Age and vitamin D affect the magnitude of the antibody response to the first dose of the SARS-CoV-2 BNT162b2 vaccine

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Key Points

Evidence before this study

Immunization is the most important strategy to facilitate the end of the global pandemic. Vaccines, authorized for use globally, have shown more than 90% efficacy against SARS-CoV-2 clinical disease in

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clinical trials. In an effort to ensure maximal coverage by vaccinating as many people as possible with one dose (of the 2-dose vaccines such as the Pfizer/BioNTech) the UK government extended the recommended interval for the second dose from three weeks to 10-12 weeks. Most studies to date only evaluate the serological response at a single time point post 1st dose or investigate the antibody response up to three weeks. Many studies simply look for the qualitative absence or presence of antibodies with a very limited number investigating the quantitative antibody response over an extended time period. In addition, most studies do not investigate factors which affect the magnitude of antibody response.

Added value of this study

This is the first study to quantitatively assess antibody concentrations at multiple time points post first dose of the BNT162b2 mRNA (Pfizer-BioNTech) COVID-19 vaccine in Healthcare workers, with no known previous infection, showing positive concentrations of IgG after 8 weeks. Moreover, younger deciles (≤40yr) showed an initial greater antibody production. Levels at week 8 were similar across ages. Vitamin D concentrations ≥50nmol/L improved the antibody response to a dose of SARS-CoV-2 BNT162b2 (Pfizer/BioNTech) vaccine.

Implications of all the available evidence

Both younger and older adults retained positive concentrations of antibodies against SARS-CoV-2, 8 weeks after the first dose of vaccine. Booster immunization should be administered following sunshine exposure or after vitamin D supplementation.

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Abstract (max 250) –250

Background Most approved vaccines utilise a two-dose strategy. To enable larger groups of patients to receive the first dose, the UK government increased the gap between the two doses from three to 12 weeks. Here we report on the immunogenicity of the first dose, including effect of age and vitamin D status on these levels over an 8 week-period.

Methods Blood was collected from healthcare workers (HCW) receiving their first BNT162b2 vaccine dose between January and February 2021. Antibody production was measured, prior to and weekly for 4 weeks post immunization, and a final measurement was performed at 8 weeks. Vitamin D were also measured at baseline.

Findings Immunization of 97 HCW induced an Ab response that peaked 3·2 weeks post immunization to decrease thereafter. Ab levels remained positive at 8 weeks. The response was significantly modified by age ($p<0·001$) and greater in younger adults. Response to immunization was significantly affected by vitamin D status ($p=0·035$), on average 29·3% greater peak value in individuals with 25(OH)D>50nmol/L. No other variable showed significant effect.

Interpretation The first dose of BNT162b2 produced Ab levels that remained positive after 8 weeks. Peak was greater in younger subjects and 25(OH)D>50nmol/L was beneficial. Booster campaigns should take into consideration vitamin D status which is at its highest following a period of sunshine exposure or following oral supplementation (400-1000IU daily).

Funding Abbott Diagnostics Ltd supplied the kits used to quantify the anti-SARS -CoV-2 Spike IgG and technical support as well as provided financial support for sample collections.

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Introduction

As SARS-CoV-2 continues to affect the world, large scale immunization programmes are having a significant impact on hospitalization rates and mortality. Pfizer/BioNTech BNT162b2, thereafter BNT162b2, was the first COVID-19 vaccine authorized for use in the UK[1]. The decision of the UK government to extend the recommended interval between the two doses from three to 12 weeks[2, 3] was received with scepticism around the potential loss or degradation of the immune response, especially as trial data was based on the shorter interval of three weeks.

Ageing is known to be accompanied by dysregulation of immune system functions and a higher incidence with more severe outcomes of respiratory infections such as pneumonia [4] and an increased occurrence of cancers [5] and autoimmune diseases [6]. A number of publications have suggested a beneficial effect of vitamin D supplementation against severe COVID-19 symptoms [7] and supplementation was recommended by the UK government in specific high risk population groups [8]. Moreover, vitamin D, specifically 1-alpha,25-dihydroxyvitamin D3, is known to modulate the innate and adaptive immune response [9, 10] and vitamin D is often used as a candidate hormone in improving immune response [11, 12].

In this study we followed the anti-SARS-CoV-2 Spike IgG production in a cohort of health care workers (HCW), over eight weeks after receiving the first dose of the BNT162b2 mRNA vaccine, using a newly developed and validated assay that can quantify the antibody concentrations. We investigated the relationship between IgG response post immunization and 25(OH)D concentrations, age and other demographics.

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Methods

Study Design and Subject Cohorts

All participants provided written informed consent. The study was approved by the Health Research Authority Health and Care Research Wales ethical committee (IRAS#292799).

We assessed the immune response generated after immunization with BNT162b2 (Pfizer Inc. [New York, USA] and BioNTech SE [Mainz, Germany]) in a cohort of 105 HCW immunized at the Norfolk and Norwich University Hospital (Norwich, UK). Serum samples were taken at baseline (within a week prior to immunization, Bo), followed by weekly (7±1 days) sampling for four weeks (W1 to W4) after the first vaccine dose was administered and at week eight (W8), prior to administration of the second dose. A questionnaire provided self-declared demographic characteristics (age, gender, ethnicity), clinical characteristics (weight, height, health issues, current medication), and the presence of COVID-19 symptoms within the last six months.

Biochemistry

Abbott Alinity i system Immunoassays (Abbott Park, IL, USA) were used to measure SARS-CoV-2 anti-spike IgG (quantitative) and anti-nucleocapsid IgG (qualitative) in serum. Quantitative results are reported in AU/mL and the positivity cut-off as per manufacturer's instruction is 50AU/mL. Qualitative results are positive for index (OD_{sample}/OD_{cal}) results above 1·4. These assays were previously validated [13, 14]. Baseline 25(OH)D was measured by LC-MS/MS and 1,25$(OH)_2$D was measured by Immunoassay (DiaSorin, Dartford, UK).

Statistical Analysis

All statistical analyses and graphical representation were performed using IBM SPSS Statistics 25.0.0.1 and/or GraphPad Prism version 9.0 (GraphPad Software, Inc., USA). Statistical significance was considered as a two-tailed P value <0·05 (*P<0·05; **P<0·01 and ***P<0·001). IgG concentrations over

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time were analyzed using a mixed model paired test. Increase and decrease of antibody concentrations were analyzed by linear regression using age (use as continuous or categorized by deciles), BMI (categorised), $1,25(OH)_2D$ (continuous variable) and 25(OH)D (continuous and categorised variable) as predictors. Circulating 25(OH)D serves as an indicator of vitamin D status. Guidelines on vitamin D in bone health as outlined by Royal Osteoporosis Society 2018[15] define circulating 25(OH)D<25nmol/L as deficient, 25(OH)D between 25 and 50nmol/L as inadequate (insufficient) and 25(OH)D>50nmol/L as adequate or replete. We used these groupings to analyse 25(OH)D as a categorical variable.

Results

Participants

A total of 105 HCW provided at least one blood sample for antibody testing prior to immunization (baseline, Bo). After excluding HCW who had a positive antibody baseline (58·1 to 5496·9 AU/mL, n=6) and those who did not seroconvert after immunization (anti-Spike IgG concentration not reaching values greater than 22·0 AU/mL, n=2), the remaining 97 HCW (mean [SD] age, 40·9 [11.0] years; 76 [78·4%] women; mean [SD] BMI, 26·4 [5·8]; 87 (89·7%) white; mean [SD] 25(OH)D; 47·5 [30·1], mean [SD] $1,25(OH)_2D$; 108·2 [34·3] pmol/L) were included in the study. The baseline characteristics of the participants appear in Table 1.

Table 1. Population characteristics. Characteristics of the HCW at baseline. Two non-responders and six HCW with a positive anti-Spike IgG were excluded.

	Baseline (pre-Dose 1)
N	97

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Age in years, mean (SD)	40·9 (11·0)
Weight in kg, mean (SD)	74·4 (18·6)
Height in cm, mean (SD)	167·4 (8·4)
BMI, mean (SD)	26·4 (5·8)
distribution: n (%)	
<25	51 (52%)
25-30	25 (25.5%)
>30	21 (21.4%)
Race, n (%)	
White	87 (89·7)
BAME	9 (9·3)
Prefer not to answer	1 (1·0)
Sex, n (%)	
Male	21 (21·6)
Female	76 (78·4)
Reported Prior COVID-19 symptoms or infection, n (%)	1 (1·0)
Antibody levels, mean (SD)	
Abbott IgG (AU/mL) [cut-off=50AU/mL]	0·3 (0·6)
Abbott IgG Index (S/C) [cut-off=1.4]	0·08 (0·13)
25(OH)D (nmol/L), mean (SD)	45·7 (30·1)
distribution: n (%)	
<25nmol/L	27·9% (19·5±3·9nmol/L)
25-50nmol/L	38·1% (36·0±6·3nmol/L)
>50nmol/L	33·0% (73·2±12·3nmol/L)
$1,25(OH)_2D$ (pmol/L), mean (SD)	108·2 (34·3)

Antibody profile post immunization

In all the remaining 97 HCW, the antibody concentration increased and peaked on average at 3·2 weeks (95% CI, 3·1-3·3). The concentrations of anti-Spike IgG did not change significantly at week 1 (p=0·3896) but increased sharply and significantly thereafter (Fig 1) (p<0·0001). IgG peak concentrations were variable and ranged between 161-12020 AU/mL. Anti-nucleocapsid IgG were measured and not detected in any of these participants, confirming Ab response was not due to natural infection.

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Association between antibody response and age and other characteristics

The group was composed of 79% females and 90% of participants were white, sex and ethnicity were therefore not included as criteria. BMI showed no association with the production of antibody (p=0·339) or with the decrease in antibody concentrations (p=0·574).

The peak in anti-Spike IgG was strongly associated with age (continuous variable p<0·001) and significantly greater in younger HCW <30yr and 30-40yr, (Spearman's p=0·001) as compared to 41-50yr and >50yrs (Fig 2). At W8, the concentrations of anti-Spike IgG had significantly decreased (p<0·001), on average by 53±11% (21-78%). All subjects who demonstrated an Ab response following the first dose had positive IgG at W8, close to W2 levels (Wilcoxon p=0·729). Antibody concentrations also decreased in an age-dependent manner (p=0·002), the younger HCW with higher peaks decreasing faster to similar concentrations between ages (Kruskal-Wallis, p=0.703) at W8.

Association between antibody response and age vitamin D status

At baseline, only 34% of HCW had adequate 25(OH)D, 27·9% had deficient and 38·1% insufficient level. As a continuous variable, 25(OH)D showed no association (p=0·303) with the production of Ab. However, there was a strong positive association between production of Ab and the categorised variable deficient/insufficient/replete (p=0·035) (Fig 3) and an even stronger association when only using below and above 50nmol/L (p=0·027), mirroring clinical cut points. Loss of antibody after peak was not associated with 25(OH)D concentrations (p=0·572).

No association was observed with $1,25(OH)_2D$ concentrations (p=0·104 peak; p=0·536 drop).

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Discussion

In this study the initial dose of BNT162b2 triggered a serological response that is likely able to protect the recipients against COVID-19 infection by priming the immune system. Our results lend some support to the decision from the UK government to delay the second dose to at least 8 weeks.

The older the subjects in this group the lower was the peak Ab response, however at W8, concentrations were similar between all ages. The age range of our subjects reflects the age of HCW currently employed with low incidence of very elderly subjects. Shroti et al., [16] reported an age dependent increase in the proportion of people who seroconvert over time. With a mean age of participants significantly higher than in our study, the study adds to the validity of our data suggesting that the effect of age is across the lifespan.

Circulating 25(OH)D concentrations are at their lowest during winter and spring seasons, lower in BAME and lower at higher latitudes. Across the UK, the expected prevalence of deficiency (<25nmol/L) in winter is 23·1% [17] and 10% in Caucasian women [18]. Almost a third (27·9%) of our group comprising close to 90% Caucasian women was 25(OH)D deficient, higher than the expected prevalence for this demographic. Repetitive lockdowns may have had a detrimental effect on the vitamin D status and may have other consequences for the health of the population (osteoporosis, other bone related disorders). There was a significantly higher Ab response observed in subjects with 25(OH)D >50nmol/L. The optimal concentration for 25(OH)D, leading to optimal $1,25(OH)_2D$ and the best Ab response remains unclear in this study and would require larger numbers of participants studied to demonstrate definitive effects. A number of trials have shown variable effects of vitamin D and the vitamin D pathway polymorphism in improving vaccine effect for infectious diseases such as influenza (A/H1N1, A/H3N2) hepatitis B, measles, rubella, tuberculosis, pneumococcal, and meningococcal disease (for review see [19, 20]). It is also important to remember that vitamin D has known effects on the immune system which are beyond the

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production of Ab [9, 21]. High dose vitamin D treatment studies, commenced when COVID-19 infection was already established, have had variable success [22, 23]. Our data would suggest that it is important to have good vitamin D status prior to COVID-19 infection or immunization to prime the immune response to be ready to combat the virus once exposure occurs. Once infected, high dose therapy may be relatively ineffective. From a Public Health point of view, a booster immunization programme would be best planned when the population's vitamin D is at its highest following a period of sunshine exposure (end of Summer or early Autumn) or after supplementation with a minimum daily dose of 400-1000 IU D_3.

The strengths of this dataset include the use of a highly performing, quantitative assay to measure anti-spike IgG across a wide range of concentrations (21-40,000AU/mL) and traceable to the WHO International Standard for anti-SARS-CoV-2 immunoglobulin, allowing robust determination of differences in concentrations over time. This is the first study describing an effect of vitamin D on the response to immunization against SARS-CoV-2. The potential implications of the observed beneficial effect of replete vitamin D [25(OH)D>50nmol/L] status is to ensure booster immunisation programs are planned when the population's vitamin D is at its highest following a period of sunshine exposure or supplementation.

Limitations

This is a single-centre study with a limited number of participants and is strongly biased towards female and white participants which might limit the generalizability of the findings.

Conclusions Amongst HCW in a single UK centre, antibody profile after a first injection of BNT162b2 was associated with age and vitamin D status at baseline. Younger people were reaching higher peaks but decreasing to similar levels as older after 8 weeks. Vitamin D was also beneficial to the production of antibodies.

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Declaration of interests

Two of the authors (SR and MB) are employees of Abbott Diagnostics Ltd who supplied the kits used to quantify the anti-SARS -CoV-2 Spike IgG and technical support as well as provided financial support for sample collections. All other authors have no conflict of interest.

Criteria for authorship

Isabelle Piec: Sample collection, data collection, data analysis, literature search, data interpretation, writing original draft and revisions.

Laura Cook: Data collection and manuscript revision

Samir Dervisevic: Conceptualisation, methodology, interpretation, reviewing and editing

William D Fraser: Conceptualisation, methodology, resource, funding acquisition, interpretation, reviewing and editing

Scott Ruetten: Resources, reviewing and editing

Marvin Berman: Resources, reviewing and editing

Emma English: Conceptualisation, methodology, resource, supervision, funding acquisition, interpretation, reviewing and editing

W Garry John: Conceptualisation, methodology, resource, supervision, funding acquisition, interpretation, reviewing and editing

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Figures

Figure 1. Anti-spike IgG concentration per week (median ± IQR)

Figure 2. Time-course of antibody production after immunization per age group

Values represent mean ±SEM; IgG concentrations per week for age groups ≤30y (empty squares and full line); 31-40y (empty diamonds and dot-dashed line); 41-50y (black circles and dotted line) and >50y (black triangles and dash-double dot line).

Figure 3. Timecourse of antibody production after vaccination depending on 25(OH)D status

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Values represent mean ±SEM; IgG concentrations per week for 25(OH)D groups ≤25nmol/L (empty squares dash-dot line); 26-50nmol/L (black diamonds and dashed line); >50nmol/L (black circle and full line).

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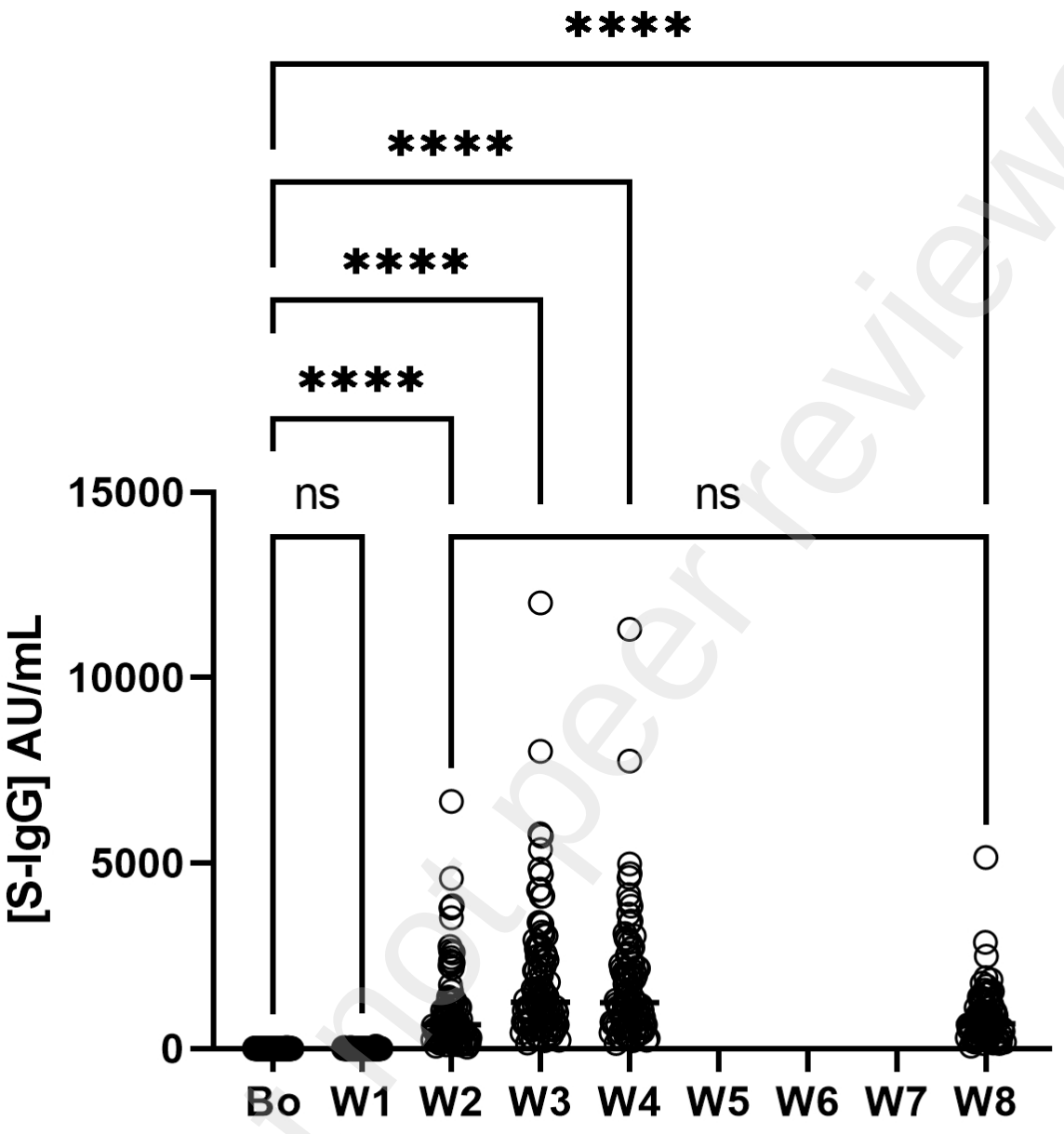

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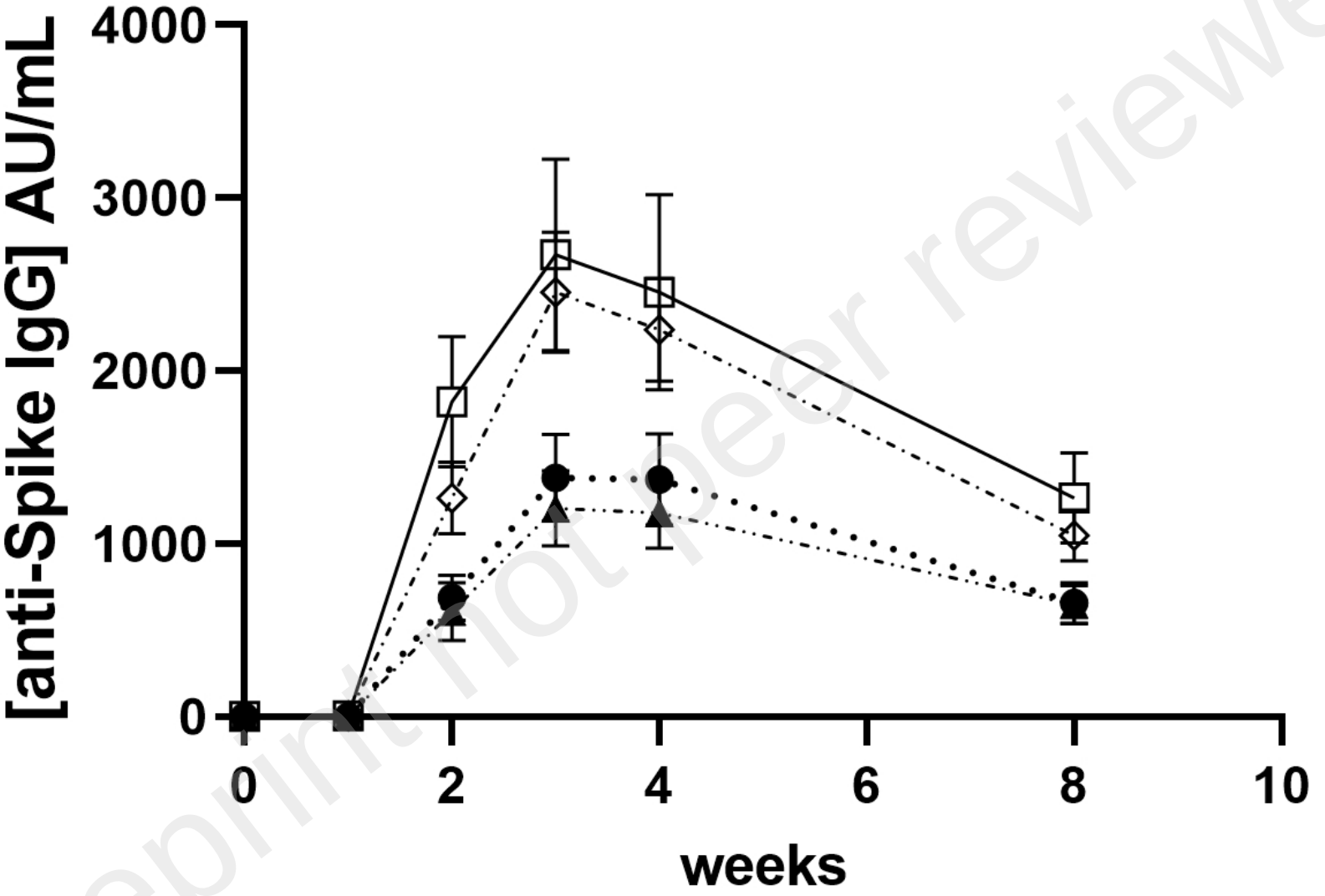

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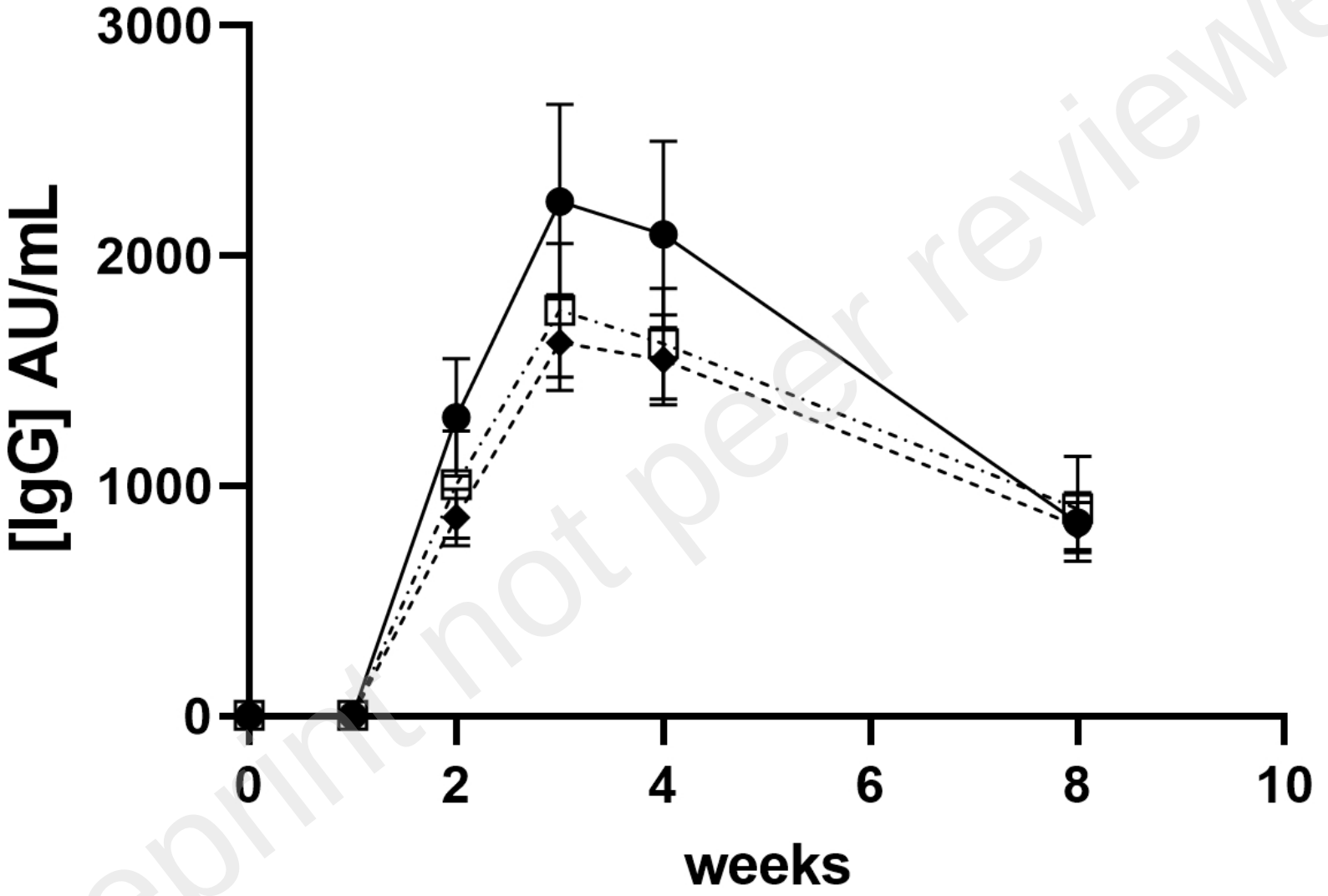

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