Determinants of early antibody responses to COVID-19 mRNA vaccines in a cohort of exposed and naïve healthcare workers

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Summary

Background Two doses of mRNA vaccination have shown >94% efficacy at preventing COVID-19 mostly in naÿve adults, but it is not clear if the second dose is needed to maximize effectiveness in those previously exposed to SARS-CoV-2 and what other factors affect responsiveness.

Methods We measured IgA, IgG and IgM levels against SARS-CoV-2 spike (S) and nucleocapsid (N) antigens from the wild-type and S from the Alpha, Beta and Gamma variants of concern, after BNT162b2 (Pfizer/BioNTech) or mRNA-1273 (Moderna) vaccination in a cohort of health care workers (N=578). Neutralizing capacity and antibody avidity were evaluated. Data were analyzed in relation to COVID-19 history, comorbidities, vaccine doses, brand and adverse events.

Findings Vaccination induced robust IgA and IgG levels against all S antigens. Neutralization capacity and S IgA and IgG levels were higher in mRNA-1273 vaccinees, previously SARS-CoV-2 exposed, particularly if symptomatic, and in those experiencing systemic adverse effects ($p<0{\cdot}05$). A second dose in pre-exposed did not increase antibody levels. Smoking and comorbidities were associated with 43% (95% CI, 19-59) and 45% (95% CI, 63-18) lower neutralization, respectively, and 35% (95% CI, 3-57%) and 55% (95% CI, 33-70%) lower antibody levels, respectively. Among fully vaccinated, 6·3% breakthroughs were detected up to 189 days post-vaccination. Among pre-exposed non-vaccinated, 90% were IgG seropositive more than 300 days post-infection.

Interpretation Our data support administering a single-dose in pre-exposed healthy individuals as primary vaccination. However, heterogeneity of responses suggests that personalized recommendations may be necessary depending on COVID-19 history and life-style. Higher mRNA-1273 immunogenicity would be beneficial for those expected to respond worse to vaccination and in face of variants that escape immunity such as Omicron. Persistence of antibody levels in pre-exposed unvaccinated indicates maintenance of immunity up to one year.

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Keywords: mRNA vaccines; SARS-CoV-2; COVID-19; Antibody; Neutralization; Avidity; Health care workers

Research in Context

Evidence before this study

The Pfizer/BioNTech BNT162b2 and the Moderna mRNA-1273 mRNA COVID-19 vaccines have shown high efficacy and immunogenicity in phase 3 clinical trials. However, clinical trials were based on individuals without history of COVID-19 and, at present, a considerable proportion of the population has already been infected by SARS-CoV-2, which may alter vaccine immunogenicity. Other determinants of early immune responses to these vaccines are also poorly defined and may be relevant to elicit a strong and persistent immunity, particularly in face of emerging variants of concern. Since the initiation of vaccine rollout, we have been searching Pubmed and medRxiv for any manuscript reporting antibody responses elicited by the mRNA COVID-19 vaccines in those naturally infected by SARS-CoV-2. To date, some studies have shown that mRNA COVID-19 vaccines elicit higher antibody responses in individuals previously exposed to SARS-CoV-2 than naïve individuals, and that a single dose boosts the immune response to high levels in pre-exposed individuals. However, previous history of COVID-19 (e.g. symptomatic infection, antibody responses, time since onset of infection) was not considered. Age had a negative impact on antibody levels and, in some studies, sex also had an effect. More recently, a couple of articles have shown that the Moderna vaccine may induce higher antibody levels than the Pfizer/BioNTech vaccine. One manuscript has also shown that adverse effects to the vaccines were positively associated with antibody levels. In general, vaccine induced antibodies recognize the variants of concern, although levels and neutralizing capacity are diminished in front of the Beta, Gamma and, particularly, the Delta and Omicron variants of concern. On the other hand, immunity induced by natural infection seems to be maintained over several months, particularly for IgG levels against the spike protein, the target antigen of the COVID-19 mRNA vaccines.

Added value of this study

We have measured early antibody responses following vaccination with Moderna or Pfizer/BioNTech vaccines in a random cohort of 578 health care workers (HCW) that have been previously followed for a year, and from whom we have detailed demographics, life-style and clinical data, including accurate history of SARS-CoV-2 infections and characterization of antibody responses to SARS-CoV-2 and coronaviruses causing the common cold. The design of our study differs from most of the reports published, many of which have small sample sizes, lack of information on COVID-19 history and immune responses and factors that could affect vaccine responses. Additionally, immune responses in other studies focus on very few antibody measurements, whereas we have quantified IgA, IgG and IgM levels to two constructs of the nucleocapsid protein and three different constructs of the spike protein, including three variants (Alpha, Beta and Gamma). As qualitative and functional measurements, we also assessed the strength of the binding of IgA and IgG to the antigens (i.e. avidity) and the neutralizing capacity of the plasma.

We found that vaccination induced high IgA and IgG levels to the vaccine immunogen, even against the variants tested. Neutralization capacity and IgA and IgG levels against spike antigens were higher in HCW who had received the Moderna vaccine, had previous SARS-CoV-2 exposure, particularly if infection was symptomatic, and in those experiencing systemic adverse effects after vaccination, independently from exposure. Importantly, a second dose in pre-exposed participants did not increase antibody levels. However, smoking and comorbidities were associated with lower neutralization and antibody levels. Among 159 fully vaccinated participants between 49 and 189 days post second dose, we

report 10 (6·3%) vaccine breakthroughs, mostly associated with the fifth wave of the pandemic in Spain and the Delta variant.

Finally, having followed up this HCW cohort for a year since the onset of the pandemic, we present the antibody kinetics in those individuals who have not been vaccinated (n=53), and show that 90% of the HCW maintained positive IgG against spike antigens for up to a year after infection.

Implications of all the available evidence

Both mRNA vaccines elicited robust antibody responses, but these were heterogeneous and correlated positively with the adverse effects after vaccination, independent of previous exposure to SARS-CoV-2. Higher immunogenicity of the Moderna vaccine suggests that this brand could be used in those individuals who may develop lower immune responses. Despite the persistence of antibody levels in unvaccinated pre-exposed HCW, vaccination is recommended but a single dose of mRNA vaccines appears to be sufficient for individuals who have recovered from COVID-19, which is relevant considering the shortage of vaccine doses worldwide. However, two doses may still be necessary for those who had asymptomatic infections, are smokers or have comorbidities, especially to mitigate breakthroughs by more contagious variants like Delta or Omicron.

Introduction

The unprecedented fast development of highly efficacious COVID-19 vaccines has changed the fate of the SARS-CoV-2 pandemic.[1] The COVID-19 vaccines from Pfizer/BioNTech (BNT162b2) and Moderna (mRNA-1273) manufacturers based on mRNA encoding the SARS-CoV-2 full-length spike (S) protein have shown vaccine efficacies of 95% and 94%, respectively, against COVID-19 disease after two doses in phase 3 trials.[2,3] Both vaccines induce good immunogenicity[4–11] and excellent effectiveness in real world population after two doses[12–15] but lower effectiveness against variants of concern (VoC) after one dose,[12,14,15] against the Delta (B.1.617.2) variant following two doses[16] and could be even lower against the emerging Omicron (B.1.1.529) variant.[17]

Unfortunately, vaccine production is limited, which has resulted in changes in immunization policies in many high- and medium-income countries, such as delays in the 2nd dose, prioritization of naÿve individuals over previously SARS-CoV-2 diagnosed individuals, or a single-dose for the latter ones. Nevertheless, evidence shows that previously infected individuals benefit from vaccination[4–11] and, therefore, the recommendation is to vaccinate the total population regardless of COVID-19 history.[18,19] However, an increasing number of studies suggest that only one dose would be sufficient to mount an optimal antibody response in previously infected individuals, as a booster response is elicited.[4–11] This has led to the recommendation in some countries to provide only one dose to those previously diagnosed,[20] and to the suggestion that two doses do not contribute to an additional improvement[7,8,11] or could even have a detrimental effect on the acquired immune response.[7,21] This would also allow an increase to the global supply of doses available to low-income countries that suffer from vaccine shortages.[22] However, further evidence is needed to guide informed decisions as most of the studies include a small sample size and it is not clear whether it applies to all individuals.[20,23] In Spain, a single-dose vaccination after at least 6 months post-infection is recommended for previously COVID-19 diagnosed individuals less than 65 years old[24] and has recently changed to 2 months in Catalonia.[25] In countries such as France or Germany, a single dose is also recommended for previously diagnosed healthy individuals.[20] However, other countries are still administering two doses to everyone and have started 6 months after primary vaccination to administer a 3rd booster dose in light of declining antibody responses and the spread of highly contagious VoCs such as Delta and Omicron, with potential immune escape and diminished vaccine effectiveness.[16,17,26]

The emergence of several fast-spreading variants since the end of 2020 may affect vaccination campaigns. Concern has been raised about the potential of some of the variants, which harbour mutations in S, to escape from neutralizing antibody immunity. Some studies have shown that antibodies from convalescent and vaccinated individuals are effective against the Alpha (B1.1.7) variant first identified in UK.[27–30] In contrast, Beta (B.1.351) and Gamma (P.1), first identified in South Africa and Brazil, respectively, have decreased sensitivity to neutralizing antibodies elicited by vaccination,[28,29,31–34] but previous exposure induces higher cross-reactivity to variants.[4,9] The Delta variant, which also presents mutations in S, was identified in India and quickly spread over the world, and data show that it may have an even lower sensitivity to convalescent and vaccine induced antibodies.[35,36] More recently, preliminary reports on the Omicron variant, which has increased number of mutations in S, shows that antibody immune escape is probably higher.[37]

Since March 2020, we have followed up a cohort of 578 health care workers (HCW) at Hospital Clínic de Barcelona (HCB), Spain.[38] After 6 months of follow-up (October 2020) the cumulative prevalence of SARS-CoV-2 infection based on real-time reverse-transcriptase polymerase chain reaction (rRT-PCR) or serology data was 19·6%, but most of the infections occurred during the first wave of the pandemic.[39] Most of the infected individuals maintained IgG levels against S antigens and their neutralizing capacity up to 7 months.[39] In the present study, we evaluated the IgA, IgG and IgM levels and their neutralizing capacity early after vaccination

with one or two doses of the BNT162b2 and mRNA-1273 vaccines, investigated the impact of previous SARS-CoV-2 infection history and antibody responses, and other variables like vaccine reactogenicity, comorbidities, or smoking habit, and report the breakthrough infections among fully vaccinated participants. In addition, we present the antibody kinetics to S and N antigens (Wuhan strain) for up to 1-year post-infection for those individuals who have not been vaccinated.

Methods

Study design, population and setting

Five hundred seventy-eight selected HCW from HCB were included in the study at baseline (month 0, M0). To assess the seroprevalence against SARS-CoV-2 at M0 and month 1 (M1), with a precision of 5% and a 95% CI, a loss to follow up between M0 and M1 of 5% and assuming that the prevalence at M0 was 30% and at M1 was 50%, with a finite population, 570 HCW were estimated as the sample size needed. Given the uncertainty about what the seroprevalence would be at M1, 50% was used, which provided the most conservative sample size.

The study population was defined as those who deliver care and services to patients, either directly as physicians or nurses, or indirectly as assistants, technicians, stretcher-bearers, or other support staff. Inclusion criteria included being an adult (>17 years) worker at HCB registered at the Human Resources department. A random sample of 1000 HCW from the Human Resources department database was extracted to identify the participants. Selected HCW were contacted telephonically following the list order. After explaining the study and assess the inclusion and exclusion criteria, the participants were invited to participate. After that, interested participants signed the informed consent. In case of no participation, the reasons were recorded. Eligible participants on quarantine were visited at their homes by study personnel. After informed consent was obtained, relevant demographic, clinical and epidemiological information were collected in the standardized case report form (CRF) and samples were also collected (oral swabs and blood depending on the study visit).

Exclusion criteria included: (a) absenteeism from workplace in the last 30 days (i.e., on vacation, sick leave, sabbatical), (b) working exclusively outside the HCB or Maternity main buildings with no interaction with patients on a daily basis, (c) retirement or end-of-contract planned within one year after the recruitment date, and (d) participating in COVID-19 clinical trials for preventive or treatment therapies.

Participants were recruited at the peak of the first wave of the pandemic in Spain (M0)[38] and performed 2 additional visits at M1 and month 6 (M6).[39] Participants with any previous evidence of SARS-CoV-2 infection were invited to participate at study months 3 (M3)[40] and 9 (M9) follow-up visits. All participants were invited again for a month 12 (M12) visit. A flow chart depicting selection of the study participants, subjects included at each time-point, and samples used in each subset analysis, is shown in Figure 1. At the M9 visit, 64 participants had already received one dose of the BNT162b2 (Comirnaty, Pfizer/BioNTech) or mRNA-1273 (Spikevax, Moderna) mRNA vaccines, with various times post vaccination. By M12, most of the participants had already received two doses of either vaccine and were invited to come for a cross-sectional visit 2 weeks (window 12-19 days) after the second dose was administered (N=342, BNT162b2 N=271, mRNA-1273 N =71).

Finger prick blood was collected from the subset who visited at M9, and 10 mL of venous blood was collected from all participants at M12. Plasma was isolated and cryopreserved at -80°C. Data on the vaccination dates, COVID-19 infection and symptoms-confirmed by the Occupational Health department at Hospital Clínic- were retrospective collected. Self-reported related adverse events (AEs) were recorded at the time of recruitment. Information on new SARS-CoV-2 infection episodes until 6 months after vaccination (M18) were also collected through the Occupational Health department at the HCB. Demographic data and information on comorbidities (heart and liver disease, diabetes, chronic respiratory and renal disease, cancers and autoimmune and other immunological disorders), chronic medication and smoking habits had been previously collected. Data for each participant were collected and managed using REDCap version 8.8.2 hosted at ISGlobal through a standardized electronic questionnaire as previously described.[38] REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies,[41,42] providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

Pre-exposure to SARS-CoV-2 was defined as having had any positive rRT-PCR or serology result any time before vaccination. rRT-PCR tests were performed at M0 and M1 visits and subsequently in several screenings at the Hospital Clínic and whenever the participant had symptoms or had been in contact with a SARS-CoV-2 infected person. The rRT-PCR performed at study visits was based on the nucleocapsid (N) gene regions 1 (N1) and N2.[38]

Quantification of antibodies to SARS-CoV-2

We measured IgA, IgG and IgM antibody levels (median fluorescence intensity, MFI) to different SARS-CoV-2 antigens using previously developed assays based

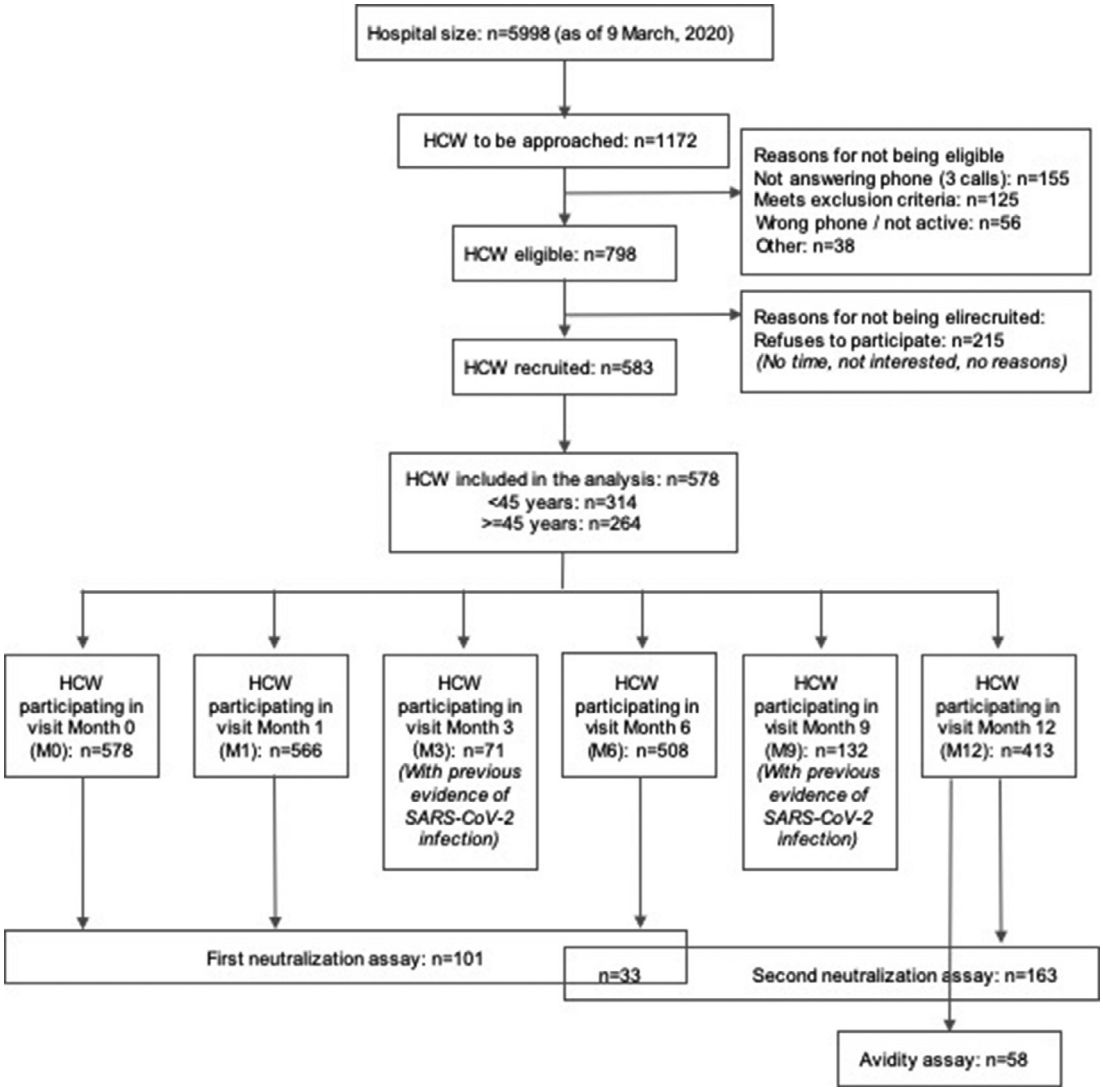

Figure 1. Study flowchart. Participants selection, recruitment, sample sizes in all study visits, and subjects used for avidity and neutralization assays. HCW, health care workers.

on the quantitative suspension array technology Luminex (Supplementary Information).[39,43] The panel of antigens included the S full length protein (aa 1-1213 expressed in Expi293 and His tag-purified) produced at Center for Genomic Regulation (CRG, Barcelona), and its subregion S2 (purchased from SinoBiological, cat. No. 40590-V08B), the receptor-binding domain (RBD) kindly donated by the Krammer lab (Mount Sinai, New York),[44] the N full length protein and the specific C-terminal region (both expressed in-house in *E. coli* and His tag-purified),[45] all from the Wuhan strain, and the full length S proteins of 3 VoCs (purchased from ACROBiosystems): Alpha (B.1.1.7; cat. No. SPN-C52H6), Beta (B.1.351; cat. No. SPN-C52Hk) and Gamma (P.1; cat. No. PN-C52Hg). Plasma samples were tested at 1:500 dilution for the 3 isotypes, and additionally at 1:5000 for IgG to avoid saturated levels in the vaccinated participants. Optimal testing dilutions were previously assessed and samples were within the quantitative range of the assay. The investigators conducted the assays blinded.

Neutralizing antibodies

For feasibility reasons, we selected 163 samples from the study visit M12 with a balanced representation of BNT162b2 and mRNA-1273 vaccinees and non-vaccinated participants (previously exposed and naive individuals) (Table S1). We already had pre-vaccination neutralization data from 33 of the selected 163 individuals.[39] Plasma neutralizing capacity was assessed as the percentage of inhibition of RBD binding to ACE2

receptor and was measured through a flow cytometric-based assay that correlates with a validated pseudovirus neutralization assay.[39] Briefly, a murine stable cell line expressing the ACE2 receptor was incubated with RBD-mFc fusion protein, composed of RBD fused to the Fc region of murine IgG1, previously exposed to the different plasma samples at a 1:400 dilution. Cells were stained with anti-mouse IgG-PE, washed, and analyzed by flow cytometry using standard procedures. Study samples were tested alongside 30 negative pre-pandemic controls, in duplicates.

Antibody avidity

For feasibility, a subset of 58 M12 samples from BNT162b2 and mRNA-1273 vaccinated participants (48 naive and 10 exposed), were randomly selected from the total cohort for the avidity assay (Table S1). Antibody avidity was determined as the percentage of IgA and IgG levels against RBD, S and S2 antigens measured with a chaotropic agent over the IgA and IgG levels measured in the same samples without chaotropic agent. Antibody levels with and without chaotropic agents were measured in plasma samples (dilution 1:5000) using the Luminex assay described above. The only difference being an incubation of the antigen-coupled beads with the chaotropic agent (urea 4M, 30 min at room temperature) after their previous incubation with the samples and subsequent washes.

Statistical analysis

MFIs were $\log_{10}$-transformed. In vaccinated participants MFIs for S-related antigen IgGs correspond to the 1:5000 dilution, except in plots where we compare pre and post vaccination levels, in which the 1:500 dilution was used. Any other MFIs correspond to the dilution 1:500, and for seropositivity calculations, only 1:500 dilution was used.

Assay positivity cutoffs specific for each isotype and analyte were calculated as 10 to the mean plus 3 standard deviations (SD) of $\log_{10}$-transformed MFI of 128 pre-pandemic controls. Positive serology was defined by being positive for IgG, IgA and/or IgM to any of the antigens tested.[39] Results were defined as undetermined when the MFI levels for a given isotype-analyte were between the positivity threshold and an upper limit defined as 10 to the mean plus 4·5 SD of the $\log_{10}$-transformed MFIs of 128 pre-pandemic samples, and no other isotype-antigen combination was above the positivity cutoff, and the participant did not have any previous evidence of seropositivity or rRT-PCR positivity.

Analysis of antibody levels after the second dose included only data from samples collected 12-19 days after vaccination, while for the first dose we included data from all samples collected 7 or more days after vaccination, as no previous visit window was established.

Groups were compared using the Wilcoxon Sum Rank test for continuous non-parametric variables and with the Wilcoxon Signed-Sum Rank Test for paired continuous data. Correlations between continuous variables were analyzed using linear regression models and Spearman's rank test. Locally estimated scatterplot smoothing (LOESS) plots were used to visualize trends in antibody levels over days post vaccination, days post-symptom onset (PSO) or post rRT-PCR diagnosis.

Univariable and multivariable linear regression models were fitted to assess factors associated with antibody responses to SARS-CoV-2 RBD and S antigens and their neutralization capacity (%) after vaccination among exposed and naive individuals, and overall. Models having both exposed and naive participants included the following independent variables: sex, age, days since first dose administration, days since second dose administration, smoking habits, chronic medication, presence of baseline illness (heart and liver disease, diabetes, chronic respiratory and renal disease, cancers and autoimmune and other immunological disorders), antibody levels ($\log_{10}$ MFI) to endemic common cold human coronaviruses (HCoV: 229E, NL63, OC43, and HKU1) at M6,[39] vaccine type, and presence of systemic or local AEs (systemic AEs included fever, arthralgia, fatigue, chills, muscle pain and headache, while local AEs included pain, erythema and/or swelling at the injection site or swollen glands near the injection site) after 1st or 2nd vaccine dose. In addition, the predictor variable "presence of any COVID-19 symptom (fatigue, cough, dyspnea and other respiratory symptoms, anosmia or ageusia, sore throat, fever, rhinorrhea, headache, chills and digestive symptoms)" was included in models having only exposed participants. Predictor variables that had a P-value of 0·2 or lower in the univariable models were selected for stepwise multivariable models performed with the function stepAIC (R package MASS). The b obtained in each model for each of the predictor variables were transformed into a percentage of antibody increase for easier interpretation. For continuous $\log_{10}$-transformed variables (log-log model) the b transformed value (%) was calculated with the formula ((10^(b*$\log_{10}$(1.1)))-1)*100. This represents the effect (in percentage) on IgG levels of a 10% increase in the corresponding predictor variable. For categorical predictor variables (log-linear models), the b transformed value (%) was calculated with the formula ((10^b)-1)*100. This gives the difference (in percentage) in IgG levels between the reference and the study group. A P-value of $< 0{\cdot}05$ was considered statistically significant and 95% confidence intervals (CI) were calculated for all estimates. We did not control for multiple testing. Missing data were not imputed and models were performed with the available data (samples sizes for each analysis are shown in Tables and Figure legends). We performed the statistical analysis in R version 4·0.3 (packages tidyverse, ggpubr and MASS).[46–48]

Ethics

Written informed consent was obtained from all study participants prior to study initiation. The study was approved by the Ethics Committee at HCB (references HCB/2020/0336 and HCB/2021/0196).

Results

Characteristics of study participants

From the 578 participants recruited at baseline, 446 came to visits at M9 and/or M12, with 414 participants sampled at M12 (Figure 1). We measured the levels of IgA, IgG and IgM to SARS-CoV-2 antigens in blood samples from both visits. Of the 414 HCW visited at M12, 360 (81%) had received one (N= 18, 1 BNT162b2 and 17 mRNA-1273) or two doses (N= 342) of the mRNA vaccines. Seventy-six percent of the 360 HCW received BNT162b2 and 24% mRNA-1273 (Table 1). Most of the study participants were females (73%) and had a mean age of 42·7 (SD 11·65) years. Around 20% had underlying comorbidities and 22% were under chronic medication (Table 1). Thirty-two per cent of all participants and 22% of those vaccinated had previously been infected by SARS-CoV-2 according to rRT-PCR or serology data (Table 1). Seventy-three per cent of the participants had AEs to vaccination, systemic in 28% after one dose and 68% after two doses (Table 1). Among the 159 participants fully vaccinated with two doses, 10 (6·3%; 95% CI, 3·5-11·1) vaccine breakthroughs were detected by rRT-PCR after 15 days post-second dose with a median of 144·5 days (49-189 days) post-vaccination. There were no differences in antibody levels after 2 vaccine doses between those who had breakthrough infections and those who did not (Fig S1), with the exception of lower levels of IgA against N and S2 ($p<0.05$, Wilcoxon Sum Rank test). Among the 53 individuals non-vaccinated at M12, 4 (7·5%; 95% CI, 3·0-17·9) had a SARS-CoV-2 infection in the same period.

Vaccination elicits high but variable antibody levels against SARS-CoV-2 and VoC

After 7 to 72 days post one dose of the BNT162b2 or the mRNA-1273 vaccines, 92·2% (95% CI, 83·0-96·6) (59/64) participants were seropositive, and seropositivity increased to 95·9% (95% CI, 86·3-98·9) (47/49) when excluding samples from less than 10 days post-vaccination. IgA and IgG levels against all S antigens tested (RBD, S full length and S2) increased in most of the participants after one dose, albeit at very heterogeneous levels (Figure 2a and Table S2). After 2 doses of the BNT162b2 or the mRNA-1273 vaccines (12-19 days post-vaccination), all participants were seropositive with the exception of a participant receiving the BNT162b2 who had renal insufficiency and was under corticoids and immunomodulatory cytokine treatments (Figure 2b). IgA, IgG and IgM levels (Figure 2b and Table S3) and neutralization capacity (Figure 3) increased in all individuals after the two doses compared to pre-vaccination but at varying levels.

IgG antibodies produced after two doses in all seropositive individuals recognized the S full length from the Alpha, the Beta and the Gamma VoCs (Fig. S2). However, the odds of being IgM seronegative were 4·7 (95% CI, 3·2-7·0) times higher for the Gamma variant, 3·8 (95% CI, 2·6-5·5) times higher for the Beta variant and 2·5 (95% CI, 1·7-3·6) times higher for the Alpha variant than for the wild-type.

Association of previous SARS-CoV-2 exposure with antibodies post-vaccination

Previously SARS-CoV-2 infected individuals produced higher IgA, IgG and IgM levels against the S antigens RBD, S full length, and S2 after 1 (5·53 median fold-change increase for IgG, all antigens pooled) and 2 doses (1·36 median fold-change increase for IgG, all antigens pooled) of the vaccine than naive participants (Figure 4). Kinetics after vaccination (Fig. S3) also show that vaccinated people who were previously exposed mounted higher antibody levels than naive individuals. Differences were larger after a 1st dose than after 2 doses (Figure 4, Fig. S3 and Table S4). Indeed, in previously infected individuals, antibody levels after the 2nd dose were similar to levels after the 1st dose with the exception of IgG against S2 that were lower after the 2nd dose while for unexposed individuals, antibody levels clearly were higher after the second dose (Figure 4 and Table S4). Differences in antibody levels between pre-exposed and unexposed individuals were similar for the S proteins of the Alpha, Beta and Gamma VoCs (Fig. S3).

Antibody neutralization capacity after 2 vaccine doses was higher in pre-exposed (median of 75·81%, 50·4 IQR) than naive individuals (median of 49·21%, 23·8 IQR) (Figure 5a). Similarly, the avidity of IgA and IgG in pre-exposed individuals after the 2nd dose was higher compared to unexposed individuals (Figure 5b, Table S5). The plasma neutralization capacity positively and strongly correlated with IgG levels (for IgG RBD and S: rho=0·81-0·76 in naive and 0·83-0·84 in pre-exposed, $p<0.001$, Spearman's rank test; Fig. S4) and moderately with IgA levels, particularly for RBD and in previously exposed participants (for IgA RBD: rho=0·45 $p=0.002$ in naÿve and rho=0·61 in pre-exposed $p<0.001$, Spearman's rank test; Fig. S4).

Prior SARS-CoV-2 infection and antibody levels affect vaccine responses

When comparing responses between HCW who had the infection more than 11 months vs less than 11 months before vaccination, the first ones induced higher levels of IgA and IgG ($p<0.05$, Wilcoxon Sum Rank test; Fig. S5). However, when using different cutoff values for the time passed between infection and vaccination, there

Variable	N at M9 and/or M12	% or mean (SD)/ median (IQR)	N Vaccinated at M12	% or mean (SD)
Sex	446[a]		360	
Male	119	26·70%	94	26·10%
Female	327	73·30%	266	73·90%
Age (years), mean (SD)	446	42·7 (11·65)	360	43·2 (11·77)
Job function	446		360	
Nurses and auxiliary health professionals	229	51·30%	177	49·20%
Physicians and psychologists	105	23·50%	89	24·70%
Laboratory and other technicians	34	7·60%	26	7·20%
Other[b]	78	17·50%	68	18·90%
Comorbidities[c]	446		360	
No	356	79·80%	289	80·30%
Yes	90	20·20%	71	19·70%
Chronic medication	446		360	
No	350	78·5%	279	77·5%
Yes	96	21·5%	81	22·5%
Smoker	445		360	
No	346	77·80%	280	77·80%
Yes	99	22·20%	80	22·20%
Number people in the household, median (IQR)	446	3 (2)	360	2·76 (1·184)
Involved in clinical care	446		360	
No	97	21·70%	88	24·40%
Yes	349	78·30%	272	75·60%
Number children co-living, median (IQR)	445	0 (1)	360	0·45 (0·8)
Vaccine type			360	
BNT162b2 (Pfizer/BioNTech)			272 (n= 1 one dose)	75·60%
mRNA-1273 (Moderna)			88 (n= 17 one dose)	24·40%
Number doses received			360	
1			18	5%
2			342	95%
Previously exposed	446		360	
No	303	67·90%	282	78·30%
Yes	143 (92 symptomatic)	32·10% (64·33% symptomatic)	78 (43 symptomatic)	21·70% (55·13% symptomatic)
Adverse events after 1st dose			358	
Local/No Adverse Events			257	71·79%
Systemic[d]			101	28·21%
Adverse events after 2nd dose			346	
Local/No Adverse Events			110	31·79%
Systemic[d]			236	68·21%

***Table 1*: Characteristics of study participants.**

[a] N at only M12 was 414

[b] Other include administration, accounting, information technology, cleaning, kitchen and maintenance staff.

[c] Comorbidities include: heart and liver disease, diabetes, chronic respiratory and renal disease, cancers and autoimmune and other immunological disorders.

[d] Systemic adverse events include fever, arthralgia, fatigue, chills, muscle pain and headache, while local adverse events include pain, erythema and/or swelling at the injection site or swollen glands near the injection site.M: Study month; SD: Standard deviation.

were no differences in antibody levels induced by the vaccines.

Previously exposed participants who had symptoms during infection produced higher IgA and IgG levels against RBD and S2 after 2 vaccine doses than asymptomatic participants ($p<0·05$, Wilcoxon Sum Rank test; Fig. S6a). Symptomatic individuals also had higher IgA and IgG levels against S full length VoCs ($p<0·05$, Wilcoxon Sum Rank test; Fig. S6b) and had higher plasma neutralization capacity ($p<0·05$, Wilcoxon Sum Rank test; Fig. S6c). In contrast, an inverse tendency was observed for IgM (Fig. S6a-b).

Pre-vaccination IgG and IgA levels in exposed participants positively and moderately to strongly

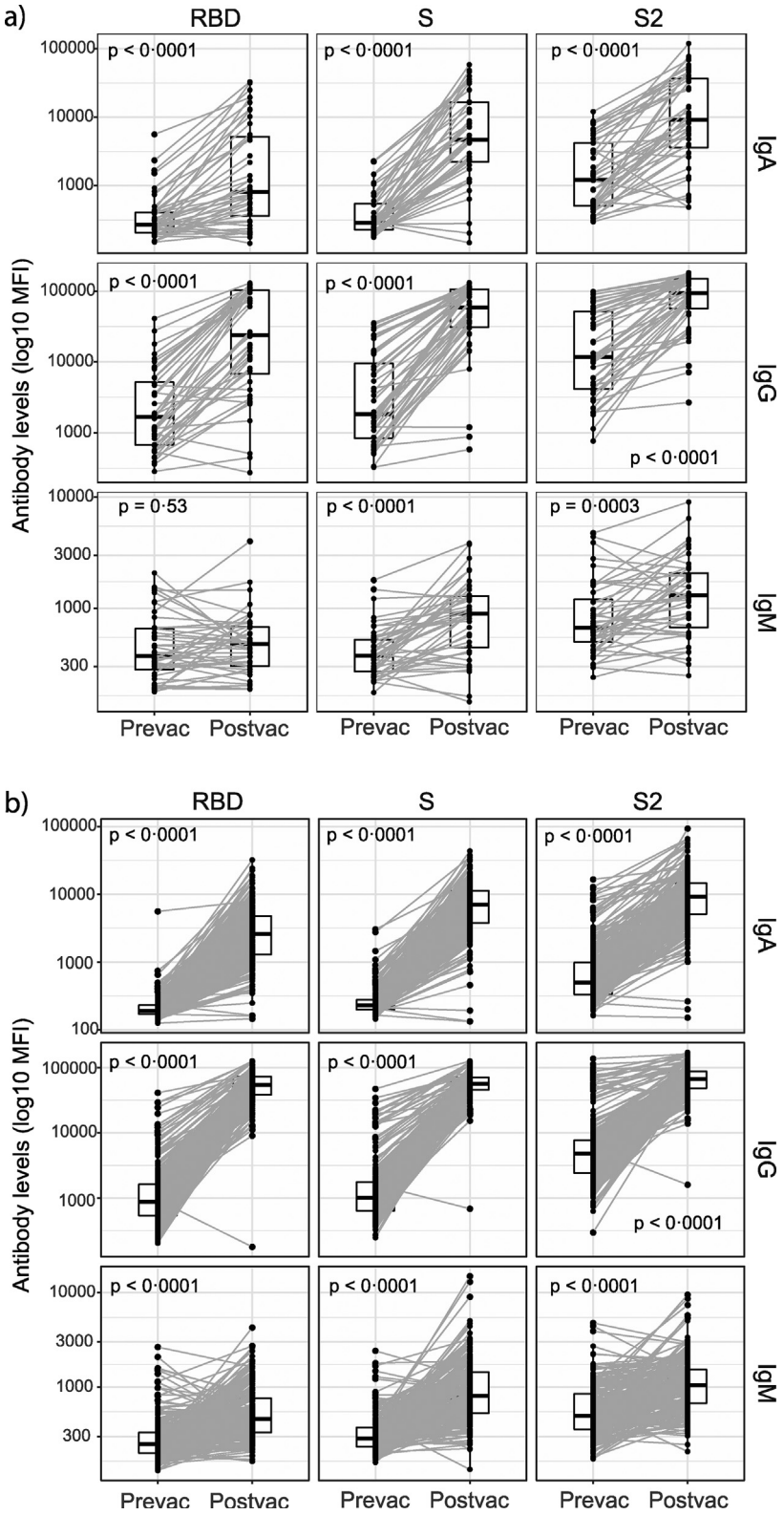

Figure 2. Pre- and post-vaccination antibody levels after 1 dose and 2 doses of the COVID-19 mRNA vaccines. Plots show IgA, IgG and IgM levels (median fluorescence intensity, MFI) against the receptor-binding domain (RBD) of the SARS-CoV-2 Spike glycoprotein (S), the S protein and its subunit S2 at pre- and post-vaccination after 1 dose (N=44) (a) and 2 doses (N=253) (b). All plasma samples were analyzed at 1:500 dilution. Pre-vaccination samples were collected at study month 6 for those who were already

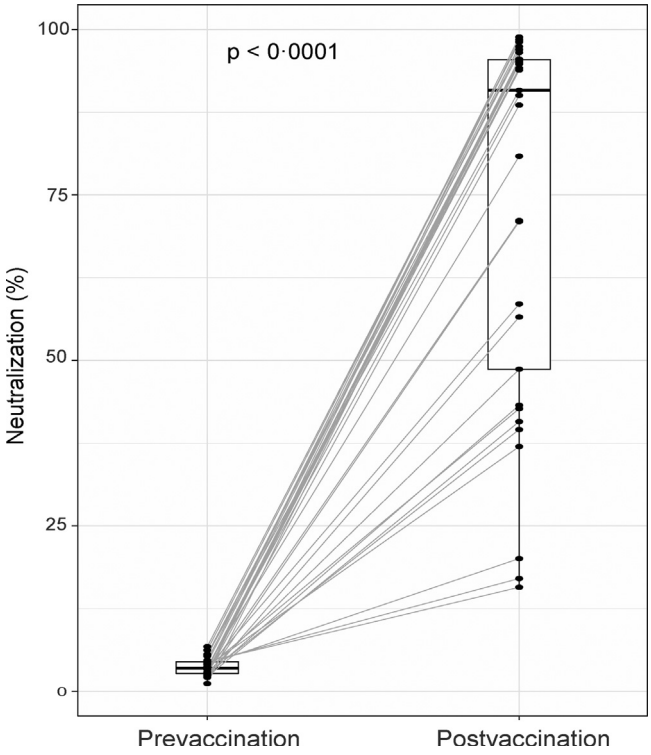

Figure 3. Neutralizing capacity of plasma samples before and after COVID-19 mRNA vaccination. Dots depict antibody neutralizing capacity, as a percentage of RBD-ACE2 binding inhibition by plasma samples from 33 participants. Paired samples are joined by grey lines. The center line of boxes depicts the median of the neutralization percentage; the lower and upper hinges correspond to the first and third quartiles; the distance between the first and third quartiles corresponds to the interquartile range (IQR); whiskers extend from the hinge to the highest or lowest value within 1·5 × IQR of the respective hinge. Wilcoxon signed-rank test was used to assess statistically significant differences between pre- and post-vaccination neutralization. Pre-vaccination levels correspond to visit M6. Pre-vaccination samples were analyzed at a 1:50 dilution while post vaccination samples were analyzed at 1:400. We standardized the post-vaccination results to make them comparable by dividing them by 8.

correlated with antibody levels post-1st and 2nd dose (Fig S7a-b). Pre-vaccination IgM levels also correlated with post-vaccination levels but to a lesser extent. Antibody levels elicited after one dose positively and moderately to strongly correlated with the antibody levels elicited after the 2nd dose among previously exposed (Fig. S7c).

mRNA-1273 vaccine elicits higher antibody responses than BNT162b2

Two doses of the mRNA-1273 vaccine elicited higher IgA ($p<0·01$, Wilcoxon Sum Rank test) and IgG ($p<0·0001$, Wilcoxon Sum Rank test) levels against RBD, S full length and the S2 subunit (Figure 6 and Table S6), and of higher neutralizing capacity [67% (43·78, IQR) vs 44·6% (38·43, IQR), Figure 6] and avidity ($p<0·05$, Wilcoxon Sum Rank test; Figure 6 and Table S7), than two doses of the BNT162b2 vaccine. Similarly, IgA and IgG levels against the S full length protein of the tested VoCs were higher after mRNA-1273 than BNT162b2 vaccination ($p<0·0001$, Wilcoxon Sum Rank test; Figure 6 and Table S6).

AEs after vaccination are associated with induction of higher antibody levels

Having had systemic AEs after 1st dose was associated with higher levels of IgA and IgG against RBD and IgG against the S protein from the wild-type and the VoCs

vaccinated at month 9, and at month 9 for those vaccinated at month 12. Post-vaccination samples analyzed are those collected >10 days after the 1st dose (a) and 2 weeks after the 2nd dose (b). Paired samples are joined by grey lines. The center line of boxes depicts the median of MFIs; the lower and upper hinges correspond to the first and third quartiles; the distance between the first and third quartiles corresponds to the interquartile range (IQR); whiskers extend from the hinge to the highest or lowest value within 1·5 × IQR of the respective hinge. Wilcoxon signed-rank test was used to assess statistically significant differences in antibody levels between pre- and post-vaccination.

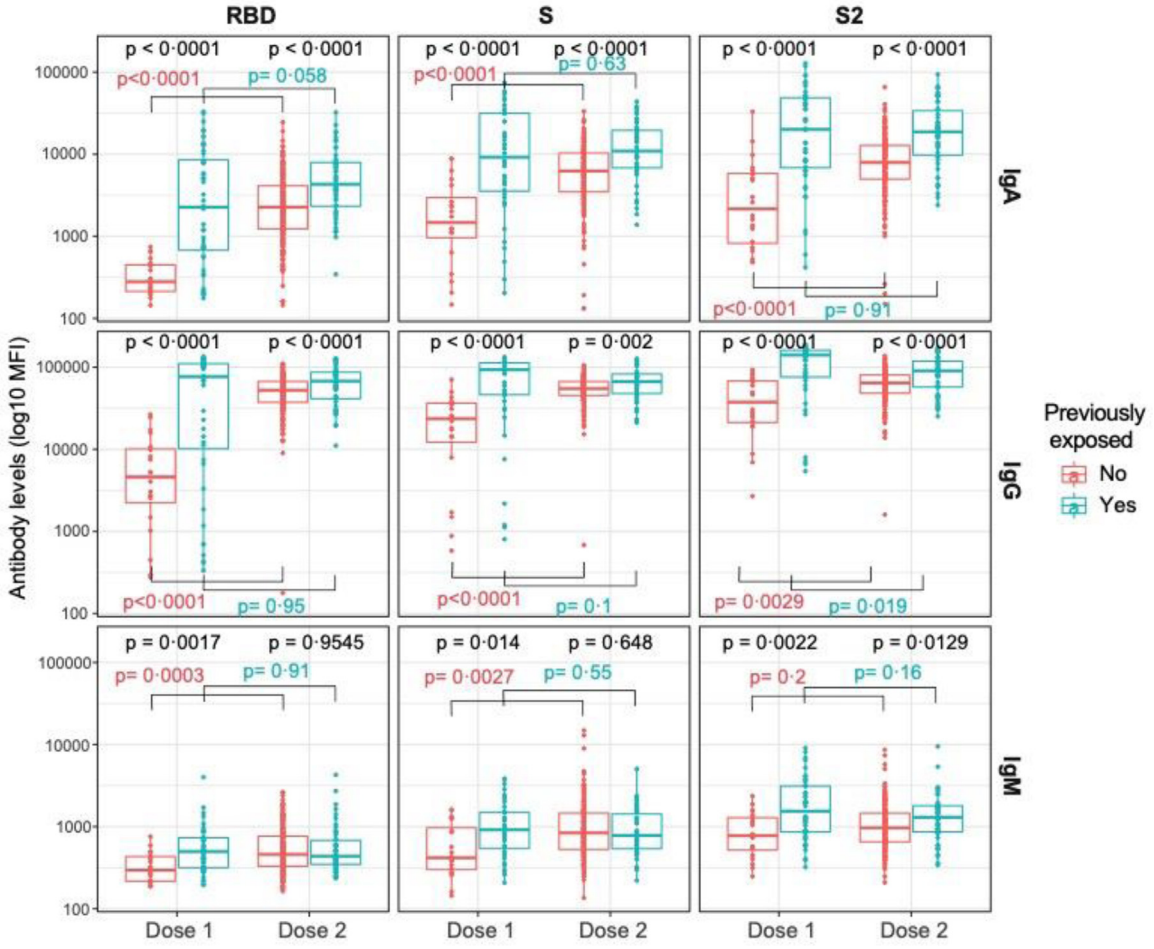

Figure 4. Antibody levels against S antigens after one and two doses of mRNA vaccines in previously SARS-CoV-2 infected and uninfected individuals. Plots show IgA, IgG and IgM levels (log_{10} MFI) against the receptor-binding domain (RBD) of the SARS-CoV-2 Spike glycoprotein (S), the S protein and its subunit S2 after 1 dose (N=64, 20 naive and 44 pre-exposed) and 2 doses (N=263, 211 naive and 52 pre-exposed). Post-vaccination samples analyzed were those collected >7 days after the 1st dose and 2 weeks after the 2nd dose. The center line of boxes depicts the median of MFIs; the lower and upper hinges correspond to the first and third quartiles; the distance between the first and third quartiles corresponds to the interquartile range (IQR); whiskers extend from the hinge to the highest or lowest value within 1·5 × IQR of the respective hinge. Wilcoxon rank test was used to assess statistically significant differences in antibody levels between naive and pre-exposed participants for a same dosage, and between 1st and 2nd dose into each group. We selected all dilutions at 1:500 to make levels comparable.

compared to having no or only local AEs (p<0·05, Wilcoxon Sum Rank test; Fig. S8a). Similarly, having had systemic AEs after the 2nd dose was associated with higher IgA, IgG and IgM levels to almost all S antigens than not having or only local AEs (p<0·05, Wilcoxon Sum Rank test; Fig. S8b). Systemic AEs were also positively associated with higher neutralization capacity and avidity after the 2nd dose (p<0·05, Wilcoxon Sum Rank test; Fig. S8c-d).

Factors independently associated with vaccine antibody responses after one dose

In univariable models, previously exposed HCW had 839% (260-2347, 95%CI; P-value<0·001) higher IgG S levels than naive HCW after a single-dose of the vaccines. BNT162b2 vaccination was associated with 78% lower IgG S levels (38-93, 95%CI; P-value=0·005) compared to mRNA-1273, whereas having had systemic AEs in contrast to local AEs or no AEs and days since 1st dose were significantly and positively associated with 350% (56-1202, 95% CI; P-value=0·006) and 10% (2-19, 95% CI; P-value=0·13) higher IgG S levels, respectively. In a stepwise multivariable model, these variables were retained but only previous exposure to SARS-CoV-2 and systemic AEs after vaccination were statistically significant (Table 2). In addition, smoking was associated with significantly less IgG S levels (63%, 6-85, 95% CI; P-value=0·038).

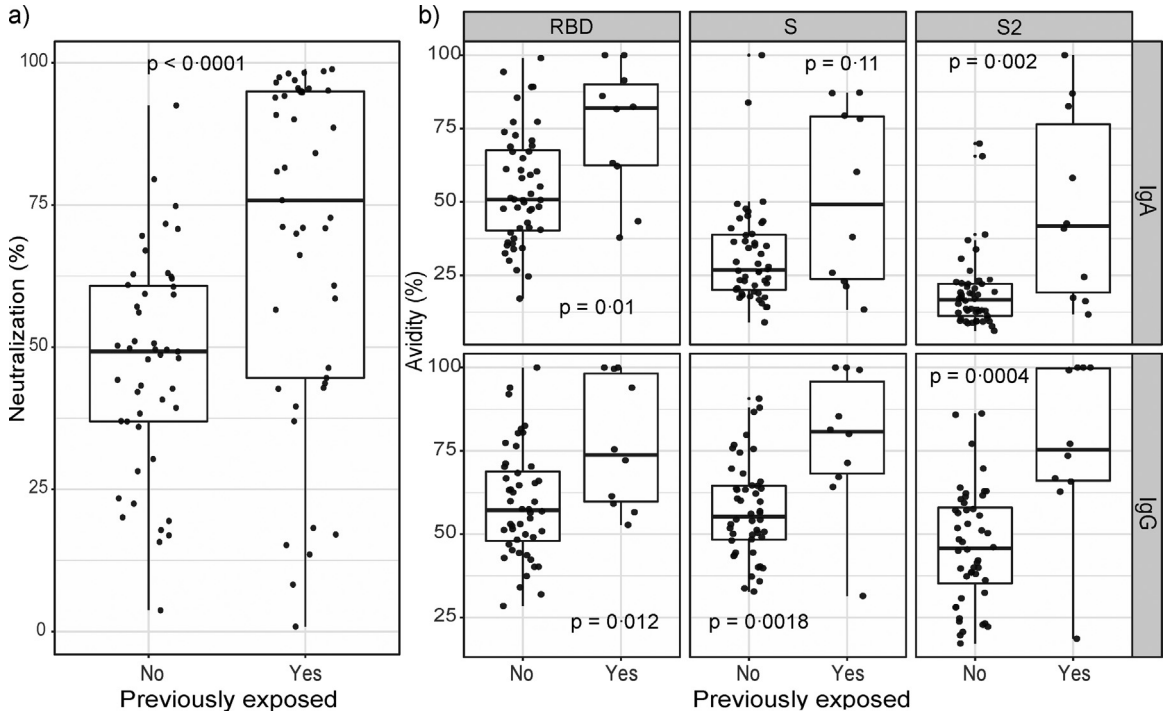

Figure 5. Antibody neutralization capacity and avidity after two doses of COVID-19 mRNA vaccines in naive and pre-exposed participants. a) Antibody neutralizing capacity, as a percentage of RBD-ACE2 binding inhibition by plasma samples assayed at 1:400 dilution (N=92, 47 naive and 45 pre-exposed). b) Antibody avidity, as % of IgA and IgG levels against RBD, S and S2 antigens measured incubating samples with a chaotropic agent over the IgA and IgG levels measured in the same samples without chaotropic agent, all at 1:5000 dilution (N=58, 48 naive and 10 pre-exposed). The center line of boxes depicts the median of MFIs; the lower and upper hinges correspond to the first and third quartiles; the distance between the first and third quartiles corresponds to the interquartile range (IQR); whiskers extend from the hinge to the highest or lowest value within 1·5 × IQR of the respective hinge. Wilcoxon rank test was used to assess statistically significant differences in antibody neutralization and avidity between naive and pre-exposed participants.

Factors independently associated with vaccine antibody responses after two doses

In univariable models, we found that males had higher IgG levels against S full length protein than females, and that IgG levels decreased by age in unexposed vaccinated participants, but not in exposed participants or when analyzing all participants together (Table S1). Comorbidities and receiving the BNT162b2 vaccine instead of the mRNA-1273 vaccine were associated with lower IgG levels (Table S1) and plasma neutralizing capacity (Table S2). Having been previously exposed, having had systemic AEs compared to local AEs or no AEs after the 1st dose (for all and pre-exposed HCW) or the 2nd dose (for all and naÿve HCW) and days since the 1st dose, were associated with higher IgG levels (Table S1). Curiously, IgG levels against the N protein of the HCoV HKU were negatively associated with post vaccination IgG levels against S full length in pre-exposed participants (Table S1). Being a smoker was also associated with lower plasma neutralizing capacity, while systemic AEs after the 2nd dose were associated with higher neutralizing capacity (Table S2).

In stepwise multivariable models, age and sex were not significantly associated with IgG levels against S protein (Table 2). Previous SARS-CoV-2 exposure was associated with 38% (13-69%, 95% CI) higher IgG levels to S, whereas BNT162b2 vaccination was associated with 43% (31-54%, 95% CI) less IgG-S levels than mRNA-1273 vaccination, regardless of exposure. In addition, in all participants and in the unexposed ones, having had systemic AEs compared to local or no AEs after the 2nd dose was associated with 23-28% higher IgG-S levels. In the pre-exposed HCW, being a smoker or having underlying comorbidities were independently associated with 35% (3-57%, 95% CI) and 55% (33-70%, 95% CI) less IgG-S levels, whereas there was a trend towards higher IgG-S levels when the HCW had a symptomatic infection compared to an asymptomatic infection. Being smoker, having comorbidities, and receiving the BNT162b2 vaccine instead of the mRNA-1273 vaccine were also associated with 43% (95% CI, 19-59), 45% (95% CI, 18-63) and 30% (95% CI, 7-48%) lower plasma neutralizing capacity, respectively (Table 3). Having had systemic AEs compared to local

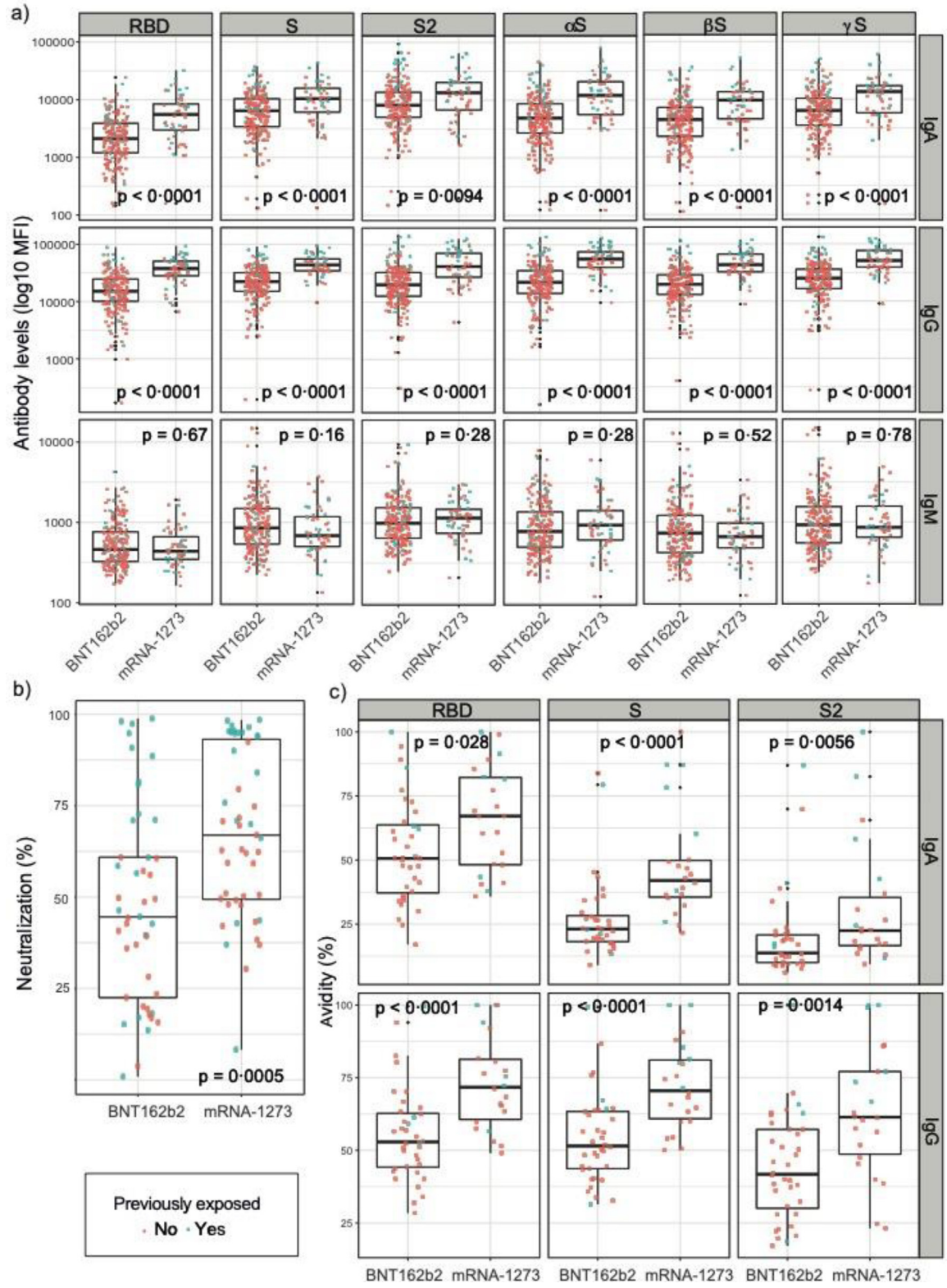

Figure 6. Comparison of antibody levels, neutralization and avidity between the two COVID-19 mRNA vaccines after two doses. a) Antibody levels elicited by BNT162b2 and mRNA-1273 among naive and pre-exposed participants (N = 263, 207 BNT162b2 / 56 mRNA-1273, 211 naïve, 52 exposed). Plasma samples were analyzed at 1:5000 dilution for IgG and 1:500 for IgA/IgM.

	b (%)[a]	Lower 95% CI	Upper 95% CI	P-value
One dose[b]				
Naive + exposed participants N=55				
Smoker	-62.45	-85.07	-5.58	**0.038**
SARS-CoV-2 pre-exposure	526.74	135.96	1564.75	**0.000**
BNT162b2 (ref: mRNA-1273)	-11.08	-74.56	210.88	0.851
Days since dose 1	5.79	-2.48	14.77	0.171
Systemic AEs dose 2 (ref: local/no AEs)	171.88	-2.05	654.64	0.055
Two doses[c]				
Naive + exposed participants N=262				
Sex (ref: male)	19.33	-0.31	42.85	0.054
Comorbidities	-17.55	-32.11	0.13	0.052
SARS-CoV-2 pre-exposure	38.18	12.97	69.02	**0.002**
BNT162b2 (ref: mRNA-1273)	-43.27	-53.58	-30.66	<**0.001**
Systemic AEs dose 2 (ref: local/no AEs)	23.27	3.70	46.54	**0.018**
Naive only N=211[d]				
Sex (ref: male)	21.97	-0.17	49.03	0.052
Age continuous	-39.64	-69.42	19.13	0.145
BNT162b2 (ref: mRNA-1273)	-45.42	-57.19	-30.41	<**0.001**
Systemic AEs dose 2 (ref: local/no AEs)	28.61	5.87	56.24	**0.012**
Exposed only N=52[e]				
Smoker	-35.40	-56.83	-3.34	**0.034**
Comorbidities	-55.05	-69.68	-33.36	**0.001**
BNT162b2 (ref: mRNA-1273)	-48.76	-61.73	-31.38	**0.001**
Symptomatic (ref. Asymptomatic)	38.72	-1.28	94.95	0.059
IgG levels against HKU1 N antigen	-1.31	-2.91	0.31	0.11

***Table 2*: Step-wise multivariable models assessing the impact of several variables on the IgG levels against the S full length protein induced after one (>7 days) and two doses of mRNA vaccines (12-19 days post-vaccine).**

Independent variables for step-wise models were selected based on univariable models (table S8)

[a] b transformed values to a percentage for an easier interpretation of variables effect

[b] The final multivariable model had smoking, SARS-CoV-2 exposure, vaccine type, days since dose 1 and AEs after dose 2 as independent variables.

[c] The final multivariable model had sex, comorbidities, SARS-CoV-2 exposure, vaccine type, and AEs after dose 2 as independent variables.

[d] The final multivariable model had sex, age, vaccine type, and AEs after dose 2 as independent variables.

[e] The final multivariable model had smoking, comorbidities, vaccine type, symptoms and IgG levels against HKU1 N antigen as independent variables.

or no AEs after the 2nd dose was associated with 60·54% (95% CI, 17-121) higher neutralizing capacity. SARS-CoV-2 exposure was also associated with 30% (95% CI, 1-79) higher plasma neutralizing capacity though the statistical significance was borderline (P-value=0·051).

IgG levels to S antigens induced by natural infection are maintained for up to a year

Antibody kinetics since the onset of symptoms along 6 time-points for 102 exposed non-vaccinated individuals are shown in Figure 7. At study month 12, 53 of the 414 HCW who visited had not received any vaccine dose yet, 36 of whom had been previously infected by SARS-CoV-2. IgM levels rapidly fell below the seropositivity thresholds. Similarly, IgA against full length N and its C-term region and IgG against N C-term decayed over time below the seropositivity thresholds. On the contrary, IgG and IgA levels against any of the S antigens tested (RBD, S or S2) remained positive over time for most of the participants for up to 1 year of follow-up, with IgG at higher levels than IgA. There were 31 exposed individuals with more than 300 days post-infection who had not been vaccinated. IgM, IgA and IgG seropositivity was 12·9% (95% CI, 5·1-28·9), 64·5% (95% CI, 46·9-78·9) and 90·3% (95% CI, 75·1-96·7), respectively, for any of the antigens tested.

Discussion

Knowledge on the antibody response induced by COVID-19 vaccines and the factors affecting it, such as

b) Plasma neutralization capacity elicited by BNT162b2 and mRNA-1273 among naive and pre-exposed participants (N=92, 45 BNT162b2r/47 mRNA-1273, 47 naive, 45 exposed). Plasma dilution used was 1:400. c) Antibody avidity elicited by BNT162b2 vs mRNA-1273 among naive and pre-exposed participants (N=58, 36 BNT162b2 and 22 mRNA-1273, 48 naive, 10 pre-exposed). Plasma dilution used was 1:5000. Red and green dots correspond to naive and pre-exposed participants, respectively.

	b (%)[a]	Lower 95% CI	Upper 95% CI	P-value
Neutralizing capacity				
All participants N=92				
Smoker	-42.80	-59.47	-19.27	**0.002**
Comorbidities	-44.81	-62.76	-18.22	**0.003**
SARS-CoV-2 exposure	30.13	-0.06	69.44	0.051
BNT162b2 (ref: mRNA-1273)	-30.19	-47.80	-6.64	**0.016**
Systemic AEs dose 1 (ref: local/no AEs)	24.85	-6.21	66.18	0.127
Systemic AEs dose 2 (ref: local/no AEs)	60.54	16.62	121.00	**0.004**

***Table 3*: Step-wise multivariable model assessing the impact of several variables on the plasma neutralizing capacity after two doses of mRNA vaccines (12-19 days post-vaccine).**
Independent variables for step-wise models were selected based on univariable models (table S9).
[a] b transformed values to a percentage for an easier interpretation of variables effect.

previous SARS-CoV-2 infection, is essential to understanding immunity elicited by vaccination and its heterogeneity in the general population, which can be used to improve the design of vaccination policies and guide personalized recommendations. We analyzed IgA, IgG and IgM responses to the COVID-19 mRNA vaccines mRNA-1273 and BNT162b2 in a well-characterized cohort of HCW with detailed demographic and clinical information, accurate history of SARS-CoV-2 exposure, and antibody responses since the beginning of the pandemic. Our results show that COVID-19 mRNA vaccines induce robust antibody responses to S antigens in most of the HCW but mRNA-1273 elicited higher antibody levels and quality than BNT162b2. Independently of the vaccine received, antibody responses were higher in previously SARS-CoV-2 exposed individuals, particularly if they had a symptomatic infection, and a 2nd dose of the vaccine in pre-exposed individuals did not increase their antibody levels, supporting the strategy of a single-dose vaccination for previously infected individuals to achieve a higher vaccination coverage and in more populations. However, our data also highlights the need for more personalized strategies as antibody responses may be diminished in asymptomatic, smokers and individuals with chronic diseases.

Higher IgG responses induced by mRNA-1273 than BNT162b2 have also recently been reported by others,[49,50] but to our knowledge, we are the first to report higher neutralizing capability and higher antibody avidity. The type of vaccine was not randomly administered, but HCW were not allowed to choose the vaccine brand since this depended on vaccine availability and did not follow a pre-established pattern. In addition, results were adjusted by relevant confounders such as previous SARS-CoV-2 exposure. Albeit very high antibody levels are induced by both vaccines, differences may be relevant for those individuals responding more poorly to vaccination or naive individuals. Although both vaccines use the same technology, they differ in the amount of mRNA per dose (100 μg vs 30 μg)[2,3] and formulation, but also in the schedule of the 2nd dose: 4 weeks after 1st dose for mRNA-1273 vs. 3 weeks for BNT162b2, which could be related to the differences observed here. A delay in the 2nd dose of the COVID-19 vaccine AstraZeneca (ChAdOx1-SARS-COV-2) showed improved immunogenicity and protection.[51,52] This suggests that there may be room for optimization in the dose quantity and schedules.

Upon vaccination, exposed participants had higher levels of IgG and IgA against S antigens than naive participants after one dose of the vaccine and, after two vaccine doses, naive individuals still had lower IgG and IgA levels against S antigens and of lower neutralizing capacity and avidity than exposed individuals. As mentioned, the 2nd dose seemed to not be beneficial in expanding the antibody response further, similarly to what has been reported by others.[7,8,11] Nevertheless, antibody responses were very heterogeneous, even among previously exposed individuals. We found that HCW who had an asymptomatic infection tended to have less IgG levels after vaccination than symptomatic HCW, and that SARS-CoV-2 antibodies before vaccination positively correlated with post-vaccination levels. Smokers and individuals with underlying comorbidities had considerably lower antibody levels and lower plasma neutralizing capacity. Therefore, a 2nd dose should be considered for exposed individuals who were asymptomatic, are smokers or people with comorbidities, especially the immunosuppressed, or could be administered depending on previous antibody titers, although this approach depends on the identification of correlates of protection and would only be feasible in high-income countries.

Nevertheless, receiving the full schedule for exposed individuals may be relevant for maintenance of responses over time assuming a decline of antibodies,[53] and to overcome the impact of new VoCs with increased transmissibility and potential immune escape, like the Omicron variant. We have detected a 6·3% vaccine breakthrough among fully vaccinated participants between 49 and 189 days post second dose, probably related to the fifth wave of the pandemic, mostly caused

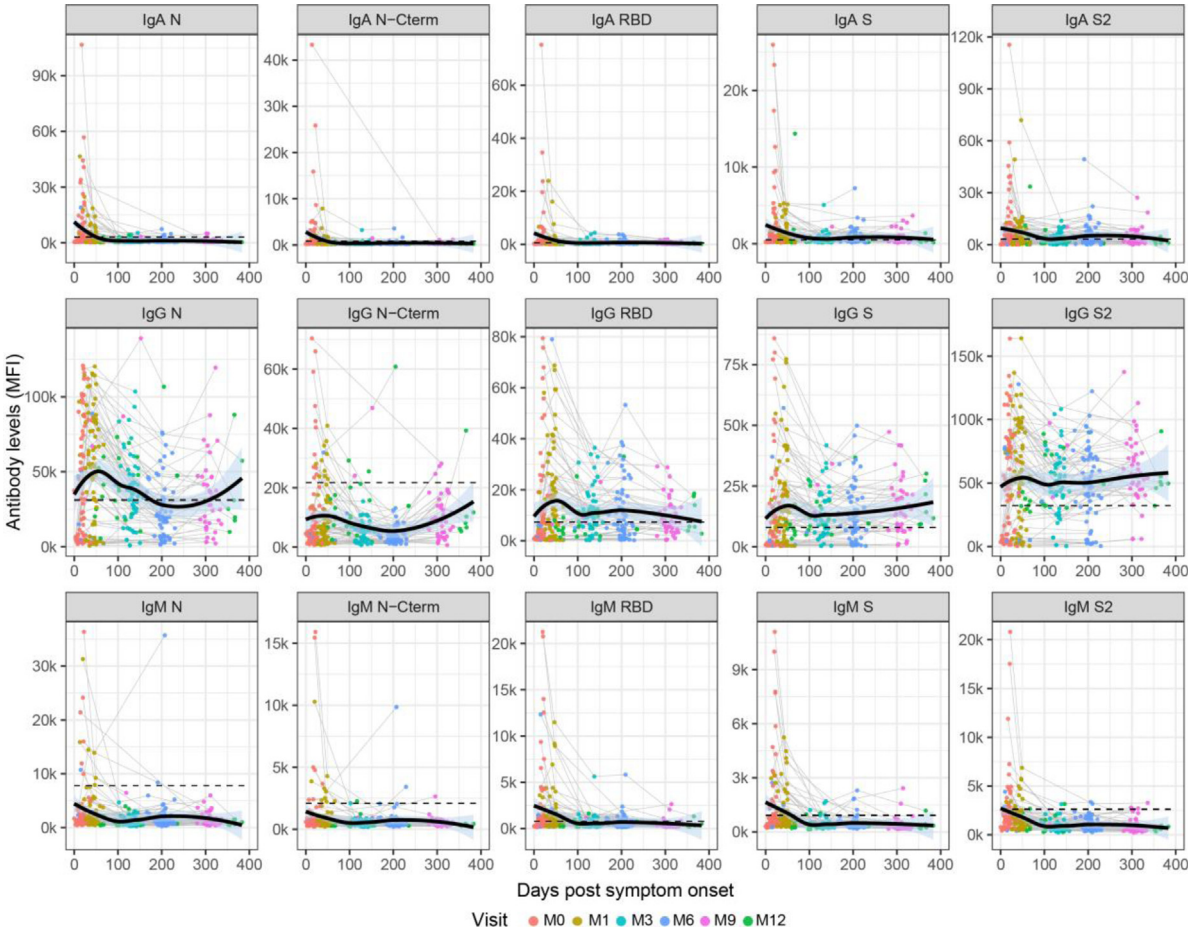

Figure 7. Kinetics of SARS-CoV-2 antibody levels since onset of symptoms in non-vaccinated participants. Levels (median fluorescence intensity, MFI) of IgA, IgG and IgM against each antigen (Nucleocapsid full length protein (N), and its C-terminal domain, the Receptor Binding Domain (RBD), full S protein and its subunit S2) measured in 338 samples from 102 symptomatic participants collected in up to 6 time points per participant (paired samples joined by lines). The black solid line represents the fitted curve calculated using the LOESS (locally estimated scatterplot smoothing) method. Shaded areas represent 95% confidence intervals. Dashed line represents the positivity threshold. Samples were analyzed at the 1:500 dilution.

by the Delta variant. In an HCW cohort from the UK (SIREN study), 7 days after the second dose of BNT162b2, there was an incidence of four infections per 10000 person-days during two months of follow-up, when Alpha variant was dominant, showing also that vaccination does not eliminate infection risk completely.[15] Here, we have not detected major differences in the IgG and IgA responses between any of the VoCs tested and the wild-type S, in contrast to other studies reporting diminished sensitivity of neutralizing antibodies against the Beta and Gamma variants.[28,29,31–34]

AEs have been associated with previous SARS-CoV-2 exposure.[23,49] Here we found that AEs, particularly after the 2nd dose, were positively associated with antibody levels and neutralizing capacity, independently of having had previous SARS-CoV-2 exposure. Another study found that clinically significant reactions to these mRNA vaccines were associated to higher IgG levels.[47] AEs may reflect a strong innate response resulting in increased acquired responses.

Despite the clear impact of SARS-CoV-2 exposure on vaccines responses, time since infection did not have a major effect. In face of shortage of vaccine doses, and based on some studies reporting maintenance of antibody responses in COVID-19 recovered patients for more than 6 months[54–57] recommendations to wait up to 6-month post-infection to get vaccinated were issued in some countries, including Spain.[24] Nevertheless, at HCB, all HCW were recommended to get the vaccine although naive individuals were prioritized.

Here, we show maintenance of IgG responses up to a year post-infection. After more than 300 days (up to 383 days) following infection among unvaccinated HCW, 90% (95% CI, 82·9 – 94·6) were still seropositive for IgG against any of the S antigens, demonstrating

persistence of immunity to natural exposure. We cannot discard SARS-CoV-2 re-exposure in some of those unvaccinated but it is unlikely as there were regular screenings in the hospital and individuals with COVID-19 related symptoms and contacts of cases were tested. This suggests maintenance of a certain level of protection irrespective of the additional role of memory T cell responses.[52,54] Maintenance of IgA was also observed in many individuals, but the role of plasma IgA in protection is unclear. Based on our data, it is difficult to recommend how long previously exposed individuals could wait to get vaccinated, although receiving at least a dose of a mRNA vaccine if previously exposed clearly increases antibody levels and neutralizing capacity regardless of the time since infection.

One of the limitations of the study is that the sample time-point post-vaccination for the first dose (7-72 days) was quite variable and different from that of the second dose (12-19 days). A long interval post first dose could affect the Ab levels, particularly IgM may decrease, in comparison to the response to the second dose. Another limitation of the study is that the HCW cohort, composed mostly by young adult women, is not representative of the general population, particularly older people. However, it is an important group to study in terms of exposure and immunity. We would expect lower antibody levels in elderly people and declining of natural immunity, but probably the same determinants affect early antibody vaccine responses. Finally, we did not analyze T cell responses, which may also be involved in protection and would provide complementary information.

Currently approved mRNA COVID-19 vaccines have proven to be highly efficacious[2,3] and effective in the real population[12–15] for at least a few months but vaccine efficacy against symptomatic infection wanes over time and vaccine escape by VoCs needs to be monitored. Optimal antibody responses elicited early after vaccination may be important for maintenance of immunity and protection and probably affect similarly responses to booster doses. We have demonstrated that responses depend on the vaccine received, number of doses, previous history of SARS-CoV-2 infection and SARS-CoV-2 immune responses, lifestyle and health of the individuals. Even in a cohort of HCW, we have found a high heterogeneity of antibody responses and highlight the need of more personalized recommendations. Moving forward, and in face of the emergence of variants with immune escape such as Omicron, differential quantitative and qualitative responses to the vaccines between exposed and naive individuals from different populations and conditions needs to be studied over time to better inform vaccination strategies.

Author contributions

G.M., R.A., A.L.G-B., C.D. designed the study. G.M., R. A. and C.D. supervised all work. R.A., C.C., P.V., S.B., M.T. and C.D. coordinated participant visits and sample and data collection. G.S., R.A., D.B, M.V., C.D. and L.P. collected samples and data at HCB. R.R., MJ.M., M.V., D.B., R.A.M. and A.J. processed the samples, developed and performed the serological Luminex assays and analyses. P.H-L, A.A. and P.E. designed and performed the flow cytometry neutralization assay. N.R.M., C.C. and L. I. produced the antigens. A.LL., A.M., A.T., A.V. contributed to design and the critical interpretation of the results. N.O., M.R., and P.R. managed and analyzed the data and N.O., M.R. and G.M. prepared the manuscript figures. S.M. managed the clinical data. G.M., R.A., N. O., M.R., C.D. interpreted the results and wrote the first draft of the paper. G.M. R.A. N.O, M.R., C.D. had access to, and verified, the data. G.M. and R.A. contributed equally. M.R., N.O., R.R., G.S. also contributed equally. A.L.G-B. & C.D. jointly supervised this work. All authors read and approved the final version as submitted to the journal.

Data sharing statement

The antibody levels, avidity and neutralization data generated in this study and metadata are deposited in the UB public repository under the title of this publication at http://diposit.ub.edu/dspace/handle/2445/55585. Protocol information will be available on reasonable request.

Declaration of interests

The authors declare no competing interests.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2021.103805.

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