1.32–2.08) and 1.33-fold (95% CI, 0.91–1.95) increased expression of neutralizing titers in HCWs 60 years or older and in HCWs with two or more comorbidities, respectively, compared with younger HCWs and HCWs with no morbidities. Concomitantly, after the third dose, no differences in IgG levels between older and younger persons, gender and those with and without comorbidities were observed (Table 1 and Supplementary Data, Section 4.3); higher levels of IgG antibodies continued to be associated with a BMI of 30 or greater following the third dose. Neutralizing antibody levels after the third dose were lower in males than females but did not differ substantially according to age group, BMI group or number of comorbidities (Table 1).

Correlation between IgG and neutralizing antibodies. We next assessed whether the increase in avidity affected the correlation between IgG and neutralizing antibodies following the third vaccine dose. A similarly strong correlation was observed following the second dose (Spearman's rank correlation of 0.62) as well as following the third dose (Spearman's rank correlation of 0.61) (Extended Data Fig. 2).

Correlates of risk. During the fourth wave of SARS-CoV-2 infections in Israel, which was predominated by the Delta VOC,

46 third-dose breakthrough infections occurred in Sheba Medical Center (SMC) HCWs between 19 August and 27 October. Third vaccine dose breakthrough infections were defined as SARS-CoV-2 infections that were diagnosed by polymerase chain reaction (PCR) at least 10 days after the receipt of the third vaccine does (range, 11–68 days). We were able to obtain peri-infection serological samples for 13 of those HCWs.

Supplementary Table 1 summarizes the demographics, symptoms, Ct value and rapid antigen testing of breakthrough cases. The results of peri-infection neutralizing and IgG antibody tests were available for 11 and 13 breakthrough cases, respectively (Supplementary Table 2). For each case, four controls were matched as described in the Supplementary Data. The antibody levels for these breakthrough cases and their matched controls are shown in Fig. 4. The neutralizing GMT titers of breakthrough cases was 1,726 (95% CI, 931–3,200) and less (P = 0.006, Wilcoxon signed rank) than the GMT of controls (5,502 (95% CI, 4,018–7,534). IgG antibody GMT levels were also lower (P = 0.012, Wilcoxon signed rank) in cases (1,995 (95% CI, 1,398–2,848) than in controls (3,543 (95% CI, 2,630–4,772).

Vaccine effectiveness of the third dose. For this analysis 12,290 naive (that were not previously infected with SARS-CoV-2) HCWs eligible for a third vaccine dose, for whom data was available, were included. These HCWs contributed together 632,759 person-days to the two-dose cohort and 339,901 person-days to the three-dose cohort. In total, 407 HCWs were found positive on PCR testing, 368 in the two-dose cohort and 39 in the three-dose cohort. The crude SARS-CoV-2 breakthrough incidence rate in the two-dose cohort was therefore 5.8 per 10,000 days at risk compared to 1.1 per 10,000 days in the three-dose cohort. After adjustment for gender, age and time (weekly period), estimated vaccine effectiveness against PCR-confirmed infection regardless of symptoms of the third dose relative to two doses was 85.6% (95% CI, 79.2–90.1) (Table 2).

Adverse events. Of 8,337 HCWs who were sent the electronic questionnaire, 3,611 responded. The differences between those who
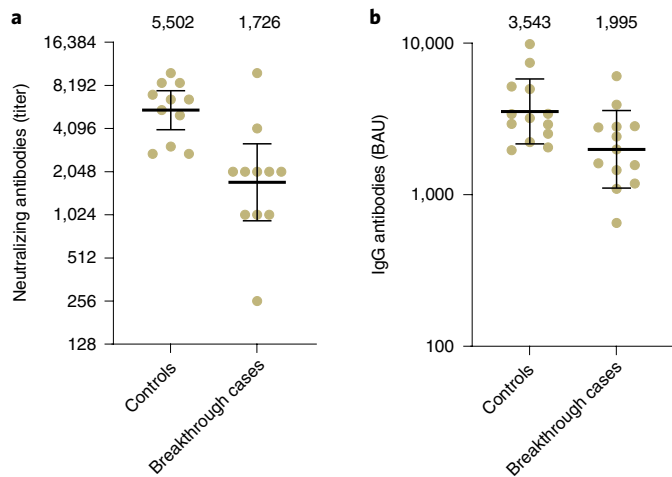
Fig. 4 | Neutralizing antibody and IgG titers among breakthrough cases and matched uninfected HCW controls. a,b, Neutralizing (a) and IgG (b) levels obtained from HCWs up to 7 days before infection ($n=11$ and 13 biologically independent samples for neutralizing and IgG levels, respectively) (breakthrough cases) or from their matched averaged HCWs who were not infected (controls) ($n=42$ and 52 biologically independent samples for neutralizing and IgG levels, respectively) were determined. The black bar indicate GMT ± 95% CI. GMT for each point is indicated.

Table 2 | Vaccine effectiveness of third dose versus two outdated doses among 12,413 HCWs

	Two-dose cohort[a]	Three-dose cohort[b]	RR (95% CI)	Relative VE (%) 100 (1 − RR) (95% CI)
Cases	368	39	n/a	n/a
PD exposure	632,759	339,901	n/a	n/a
Crude breakthrough infection rate/10,000 PD	5.82	1.15	5.07 (3.64–5.07)	80.3 (72.6–85.8)
Adjusted breakthrough infection rate/10,000 PD	6.63	0.95	6.96 (4.82–10.05)	85.6 (79.2–90.1)

[a]Second dose of vaccine given at least 5 months previously (no third dose). [b]Third dose given at least 10 days previously. n/a, not applicable; PD, person-days; RR, rate ratio; VE, vaccine effectiveness.

responded to the questionnaire and those who did not are summarized at Supplementary Table 3. Extended Data Fig. 3 summarizes the local and systemic reactions reported among responders.

The proportion of females among responders was significantly higher ($P < 0.0001$), and the median age of the responders group was significantly lower (51.7, IQR 39.4–66.5, versus 55.9, IQR 41.6–68.5, in nonresponders). We thus stratified adverse events by gender and age group.

Local reactions were very common, with nearly all (95%) young females (age <60 years) and two thirds (68%) of the older male group reporting local reactions (mostly pain at the injection site). Systemic reactions were also frequently reported by young females, with 76% reporting any systemic reaction, including fatigue and myalgia, and 19% reporting fever. Yet, only 31% of the older males reported any systemic reaction, and only 3% reported fever (Fig. 5 and Extended Data Fig. 3). Only two HCWs required hospital admission due to symptoms that occurred in proximity to the receipt of the third vaccine dose; one suffered from a migraine with sensory loss and was hospitalized for 2 days, and the other had unexplained hyponatremia and was discharged from the hospital after 4 days.

Discussion

In this study, we found that the humoral response generated by the third BNT162b2 vaccine dose is substantially superior to the response to the second dose, resulting in overall increased IgG levels, avidity and neutralizing antibody titers, eliminating the differential lower IgG and neutralizing levels observed in older and morbid populations. These third dose-generated antibodies are correlated with risk from infection against the Delta VOC. With mostly self-limiting adverse reactions, the third vaccine dose boosted vaccine effectiveness against infection, which was diminished 5–6 months after the second vaccine dose.

Accumulating data regarding immunogenicity demonstrate a significant increase in antibody and neutralizing levels in the first weeks following two BNT162b2 vaccine doses[17], which decline over a period of at least 6 months, with IgG antibodies decreasing at a consistent rate and neutralizing antibody titers plateauing after

3 months[9]. In conjunction with this finding, vaccine effectiveness was shown to be 90–95% in the first months and rapidly waning in all age groups with time following the second vaccine dose[3,7,8]. Antibody and neutralizing titers were both demonstrated to be correlated with protection from infection[10,18,19], suggesting that immunogenicity levels observed following the second dose can serve as a marker for immunity. With the administration of the third vaccine dose, key questions arising are whether antibody and neutralizing response after the third dose as well as immunogenicity kinetics will be similar to those of the second dose. Our results show that although a significant yet small (~1.7-fold) increase in IgG antibody levels was observed, there was a ~6-fold increase in neutralizing titers comparing with those after the second dose. This discrepancy between antibody levels and neutralization response is most likely due to the significant increase in the strength of interaction between IgG antibodies and SARS-CoV-2 antigen (avidity) observed after the third dose compared to that observed after the second dose. Interestingly, the increase in avidity after the third dose did not alter the correlation between IgG antibodies and neutralizing levels. Thus, our results demonstrate that the third vaccine dose elicits IgG affinity maturation, which specifically impacts neutralization capacity. This finding suggests that lower IgG antibody levels will be required to maintain high neutralizing titers, and as a result, immunity may be sustained for a longer period of time following administration of the third vaccine dose, despite a similar rate of IgG waning. Accordingly, the high-avidity antibodies generated by the third vaccine dose may induce higher neutralizing protection against VOCs, including the newly emerging Omicron VOC.

Although the third vaccine dose significantly induced humoral response, a critical priority is to assess whether low IgG and neutralizing antibodies may serve as correlates of risk after a booster dose and toward the Delta VOC. Our comparison of peri-infection antibody levels in breakthrough cases with their matched uninfected controls demonstrated that both antibody markers were significantly lower in breakthrough cases. Interestingly, we previously observed that following two vaccine doses, substantially lower peri-infection IgG and neutralizing antibody levels among Alpha VOC breakthrough cases and uninfected controls[10] were correlated with protection from infection against the Alpha variant. These data suggest that even at this very early post-booster dose, where antibody levels are high, there is a correlation between antibody and infection. Our results provide evidence that individuals with lower antibody levels are less protected against infection than
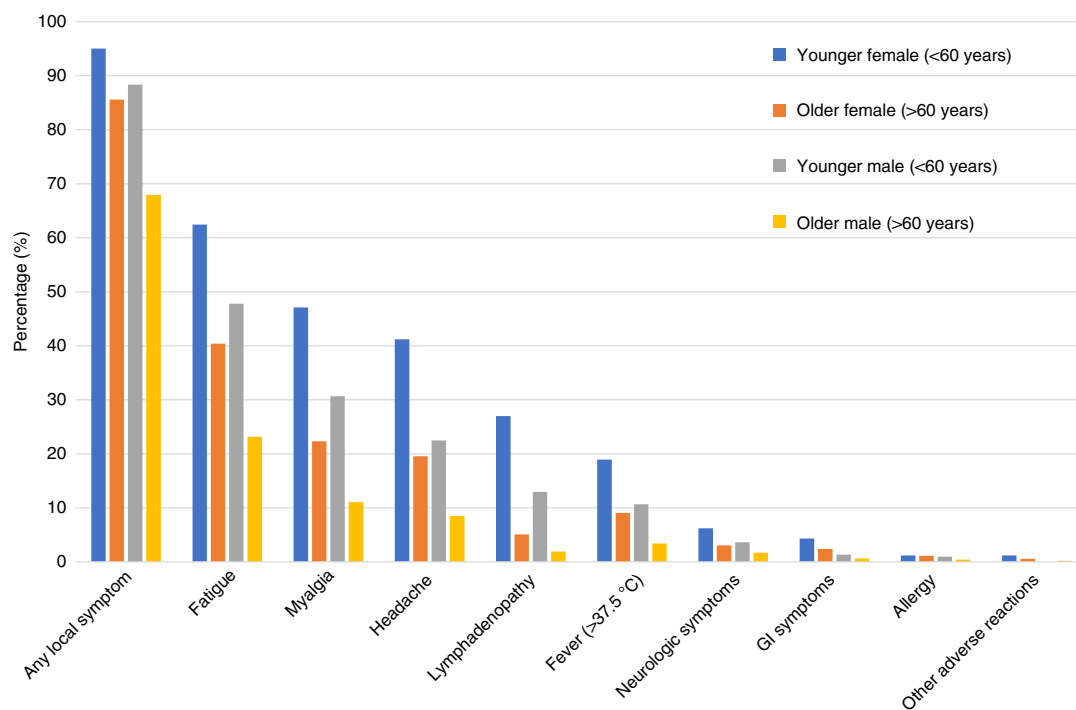
Fig. 5 | Local and systemic reactions reported after the third vaccine dose. Local symptoms included local pain (reported by 3,058 of 3,611 HCWs), swelling (*n* = 685), redness (*n* = 445) and itching (*n* = 11). Neurologic symptoms included paresthesia (*n* = 138) and facial nerve palsy (*n* = 17). Other adverse reactions included abnormal laboratory results, such as elevated C-reactive protein, elevated thyroid-stimulating hormone, hyponatremia or leukopenia (*n* = 1 each). Other symptoms included herpes labialis (*n* = 3) and report of new-onset psoriasis (*n* = 1). GI, gastrointestinal.

individuals with higher levels and that overall antibodies are highly correlated with risk from infection after the third dose and against the Delta VOC.

Using a mixed model, we analyzed the association of age, sex and coexisting conditions with immunogenicity 1 month after the second and third doses. Separately, we also investigated the immune response before and after the third dose. Consistent with our previous results[9], a significantly lower antibody response was found among older HCWs, males and HCWs with two or more comorbidities 1 month after the second dose. Interestingly, HCWs 60 years or older and HCWs with two or more comorbidities had increased reaction after the third dose compared to younger HCWs and HCWs with no morbidities, respectively. As a consequence, no significant difference was observed between old and young populations as well as between HCWs with and without comorbidities 1 month after the third vaccination, indicating that these vulnerable populations mount an immune response similar to that of healthy HCWs. Indeed, a similar phenomenon of increased response to vaccination in older HCWs was observed after the second BNT162b2 vaccine dose[17]. It is interesting to speculate that although generation of a primary immune response may be hampered in vulnerable individuals, their secondary response is intact and able to compensate following sequential booster doses.

The relative vaccine effectiveness measured in this study of the third dose against PCR-confirmed infection regardless of symptoms of 85.6%, comparing incidence rate among third dose recipients to that among those who were eligible for a third dose but did not receive it, is slightly lower than those from three other observational studies from Israel, which reported vaccine effectiveness of 88–92%[12,13,16]. A limitation of this study is the relatively small number of participating individuals, which prevented us from investigating vaccine effectiveness against severe COVID-19. However, the meticulous follow-up of Sheba HCWs allowed us to examine the effectiveness of the third dose against infection even in

asymptomatic and very mild symptomatic cases, which are usually not identified in observational studies. As a result, we believe our data more accurately reflect the vaccine effectiveness of symptomatic and asymptomatic individuals. Furthermore, our vaccine effectiveness data were generated in the same cohort used for conducting the immunogenicity assays, thus enabling us to better associate vaccine effectiveness with immune response. Continued monitoring of the Sheba HCW cohort will allow us to assess vaccine effectiveness for Omicron and other future VOCs.

Reactogenecity in our study was actively pursued, and therefore, we believe it did not miss adverse events, particularly not serious ones. Yet, our estimations of proportion of individuals reporting adverse events may be overestimated due to reporting bias. Furthermore, although the data from this study seem to show that there is no alarming or life-threatening signals after the third dose, the small size does not allow complete assessment of adverse events. In our study, most third vaccine dose recipients reported mild to moderate and transient local and systemic reactions. The rate of adverse events here is similar to those reported in several studies that monitored the safety of one and two doses of BNT162b2 administration among healthy individuals[20,21]. The rate is also similar to that of a recent study that examined the safety of a third dose of COVID-19 vaccine among vaccine recipients in the United States using a self-monitoring surveillance system[22]. Overall, these results suggest that the reactogenicity to the third dose is frequent yet limited to mild local and systemic events and not different from the adverse events identified in the first and second vaccine doses.

This study was conducted in HCWs and therefore does not represent the general population. However, the continuous monitoring of this cohort even before the administration of the first vaccine dose in December 2020 allowed us to thoroughly examine the impact of the BNT162b2 vaccine using repeated measurements on the same population, which resulted in obtaining valuable immunogenicity data across age, gender and comorbidities. It is important

to keep in mind that the chief VOC circulating during the study period was Delta, and therefore, vaccine effectiveness can be different against other VOCs that may emerge, such as the newly discovered Omicron variant. Nevertheless, our immunogenicity data can be of great importance once vaccine penetration and correlates of protection levels are determined.

Taken together, our immunogenicity, vaccine effectiveness and safety data clearly demonstrate that the third BNT162b2 vaccine dose, given at least 5 months after the second dose, safely boosts protection from SARS-CoV-2 infection by substantially inducing broad humoral and cellular responses. The antibodies generated as a result of this booster dose are of high avidity and as such are superior and will most probably protect from infection also vulnerable populations longer than second dose-generated antibodies. Even at the early post-booster dose stage, when antibody response is at its peak, we report a correlation between humoral markers and risk against infection with the Delta VOC, thus providing more evidence of the importance of the humoral response to protection against SARS-CoV-2 infection.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41590-022-01212-3.

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Methods

Ethics. The protocol was approved by the Institutional Review Board of the SMC, and written informed consent was obtained from all study participants. Data collection and analysis were not performed blind to the conditions of the experiments.

Study setting and period. The Sheba HCW COVID cohort study is an ongoing prospective cohort study following SMC HCWs that has been conducted since vaccination rollout first began on December of 2020 (refs. [9–11,17]). SMC is the largest tertiary medical center in Israel, with 1,600 beds and 14,479 HCWs, including employees, students, volunteers and retired personnel. Between December 2020 and July 2021, a total of 95% of eligible HCWs received two doses of the BNT162b2 vaccine. On 28 July 2021, the Israeli ministry of health decided to administer a third vaccine dose to individuals aged 60 years or older on 15 August 2021 to younger HCWs who received their second vaccine dose more than 5 months prior and on 29 August 2021; this decision was expanded to general population. Of all SMC HCWs, 12,243 received two COVID-19 vaccine doses by May 2021, did not acquire SARS-CoV-2 by 28 July 2021 and were eligible to receive the third dose, which was offered at SMC.

This study included immunogenicity data from February 2021 until October 2021. The vaccine effectiveness substudy took place during the fourth surge of SARS-CoV-2 infections in Israel, which was predominated by the Delta VOC, between 8 July and 1 October 2021. Safety data were collected between 7 August and 2 September 2021.

Study design and population. The Sheba serology study, which was initiated before the rollout of the first COVID-19 vaccine dose and recruited 6,466 HCWs, consisted of monthly serological follow-up. Here, we included HCW who fulfilled the following criteria: (1) 18 years or older and COVID-19 naive (that is, no history of SARS-CoV-2 infection determined by previous positive PCR result, positive anti-S IgG before receiving the first dose or positive anti-N IgG at any time point), (2) had available sera 14–45 days after the second dose or/and (3) had available sera 45 days or less before the third dose or/and (4) had available sera 14–45 days after third dose. For subjects who had more than one eligible sample in the post-second or post-third dose period, the sample with highest IgG levels was included in the analysis, as a best measure of the peak level after vaccination. For subjects who had more than one eligible sample in the pre-third dose period, the sample closest to receipt of the third vaccine dose was chosen. Baseline characteristics of the study population are presented in Supplementary Table 4.

Neutralizing antibody assays were performed on a selected subgroup that included higher proportions of persons with risk factors of interest, such as an age of 65 years or older and coexisting conditions. Criteria for the selection of participants for the neutralizing antibody subgroup are listed in Supplementary Data section 2. Comparisons of antibody levels at different time points relative to the second and third vaccines were adjusted for these selection criteria (Methods, Statistical analysis), although the impact of selection on such within-cohort comparisons is not expected to be large.

Breakthrough infections were defined as the detection of SARS-CoV-2 on quantitative real-time PCR (qRT-PCR) assay performed 11 or more days after receipt of a third dose of BNT162b2. For each breakthrough case, we matched samples that had been obtained from four uninfected controls based on age (with an age difference of ±5 years), sex, immunosuppression and time interval between receipt of the third vaccine dose and serological test. We compared neutralizing antibody titers and S-specific IgG antibodies against SARS-CoV-2 obtained within a week before SARS-CoV-2 detection on RT-PCR testing (preinfection period).

For the vaccine effectiveness study, all HCW without history of SARS-CoV-2 infection by 15 June 2021, who received the second vaccine dose by 1 May 2021 and were eligible to receive the third dose between 29 July and 2 October 2021 were included (Extended Data Fig. 4b). Data on PCR testing methods are provided in Supplementary Data.

Data on age and sex were available for all study participants. A computer-based questionnaire about demographic characteristics and coexisting conditions was sent electronically to all serology study participants. The questionnaire and definitions of the study variables are provided in Supplementary Tables 5 and 6.

Serology assays. Samples from vaccinated participants were tested before receipt of the third dose using the Access SARS-CoV-2 receptor-binding domain (RBD) IgG assay (C58961, Beckman-Coulter) or after receipt of the third dose using the SARS-CoV-2 IgG II Quant (6S60, Abbott) test. These commercial tests were performed according to the manufacturer's instructions. To present all IgG antibody levels in BAUs per the World Health Organization standard measurements, we imputed the Abbott-based BAU values from the Beckman-Coulter assay results based on an independent sample of individuals with both Abbott BAU and Beckman-Coulter levels (see Supplementary Data 4.1). The cutoff values for positivity for IgG are 21.4 BAUs.

A SARS-CoV-2 pseudovirus (psSARS-2) neutralization assay was performed[17] using a propagation-competent vesicular stomatitis virus spike (kindly provided by G. Zimmer, University of Bern, Switzerland) shown to be highly correlative to authentic SARS-CoV-2 virus microneutralization assay. Following titration, 100 focus-forming units of psSARS-2 were incubated with twofold serial dilution of heat-inactivated (56 °C for 30 min) tested sera. After incubation for 60 min at 37 °C, virus/serum mixture was transferred to Vero E6 cells (CRL-1586, ATCC) that have been grown to confluency in 96-well plates and incubated for 90 min at 37 °C. After the addition of 1% methyl cellulose (M0512, Sigma-Aldrich) in Dulbecco's modified Eagle's medium (Biological Industries) with 2% of fetal bovine serum (Biological Industries), plates were incubated for 24 h, and 50% plaque reduction titer was calculated by counting green fluorescent foci using a fluorospot reader (AID Autoimmun Diagnostika). Sera not capable of reducing viral replication by 50% at 1:8 dilution or below were considered nonneutralizing. For clear presentation, nonneutralizing samples were marked as a titer of 2.

Avidity assay was based on an in-house RBD-IgG enzyme-linked immunosorbent assay with the addition of 6 M urea (U5378, Sigma-Aldrich) or PBS (Biological Industries) for 10 min for each sample. Briefly, a 96-well microtiter Polysorb plate (Nunc, Thermo) was coated overnight at 4 °C with 50 μl per well of 1 μg ml⁻¹ RBD antigen. After blocking with 5% skimmed milk at 25 °C for 60 min, serum samples diluted 1:100, 1:400 and 1:1,000 with 3% skimmed milk were added to antigen-coated wells. The plate was incubated at 25 °C for 120 min, and following washing, each sample was incubated either with the addition of 6 M urea or PBS for 10 min. After washing, a goat anti-human IgG horseradish peroxidase conjugate (catalog 109–035–088, Jackson ImmunoResearch) (diluted 1:15,000) was added to each well for 60 min. After washing, incubation of TMB Substrate Solution (Abcam) for 5 min and the addition of stop solution (2 N HCl), the optical density (OD) of each well was measured at 450 nm using a microplate reader (Sunrise, Tecan). Avidity index was calculated as the ratio (in percentage) between sample OD with 6 M urea and sample OD with PBS.

To assess for T cell response, peripheral blood mononuclear cells were isolated by density gradient centrifugation using UNI-SEP+ (Novamed). Plasma was collected and spun at $1,000 \times g$ for 20 min to remove platelets before collection of peripheral blood mononuclear cells. Following one wash with PBS and one wash with 4Cell Nutri-T-Medium (Sartorius), cells were resuspended in 4Cell Nutri-T-Medium and counted using the Countess II Cell counter (Invitrogen).

Interferon-γ (IFN-γ)-secreting cells were enumerated using Elispot IFN-γ kits (IFN-γ kit, ELSP5000/5500, AID Autoimmun Diagnostika) according to manufacturer instructions. For antigen stimulation, 50 μl SARS-CoV-2 peptide pools (S-complete, 130–127–953, Miltenyi Biotech) was used. Test medium was used as negative control, and phytohaemagglutinin (Mabtech) was used as positive control. IFN-γ-secreting cell frequency was quantified using a fluorospot reader (AID Autoimmun Diagnostika). The unspecific background (mean spot forming units from negative control wells) was subtracted from experimental readings.

Adverse event active surveillance. All HCWs who received the third vaccine dose before 2 September were sent a short electronic questionnaire regarding side effects of the third vaccine dose. They were asked about various localized and systemic side effects, the duration of these symptoms and whether they required medical care or hospitalization. Additionally, HCWs and their treating physicians were encouraged to report any serious adverse event or hospitalization. To identify reporting bias of those answering the questionnaire, we compared demographic variables of responders and nonresponders and accordingly stratified the outcome by under- or overrepresented subpopulations.

Statistical analysis. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications[9,17]. No data points were excluded from the analyses.

Statistical inference was based mostly on nonparametric methods, including nonparametric bootstrap CIs or Wilcoxon rank-sum tests. (When bootstrap methods were used, the bootstrap distributions of the estimates were examined. In all cases they appeared close to normal.) The exception was the modeling of the neutralizing antibody levels, which was based on the usual application of linear mixed models. For these analyses, the distribution of residuals of the models were checked visually for normality, and when they appeared nonnormal, the confidence limits of the estimates were recalculated using nonparametric bootstrap methods and were compared to the model-based confidence limits. In no cases were the two sets of confidence limits appreciably different.

Binding antibody levels measured in BAUs were available only after receipt of the third dose. Before the receipt of the third dose, binding antibodies were assessed using the Beckman-Coulter RBD assay. To make comparisons of before and after the third dose, Abbott-based BAU values were imputed from the Beckman-Coulter assay levels on the basis of an independent sample of individuals who had both measurements. For details of the imputation, see Supplementary Data, section 4.1. To account for the extra uncertainty due to using imputed values, bootstrap methods were used to calculate CIs.

All IgG antibody levels were analyzed on the natural logarithmic scale. Pre-third vaccine neutralizing antibody levels were compared to post-third vaccine levels, and the difference was expressed as a ratio between geometric means, with the 95% CI based on matched pre- and post-samples.

Pre-and post-third vaccine IgG levels were compared in the same way, but the CI was based on taking bootstrap samples of both the imputation sample and the sample of HCWs with pre- and post-third vaccine levels. See Supplementary Data, section 4.2 for more details.

This comparison of post-second with post-third vaccine antibody levels included three subgroups of HCWs: those with antibody levels measured after the second dose only, those with antibody levels measured after the third dose only and those with measurements at both time points. Both neutralizing antibody and IgG levels at these time points were compared using linear mixed models. Each individual's level was modeled as a random effect, and time point (after third vaccine versus after second vaccine) was modeled as a fixed effect. Individuals' characteristics were included as fixed-effect adjusting covariates and included gender, age group (<60 years, ≥60 years), BMI (<30, ≥30, missing) and number of comorbidities (0, 1, ≥2, missing), where the comorbidities considered were hypertension, diabetes mellitus, dyslipidemia, heart disease, lung disease, kidney disease and liver disease. Interaction terms between each adjusting covariate and the time-point variable were also included. Parameter estimates from the model were then used to compare post-third dose to post-second dose levels, overall and in subgroups of adjusting covariates, as well as comparing post-second dose levels according to covariates and post-third dose levels according to covariates.

Results are expressed as ratios of geometric means. For neutralizing antibodies, P values and 95% confidence limits were calculated directly from the model output. For binding antibodies, they are based on bootstrapping of both the imputation sample and the HCW sample. See Supplementary Data, section 4.3 for more details.

For correlates of risk analysis, the distribution of antibody levels (both neutralizing and IgG) was compared between cases and matched controls using the Wilcoxon matched rank-sum test.

We investigated vaccine effectiveness of the third vaccine dose relative to two doses given at least 5 months previously for the period 1 July to 2 October. Two cohorts of HCWs were defined: the 'two-dose' cohort (including those eligible for the third dose (previously uninfected and having received their second dose at least 5 months previously) but not having received it) and the 'three-dose' cohort (including those who had received their third dose (10 or more days previously)). Individuals in the two-dose cohort exited that cohort on the day that they were diagnosed with a positive PCR test or on the day they received the third dose. Individuals entered the three-dose cohort on the tenth day following receipt of the third dose and exited on the day that they were diagnosed with a positive PCR test. Follow-up terminated on 2 October 2021.

Incidence rates were analyzed using a Poisson regression model. The follow-up period was divided into weekly periods; for each period, the number of positive diagnoses and the number of follow-up days were calculated for each cohort and subdivided into four subgroups according to gender and age (<60 years and ≥60 years). From this model, we estimated the ratio of incidence rates in the two cohorts (third dose versus two doses only), adjusted for gender, age and period. CIs (95%) were calculated based on model standard errors of the estimated log ratio.

To determine the representability of those who responded to the adverse events questionnaire, we compared demographic characteristics of responders to nonresponders using chi-squared and two-sample Student's t-test.

Scatter plots of IgG and neutralizing antibody levels since the receipt of the second and third doses were created with the use of GraphPad Prism software, version 9.0 (GraphPad Software). Excel 2016 was used to collect all clinical, demographic and immunogenicity data.

Correlations between IgG and neutralizing antibody levels for each period were assessed by Spearman's rank correlation. Paired pre- and post-third vaccine dose avidity, neutralization and T cell activation were compared using the Wilcoxon signed rank test. Statistical analysis was performed using SAS software, version 9.4 (SAS Institute), and linear mixed model analyses were performed using R software, version 3.6.2 (R Foundation for Statistical Computing).

PCR testing. Hospital personnel were tested in several scenarios: upon every symptom suspected to be COVID-19, following exposure to a positive COVID-19 contact (hospital or community contacts) or as part of a 'return to work' protocol during the end of isolation period following exposure or disease.

For qRT-PCR, nasopharyngeal swabs were placed in 3 ml universal transport medium or viral transport medium. Test was performed according to manufacturers' instructions on various platforms: Allplex 2019-nCoV (Seegene), NeuMoDx SARS-CoV-2 assay (NeuMoDx Molecular), Xpert or Xpress SARS-CoV-2 (Cepheid).

Inclusion criteria for selecting the neutralizing antibody subgroup. Not all sera samples collected from the study participants could be tested for neutralizing antibodies. However, the following criteria were used to choose those who were tested for neutralizing antibodies from the entire cohort: age ≥65 years, BMI ≥30, pregnancy, history of any allergic reaction, hypertension, diabetes, dyslipidemia, heart disease, lung disease, kidney disease, liver disease, autoimmune disease or immunosuppression. Additionally, 50% of healthy HCWs were randomly selected for the neutralizing antibody subgroup.

Imputation of binding antibody units based on Beckman-Coulter assay. We developed a method for imputing Abbott IgG levels from Beckman-Coulter IgG levels using data on 215 selected serum samples taken from individuals who had not received a booster dose, were not included in the HCW cohort and were

measured by both methods. We fitted a cubic polynomial regression model where log (to the base e) of IgG measured in BAUs by the Abbott kit was regressed on the log (to the base e) of IgG measured by the Beckman-Coulter kit, its squared value and its cubed value. The fitted regression equation, using the glm procedure in R (output presented in Supplementary Table 8), was:

$$logIGG\_Abbott = 4.506 + 0.6634 \times logIGG\_Beckman - 0.0852$$
$$\times (logIGG\_Beckman)^2 + 0.0403 \times (logIGG\_Beckman)^3$$

A simple linear model has an R^2 of 0.905, compared to 0.919 for the cubic polynomial. One can see from Extended Data Fig. 4 that the cubic polynomial fits the data much better at the lower and upper ends of the scale.

Comparison of pre-third with post-third vaccine IgG levels. We included only participants who were measured before (Beckman-Coulter) and after (Abbott) the third vaccine and excluded the very few participants who had an Abbott kit measurement before the third vaccine. The number of participants included in the analysis was 1,047. We used bootstrapping of both the imputation sample and the before or after third vaccine sample (2,000 repeats) to obtain CIs for the mean change on the log scale from before to after vaccination. Having established that the bootstrap distribution of estimates appeared very symmetric, we used the percentile method to estimate the CI. In addition to the average change, we computed the geometric mean IgG levels at before and after the third vaccine for the total sample and stratified by either gender or age group. The CIs of these means were also obtained by the bootstrap percentile method.

The results are given in Supplementary Table 9. The 'ratio' row is the ratio of the post-vaccine IgG level to the pre-vaccine level. The 'GM' column gives the geometric means. In the tables stratified by gender and age group, the ratio column compares geometric means of females versus males or individual younger than 60 years versus those aged 60 y or older. Levels for females, both before and after the third dose, were higher than for males. Prevaccine levels were higher for those aged younger than 60 years than for those 60 years or older, but post-vaccine levels for the two subgroups were similar.

Comparison of post-second with post-third vaccine IgG levels. We included subjects who were measured either after the third (Abbott) or the second (Beckman-Coulter) vaccination. There were 3,787 subjects included; 922 were measured after both second and third vaccinations, 2,555 were measured after the second vaccination only and 310 were measured after the third vaccination only. We estimated the difference between the post-third vaccine value and the post-second vaccine value on the log Abbott BAU scale using a linear mixed model, where each person's level was modeled as a random effect, and post-third dose versus post-second dose was modeled as a fixed effect. Individuals' characteristics were included as fixed-effect adjusting covariates and included gender, age group (<60 years, ≥60 years), BMI (<30, ≥30, missing) and number of comorbidities (0, 1, ≥2, missing), where the comorbidities considered were hypertension, diabetes, dyslipidemia, heart disease, lung disease, kidney disease and liver disease. Interaction terms between each adjusting covariate and the time-point variable were also included. BMI and comorbidity data were missing in 23% of the sample, and, to include these persons in the analysis, we included a missing category for each of these variables. We used bootstrapping of both the imputation sample and the post-vaccination sample (2,000 repeats) to obtain CIs, stratifying the latter sample into three strata: those measured after second dose only, those measured after third dose only and those measured at both time points. Confidence limits were calculated using the bootstrap percentile method, having found that the bootstrap distributions were very symmetric.

The estimates of the parameters in the model, obtained from the statistical package in R software, version 3.6.2 (R Foundation for Statistical Computing) are shown in Supplementary Table 10.

Aside from the intercept term, which is on the BAU scale, each coefficient is a multiplicative factor associated with that covariate. Reference categories for the covariates are female, aged <60 years, BMI < 30 and no comorbidities. The parameters of interest are The third dose (v3) and its interaction with the covariates. For example, v3 measures the factor increase in post-vaccine 3 level compared to post-vaccine 2 level for a woman aged <60 years with BMI < 30 and no comorbidities (48% increase). The interaction term 'v3:agec60+' measures how different is the change in the IgG levels from post-vaccine 2 to post-vaccine 3 between those aged ≥60 years and those aged <60 years (47% increase). If the value 1 is within the 95% CI, then the effect of the covariate is regarded as nonsignificant at the 5% level. One can see that the multiplicative change between post-vaccine 2 and post-vaccine 3 levels is larger for those aged older than 60 years and for those with BMI greater than or equal to 30.

The terms 'male', 'agec60+', 'bmi30', 'ndis1' and 'ndis2' give the ratios of the IgG levels at post-vaccine 2 between the subgroup and its reference subgroup. For example, males had IgG levels after vaccine 2 that are 89.5% of the levels of females, and the confidence limits exclude the value 1, meaning that the female levels were statistically significantly higher than those of males. Post-vaccine 2 IgG levels appeared to be higher also in older persons and those who were obese. Supplementary Table 11 gives the corresponding ratios for the post-vaccine 3 measurement.

It can be seen that the levels of IgG after vaccine 3 differ statistically significantly according to BMI (with obese patients having higher levels), but not according to gender, age or comorbidity.

We also examined the geometric mean IgG levels after vaccine 2 and after vaccine 3 of the full sample and by gender and age group, adjusted for all covariates. They were obtained by estimating the expected IgG (using the estimated coefficients of the fixed effects from the model) of all participants in post-vaccine periods and averaging their expected levels. The CIs are obtained by the bootstrap procedure. The results are given in Supplementary Table 12.

Similar methods were used for analyzing neutralizing antibody levels post-vaccine 2 and post-vaccine 3, but CIs were calculated from model-based standard error estimates, as no imputation was necessary for these levels.

Vaccine effectiveness. We used a Poisson regression model to evaluate the effectiveness of the third dose (10 or more days after administration) compared to two doses given at least 5 months previously. The parameter estimates from the model, obtained from the *glm* procedure in R software, are shown in Supplementary Table 13.

The log incidence rate ratio for the third dose versus two doses is given by the coefficient for the 'booster' variable (see the row in bold type). The rate ratio estimate is therefore 0.144 (exponent of −1.9396), which translates into a relative vaccine effectiveness of 85.6%.

The other rows show the relationship of the adjusting covariates, age, gender and period to the incidence rate. It can be seen that in this study, those aged 60 years and older were at lower risk than younger workers. It can also be seen that the incidence surge reached its peak between 19 August and 16 September.

Adjusted incidence rates were calculated by applying the above model parameters to calculate the expected incidence rates for each covariate profile, first under receipt of the third dose and then under no receipt of the third dose. A weighted average of these expected incidence rates was then taken, with weights equal to the person-days at risk on each profile.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The de-identified datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. Source data are provided as source data files. Source data are provided with this paper.

Author contributions

Y.L. contributed to concept and design, data collection, analysis and interpretation of data and drafting of the manuscript. T.G. contributed to concept and design, data collection, analysis and interpretation of data and drafting of the manuscript. L.M. and M.G. contributed to data collection, analysis and interpretation of data. V.I. contributed to analysis of data and critical revision of the manuscript for important intellectual content. C.C. contributed to administrative, technical or material support and critical revision of the manuscript for important intellectual content. S.A. contributed to critical revision of the manuscript for important intellectual content. H.J. contributed to administrative, technical, or material support and critical revision of the manuscript for important intellectual content. R.D. and K.A. contributed to data collection and critical revision of the manuscript for important intellectual content. contributed to data collection, critical revision of the manuscript for important intellectual content. C.R. and R.F. contributed to statistical analysis and critical revision of the manuscript for important intellectual content. E.M. contributed to critical revision of the manuscript for important intellectual content. L.F. contributed to statistical analysis and critical revision of the manuscript for important intellectual content. G.R.-Y. contributed to concept and design, analysis and interpretation of data and drafting of the manuscript. Y.K. contributed to concept and design, critical revision of the manuscript for important intellectual content and supervision. All authors reviewed and approved the final manuscript. Y.L. and G.R.-Y. accessed and verified the data and had final responsibility for the decision to submit for publication.

Competing interests

The authors declare no competing interests.

Additional information

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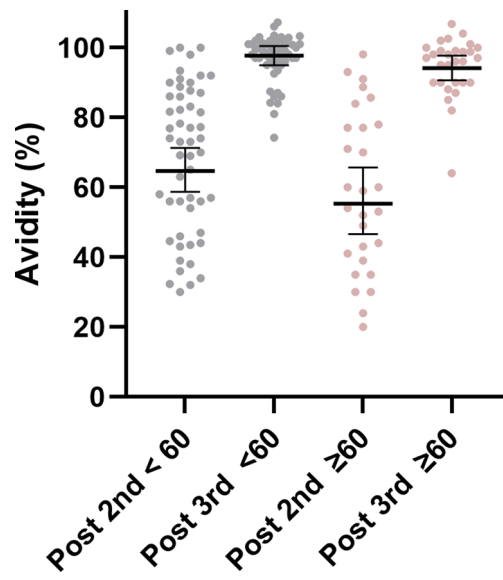
Extended Data Fig. 1 | Avidity 14–45 days after the second and third vaccine. Scatter plot analysis of avidity levels in HCW equal or above 60 (>59, n=29 biologically independent samples) or below 60 (<60, n= 52 biologically independent samples) years old. The black bar indicates GMT ± 95%CI. Significance between younger and older populations were tested by two-sided Mann-Whitney test.

Peak 2nd

Peak 3rd
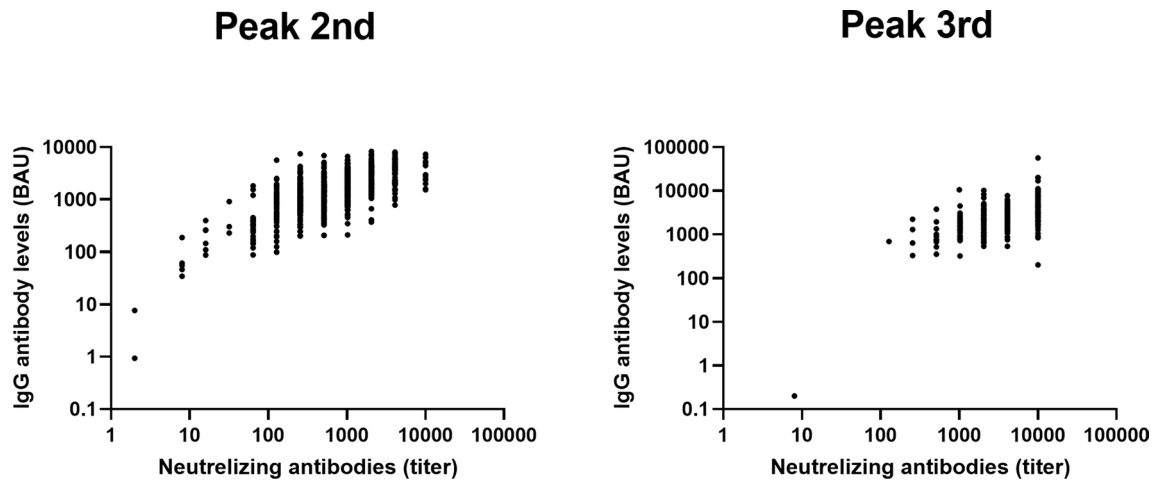
Extended Data Fig. 2 | Correlation of IgG and Neutralizing antibodies. The correlation was analyzed 14–45 days after the second (peak second) and third (peak third) vaccine dose.
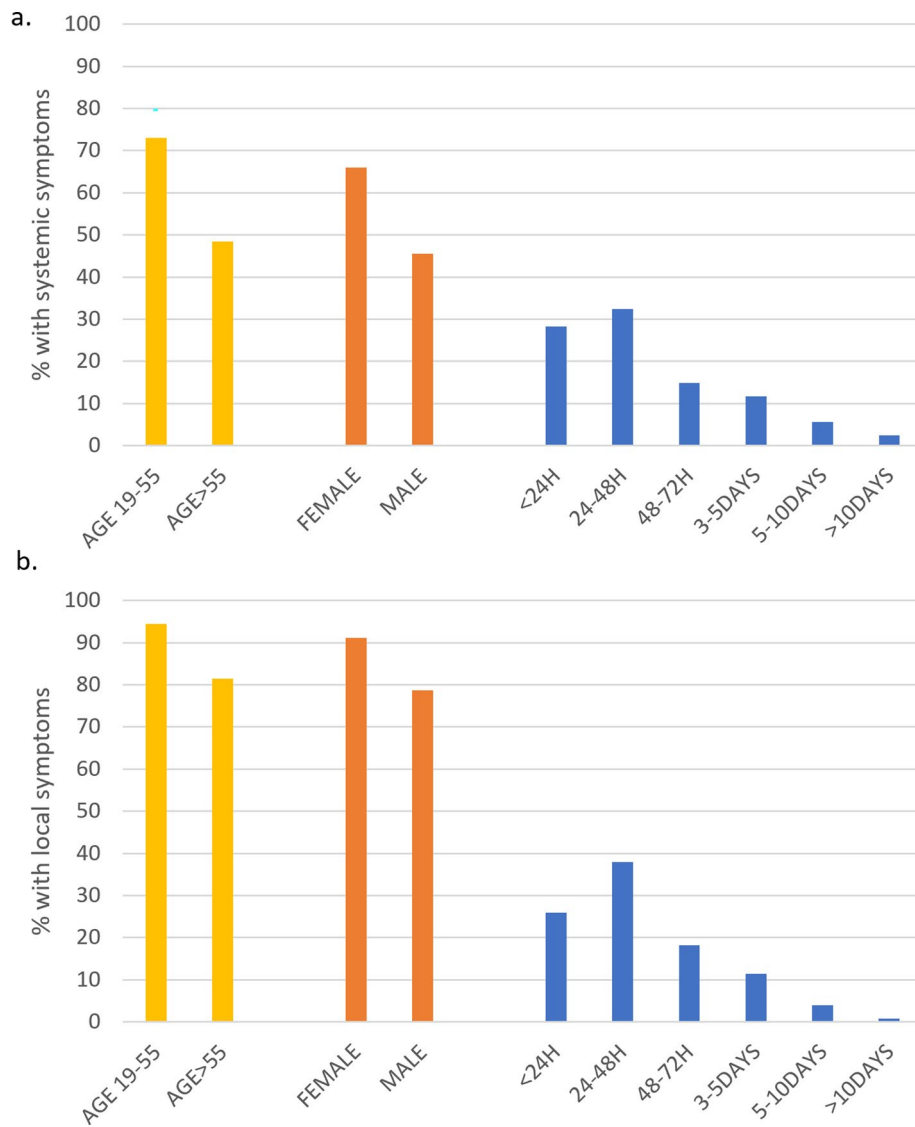
Extended Data Fig. 3 | Local and systemic reactions reported following the third vaccine dose. Local (**a**) and systemic (**b**) reactions with time and among ages and gender.
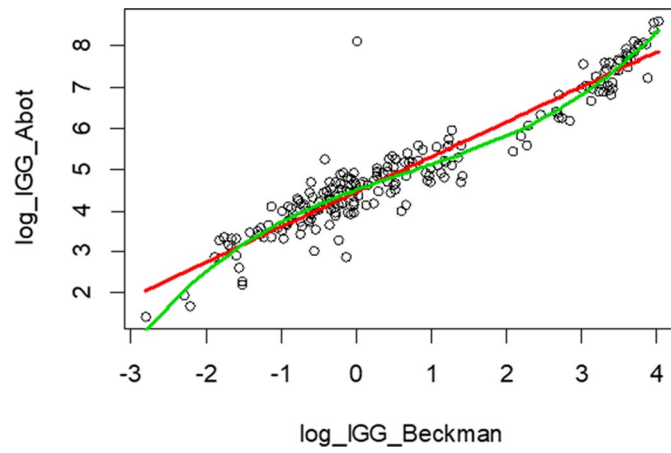
Extended Data Fig. 4 | Visualization of the simple linear method suggested for imputation compared with the cubic polynomial. The regression equation described in the methods was used to impute the values of Abbott BAU for samples taken before the third (booster) dose.

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Reporting Summary

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☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
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☐	☒	For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Excel 2016
Data analysis	GraphPad Prism software, version 9.0, SAS software, version 9.4 , R software, version 3.6.2

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was determined based on the agreement of Health Care workers (HCW) to participate in the study. Statistical analysis was performed with confidence intervals to make sure the results provided enough power to draw conclusions.
Data exclusions	No data was excluded. Only samples obtained from HCW which were excluded from the study. Exclusion criteria included no history of SARS-CoV-2 infection determined by previous positive PCR, positive anti-S IgG before receiving the first dose or positive anti-N IgG at any time point.
Replication	Not Applicable. Only one sample per time point per individual can be obtained on this prospective study
Randomization	Study groups were based on number of vaccines, gender, age and co-morbidities
Blinding	Not Applicable. It is a prospective study and not a clinical study. No blinding was used.

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Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Goat anti-human IgG horseradish peroxidase (HRP) conjugate (Jackson ImmunoResearch, PA, USA Code: 109-035-088) (diluted 1:15000)
Validation	Validation was preformed by manufacturer and also during the development of the assay (PMID:33227020).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Vero cells derived from African green monkey were obtained from ATCC
Authentication	By ATCC and regularly by assessment of characteristic morphology and growth rate in culture.
Mycoplasma contamination	Cells were tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	None

Human research participants

Population characteristics	The Sheba serology study, initiated before the rollout of the first COVID-19 vaccine dose and recruited 6,466 HCW, consisted of monthly serological follow-up. Here, we included HCW who fulfilled the following criteria: (i) 18 years or older, COVID-19-naïve, i.e., no history of SARS-CoV-2 infection determined by previous positive PCR, positive anti-S IgG before receiving the first dose or positive anti-N IgG at any time point, (ii) had available sera 14-45 days after second (Post 2nd) or/and (iii) had available sera 45 days or less before the third (Pre 3rd) or/and (iv) had available sera 14-45 days after third (Post 3rd) dose.
Recruitment	All participants were HCW invited to participate in the study and provide peripheral blood samples for serological assays . A computer-based questionnaire about demographic characteristics and coexisting conditions was sent electronically to all serology-study participants. All analysis was deidentified.
Ethics oversight	Sheba Medical Center, IRB committee

Note that full information on the approval of the study protocol must also be provided in the manuscript.