Antibody response to BTN162b2 mRNA vaccination in naïve versus SARS-CoV-2 infected subjects with and without waning immunity

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3

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25 **Abstract**

26 *We profiled antibody responses in a cohort of recipients of the BTN162b2 mRNA*
27 *vaccine who were either immunologically naïve (n=50) or had been previously*
28 *infected with SARS-CoV-2 (n=51). Of the previously infected, 25 and 26 were*
29 *infected during the first and second pandemic waves in Italy, respectively; the*
30 *majority of those from the first wave had corresponding waning immunity with low to*
31 *undetectable levels of anti-S antibodies and low anti-N antibodies. We observed in*
32 *recipients who had been previously infected that spike-specific IgG and pseudovirus*
33 *neutralization titers were rapidly recalled by a single vaccine dose to higher levels*
34 *than those in naïve recipients after the second vaccine dose, irrespective of waning*
35 *immunity. In all recipients, a single vaccine dose was sufficient to induce a potent*
36 *IgA response that was not associated with serum neutralization titers.*

37

38 As we write, four COVID-19 vaccines have been authorized for use by FDA and/or
39 EMA, and additional vaccine candidates are under evaluation. The authorized
40 vaccines are based on the use of mRNA ¹ or adenoviral vectors ² that induce the
41 expression of the SARS-CoV-2 spike protein. Apart from the Johnson & Johnson
42 adenovirus-based vaccine that requires only a single dose, all other vaccines are based
43 on a double dose regimen to maximize their efficacy.

44 Increasingly available anti-SARS-CoV-2 antibody and virus-specific T cell data
45 support a strategy that previously infected (P.I.) vaccine recipients have sufficient
46 immune response from only one vaccine dose;³⁻⁶ this would have significant impact
47 on global vaccine supply. Spike-specific IgG antibody levels and ACE2 antibody
48 binding inhibition responses elicited by a single vaccine dose in individuals with prior
49 SARS-CoV-2 infection (P.I.) were similar to those seen after two doses of vaccine in

50 individuals without prior infection (naïve) ⁷. In another study, the antibody titers of
51 recipients with preexisting immunity were 10 to 45-fold higher than naïve recipients
52 at the same time points after the first vaccine dose of vaccine, and no increases in
53 antibody titers were observed in P.I. recipients who received the second vaccine dose
54 ⁸. Similarly, Bradley et al reported that after the first vaccine dose, recently infected
55 P.I. recipients had higher titers of antibodies to the S1 and S2 subunit and the
56 receptor-binding domain (RBD) of the spike protein compared to naïve recipients
57 who had received the two doses of vaccine ⁹. Interestingly, at baseline, naïve
58 recipients exhibited a significant level of reactivity to the S2 subunit, suggesting a
59 pre-existing cross-reactive response to common coronavirus infections ⁹. In another
60 study, antibody binding to a trimeric spike protein and live-virus neutralization assays
61 performed on a cohort of volunteers who received one dose of an mRNA vaccine
62 showed a rapid response in P.I. recipients with antibody titers raising at 7 days and
63 reaching a peak 10-14 days post-vaccination, a kinetic that was significantly faster
64 than that observed in naïve individuals ¹⁰. In addition, two of these studies reported
65 that vaccine reactogenicity was more prominent in P.I. individuals after the first dose
66 but similar between the two groups after the second dose ^{7,8}. A cautionary tale for the
67 use of a two doses regimen in P.I. individuals was raised by Levi et al who considered
68 the possibility of antibody-dependent enhancement ¹¹ or antigen exhaustion as a result
69 of an over-boosting of immune responses ¹². The lack of an established correlate of
70 protection against disease and/or infection adds further complexity. The emergence of
71 SARS-CoV-2 variants is also a concern, and Stamatatos et al. highlighted the
72 importance of two dose regimens in both naïve and P.I. individuals to achieve cross-
73 variant neutralizing antibodies ¹³. An additional component of the immune response to
74 SARS-CoV-2 that could influence the outcome of vaccination is the presence in most

75 SARS-CoV-2 naïve individuals of variable levels of pre-existing immunity to spike
76 protein epitopes that are shared with other common human coronaviruses (hCoVs);
77 ^{14,15} these have been suggested to be potentially protective or pathogenic and may
78 shape the kinetic and potency of the immune response to the vaccine ¹⁶⁻¹⁹.
79 Here we report data from a serological profile of a cohort of 101 naïve and P.I.
80 recipients who received both doses of Pfizer-BioNtech BNT162b2 mRNA vaccine.
81 We took advantage of the availability of two different sub-groups of P.I. recipients
82 who experienced a SARS-CoV-2 infection during the first wave (Spring 2020) and
83 the second wave (Autumn 2020) of the pandemic in Northern Italy to investigate the
84 different effects of vaccination in recipients with recent-active or past-waning
85 immunity. Antibody levels were measured at three time points: prior to first
86 vaccination (T0), prior to second vaccination (T1), and three weeks after the second
87 vaccination (T2). As the BNT162b2 vaccine is expected to elicit only IgG-S
88 antibodies, we also tested all subjects for the presence of IgG-nucleocapsid (IgG-N)
89 antibodies to identify recipients with past infection. The IgG-N antibody test is also a
90 reliable marker of enduring immunity to SARS-CoV-2 ²⁰ and as such can be used to
91 monitor waning immunity. Since natural infection with SARS-CoV-2 is often
92 followed by a rapid rise in IgG antibodies that can occur concomitantly or even before
93 the appearance of IgM antibodies ^{14,21-23} we tested whether a similar pattern would
94 follow vaccination by measuring both IgG antibodies specific for the RBD of the
95 spike protein (IgG-S(RBD)) and IgM spike-specific antibodies (IgM-S).
96 IgA have also been implicated in protective immunity to SARS-CoV-2, but, to the
97 best of our knowledge, there are no available data on the IgA response following
98 vaccination and no potential association between vaccine-induced IgA response and
99 serum virus-neutralizing activity. During natural SARS-CoV-2 infection, IgA

responses precede IgG responses²⁴⁻²⁶. Whether this is also the case after vaccination is unknown. We therefore tested all sera for the presence of IgG-N, IgM-S, IgG-S(RBD), and IgA-S as well as for the presence of virus-neutralizing activity as measured in a pseudovirus neutralization assay.

We enrolled 101 healthcare workers with (P.I.) or without (naïve) preexisting immunity to SARS-CoV-2. Of the 51 P.I. vaccinees, 25 had been infected during the first wave and 26 during the second wave. All subjects received the first vaccine dose (BNT162b2 mRNA, Pfizer-BioNTech) in January 2021. The two groups were homogeneous in age and sex (Table 1).

IgG-N antibody testing was negative in all naïve recipients and positive in 24/51 (47%) P.I. recipients (Fig. 1A). A single subject, originally classified as naïve, who resulted negative at baseline but highly positive at T1 and T2 for the presence of IgG-N was excluded from the analysis. In the P.I. group, at baseline, 7/25 (28%) and 17/26 (65%) of those who were infected during the first and second waves, respectively, were positive for IgG-N antibodies, consistent with a trend toward waning immunity in recipients infected during the first wave (Fig. 2A and Table 2).

IgM-S antibodies measured before vaccination (T0) showed a similar pattern with 8/25 (32%) and 16/26 (61%) testing positive at baseline in the first and second wave P.I. recipients, respectively (Fig. 2B and Table 2). Following vaccination, 27/50 (54%) naïve recipients became IgM-S positive after the first dose, and 29/49 (59%, 20 of whom were already positive after the first dose) were positive after the second dose with no significant increase in antibody titers compared to baseline (Fig. 1B and Table 2). In P.I. recipients from the first or second wave, there was no significant difference in the IgM-S response after vaccination (Fig. 2B and Table 2).

124 IgG-S(RBD) were detectable after the first vaccine dose in 49/50 (98%) naïve
125 recipients but with very low titers that were boosted by the second vaccine dose
126 resulting in a highly significant increase ($p<0.0001$) (Fig. 1D and Table 2). Forty-
127 six/51 (90%) P.I. recipients showed low IgG-S(RBD) titers at baseline (Fig. 1D and
128 Table 2), and the first vaccine dose induced a strong increase in IgG-S(RBD) reaching
129 levels that were 12-fold higher than titers observed in naïve subjects after the first
130 dose (naïve T1: 1673 ± 1787 ; P.I. T1: 20131 ± 18990 ; $p<0.0001$) and 1.9-fold higher
131 when comparing P.I. and naïve recipients after the second dose (naïve T2:
132 19551 ± 10941 ; P.I. T2: 37607 ± 22895 ; $p=0.4583$, ns), whereas it was similar when
133 comparing P.I. recipients after the first dose to naïve recipients after the second dose
134 (naïve T2 19551 ± 10941 ; P.I. T1: 20131 ± 18990 , $p>0.9999$, ns). The second vaccine
135 dose in P.I. recipients did not result in a significant increase in antibody titers
136 consistent with data reported by other authors ^{7-9,27}. Comparison of P.I. recipients
137 infected during the two waves did not show any significant difference in the responses
138 to the first and second vaccine dose (Fig. 2D and Table 2), although there was an
139 unexpectedly higher (although not statistically significant) response to the first dose in
140 those infected during the first wave compared with those infected during the second
141 wave, with recipients showing binding titers as high as 80000 AU/ml.

142 The pseudovirus neutralization assay (TCID₅₀) essentially mirrored the results of the
143 IgG-S(RBD) assay with 47/50 (94%) naïve recipients showing a weak but positive
144 score (>120 for TCID₅₀) after the first dose and 49/49 (100%) after the second dose,
145 but with a highly significant ($p<0.0001$) increase in neutralizing titers (Fig. 1E). In
146 P.I. recipients, we observed the same pattern seen in the IgG-S(RBD) assay with an
147 efficient boost of neutralizing antibodies after the first dose ($p<0.0001$) and no further
148 increase after the second dose (Fig. 1E).

A correlation analysis between the IgG-S(RBD) and the pseudovirus neutralization assay (TCID₅₀) confirmed a strong association between serum IgG-S(RBD) and neutralizing titers (Fig. 3A) consistent with other reports showing a major role played by RBD-specific antibodies in virus neutralization²⁸. Results of the two waves P.I. cohorts also mirrored those obtained with the IgG-S(RBD) and evidenced a surprisingly, although not statistically significant, higher virus-neutralization response in P.I. recipients of the first wave compared with the second, with TCID₅₀ titers as high as 1/4000 (Fig. 2E). These data are strongly suggestive of the persistence of memory B cell responses that can be rapidly recalled by a single vaccine dose after 9-10 months from primary infection even in the absence of detectable serum IgG-S(RBD) antibodies. It is also conceivable that natural infection with SARS-CoV-2 may prime the immune system to produce antibody specificities other than RBD that can be readily recalled by a single dose of vaccine.

The potential implication of cross-reactive immunity to other coronaviruses in the response to vaccination is supported by an unexpected feature that emerged from our data: the unconventional isotype pattern observed in both naïve and P.I recipients. In the naïve recipients, after the first dose, when the canonical primary immune response is expected to generate IgM first followed by IgG, only 27/50 (54%) recipients were positive for IgM-S with no further increase after the second dose (29/49; 59%), whereas 49/50 (98%) and 49/49 (100%) naïve recipients scored positive for IgG-S(RBD) after the first and second dose respectively (Table 2). Twenty-three/50 (46%) naïve recipients showed an IgG-S(RBD) positive test but were negative for IgM-S, 27/50 (54%) were positive for both IgG-S(RBD) and IgM-S, and none were positive for IgM-S and negative for IgG-S(RBD) (Table 3). This isotype pattern is consistent

with that of an anamnestic response sustained by memory B cells specific for spike epitopes shared with other common human coronaviruses ^{14,15}.

At baseline, IgA-S were detected in none of the naïve recipients, in 18/25 (72%) of the P.I. recipients who were infected during the first wave, and in 22/26 (84%) of those infected during the second wave (Fig. 1C and Table 2). The first vaccine dose resulted in a significant increase ($p<0.0001$) in IgA-S titers in both naïve and P.I. recipients (Fig. 1C). The second dose further boosted the IgA-S titers in naïve recipients ($p<0.0001$) but led to a significant reduction in P.I. recipients ($p=0.0099$). The decline in IgA titers after the second vaccination in P.I. recipients was not related to the time from infection, as it was observed in both subjects infected during the first and second wave and most likely represents a response to the vaccination (Fig. 2C). A correlation analysis between the IgA-S and IgG-S(RBD) titers at baseline and T1 and T2 revealed in naïve subjects the appearance of high IgA titers after the first vaccine dose followed by a significant increase in IgG-S(RBD) titers only after the second dose (Fig. 3B). In P.I. recipients, the first and second dose boosted both IgA-S and IgG-S(RBD) titers (Fig. 3B). We did not observe a correlation between IgA-S titers and virus-neutralization titers (TCID₅₀) both in naïve and P.I. recipients (Fig. 3C). On the other hand, the early increase in IgA after the first dose that preceded the increase in IgG-S(RBD) titers after the second vaccine dose is consistent with what has been observed during natural SARS-CoV-2 infection where IgAs precede IgGs ^{25,26,29}. The presence of IgA-S in a significant proportion of P.I. recipients at baseline is also consistent with the slower waning of IgA compared to IgG observed in convalescent patients ³⁰.

We next examined the influence of gender in the IgG-S(RBD) and IgA-S responses to vaccination. In the naïve recipients, there were no differences in the kinetic and size

of IgG-S(RBD) responses between males and females. In contrast, in the P.I group, males responded to the vaccine by producing higher titers of IgG-S(RBD) than females ($p<0.05$) (Fig. 4A). This difference was reflected by the neutralization assay, which showed higher neutralizing titers in males than in females (Fig.4B). The differences between the two groups were significant in male and female naïve IgA-S responses at T1 ($p<0.05$) and T2 ($p<0.01$) (Fig.4C).

Taken together these data show that: 1) immunologically naïve recipients react to the first dose of vaccine with a low-titer IgG-S(RBD) response that is then boosted by the second dose; 2) in previously infected recipients one dose of vaccine is sufficient to induce antibody titers that are higher than those observed in naïve recipients after the second vaccine dose; 3) there is a good correlation between IgG-S(RBD) titers and virus neutralizing titers, confirming that the IgG-S(RBD) testing is a proxy for virus neutralization; 4) recipients who were infected with SARS-CoV-2 during the first pandemic wave exhibit a rapid response to vaccination measured as both IgG-S(RBD) binding titers and virus neutralizing titers; 5) in 46% of naïve recipients, IgG-S(RBD) appear in the absence of IgM-S as if the response to vaccination was influenced by previous antigen exposures; 6) in naïve recipients IgA-S appear before IgG-S(RBD) after the first vaccine dose and are further boosted by the second dose; 7) in previously infected recipients, IgA show a rapid, high titer response to the first vaccine dose that is followed by a decline with the second dose; 8) there is no correlation between IgA-S antibody titers and virus neutralization; 9) neutralization titers and IgG-S(RBD) were higher in previously infected male recipients compared to female but not in naïve recipients.

Our findings expand on previous studies that indicated higher levels of anti-S antibodies at baseline and after a single mRNA vaccine dose in previously infected

223 individuals compared with those without prior infection, suggesting that a second
224 vaccine dose does not offer P.I. recipients a substantially greater benefit over a single
225 dose in antibody neutralization. The availability of two sub-groups of recipients who
226 had been infected during the first and second waves of the pandemic in Italy gave us
227 the additional opportunity to evaluate the effects of one dose versus two doses of
228 vaccination in the context of a past-waning immunity and compare it with that of
229 recent-active immunity. The lower IgG-N and IgG-S(RBD) antibody titers observed
230 at baseline in the first wave P.I. vaccines compared to the second wave P.I. vaccines
231 (Fig. 2A) gave us confidence that the two cohorts were in two different stages of post-
232 infection immunity. However, when the IgA-S antibody titers were considered, no
233 substantial differences were observed between the two cohorts at baseline, indicating
234 that serum IgAs may be a better marker of long-lasting immunity and that
235 immunological memory may be more long-lasting than what previously estimated on
236 the basis of IgG antibody levels alone. This was further substantiated by the surprising
237 rapid recall of high IgG-S(RBD) and virus neutralizing titers observed in first wave
238 P.I. recipients even in individuals with absent or very low serum IgG-S(RBD) levels.
239 Although not statistically significant, the response observed in the first wave P.I.
240 recipients was greater than that observed in the second wave P.I. recipients, probably
241 as a result of the persistence of memory B cell responses that can be rapidly recalled
242 by a single dose and consistent with recent data on the appearance in convalescent
243 patients of memory B cells with a turnover time of 6 months that express antibodies
244 with increased somatic hypermutations, neutralizing breadth, and potency ³¹. The
245 possibility that natural infection with SARS-CoV-2 may prime the immune system to
246 produce antibody specificities other than RBD, further expanding the antibody
247 repertoire induced by vaccination, should also be considered. Our study did not

248 address the epitope specificities of vaccination-induced antibodies, and additional
249 studies addressing the fine specificities of vaccine-induced antibodies are warranted.
250 A priming effect of previous exposures to common human coronaviruses on the
251 immune response to the vaccine is suggested by our findings in the cohort of naïve
252 recipients showing IgG-S(RBD) antibodies in the absence of IgM-S antibodies, an
253 isotype pattern typical of anamnestic responses. Antibodies that cross-neutralize
254 SARS-CoV-1, SARS-CoV-2, and other coronaviruses have been described that bind
255 conserved epitopes of the hACE2 binding site showing extensive conservation among
256 the SARS-like coronaviruses ³². It remains to be determined whether the BTN162b2
257 mRNA vaccine is capable of eliciting these types of antibodies.

258 Our findings on the more rapid and potent IgA response compared with IgG responses
259 to the first vaccine dose parallel those in SARS-CoV-2 infected patients, where IgA
260 antibodies that bind to SARS-CoV-2 are produced rapidly after infection and remain
261 elevated in the plasma for at least 40 days after the onset of symptoms ³³. We did not
262 see a significant association between serum IgA and virus-neutralizing activity post-
263 vaccination, which, in contrast, has been reported in COVID-19 patients ²⁸. However,
264 it is plausible that the types of IgA antibodies elicited by intramuscular vaccination
265 may differ from the compartmentalized, mucosal immune response to natural
266 infection. Accordingly, SARS-CoV-2 specific plasma IgA monomers have been
267 shown to be two times less potent than IgG equivalents and, in contrast, IgA dimers,
268 the principal type of antibody in the nasopharynx, are 15 times more potent against
269 the same target as IgA monomers.³³

270 Our data clearly show a rapid and potent induction of IgG-S(RBD) after a dose of
271 vaccine in individuals with past-waning immunity. Although a clear correlate of
272 protection from COVID-19 has not yet been identified, the recent findings by Zohar

and colleagues that, notwithstanding equivalent IgM and IgA immunity to the virus observed in different disease severity levels, rapid and potent IgG class switching is associated with survival ³⁴ provide an additional argument in support of the use of a single-dose vaccine regimen in individuals with prior SARS-CoV-2 infections. Limitations of our study include the design (cross-sectional), the limited sample size, the use of only one type of vaccine (Pfizer-BioNTech BNT162b2), the lack of information on T-cell responses, and neutralization response against emerging SARS-CoV-2 variants of concern.

Methods

The sera of 101 healthcare workers with and without pre-existing immunity for SARS-CoV-2 (as per former nasal swab positivity) who received their first vaccine dose (BNT162b2 mRNA, Pfizer-BioNTech) in January 2021 were analyzed. Samples had been collected and stored in the University of Verona biobank (Ethics Committee approval prot. N. 1538) and in Tropica Biobank of the IRCCS Sacro Cuore Don Calabria Hospital (Ethics Committee approval prot. N. 50950). All participants signed informed consent.

The SARS-CoV-2 IgG-N assay and the SARS-CoV-2 IgM-S assay (Abbott, Ireland) are chemiluminescent microparticle immunoassays (CMIA) used to detect IgG antibodies to the nucleocapsid protein and IgM antibodies to the spike protein, respectively, of SARS-CoV-2 in human serum. The automated assay was performed according to the manufacturer's procedure, using the ARCHITECT I System (Abbott). The resulting chemiluminescent reaction was measured as a relative light unit (RLU) by the system optics. The RLU of the sample (S) was automatically compared with the RLU of a specific calibrator I, resulting in an assay index (S/C).

298 As per manufacturer's instructions, the interpretation of the results were as follow:
 299 index (S/C)<1.4 = negative, index (S/C)≥1.4 = positive for IgG-N, and index (S/C)<1
 300 = negative, index (S/C)≥1 = positive, for IgM-S.
 301 Serum samples were tested for the presence of SARS-CoV-2 IgA using the Anti-
 302 SARS-CoV-2 ELISA kit (EUROIMMUN Medizinische Labordiagnostika AG,
 303 Germany). The assay detects IgA antibodies in serum binding the S1 domain of the
 304 SARS-CoV-2 spike protein. Samples were tested and analyzed as recommended by
 305 the manufacturer, and results reported as a ratio based on sample O.D. divided by the
 306 O.D. of the calibrators. Antibodies were considered undetectable (negative result) if
 307 the ratio was less than 0.8, borderline (inconclusive) between 0.8 and 1.1, and positive
 308 if greater 1.1.
 309 The SARS-CoV-2 IgG II Quant assay (Abbott, Ireland) is a CMIA used for the
 310 quantitative measure of IgG-S(RBD) antibodies (including neutralizing Abs) in
 311 human serum. The automated assay was performed according to the manufacturer's
 312 procedure, using the ARCHITECT I System (Abbott). Results were reported as
 313 Arbitrary Unit (AU)/mL, according to the following interpretation: AU/mL<50 =
 314 negative, AU/mL≥50 = positive. According to the WHO International Standard for
 315 anti-SARS-CoV-2 immunoglobulin binding antibody units (BAU), the AU/mL are
 316 converted into BAU by the equation: AU/mL* 0.142 = BAU/mL.
 317 Lentiviral particles pseudotyped with SARS-CoV-2 spike were produced in 10 cm
 318 plates seeded the day before with 3 million HEK293T cells in 10 ml of complete
 319 DMEM, supplemented with 10% FBS. Cells were transfected using the Calcium
 320 Phosphate technique with 15 µg of an Env-defective SIV-Mac239 provirus construct
 321 expressing GFP in place of Nef³⁵ and 1.5 µg pCDNA3.1 expression vector encoding
 322 the WT SARS-CoV-2 spike (reference sequence Wuhan-Hu-1, accession number

YP_009724390) with a truncation of the C-terminal 19 amino acids. Supernatants containing pseudotyped virions were harvested 48 hours post-transfection, filtered through a 0.45- μ m filter, and frozen at -80°C until used. Sera neutralization titers were assayed on Huh-7 cells engineered to overexpress the SARS-CoV-2 receptor ACE2 upon stable transduction with a lentiviral expression vector. Target cells were seeded on 384-well tissue culture plates one day before neutralization. Virus inoculum was adjusted to produce no more than 10% of monolayer transduction to ensure a linear working range of the assay. Sera dilutions were added to target cells using an acoustic dispenser (Beckman Echo 650) to reach the indicated dilution in DMEM with 10% FBS. Pseudotyped virus was then added to wells using a Tecan Evo® 200 liquid handler. After 48 hours, transduction was assessed by calculating the percentage of GFP-expressing cells upon nuclei counterstaining with Hoechst 33342 and measuring using the High Content Molecular Device Image Xpress® Micro Confocal. Each serum dilution was evaluated in triplicate. Neutralization was measured by calculating the residual transduction activity of the pseudovirus considering the untreated sample as 100%. Fitted sigmoidal curves and IC₅₀ were obtained using Prism (Graphpad) with the least square variable slope method and using the normalized dose response protocol.

Statistical analysis

P values were calculated using the non-parametric two-tailed unpaired Kruskal-Wallis test (Fig. 1 and Fig. 2), the two-sided Spearman rank-correlation test (Fig. 3), the Wilcoxon matched-pairs signed ranked test (Fig. 4), and the chi-squared test (Table 1) using SPSS (version 22, SPSS Inc.) and Prism 9 (GraphPad Software, LLC).

Reporting Summary

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

Data availability

Requests for data may be directed to the corresponding authors. Data limitations are designed to ensure patients and participant confidentiality.

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Author Contributions:

L.D.C. designed the study, enrolled patients, analyzed data and discussed results; M.T.V. designed the study, collected samples and clinical data; Z.B. and C.P. designed the study, collected data, managed data and critically revised the manuscript; M.P. and S.A. performed and analyzed pseudovirus neutralization assays; S.M., S.F., D.A. L.M., N.T. and S.S.L. participated in data collection and analysis; A.M., J.B., V.L.V., G.D. and E.C. collected samples and clinical data; L.L. analyzed and

discussed data and critically revised the manuscript; A.B. analyzed and discussed data, drafted and wrote the manuscript; D.Z. designed and coordinated the study, analyzed data and results, prepared figures and tables, drafted and wrote the manuscript.

Competing Interests Statement

All authors declare no competing financial interests.

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471

Tables

Table 1. Characteristics of the study population

	Naïve (50)	P.I. (51)	p
Males/Females	13/37	19/32	p=0.23 (ns)
Age (yrs)	43±13	46±12	p=0.21 (ns)
Fever after 1 st dose (%)	7.7	0	p=0.40 (ns)
Discomfort after 1 st dose (%)	26.9	22.2	p=0.78 (ns)
Fever after 2 nd dose (%)	38.5	33.3	p=0.79 (ns)
Discomfort after 2 nd dose (%)	53.8	66.7	p=0.51 (ns)

P.I: Previously infected; ns: not significant

479 **Table 2. Antibody and neutralization assays (positives/total and percent)**

Test	Time	Naïve	P.I.	1 st wave	2 nd wave
IgG-N	T0	0/48 (0%)	24/51 (47.1%)	7/25 (28%)	17/26 (65.4%)
	T1	0/50 (0%)	23/51 (45.1%)	7/25 (28%)	16/26 (61.5%)
	T2	0/49 (0%)	21/49 (42.9%)	6/25 (24%)	14/24(58.3%)
IgM-S	T0	0/48 (0%)	24/51 (47.1%)	8/25 (32%)	16/26 (61.5%)
	T1	27/50 (54%)	23/51 (45.1%)	8/25 (32%)	15/26 (57.7%)
	T2	29/49 (59.2%)	15/49 (30.6%)	5/25 (20%)	10/24 (41.7%)
IgG-S(RBD)	T0	0/48 (0%)	46/51 (90.2%)	23/25 (92%)	23/26 (88.5%)
	T1	49/50 (98%)	50/51 (98%)	25/25 (100%)	25/26 (96.2%)
	T2	49/49 (100%)	49/49 (100%)	25/25 (100%)	24/24 (100%)
IgA-S	T0	0/49 (0%)	40/51 (78.4%)	18/25 (72%)	22/26 (84.6%)
	T1	44/50 (88%)	51/51 (100%)	25/25 (100%)	26/26 (100%)
	T2	49/49 (100%)	49/49 (100%)	25/25 (100%)	24/24 (100%)
TCID50	T0	2/48 (4.2%)	46/51 (90.2%)	23/25 (92%)	23/26 (88.5%)
	T1	47/50 (94%)	51/51 (100%)	25/25 (100%)	26/26 (100%)
	T2	49/49 (100%)	50/50 (100%)	25/25 (100%)	25/25 (100%)

494 P.I.: Previously Infected

495 **Table 3. Number and percent of IgM-S and IgG-S(RBD) positive subjects**

496

		Naïve			P.I.			1 st wave			2 nd wave		
IgM	IgG	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
+	+	0 (0%)	27 (54%)	29 (59.2%)	24 (47.1%)	22 (43.1%)	15 (30.6%)	8 (32%)	8 (32%)	5 (20%)	16 (61.5%)	14 (53.8%)	10 (41.7%)
+	-	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3.8%)	0 (0%)
-	+	1 (2.1%)	23 (46%)	20 (40.8%)	22 (43.1%)	28 (54.9%)	34 (69.4%)	15 (60%)	17 (68%)	20 (80%)	7 (26.9%)	11 42.3%)	14 (58.3%)
-	-	47 (97.9%)	0 (0%)	0 (0%)	5 (9.8%)	0 (0%)	0 (0%)	2 (8%)	0 (0%)	0 (0%)	3 (11.5%)	0 (0%)	0 (0%)

497

498 P.I.: Previously Infected

499

Figure legends

Fig. 1: analysis of the antibody response profile at the time of first vaccination (T0), second vaccination (T1), and 3 weeks after the boost (T2) in naïve and previously infected (P.I.) recipients. Median values with the interquartile range are displayed. The horizontal dot lines indicate the limit of each assay, according to the manufacturer's instructions. Panel A: IgG for the SARS-CoV-2 nucleocapsid protein N; panel B: IgM for the spike glycoprotein; panel C: IgA for the spike glycoprotein; panel D: IgG for the receptor-binding domain (RBD) of the spike; panel E: pseudoviruses neutralization assay, expressed as median tissue culture infectious dose (TCID50). Sample sizes are reported in Table 2. P values were calculated using the non-parametric, two-tailed unpaired Kruskal-Wallis test. Differences were considered significant if $p < 0.05$.

Figure 2: analysis of the antibody response profile at the time of first vaccination (T0), second vaccination (T1) and 3 weeks after (T2) in subjects infected during the first (orange dots) and the second (red dots) COVID-19 wave. Median values with the interquartile range are displayed; the horizontal dot lines indicate the limit of each assay, according to the manufacturer's instructions. Panel A: IgG for the SARS-CoV-2 nucleocapsid protein N; panel B: IgM for the spike glycoprotein; panel C: IgA for the spike glycoprotein; panel D: IgG for the receptor-binding domain (RBD) of the spike; panel E: neutralization assay, expressed as median tissue culture infectious dose (TCID50). Sample sizes are reported in Table 2. P values were calculated using the non-parametric, two-tailed unpaired Kruskal-Wallis test. Differences were considered significant if $p < 0.05$.

525

526 **Figure 3: analysis of the correlation at time of first vaccination (T0), second**
527 **vaccination (T1), and 3 weeks after (T2) in naïve (top row) and previously**
528 **infected (P.I.) recipients (bottom row).** Panel A: correlation between IgG-S(RBD)
529 and neutralization (TCID50); panel B: correlation between IgG-S(RBD) and IgA;
530 panel C: correlation between neutralization (TCID50) and IgA-S. Sample sizes are
531 reported in Table 2. The correlation was calculated using the two-sided Spearman
532 rank-correlation test.

533

534 **Figure 4: antibody response in naïve and previously infected (P.I.) male (blue**
535 **dots) and female (red dots) subjects following vaccination.** Panel A: IgG-S(RBD)
536 titers at the time of first vaccination (T0, n. males/females 12/36 for naïve, 19/32 for
537 P.I.), second vaccination (T1, n. males/females 13/37 for naïve, 19/32 for P.I.), and 3
538 weeks after the boost (T2, n. males/females 13/36 for naïve, 19/30 for P.I.); panel B:
539 neutralization activity expressed as TCID50 (n. males/females: T0 12/36, T1 13/37,
540 T2 13/36 for naïve, T0 19/32, T1 19/32, T2 19/31 for P.I.); panel C: IgA-S antibody
541 titers (n. males/females: T0 12/37, T1 13/37, T2 13/36 for naïve, T0 19/32, T1 19/32,
542 T2 18/31 for P.I.). P-values were calculated using the Wilcoxon matched-pairs signed
543 ranked test and considered significant if $p < 0.05$.

Figures

Figure 1

analysis of the antibody response profile at the time of first vaccination (T0), second vaccination (T1), and 3 weeks after the boost (T2) in naïve and previously infected (P.I.) recipients. Median values with the interquartile range are displayed. The horizontal dot lines indicate the limit of each assay, according to

the manufacturer's instructions. Panel A: IgG for the SARS-CoV-2 nucleocapsid protein N; panel B: IgM for the spike glycoprotein; panel C: IgA for the spike glycoprotein; panel D: IgG for the receptor-binding domain (RBD) of the spike; panel E: pseudoviruses neutralization assay, expressed as median tissue culture infectious dose (TCID50). Sample sizes are reported in Table 2. P values were calculated using the non-parametric, two-tailed unpaired Kruskal-Wallis test. Differences were considered significant if $p < 0.05$.

Figure 2

analysis of the antibody response profile at the time of first vaccination (T0), second vaccination (T1) and 3 weeks after (T2) in subjects infected during the first (orange dots) and the second (red dots) COVID-19 wave. Median values with the interquartile range are displayed; the horizontal dot lines indicate the limit of each assay, according to the manufacturer's instructions. Panel A: IgG for the SARS-CoV-2 nucleocapsid protein N; panel B: IgM for the spike glycoprotein; panel C: IgA for the spike glycoprotein; panel D: IgG for the receptor-binding domain (RBD) of the spike; panel E: neutralization assay, expressed as median tissue culture infectious dose (TCID₅₀). Sample sizes are reported in Table 2. P values were calculated using the non-parametric, two-tailed unpaired Kruskal-Wallis test. Differences were considered significant if $p < 0.05$.

A - TCID50 vs IgG-S(RBD)

B - IgA-S vs IgG-S(RBD)

C - IgA-S vs TCID50

Figure 3

analysis of the correlation at time of first vaccination (T0), second vaccination (T1), and 3 weeks after (T2) in naïve (top row) and previously infected (P.I.) recipients (bottom row). Panel A: correlation between IgG-S(RBD) and neutralization (TCID50); panel B: correlation between IgG-S(RBD) and IgA; panel C: correlation between neutralization (TCID50) and IgA-S. Sample sizes are reported in Table 2. The correlation was calculated using the two-sided Spearman rank-correlation test.

Figure 4

antibody response in naïve and previously infected (P.I.) male (blue dots) and female (red dots) subjects following vaccination. Panel A: IgG-S(RBD) titers at the time of first vaccination (T0, n. males/females 12/36 for naïve, 19/32 for P.I.), second vaccination (T1, n. males/females 13/37 for naïve, 19/32 for P.I.), and 3 weeks after the boost (T2, n. males/females 13/36 for naïve, 19/30 for P.I.); panel B: neutralization activity expressed as TCID50 (n. males/females: T0 12/36, T1 13/37, T2 13/36 for naïve, T0 19/32, T1

19/32, T2 19/31 for P.I.); panel C: IgA-S antibody titers (n. males/females: T0 12/37, T1 13/37, T2 13/36 for naïve, T0 19/32, T1 19/32, T2 18/31 for P.I.). P-values were calculated using the Wilcoxon matched-pairs signed ranked test and considered significant if $p < 0.05$.