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How do successive vaccinations and SARS-CoV-2 infections impact humoral immunity dynamics: an 18-month longitudinal study.

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The authors declare no competing interest.

Dear editor,

Informed decisions regarding COVID-19 vaccine administration and pandemic management strategies have been substantiated by empirical evidence concerning immune responses and vaccine effectiveness. Thus, recent studies published in your journal showed the advantages of using mixed vaccine strategies instead of homogenous ones[1] or that the benefit of hybrid immunity varied in function of vaccination history[2]. In the post-pandemic period, an increasing number of infection and vaccination history profiles are emerging within the population, leading to the formation of groups with distinct susceptibilities to

new infections. It is therefore essential to continue informing health policies with real-world data collected on long-term studies from representative cohorts in order to rationalize administration of future booster doses to subgroups of the population, particular at the time when their immune defenses are expected to drop below a protective threshold.

Here, we examined the influence of the type and number of vaccines, breakthrough infection and their chronological occurrence on humoral immune responses against SARS-CoV-2 within groups characterized by various sociodemographic factors and comorbidities. Employing linear mixed models, we examined the dynamics of anti-Spike IgG and SARS-CoV-2 neutralizing antibodies titers in a cohort of 1347 students and staff members from the University of Liège, Belgium, from April 2021 to December 2022.[3] Blood samples were collected at several time points after exposure to SARS-CoV-2 antigens, defined as either an infection (I), complete primary vaccination (P) or an mRNA-1273 or BNT162b2 booster dose (B). SARS-CoV-2 infections were monitored by quantifying anti-SARS-CoV-2 nucleocapsid antibodies and by weekly saliva RT-qPCR testing. The participant flowchart and description are presented in Supplementary Methods, Figures S1-2, and Tables S1-6.

After the first exposure, controlling for Rhesus status, diabetes, and autoimmune diseases, older age was significantly associated with lower humoral immune response.[4-5] Overall, the groups with the highest antibody levels (anti-Spike and neutralizing) were, in decreasing order, those vaccinated with mRNA-1273, BNT162b2, ChadOx1, infected once, and finally those vaccinated with Ad26.COV2.S.[6] (Figure 1; Supplementary Table S7). Participants vaccinated with mRNA-1273 and BNT162b2 presented a faster decline of humoral immunity than people infected once or those vaccinated with Ad26.COV2.S. (Figure 1; Supplementary Tables S8-9).

After two exposures, participants infected after primary vaccination (PI) showed the highest antibody levels and better maintained their humoral immunity. Conversely, the infected twice (II) group displayed the lowest antibody levels, but a slower immunity waning when compared to participants infected before their primary vaccination (IP group) or primary vaccinated and boosted (PB group) (Figure 1; Supplementary Table S7). Further analyses revealed an interaction effect between primary vaccine types and groups. Yet, the only significant effect was the induction of higher titers in previously infected participants after a subsequent vaccination with mRNA-1273 or BNT162b2, compared with ChAdOx1 (Supplementary Table S10-11; Figure S3).

After three exposures, participants with the highest anti-Spike IgG and neutralizing antibody titers were those primary vaccinated, boosted and infected after boosting (PBI). They also appeared to maintain their anti-Spike IgG and neutralizing antibodies better than boosted individuals infected prior to primary vaccination (Figure 1; Supplementary Tables S7-9). This suggested that, in hybrid immunity participants, infection after primary vaccination (two exposures) or after boosting (three exposures) induced stronger immune responses, at least against Spike protein, in agreement with previous results.[7]

One explanation could relate to the fact that infection provided a different source of antigens that were probably presented in another context and had therefore an increased boosting effect.

After four exposures, lower levels of anti-Spike IgG and neutralizing antibodies were observed in older people, particularly those over 50 years. The group of primary vaccinated, boosted, infected and reboosted (PBIB) participants displayed the highest antibody levels compared to the three other groups. Next, participants that were infected before their primary vaccination and after their boost (IPBI) showed more stable anti-Spike IgG levels than the two other groups, namely participants primary vaccinated and infected before and after their boost (PIBI), and participants primary vaccinated, boosted and then infected twice (PBII) (Supplementary Table S7).

We finally compared the anti-Spike IgG and neutralizing antibodies levels between groups of different numbers and type of exposures. As expected, anti-Spike IgG and neutralizing antibody levels were higher in participants with multiple exposures (Figure 2), with notable exceptions. We observed that participants infected twice (II) and those infected before and after their primary vaccination (IPI) displayed similar anti-Spike IgG levels compared to people only primary vaccinated with an mRNA-based vaccine (i.e., mRNA-1273 or BNT162b2) (Figure 2A-B). Interestingly, in those groups (II and IPI), a single additional exposure was almost always beneficial (Figure 2C-F).

An infection before the primary vaccination did not always guarantee a better immune response, or could even have a detrimental effect, highlighted by lower or at least similar, levels of anti-Spike IgG in IPB, IPI as compared to PB or PI groups (Figure 2C); or lower neutralizing and anti-Spike IgG levels in participants infected before vaccination, boosted and then infected (IPBI) compared to the participants who presented the same pathway without an initial infection (PBI) (Figure 2E-F). However, an infection after primary vaccination or boost or the occurrence of a second infection in unvaccinated participants enhanced the levels of anti-Spike IgG and neutralizing antibodies (Figure 2A-D). This could be related to the competition between memory B cells for antigens upon infection[8] or immune imprinting by infections with different viral strains that could shape the following hybrid immune responses.[9] In particular, it had been shown that infection by the ancestral Wuhan Hu-1 strain before vaccination prevented the enhancement of immune responses observed in previously uninfected, vaccinated and boosted individuals who experienced a breakthrough infection with Omicron after vaccination.[10] Our results importantly showed that the benefit of this hybrid immunity was observed when the first exposure to SARS-CoV-2 was a vaccination and the infection then occurs, not the other way round.

The strengths of our study are the longitudinal follow-up, the diversity of individual profiles including the nature of the vaccines and the rigorous characterization of the infection status of each participant through routine PCR-based testing and serology. Altogether, our results highlight the benefit of (re)-vaccination for those uninfected-primary vaccinated, infected-only, and immune-vulnerable participants. These results also highlight that a first exposure to SARS-CoV-2 antigens through a

massive campaign of vaccination followed by virus circulation and revaccination of targeted populations was the best strategy to maintain a high immune response, both at the population and individual level.

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Author contributions:

Conceptualisation and methodology: D.A.N., S.J., G.C., G.M., D.G., S.C., D.D., D.A.F., B.F., L.G.; data curation: D.A.N., G.N., H.E.; formal analysis: D.A.N.; funding acquisition: F.B., L.G.; investigation: S.J., G.C., G.E., M.F., E.O., B.G.; project administration: P.M., G.N., H.E.; supervision: G.M., D.A.F., B.F., L.G.; writing – original draft: D.A.N, S.J., G.C., L.G.; and writing– review & editing: all authors.

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Competing interests

The authors declare no competing interest.

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Figure Legends

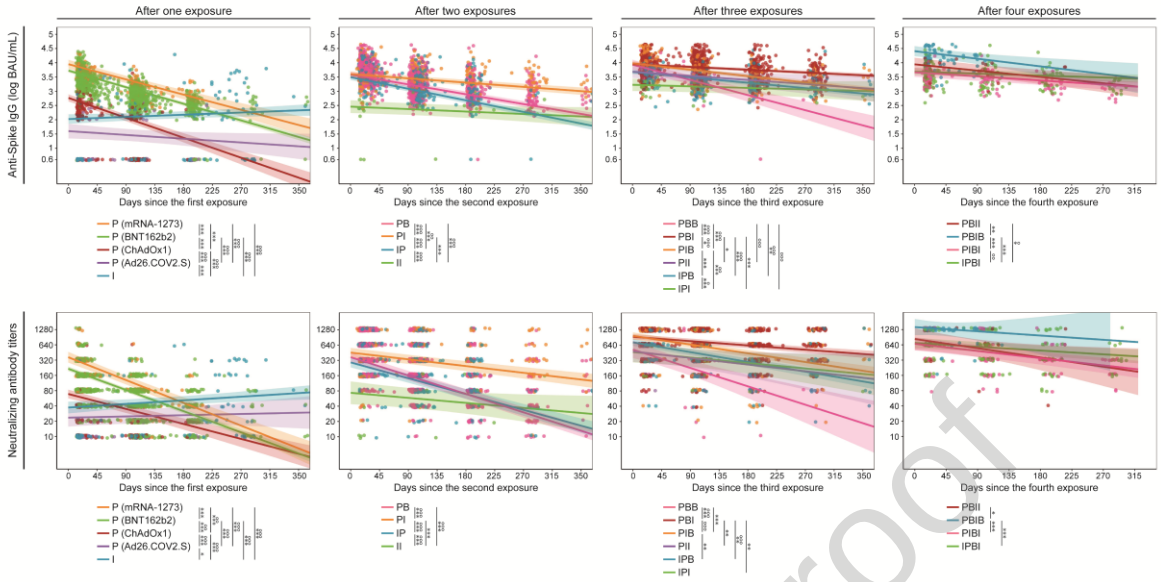

Figure 1. Trends of anti-Spike IgG and neutralizing antibodies over time among groups with different numbers, types and sequences of exposures. Data points represent individual participants, and the solid lines represent trend-estimates from a linear mixed model, with shaded areas showing the 95% confidence interval. Acronyms are used to indicate the sequence of antigen exposure with I = infection, P = primary vaccination and B = booster vaccination. Statistically significant differences between antibody levels (*) and waning slopes (°) are displayed. $*^{/\circ}$ $p < 0.05$; $**^{/\circ\circ}$ $p < 10^{-2}$; $***^{/\circ\circ\circ}$ $p < 10^{-3}$.

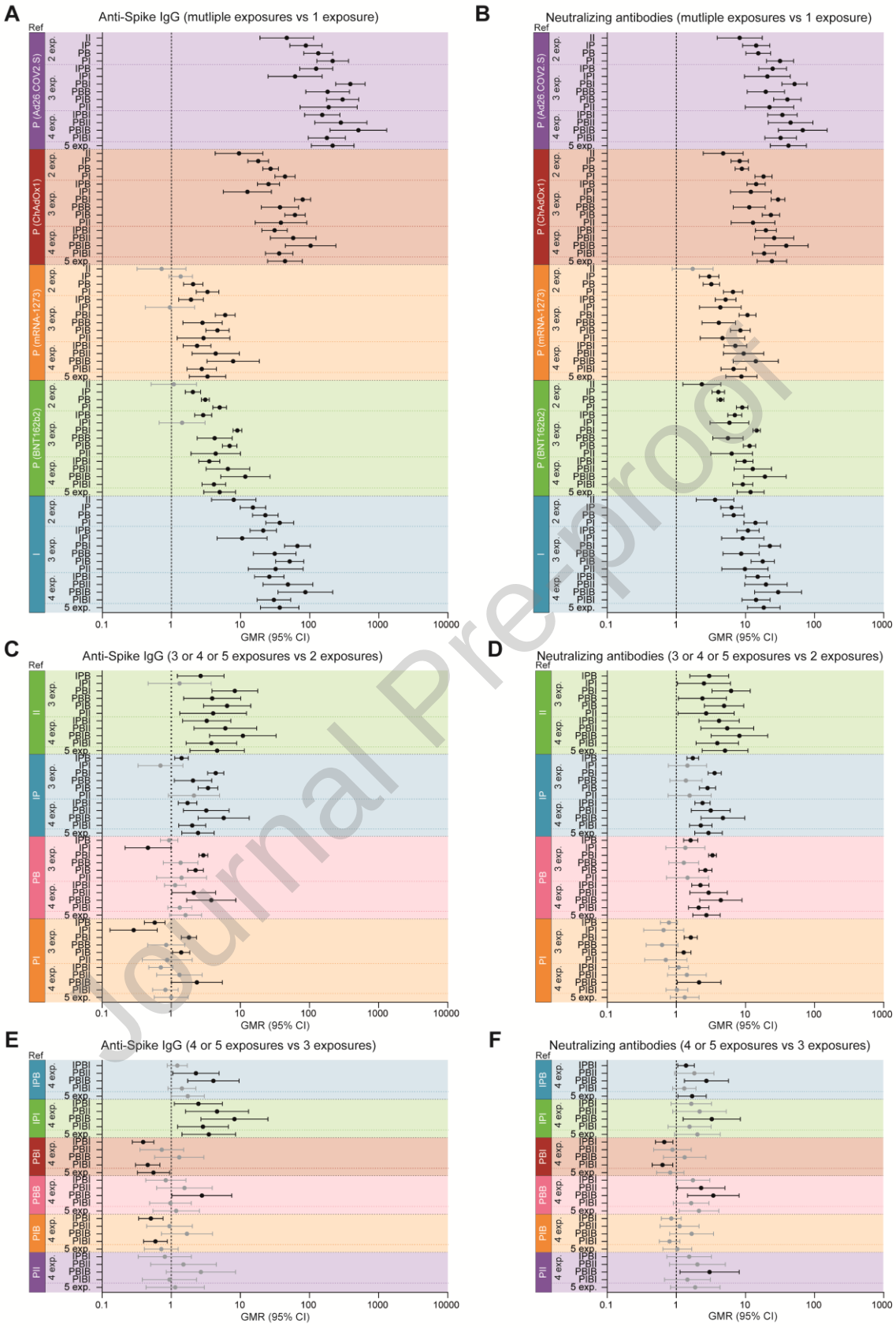

Figure 2. Comparisons of anti-Spike IgG (A, C, E) and neutralizing antibodies (B, D, F) levels between groups with different numbers of exposures. The data incorporated all the data analyzed at each level of exposure. Acronyms are used to indicate the sequence of antigen exposure with I = infection, P =

primary vaccination and B = booster vaccination. Global mean ratios and 95% confidence intervals are shown, and ratios that are not significant ($p \geq 0.05$ and the CI containing the value 1) are highlighted in grey.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: