Eleven-month longitudinal study of antibodies in SARS-CoV-2 exposed and naïve primary health care workers upon COVID-19 vaccination

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ABSTRACT

We evaluated the kinetics of antibody responses to SARS-CoV-2 spike (S) and nucleocapsid (N) antigens over 5 cross-sectional visits (January-November 2021), and the determinants of pre-booster immunoglobulin levels, in a prospective cohort of vaccinated primary health care workers in Catalonia, Spain. Antibodies against S antigens after a full primary vaccination course, mostly with BNT162b2, decreased steadily over time and were higher in pre-exposed (n=247) than naïve (n=200) individuals, but seropositivity was maintained at 100% (100% IgG, 95.5% IgA, 30.6% IgM) up to 319 days after the first dose. Antibody binding to variants of concern was highly maintained for IgG compared to wild type but significantly reduced for IgA and IgM, particularly for Beta and Gamma. Factors significantly associated with longer-term antibodies included age, sex, occupation, smoking, adverse reaction to vaccination, levels of pre-vaccination SARS-CoV-2 antibodies, interval between disease onset and vaccination, hospitalization, oxygen supply, post COVID and symptomatology. Earlier morning vaccination hours were associated with higher IgG responses in pre-exposed participants. Symptomatic breakthroughs occurred in 9/447 (2.01%) individuals, all among naïve (9/200, 4.5%) and generally boosted antibody responses. Additionally, an increase in IgA and/or IgM seropositivity to variants, and N seroconversion at later time points (6.54%), indicated asymptomatic breakthrough infections, even among pre-exposed.

Seropositivity remained highly stable over almost a year after vaccination. However, gradually waning of anti-S IgGs that correlate with neutralizing activity, coupled to evidence of an increase in breakthrough infections during the Delta and Omicron predominance, provides a rationale for booster immunization.

Keywords: SARS-CoV-2; COVID-19; vaccine; antibodies; duration; kinetics; health care workers; cohort; spike; variants; baseline determinants, vaccine breakthroughs.

SUMMARY

Antibodies against SARS-CoV-2 spike antigens after a primary vaccination course, mostly with BNT162b2, decreased steadily over 5 cross-sectional visits (January-November 2021), being higher in pre-exposed (n=247) than naïve (n=200) primary health care workers in Catalonia, Spain, but seropositivity was maintained at 100% up to 319 days after the first dose. Factors significantly associated with longer-term antibodies included age, sex, occupation, smoking, adverse reaction to vaccination, levels of pre-vaccination antibodies, hour of vaccination, interval between disease onset and vaccination, hospitalization, oxygen supply, post COVID and symptomatology. Antibody binding to variants of concern was highly maintained for IgG compared to wild type but significantly reduced for IgA and IgM, and symptomatic breakthroughs occurred in 2% individuals, all among naïve, and generally boosted antibody responses.

INTRODUCTION

Two years into the COVID-19 pandemic and one year after the start of vaccination rollout, the world faced a peak of cases associated with the highly contagious Omicron variant of concern (VoC) of SARS-CoV-2. Immunity achieved through natural infection and vaccination has had a large impact in containing disease severity and deaths, but transmission has not been interrupted and breakthrough infections are common, though often asymptomatic or mild [1]. Key questions remain regarding the correlates of protection [2–4], durability of immunity and evasion capacity of emerging VoCs, which prevent a rational prioritization of second-generation vaccines and the design of booster immunization policies. The mRNA-1273 (Moderna) [5] and BNT162b2 (Pfizer/BioNTech) [6] vaccines remain 93% and 84% effective, respectively, 6 and 4 months after a second dose, but an apparent decline of protection in subsequent months led to the implementation of 3rd doses, even when a substantial number of individuals may still have high antibody and cellular immune responses. Most vaccines

maintain binding and functional antibodies against many SARS-CoV-2 variants, with Beta (B.1.351) and Omicron (B.1.1.529) having the lowest antibody recognition [7, 8]. However, data on primary vaccination effectiveness are lacking beyond 9 months, even though booster administrations (3rd and beyond) were implemented in many countries [9–13].

There is significant individual heterogeneity in the immune response to natural infection and to partial immunization, less so at the peak response after a full primary vaccination course [14], and immunity differs by vaccine [15]. A more potent response, so-called hybrid immunity, is achieved following SARS-CoV-2 infection (more so if symptomatic) and vaccination, even with only one dose [15–17]. Other factors affecting primary vaccine responses include interval between doses [18], comorbidities and smoking [15]. However, it is less clear what determinants affect the maintenance of immune responses as time progresses and thus who should be revaccinated and when. Moreover, it is likely that, as antibodies decline months after vaccination, there is variability also in their decay rate. Therefore, it is important to identify the determinants of sustained immunity to move towards more personalized evidence-based vaccine strategies if and when protective immunity wanes.

To better understand determinants of durability of vaccine antibody responses, we performed a longitudinal cohort study between January and November 2021 in 447 health care workers (HCW) with and without prior COVID-19, and assessed demographic, clinical (symptoms, comorbidities) and epidemiological factors affecting the levels of antibodies almost a year after vaccination, just before the implementation of the 3rd booster and the onset of the Omicron wave (sixth) in Spain. Such data are relevant to establish what factors impact resistance to breakthrough infections, and rationally define when revaccination may be warranted.

METHODS

Study subjects

The CoviCatCentral cohort is composed of two groups of primary HCW, recruited in three primary care counties in Barcelona, Spain, who were offered COVID-19 vaccination starting December 2020. The first group was composed of individuals recruited since the first wave of the COVID-19 pandemic (March-April 2020, n=247) with symptomatic SARS-CoV-2 infection confirmed by RT-PCR and/or antigen RDT; all HCW with COVID-19 were invited to participate. HCW were subsequently visited at seven cross-sectional surveys up to end November 2021, with venous blood collection since September 2020. The second group was composed of naïve HCW recruited since March-April 2021 after full primary vaccination (n=200), having similar characteristics (age, sex, professional category, smoking habits) to the pre-exposed group; participants were visited at four cross-sectional surveys, with venous blood collection up to end November 2021. Demographic and clinical data were collected at baseline and during follow-up visits through telephone interviews and questionnaires by study physicians and nurses. Recorded baseline information included history of previous environmental allergies, smoking habits, and symptoms in the SARS-CoV-2 infected ones (fever, shivers, headache, asthenia, myalgia, arthralgia, dyspnea, chest pain, cough, sputum production, anosmia, hypogeusia, odynophagia, tachycardia, dizziness and thrombosis). For the multivariable regression analysis, symptoms were grouped into categories: digestive, otolaryngology, neurological, ophthalmology (conjunctival hyperemia, tearing, dry eyes, blurred vision), and skin disorders. Follow-up questionnaires registered comorbidities, including chronic kidney disease, chronic obstructive pulmonary disease, asthma, cardiovascular disease, neurological diseases, digestive diseases, autoimmune diseases, cancer, immunosuppression (disease or drug-related), diabetes mellitus, dyslipidemia, hypertension, depression and/or anxiety and hypothyroidism, as well as pregnancy status, obesity and other cardiovascular risk factors (smoking habits), and new or persistent symptoms, including sequelae post COVID-19 condition (occurrence of symptoms three months after COVID-19, with

138 symptoms and effects lasting for at least two months). Reinfections and vaccine
139 breakthroughs were captured by passive case detection.

140 The study protocol was approved by the IRB *Comitè Ètic d'Investigació Clínica IDIAP*
141 *Jordi Gol* (codes 20/094-PCV and 20/162-PCV) and written informed consent was
142 obtained from participants.

143

144 **Antibody measurements**

145 Levels of IgM, IgA and IgG were quantified in plasma by Luminex. The performance of
146 these Luminex assays to detect seropositivity has been previously reported being of a
147 100% specificity and 95.78% sensitivity at ≥14 days, and 95.65% sensitivity at ≥21
148 days since the onset of symptoms, with areas under the curve (AUCs) of 0.977 and
149 0.999, respectively [19]. Recombinant proteins included the nucleocapsid (N) full length
150 (FL) protein and the C-terminal fragment (N CT), both produced at ISGlobal, the spike
151 (S) full length protein produced at CRG, its subregion S2 (SinoBiological), the receptor-
152 binding domain (RBD) donated by the Krammer lab (Mount Sinai, New York), and the
153 RBD proteins of four VoCs produced at CRG: Alpha, Beta, Delta and Gamma. Antigen-
154 coupled microspheres were added in multiplex to a 384-well µClear® flat bottom plate
155 (Greiner Bio-One, Frickenhausen, Germany) in 90 µL of Luminex Buffer (1% BSA,
156 0.05% Tween 20, 0.05% sodium azide in PBS) using an Integra Viaflo semi-automatic
157 device. Positive control pools were added to each assay plate as serially diluted
158 titration curves for QA/QC purposes. Pre-pandemic samples (n=128) were used as
159 negative controls. Test and control plasma samples were added to the 384-well plate
160 using an Assist Plus Integra device. All samples were tested at 1:500 dilution, and
161 additionally at 1:5000 dilution for anti-S IgG antibodies to avoid saturation upon
162 vaccination. For IgM and IgA, samples were pre-treated with anti-Human IgG
163 (Gullsorb) at 1:10 dilution, to avoid IgG interferences. Technical blanks (Luminex Buffer
164 and microspheres without samples) were added to control for non-specific signals.

Plates were incubated for 1 h at room temperature in agitation at 900 rpm and protected from light. Then, plates were washed three times with 200 µL/well of PBS-T (0.05% Tween 20 in PBS), using a BioTek 405 TS. Twenty five µL of goat anti-human IgG-phycoerythrin (PE) (GTIG-001, Moss Bio) at 1:400, goat anti-human IgA-PE (GTIA-001, Moss Bio) at 1:200, or goat anti-human IgM-PE (GTIM-001, Moss Bio) at 1:200 in Luminex buffer, were added to each well and incubated for 30 min. Plates were washed and microspheres resuspended with 80 µL of Luminex Buffer and acquired on a Flexmap 3D® reader (at least 50 microspheres per analyte per well), and median fluorescence intensity (MFI) was reported for each analyte. The cutoff for seropositivity was calculated with pre-pandemic plasma samples as 10 to the mean plus 3 standard deviations of $\log_{10}$-transformed MFI values.

Data analysis

The percentage decrease in seropositivity for RBD VoCs compared to the RBD wild type (WT) Wuhan was calculated as: ([seropositivity RBD WT – seropositivity RBD VoC]/seropositivity RBD WT)*100. The changes in post-vaccination levels in relation to days since first dose were expressed as Spearman coefficient (rho) with p-values. Univariable and multivariable stepwise linear regression models were fit to identify the variables affecting the antibody levels ($\log_{10}$ MFI) up to 11 months after vaccination. Multivariable models were selected based on the Akaike information criterion, Bayesian information criterion and adjusted r-square parameters. For an easier interpretation of the results, a transformed beta value (%) of the log-linear model was calculated with the formula: ([10^beta]-1)*100, giving the difference (in percentage) in antibody levels when comparing to the reference group for categorical variables or for a one-unit increase for continuous variables. Likewise, a transformed beta value (%) of the log-log model was calculated with the formula: ([10^(beta*$\log_{10}$(1.1))]-1)*100, giving the difference (in percentage) in antibody levels for a 10% increase of the predictor

192 variable, for continuous variables. P-values were considered statistically significant at
193 the 5% level. All data collected were managed and analyzed using the R software
194 version 4.1.2.

195

196 **RESULTS**

197 The characteristics of the study population at baseline and at the later timepoint 8 (T8)
198 visit, when the determinants of long-lasting antibody levels were evaluated, are
199 included in **Tables 1** and **S1**. Most HCW (97.6%) were vaccinated with BNT162b2
200 Comirnaty (Pfizer/BioNTech), and a minority received mRNA-1273 Spikevax
201 (Moderna), ChAdOx1 nCov-19 Vaxzebria (AstraZeneca) or Ad26.COV2.S (Janssen,
202 Johnson & Johnson), or combinations.

203 **SARS-CoV-2 seropositivity over time**

204 Seropositivity rates over five cross-sectional visits (T4-T8) following COVID-19
205 vaccination for each antibody isotype /antigen pair are shown in **Table S2**, and
206 stratified by prior COVID-19 disease in **Table 2**. All participants had seroconverted for
207 at least one immunoglobulin/S antigen pair after vaccination, and this was maintained
208 up to 11 months, with no seroreversions. IgG responsiveness was the highest,
209 reaching 100% in all timepoints except the first visit (T4) when some exposed
210 participants had only received one vaccine dose. For IgA and IgM, the percentage of
211 responders was higher in the vaccinated pre-exposed compared to the naïve
212 individuals, reflecting hybrid immunity. Thus, non-responders or hypo-responders were
213 not identified in these HCW cohorts. The most immunogenic antigen was the full-length
214 S, followed by RBD and by S2, which were shown to highly correlate with neutralizing
215 antibodies in our prior studies [20][15]. The inclusion of N antigens also allowed
216 assessing the evolution of seropositivity over time in those with prior COVID-19 history,
217 which generally decreased over time. Furthermore, N serology allowed to identify

about 1% (for IgG and IgM) to 3% (for IgA) prior unnoticed SARS-CoV-2 exposures, according to N FL seropositivity, in the infection-naïve cohort where individuals did not have prior documented COVID-19 diagnosis. Seropositivity to N CT was excluded from this analysis as we have previously identified a potential antibody cross-reactivity with S following vaccination [21]; indeed seropositivities in naïve individuals post vaccination (T5) were higher for N CT than for N FL (2.5% for IgM, 6.5% for IgA and 10% for IgG) but decreased over time, thus suggesting a transient nature. Finally, N serology also allowed to monitor potential breakthrough infections (see below), with an increase in N FL seroprevalence noted from T7 to T8 for both naïve and pre-exposed individuals (up to 1.8% IgM, 14.6% IgA, and 17.3% IgG). The increase in N FL seroprevalence was accompanied by an increase in IgM and IgA to S antigens, coinciding with the onset of the sixth wave in Spain [22], in face of waning antibody levels (see kinetics below).

Antibody recognition of SARS-CoV-2 variants

Seropositivity rates to SARS-CoV-2 RBD from VoCs in comparison to WT RBD over three cross-sectional visits (T6-T8) after COVID-19 vaccination, are shown in **Tables 3** and **S3**. There was little variation in IgG seropositivity against RBD from VoCs, with an overall decrease between 4.7-5.8% against Beta (9.0-11.7% among COVID-19 pre-exposed) and negligible (up to 1%) against Alpha, Delta, or Gamma. However, there was less cross-variant antibody recognition for IgA, with a greater percentage decrease in seropositivity against RBD from Beta (~50%) > Gamma (~30%) > Delta (~5%) > Alpha (~1%), being about 2-fold higher or more in pre-exposed. There was higher IgA seropositivity to the Alpha variant compared to the WT at T6 when Alpha was the predominant VoC circulating, and higher IgA seropositivity to the Delta variant compared to the WT at T8 when Delta became the predominant VoC (**Tables 3 & S3**). A similar gradient of VoC cross-reactivity with WT was seen for IgM among pre-

exposed individuals, with lower variant-transcending recognition compared to IgG (particularly for Beta and Gamma, with ~80% decrease in seropositivity), but seroprevalences were much lower (**Table S3**). However, among vaccinated naïve individuals, IgM seropositivity rates increased for Alpha (predominant at T6) and Delta (predominant at T7 and T8) RBD, with a general most prominent increase at T8 when IgG antibodies were the lowest, coinciding with the onset of the sixth wave in Spain (just pre-Omicron), possibly indicative of subclinical incident infection breakthroughs.

Antibody kinetics following vaccination

The evolution of SARS-CoV-2 antibody levels (log_{10} MFI) over up to 319 days for each immunoglobulin isotype/antigen pair, including RBD to VoC, is shown in **Figure 1**. The correlation between antibody levels with days since the first vaccine dose resulted in negative and significant Rho Spearman correlation coefficients for IgG and IgA, indicative of waning. IgA appeared to have somewhat slower antibody decay than IgG, and the slope of IgG decay seemed more pronounced post-vaccination (Rho≅-0.5) compared to post-natural infection (Rho≅-0.25) in a prior analysis of this cohort over a similar follow-up time [23]. In multivariable regression models, time since vaccine dose 1 was negatively and significantly associated with IgG to S and RBD antigens at T8 (**Table 4**). Regarding IgM, even though most participants were seronegative at T8, the slopes of the correlation lines were more stable and, for some antigens (N FL, RBD), Rho values were positive due to increases in levels in T8, as seen with seropositivity. Stratifying by COVID-19 status pre-vaccination, IgA and IgG levels were higher for individuals with prior symptomatic diagnosis of SARS-CoV-2 but the slopes of the waning curves did not seem to diverge substantially from those of naïve individuals (**Figure 2**). Consistently, the strongest determinant for T8 antibody levels in multivariable regression models was having had COVID-19 before vaccination **(Table 4)**. At the individual level, a 10% increase in pre-vaccination IgG levels significantly

increased T8 post-vaccination levels by 3.85% (95%CI 1.03-3.05) against S, 4.16% (95%CI 1.03-3.25) against RBD, and 6.02% (95%CI 1.05-4.92) against S2 (all p<0.0001) antigens.

The pattern for anti-N antibodies differed between groups, as expected, with waning in levels for pre-exposed individuals while naïve had flat negative values, with an increase also noted at T8 for N FL IgM and IgA (**Figure 2**).

Other factors affecting late post-vaccination antibody levels

Baseline and follow-up variables significantly associated with lower T8 post-vaccination antibody levels by multivariable regression models included older age, male sex, being a physician or a nurse compared to other occupations, smoking, and not having had adverse events (AEs) during primary vaccination (**Table 4**).

Among individuals with prior history of COVID-19, variables significantly associated with higher T8 post-vaccination antibody levels by multivariable regression models included longer intervals since disease onset, hospitalization, not having received supplementary oxygen, as well as having had anosmia/ageusia, fever, dyspnea, or shivers (**Table 4**). Post COVID condition was associated with lower IgM levels to RBD. In addition, the hour of first dose vaccination had an effect, with significantly lower levels of IgG to S and all RBD variants with increased time, i.e, each hour increase in the vaccination time (from 8:30 am) reduced post-vaccination T8 IgG levels between 3.8-5.7%, adjusted by other significant covariates (**Table 5**). The determinants associated with T8 IgG levels to RBD VoCs were very similar to those shown in Table 4 for the RBD WT (data not shown).

Type of vaccine had minor effects because very few HCW received products other than Pfizer/BioNTech. In spite of this, in multivariable models, adenoviral (AstraZeneca and/or Janssen) and Pfizer/BioNTech vaccines induced 56.7% (p=0.005) and 37.5% (p=0.015) lower cross-reactive N CT IgA levels at T8, respectively, than vaccination schedules including Moderna [21]. Among pre-exposed HCW, adenoviral vaccination

induced 51.2% (p=0.029) lower cross-reactive N CT IgA levels than Moderna, and Pfizer/BioNTech 74% (p=0.028) higher RBD IgM levels than Moderna.

Breakthrough infections and antibody boosting

There were 9 symptomatic vaccine breakthroughs detected, all between HCW with no history of COVID-19, all at the time of Delta predominance during the fifth wave in Spain (Summer-Autumn 2021). **Figures 3** and **S1** show the boost in antibodies following those patent infections. An increase in N seropositivity at T8 (**Figure 1**) was also indicative of asymptomatic breakthroughs, still during the Delta predominance and when the Omicron wave just started. Thus, there were 25/382 seroconverters to N FL (6.54% asymptomatic breakthroughs), of whom 11 (44%) had prior COVID-19 history (i.e. 2.9% asymptomatic breakthroughs among pre-exposed).

DISCUSSION

Our study shows that seropositivity after 1 or 2 vaccine doses was maintained in all participants of a prospective HCW cohort up to 11 months after initial immunization, although levels gradually decreased but with high heterogeneity. Infection breakthroughs (2.01% symptomatic and 6.54% asymptomatic) were more frequent towards the end of the follow up and coinciding with predominance of more highly transmissible VoCs. We confirmed that individuals with prior history of COVID-19 still had higher responses almost a year after vaccination, and that post-vaccination levels were positively associated with pre-vaccination levels. Remarkably, compared with our prior analysis post one-year in the same cohort [23], a more steep decay in antibodies was seen after vaccination than following natural exposure, indicating that SARS-CoV-2 infection may induce better memory responses or longer-lived plasma cells.

Although we do not have an absolute correlate of protection to ascertain what circulating levels may suffice to prevent infection, and to rationally indicate when

boosters would be beneficial, the occurrence of breakthrough infections suggests that such threshold was likely crossed for a number of individuals, more over in face of a more contagious VoC (Delta) with a high degree of immune escape [24, 25]. Of note, none of the breakthroughs were related to severe disease. In addition to symptomatic cases, seroconversion of N FL antibodies (including IgM) was indicative of asymptomatic infections that appeared to increase further at the later timepoints (T8) when the lowest levels of IgG were attained. Although advances are underway to establish correlates of protection [3, 26–30], not having them also makes it more difficult to link analysis of antibody waning with effectiveness [31], and to predict the impact of VoC like Omicron. In prior studies, one month after the second dose of Moderna, 85% of participants had neutralizing activity against Omicron but after 7 months this was reduced to 55% [32].

Here, factors affecting the levels of longer-term antibodies were similar to those involved in early peak responses (age, sex, smoking, time intervals), but with some additional results. We consistently found that physicians and nurses had lower responses than other occupations in the primary care health sector, and this could be due to work-related stress or burn out [33][34]. The finding that patients who received oxygen supplement also had lower levels is somewhat surprising as more COVID-19-like symptoms (e.g. fever, dyspnea, shivers) and severe disease are associated with higher antibody levels [35][36]. Oxygen requirement could be indicative of a status of immune suppression and poorer response to vaccination, or it could be related to a worse recovery, or have a long-term impact that affects vaccine responses months later. As models were controlled for time between infection and vaccination, this factor would not be a confounder. Interestingly, lower RBD IgM levels were associated with post COVID condition, and this could be related to the pathophysiological mechanisms of this heterogeneous syndrome that at the moment remain unknown.

353 As reported in our prior hospital-based studies [15], having AEs after vaccination was
354 associated with higher antibody levels; consistently with the age pattern of lower
355 responses with age, more frequent AEs were seen in younger than older individuals.
356 Of note, this impact of AEs on antibody levels appears to affect not only the early peak
357 responses but also the long-term steady phases. Interestingly, we found a significant
358 association between earlier vaccination and better immune response, but only in those
359 pre-exposed. Previous studies have reported similar results for COVID-19 and other
360 vaccines [37][38], while one study reports a better response if vaccination is in the
361 afternoon [39]. Our data indicate a benefit of early morning vaccination to attain better
362 immunogenicity and durability.

363 Information on when antibodies wane below protective levels is needed to avoid too
364 frequent booster immunizations in high-income countries that may not be necessary in
365 younger or healthier populations or even be counter-productive [40], and to direct
366 limiting vaccine supplies for more vulnerable populations (unvaccinated, elderly and
367 immune compromised). Ensuring maximum vaccine coverage worldwide will, in turn,
368 slow down the emergence of VoC that may threaten vaccine effectiveness. In
369 individuals boosted with a 3rd dose, neutralizing titers against Omicron variant were
370 much higher, and remained detectable 6 months after the booster [32]. However, there
371 are also memory B and T lymphocytes that would respond rapidly after an infection
372 and control it to not progress to severe forms, therefore only antibodies may not fully
373 predict protection against severe COVID-19.

374 The study has some limitations, like the focus on HCW that may not be representative
375 of the overall population, particularly not including the elderly in whom seroreversions
376 in the 11-month period would have been expected. In addition, we do not report
377 neutralizing activity, but our prior studies have shown a very strong correlation with
378 anti-S IgG and IgA levels. Our investigation precedes the implementation of vaccine
379 boosters and the rise of Omicron, but few studies have performed such long and depth

analyses of long-term antibodies after primary vaccination and including all the main VoC up to the Delta waves. Ongoing experiments will address the role of cellular immunity in this population in relation to antibody maintenance and immune escape.

In conclusion, data indicate that older physician or nurse males not having vaccine reactogenicity will be more likely to have lower antibodies within a year timeframe, and thus be prioritized for booster vaccinations. On the other hand, non-smoking younger individuals with hybrid immunity as a result of a prior COVID-19 episode with certain features (anosmia/ageusia, fever, dyspnea and hospitalization but not oxygen supply) would maintain higher antibody levels and be less likely to need vaccine boosters in the timeframe that many Western countries have adopted them. The study also manifests the benefit of administering the vaccines earlier in the morning and as late as possible after a COVID-19 episode, and confirms that Moderna is superior to Pfizer/BioNTech and AstraZeneca vaccines, despite the asymmetrical distribution. Future biannual longitudinal follow-up studies will address further the breadth and maintenance of immunity and the waning of antibodies following the booster doses, including the impact on emerging variants like Omicron on breakthrough infections.

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Author contributions

Concept and design: CD, GM, ARM, JVA, ARC. Acquisition, analysis, or interpretation of data: ARM, SA, MV, RR, AJ, EP, CD, GM, JVA, ARC. Produced antigens: NRM, CC, LI. Sample processing: RAM, CJ, DB, RR, SA. Statistical analysis: GRO, SA, RR. Supervision: CD, JZ, GM, RA, ARC, JVA. Obtained funding: CD. Had full access to the study data and take responsibility for the integrity and the accuracy of the data and analysis: CD, GM, ARC and JVA. Drafting of the first manuscript: CD. Critical revision of the manuscript for important intellectual content: ARM, SA, GRO, MV, RR, EP, CJ, RAM, DB, AJ, JZ, LI, NRM, CC, JVA, RA, GM, GVA, ARC.

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List of Figures

Figure 1. SARS-CoV-2 antibody levels by days since first dose of COVID-19 vaccination. IgA (1/500 dilution), IgG-N FL & N CT (1/500 dilution), IgG-S, S2, RBD, RBD α, RBD β, RBD δ & RBD γ (1/5000 dilution) and IgM (1/500 dilution) antibody levels (log_{10} median fluorescence intensity [MFI]). Black dots represent seropositive and grey squares seronegative individuals. Data from the same individual are linked by grey lines (continuous lines in seropositive and dashed in seronegative). The blue solid lines represent the fitted linear regression models. The Spearman correlation coefficients and p-values are reported in each plot. Antigens: nucleocapsid full length (N FL) and C-terminus (N CT), spike full length (S), S2 subunit, and receptor-binding domain (RBD) for wild type and variants of concern.

Figure 2. SARS-CoV-2 antibody levels by days since first dose of vaccination and by previous COVID-19 status. IgA (1/500 dilution), IgG-N FL & N CT (1/500 dilution), IgG-S, S2, RBD, RBD α, RBD β, RBD δ & RBD γ (1/5000 dilution) and IgM (1/500 dilution) levels (log_{10} median fluorescence intensity [MFI]). Red dots are individuals with prior COVID-19 diagnosis and blue dots individuals without (open circles for 1st doses and full circles for 2nd doses). Data from the same individual are linked by colored lines. The red and blue solid LOESS lines represent the fitted curves of the pre-exposed and naïve individuals, respectively. The Spearman correlation coefficients and p-values are reported for pre-exposed and naïve individuals separately. Antigens: nucleocapsid full length (N FL) and C-terminus (N CT), spike full length (S), S2 subunit, and receptor-binding domain (RBD) for wild type and variants of concern.

Figure 3. **Dynamic changes in IgG levels in nine patients with symptomatic breakthroughs.** Vaccine doses as dashed lines and infections as red lines. IgG levels

as log_{10} median fluorescence intensity (MFI) (1/500 dilution). Antigens: nucleocapsid full length (N FL) and C-terminus (N CT), spike full length (S), S2 subunit, and receptor-binding domain (RBD) for wild type and variants of concern. IgA and IgM in Figure S1.

583 **Table 1**. Population characteristics of the entire study cohort according to COVID-19
584 pre-exposure (up to timepoint 8), and when antibodies were assessed (11 months after
585 vaccination).

	Full study cohort		Timepoint 8	
	Pre-exposed N=247 (55.3%)	**Naive** N=200 (44.7%)	**Pre-exposed** N=194 (50.8%)	**Naive** N=188 (49.2%)
Age[1]	47 (17)	46 (17)	48.5 (17.8)	47 (16.3)
Sex (female)	205 (83.0%)	170 (85.0%)	160 (82.5%)	160 (85.1%)
Occupation				
Physician or dentist	86 (34.8%)	60 (30.0%)	70 (36.1%)	56 (29.8%)
Nurse or auxiliary nurse	111 (44.9%)	84 (42.0%)	82 (42.3%)	79 (42.0%)
Other[2]	50 (20.2%)	56 (28.0%)	42 (21.6%)	53 (28.2%)
Site				
Bages	122 (49.4%)	105 (52.5%)	92 (47.4%)	99 (52.7%)
Osona	72 (29.1%)	37 (18.5%)	65 (33.5%)	35 (18.6%)
Anoia	53 (21.5%)	58 (29.0%)	37 (19.1%)	54 (28.7%)
Type vaccine (dose 1 - dose 2)[3]				
Moderna alone or combined	2 (0.8%)	0 (0.0%)	2 (1.0%)	0 (0.0%)
Pfizer alone	207 (83.8%)	200 (100.0%)	185 (95.4%)	188 (100.0%)
Astrazeneca or Janssen	6 (2.4%)	0 (0.0%)	6 (3.1%)	0 (0.0%)
Astrazeneca and Pfizer combined	2 (0.8%)	0 (0.0%)	1 (0.5%)	0 (0.0%)
Number doses[3]				
One	76 (30.8%)	0 (0%)	71 (36.6%)	0 (0.0%)
Two	141 (57.1%)	200 (100%)	123 (63.4%)	188 (100%)
Days since 1st vaccination[1]	na	na	289.5 (105.2)	307 (11)
Days since 2nd vaccination[1,4]	na	na	281 (25)	286 (11)
Hour vaccine dose 1[1,5]	16 (4)	17 (3)	16 (4)	18 (3.3)
Hour vaccine dose 2[1,6]	16 (3)	17 (2)	17 (3)	17 (2)
Adverse events dose 1[7]				
None	65 (26.3%)	122 (61.0%)	58 (29.9%)	115 (61.2%)
Local	53 (21.5%)	36 (18.0%)	48 (24.7%)	32 (17.0%)
Systemic	99 (40.1%)	42 (21.0%)	88 (45.4%)	41 (21.8%)
Adverse events dose 2[8]				
None	55 (22.3%)	83 (41.5%)	44 (22.7%)	79 (42.0%)
Local	10 (4.0%)	19 (9.5%)	10 (5.2%)	19 (10.1%)
Systemic	76 (30.8%)	98 (49%)	69 (35.6%)	90 (47.9%)
Smoking				
No	175 (70.9%)	117 (58.5%)	132 (68.0%)	109 (58.0%)
Ex-smoker	54 (21.9%)	30 (15.0%)	50 (25.8%)	29 (15.4%)
Yes	18 (7.3%)	53 (26.5%)	12 (6.2%)	50 (26.6%)
Baseline comorbidities	148 (59.9%)	120 (60%)	124 (63.9%)	112 (59.6%)
Days between onset symptoms and vaccination[1,9]	304 (69)	na	303.5 (69.8)	na
Hospitalization first COVID-19 episode	25 (10.1%)	na	21 (10.8%)	na
Intensive care unit first COVID-19 episode	1 (0.4%)	na	1 (0.5%)	na
Oxygen first COVID-19 episode	16 (6.5%)	na	15 (7.7%)	na
Any symptoms first COVID-19 episode	247 (100%)	na	194 (100%)	na
Duration symptoms (days)[1]	22 (21.5)	na	20.5 (19.8)	na
Post COVID condition	117 (47.4%)	na	95 (49%)	na

na = not applicable; NA = missing data. See Table S1 for detailed list of comorbidities and symptoms.

[1] Median (IQR)

[2] Social worker, customer service, technician, driver, maintenance worker, IT worker, X-ray technician, others

[3] na (no doses received): in the full study cohort, 30 (6.7%) among all, 30 (12.1%) among exposed

[4] na (without dose 2): 71 (all), 71 (pre-exposed)

[5] Full study cohort: na (without dose 1): 30 (all), 30 (pre-exposed). NA (missing data from dose 1): 22 (all), 17 (pre-exposed), 5 (naive)
T8 only: NA (missing data from dose 1): 18 (all), 14 (pre-exposed), 4 (naive)

[6] Full study cohort: na (without dose 2): 106 (all), 106 (pre-exposed). NA (missing data from dose 2): 10 (all), 3 (pre-exposed), 7 (naive)
T8 only: na (without dose 2): 71 (all), 71 (pre-exposed). NA (missing data from dose 2): 8 (all), 2 (pre-exposed), 6 (naive)

[7] Full study cohort: na (without dose 1): all 30 (6.7%), pre-exposed 30 (12.1%)

[8] Full study cohort: na (without dose 2): All 106 (23.7%), pre-exposed 106 (42.9%); T8 only: All 71 (18.6%), pre-exposed 71 (36.6%)

[9] Full study cohort: na (without dose 1): 30

586

587 **Table 2.** Percentage seropositivity after vaccination stratified by prior COVID-19 status.

	T4	T5		T6		T7		T8	
	Exposed (n=79)	**Naive (n=200)**	**Exposed (n=133)**	**Naive (n=198)**	**Exposed (n=165)**	**Naive (n=188)**	**Exposed (n=187)**	**Naive (n=188)**	**Exposed (n=194)**
Positive	97.47%	100%	100%	100%	100%	100%	100%	100%	100%
IgA	96.20%	98%	96.99%	89.90%	96.97%	81.38%	95.72%	93.09%	97.94%
IgA N CT	84.81%	6.50%	44.36%	2.02%	15.76%	2.66%	12.83%	4.79%	11.34%
IgA N FL	50.63%	3%	20.30%	2.02%	9.09%	3.72%	6.95%	10.11%	19.07%
IgA RBD	94.94%	87%	93.98%	39.90%	86.67%	31.91%	86.63%	62.23%	95.36%
IgA S	96.20%	97.50%	96.99%	88.89%	96.97%	80.32%	95.72%	93.09%	97.94%
IgA S2	93.67%	75%	93.23%	26.77%	80.61%	17.55%	78.61%	43.62%	93.30%
IgG	97.47%	100%	100%	100%	100%	100%	100%	100%	100%
IgG N CT	88.61%	10%	67.67%	0%	19.39%	2.13%	12.30%	0.53%	0.52%
IgG N FL	59.49%	1%	36.84%	1.01%	12.73%	1.06%	4.28%	6.91%	27.32%
IgG RBD	96.20%	100%	100%	100%	100%	100%	100%	100%	100%
IgG S	97.47%	100%	100%	100%	100%	100%	100%	100%	100%
IgG S2	96.20%	99.50%	100%	92.93%	98.18%	73.94%	96.26%	67.02%	96.39%
IgM	68.35%	42.50%	65.41%	9.60%	17.58%	5.85%	12.30%	22.34%	38.66%
IgM N CT	1.27%	2.50%	2.26%	1.01%	1.21%	0%	1.07%	3.19%	2.06%
IgM N FL	1.27%	1%	0%	1.52%	0%	0.53%	0%	2.66%	1.03%
IgM RBD	48.10%	17%	45.86%	1.52%	11.52%	1.60%	8.02%	7.98%	30.93%
IgM S	58.23%	38.50%	48.12%	7.58%	6.67%	3.72%	3.21%	11.17%	13.92%
IgM S2	40.51%	7%	20.30%	0%	2.42%	0%	2.14%	1.06%	8.25%

588
589 T4 – January/February 2021, T5 – March/April 2021, T6 – May/June 2021, T7 – July 2021, T8 – November 2021.
590 n, number of individuals who donated samples per timepoint.
591 Participants with diagnosis of COVID-19 before vaccination were classified as exposed, and those without prior COVID-19 diagnosis (recruited at T5) as naïve.
592

Table 3. Percentage decrease in seropositivity to receptor binding domain (RBD) after vaccination for variants of concern (VoC) compared to Wuhan, stratified by COVID-19 status.

	T6		T7		T8	
	Naive (n=198)	**Exposed (n=165)**	**Naive (n=188)**	**Exposed (n=187)**	**Naive (n=188)**	**Exposed (n=194)**
IgA RBD Alpha	-2.53[1]	-0.69	3.32	0.62	-3.42	0.53
IgA RBD Beta	87.34	42.66	78.35	47.54	72.65	35.68
IgA RBD Gamma	62.03	19.58	58.32	24.08	34.18	11.89
IgA RBD Delta	5.06	0.7	21.65	1.24	-15.39	0
IgG RBD Alpha	0	0	1.06	0	0	0
IgG RBD Beta	9.6	1.21	11.7	3.21	9.04	0.52
IgG RBD Gamma	0	0	1.6	1.07	1.06	0
IgG RBD Delta	0	0	1.06	1.07	0	0
IgM RBD Alpha	-165.79	26.39	-66.25	33.29	-19.92	-1.75
IgM RBD Beta	33.55	89.5	100	100	73.31	91.65
IgM RBD Gamma	66.45	89.5	66.88	93.39	53.38	76.63
IgM RBD Delta	-199.34	36.89	-99.38	19.95	-79.2	-33.46

Calculated as (seropositivity RBD Wuhan – seropositivity RBD VoC) / seropositivity RBD Wuhan * 100

[1] Negative percentage values indicate increase in seropositivity

Table 4. Variables significantly associated with antibody levels to spike antigens post vaccination (T8) by multivariable linear regression models.

	IgM RBD		IgM S		IgM S2		IgA RBD		IgA S		IgA S2		IgG RBD		IgG S		IgG S2	
	Beta[1]	p-value	Beta	p-value	Beta	p-value	Beta	p-value	Beta	p-value	Beta	p-value	Beta	p-value	Beta	p-value	Beta	p-value
FULL COHORT																		
Age	-1,06	0,0002	-1,50	<0,0001	-1,89	<0,0001									0,77	0,0427		
Sex (ref: male)			23,95	0,0074														
Cohort (ref: exposed)	-38,29	<0,0001	-13,51	0,03	-34,87	<0,0001	-78,05	<0,0001	-70,47	<0,0001	-78,67	<0,0001	-74,75	<0,0001	-63,99	<0,0001	-74,57	<0,0001
Occupation (ref: other[2])																		
Physician or dentist									-25,80	0,0484	-26,16	0,0492			-21,13	0,032		
Nurse or auxiliary nurse															-22,40	0,0186	-22,42	0,0205
Smoking (ref: no)													-39,97	0,0006	-24,80	0,0164	-21,53	0,0443
Days since 1st vaccination													-0,36	0,0046	-0,28	0,0072	-0,24	0,0226
Adverse events dose 1 (ref: none)																		
Systemic													32,88	0,0224				
Adverse events dose 2 (ref: none)[3]																		
Local																	56,68	0,0189
Systemic													43,06	0,0125	42,66	0,0018	45,21	0,0017
Days between onset symptoms and dose 1[4]							0,38	<0,0001	0,30	<0,0001			0,19	0,0084	0,15	0,0123		
PRE-EXPOSED COHORT																		
Age	-1,38	0,0037	-1,51	0,0002	-1,69	0,0036									1,02	0,0371		
Occupation (ref: other)																		
Physician or dentist																		
Days between onset symptoms and dose 1							0,32	0,0001	0,24	0,0019			0,17	0,0042	0,14	0,0044	0,11	0,045
Adverse events dose 1 (ref: none)																		
Systemic													34,17	0,042				
Adverse events dose 2 (ref: none)																		
Local													147,75	0,0043	99,88	0,0112	105,21	0,0226
Systemic													61,03	0,0106	36,93	0,0493	43,87	0,0495
Hospitalization first COVID-19 episode									190,0	0,0242	170,12	0,0418	321,31	0,0001	200,69	0,0003	144,39	0,0067
Oxygen first COVID-19 episode									-66,4	0,041	-71,24	0,0242						
Post COVID condition	-19,41	0,0387																
Symptoms first COVID-19 episode																		
Anosmia/Ageusia													37,66	0,0097	36,31	0,003	47,89	0,0006
Dispnea													42,13	0,0098	28,31	0,0289	35,90	0,0139
Fever													40,29	0,0153	31,52	0,0195	38,09	0,0118
Shivers			18,00	0,0454														

[1] Beta coefficient has been transformed into percentage for easier interpretation (see Materials & Methods)
[2] Other: social worker, customer service, technician, driver, maintenance worker, IT worker, X-ray technician, others.
[3] 71 na (not applicable) values: not vaccinated with dose 2. Separate model removing the variable number of doses because all individuals included had received 2 doses.
[4] 188 na (not applicable) values: naive cohort, without onset of symptoms. Separate model removing variable cohort because all individuals included were pre-exposed.

610 **Table 5.** Effect of one hour increase in the dose 1 vaccination time (from 8.30 am) on
611 levels of IgG antibodies up to 11 months later in health care workers with prior history
612 of COVID-19.

613

Antigens	%Beta	95% CI	P-value
S	-3.81	-0.0325; -0.0013	0.034
RBD Wuhan	-5.45	-0.0429; -0.0057	0.011
RBD Alpha	-5.66	-0.0442; -0.0064	0.009
RBD Beta	-4.38	-0.0381; -0.0008	0.041
RBD Delta	-5.00	-0.0406; -0.0039	0.018
RBD Gamma	-4.95	-0.0418; -0.0023	0.029
S2	-3.21	-0.032; 0.0037	0.118

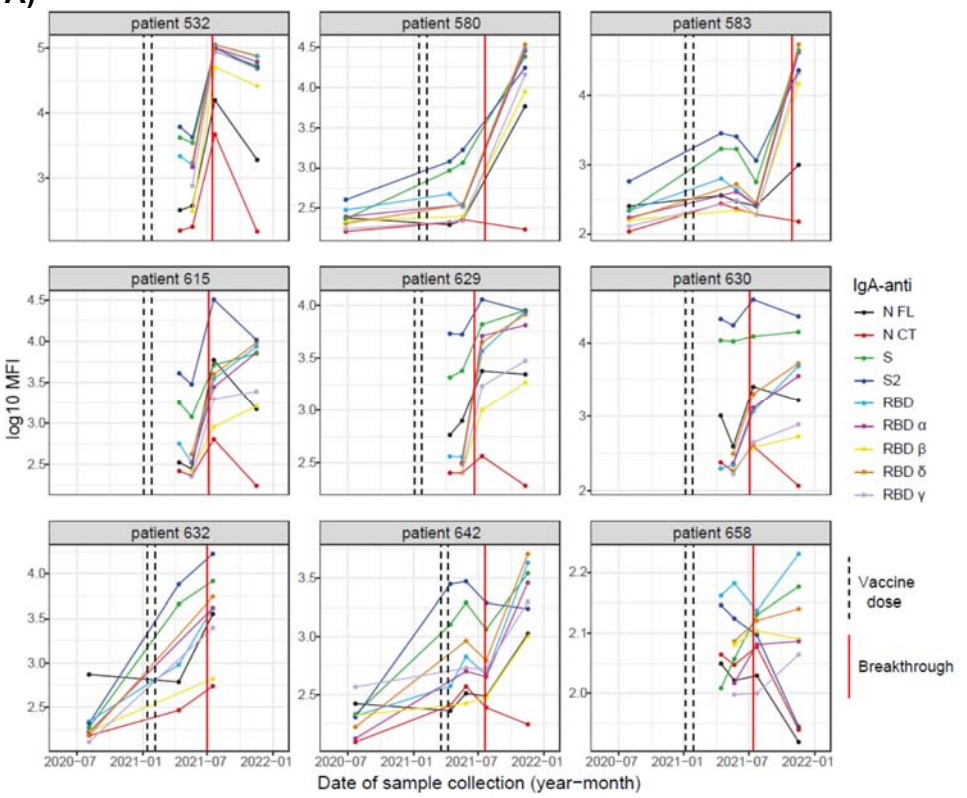
614 Multivariable models including as regression variables also age, sex, professional category,
615 vaccine adverse events, time between onset of symptoms and dose 1, hospitalization, oxygen
616 support, symptoms (fever, shivers, dyspnea, anosmia/ageusia, digestive, dyslipidemia) and
617 their duration. Significant associations or trends are shown.

618 Negative transformed beta coefficient means that there is a decrease in antibodies with
619 increased hours.
620

SUPPLEMENTARY MATERIAL

Figure S1. Changes in IgA **(A)** and IgM **(B)** levels (log_{10}MFI) in nine vaccinated patients after symptomatic breakthrough infection. Antigens: nucleocapsid full length (N FL) and C-terminus (N CT), spike full length (S), S2 subunit, and receptor-binding domain (RBD) for wild type and variants of concern.

A)



B)

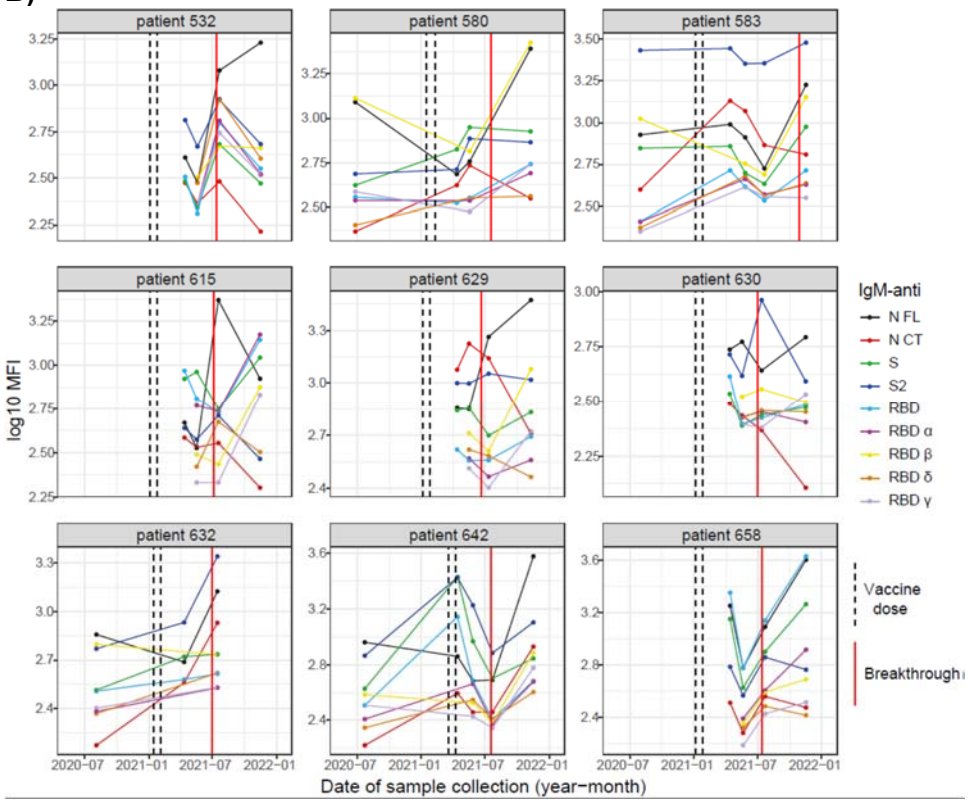


Table S1. Detailed baseline comorbidities and symptoms of the entire study cohort (up to timepoint 8), and when antibodies assessed (11 months after vaccination).

	Full study cohort		Timepoint 8	
	Pre-exposed	**Naive**	**Pre-exposed**	**Naive**
	N=247 (55.3%)	N=200 (44.7%)	N=194 (50.8%)	N=188 (49.2%)
Baseline comorbidities				
Allergy	41 (16.6%)	16 (8.0%)	33 (17.0%)	16 (8.5%)
Asthma	16 (6.5%)	15 (7.5%)	12 (6.2%)	14 (7.4%)
Autoimmune	23 (9.3%)	15 (7.5%)	19 (9.8%)	15 (8.0%)
Cancer	10 (4.0%)	10 (5.0%)	9 (4.6%)	10 (5.3%)
Cardiac disease	4 (1.6%)	3 (1.5%)	2 (1.0%)	3 (1.6%)
Depression	51 (20.6%)	47 (23.5%)	42 (21.6%)	42 (22.3%)
Digestive disease	22 (8.9%)	13 (6.5%)	19 (9.8%)	13 (6.9%)
Dyslipidemia	35 (14.2%)	25 (12.5%)	32 (16.5%)	23 (12.2%)
Diabetes Mellitus	6 (2.4%)	3 (1.5%)	4 (2.1%)	3 (1.6%)
Hypotiroidism	26 (10.5%)	13 (6.5%)	23 (11.9%)	12 (6.4%)
Hypertension	20 (8.1%)	13 (6.5%)	16 (8.2%)	12 (6.4%)
COPD	1 (0.4%)	2 (1.0%)	1 (0.5%)	2 (1.1%)
Chronic kidney disease	2 (0.8%)	0 (0.0%)	1 (0.5%)	0 (0.0%)
Neurological disease	24 (9.7%)	26 (13.0%)	22 (11.3%)	23 (12.2%)
Immunodepression	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Pregnancy	3 (1.2%)	0 (0.0%)	3 (1.5%)	0 (0.0%)
Obesity	26 (10.5%)	16 (8.0%)	22 (11.3%)	16 (8.5%)
Symptoms first COVID-19 episode				
Anosmia/Ageusia	147 (59.5%)	na	116 (59.8%)	na
Fever	178 (72.1%)	na	136 (70.1%)	na
Shivers	121 (49.0%)	na	94 (48.5%)	na
Headache	179 (72.5%)	na	138 (71.1%)	na
Dizziness	44 (17.8%)	na	32 (16.5%)	na
Myalgia/Arthralgia	144 (58.3%)	na	114 (58.8%)	na
Cough	145 (58.7%)	na	118 (60.8%)	na
Dispnea	81 (32.8%)	na	63 (32.5%)	na
Thorax pain	44 (17.8%)	na	29 (14.9%)	na
Digestive	136 (55.1%)	na	102 (52.6%)	na
Asthenia	192 (77.7%)	na	149 (76.8%)	na
Oftalmological	43 (17.4%)	na	31 (16.0%)	na
Cutaneous	35 (14.2%)	na	30 (15.5%)	na
Odynophagia	82 (33.2%)	na	64 (33.0%)	na
Sputum	16 (6.5%)	na	10 (5.2%)	na
Tachycardia	33 (13.4%)	na	24 (12.4%)	na
Neurological	22 (8.9%)	na	15 (7.7%)	na
Otorhino	113 (45.7%)	na	89 (45.9%)	na
Thrombosis	1 (0.4%)	na	1 (0.5%)	na

na = not applicable

Table S2. Seropositivity over time after vaccination.

	T4 (n=79)		T5 (n=333)		T6 (n=363)		T7 (n=375)		T8 (n=382)	
	n	%	n	%	n	%	n	%	n	%
Total	77	97.47%	333	100%	363	100%	375	100%	382	100%
IgA	76	96.20%	325	97.60%	338	93.11%	332	88.53%	365	95.55%
IgA N CT	67	84.81%	72	21.62%	30	8.26%	29	7.73%	31	8.12%
IgA N FL	40	50.63%	33	9.91%	19	5.23%	20	5.33%	56	14.66%
IgA RBD	75	94.94%	299	89.79%	222	61.16%	222	59.20%	302	79.06%
IgA S	76	96.20%	324	97.30%	336	92.56%	330	88.00%	365	95.55%
IgA S2	74	93.67%	274	82.28%	186	51.24%	180	48.00%	263	68.85%
IgG	77	97.47%	333	100%	363	100%	375	100%	382	100%
IgG N CT	70	88.61%	110	33.03%	32	8.82%	27	7.20%	2	0.52%
IgG N FL	47	59.49%	51	15.32%	23	6.34%	10	2.67%	66	17.28%
IgG RBD	76	96.20%	333	100%	363	100%	375	100%	382	100%
IgG S	77	97.47%	333	100%	363	100%	375	100%	382	100%
IgG S2	76	96.20%	332	99.70%	346	95.32%	319	85.07%	313	81.94%
IgM	54	68.35%	172	51.65%	48	13.22%	34	9.07%	117	30.63%
IgM N CT	1	1.27%	8	2.40%	4	1.10%	2	0.53%	10	2.62%
IgM N FL	1	1.27%	2	0.60%	3	0.83%	1	0.27%	7	1.83%
IgM RBD	38	48.10%	95	28.53%	22	6.06%	18	4.80%	75	19.63%
IgM S	46	58.23%	141	42.34%	26	7.16%	13	3.47%	48	12.57%
IgM S2	32	40.51%	41	12.31%	4	1.10%	4	1.07%	18	4.71%

T4 – January/February 2021, T5 – March/April 2021, T6 – May/June 2021, T7 – July 2021, T8 – November 2021.
n, number of individuals who donated samples per timepoint

Table S3. Seropositivity over time after vaccination for variants of concern.

	T6			T7			T8		
	Naive (n=198)	Exposed (n=165)	Total (n=363)	Naive (n=188)	Exposed (n=187)	Total (n=375)	Naive (n=188)	Exposed (n=194)	Total (n=382)
IgA RBD Wuhan	39.9	86.67	61.16	31.91	86.63	59.2	62.23	95.36	79.06
IgA RBD Alpha	40.91	87.27	61.98	30.85	86.09	58.4	64.36	94.85	79.84
IgA RBD Beta	5.05	49.7	25.34	6.91	45.45	26.13	17.02	61.34	39.53
IgA RBD Gamma	15.15	69.7	39.94	13.3	65.77	39.47	40.96	84.02	62.83
IgA RBD Delta	37.88	86.06	59.78	25	85.56	55.2	71.81	95.36	83.77
IgG RBD Wuhan	100	100	100	100	100	100	100	100	100
IgG RBD Alpha	100	100	100	98.94	100	99.47	100	100	100
IgG RBD Beta	90.4	98.79	94.21	88.3	96.79	92.53	90.96	99.48	95.29
IgG RBD Gamma	100	100	100	98.4	98.93	98.67	98.94	100	99.48
IgG RBD Delta	100	100	100	98.94	98.93	98.93	100	100	100
IgM RBD Wuhan	1.52	11.52	6.06	1.6	8.02	4.8	7.98	30.9	19.63
IgM RBD Alpha	4.04	8.48	6.06	2.66	5.35	4	9.57	31.44	20.68
IgM RBD Beta	1.01	1.21	1.1	0	0	0	2.13	2.58	2.36
IgM RBD Gamma	0.51	1.21	0.83	0.53	0.53	0.53	3.72	7.22	5.5
IgM RBD Delta	4.55	7.27	5.79	3.19	6.42	4.8	14.3	41.24	28.01

T6 – May/June 2021, T7 – July 2021, T8 – November 2021.
n, number of individuals who donated samples per timepoint