Heliyon 8 (2022) e09438

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Antibody response after two doses of the BNT162b2 vaccine among healthcare workers of a Greek Covid 19 referral hospital: A prospective cohort study

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ARTICLE INFO

Keywords:
BNT162b2
Humoral immunity
Health care workers
SARS-CoV-2

ABSTRACT

The global vaccination against SARS-CoV-2 has highlighted the need of assessing vaccines' immunogenicity against COVID-19. To evaluate humoral immunity induced by the BNT162b2 vaccine, we enrolled health care workers at AHEPA University Hospital of Thessaloniki, Greece to measure Anti-S SARS-CoV-2, anti-RBD SARS-CoV-2 and neutralizing antibodies. A total of 955 individuals with a median age of 50 years, were included in the study. Median values of antibodies were 1947.27 BAU/mL (Abbott SARS-CoV-2 IgG II Quant), 2064.98 BAU/mL (MAGLUMI SARS-CoV-2 S-RBD IgG) and 2464.63 IU/mL (MAGLUMI SARS-CoV-2 Neutralizing Antibodies). Individuals previously infected had greater antibody responses than infection naive ones and a 7-fold higher neutralizing antibodies titre. Antibodies degreased by age but not sex. Spearman's correlation coefficient among the three assays ranged from 0.903 to 0.969.

The BNT162b2 vaccine was highly immunogenic in our cohort. Further research is needed to evaluate the vaccine's immunogenicity through time as well as in different populations.

1. Introduction

After its emergence in December 2019 [1], Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has spread worldwide causing a pandemic which accounts for more than 4.5 million deaths [2] and devastating economic consequences because of the intense and long lockdowns that have been implemented in order to halt its spread.

The European Medicines Agency (EMA), since 21 December 2020, has authorized five vaccines for emergency use in Europe, including the Comirnaty (BNT162b2), Spikevax, Vaxveria and COVID-19 Vaccine Janssen [3].

The BNT162b2 is an m-RNA vaccine that contains nucleoside-modified messenger RNA, coding the spike (S) viral protein, encapsulated in lipid nanoparticles that enable its delivery into host cells. As a result, the SARS-CoV-2 S antigen is expressed eliciting both humoral and T-cell responses. The vaccine is administered by intramuscular injection in two doses of 30μg 21 days apart [4].

On December 27, 2020 vaccination began in Greece while as of August 29, 2021 57.95% of the population had been administered at least one vaccine dose [5]. Healthcare workers (HCWs) were the first group of the population to be vaccinated with the BNT162b2, developed by BioNTech and Pfizer and indicated for active immunization against SARS-CoV-2 virus, in individuals 12 years of age and older [6]. The vaccine has shown excellent efficacy against severe infection both in phase 3 trials [4] and in a real-world setting [7].

Neutralizing antibodies elicited after vaccination, have shown the ability to neutralize the virus and their presence correlates well with protection against reinfection, both in humans and in non-human primates [8, 9]. Additionally, neutralizing antibodies target the spike protein and more robustly the SARS-CoV-2 receptor-binding domain (RBD) thus being highly immunogenic, accounting for 90% of the serum neutralizing activity [10].

Although seropositivity to SARS-CoV-2 can reduce the incidence rate against SARS-CoV-2 infection by 82%, the presence of antibodies in

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https://doi.org/10.1016/j.heliyon.2022.e09438
Received 4 November 2021; Received in revised form 13 April 2022; Accepted 11 May 2022

general cannot guarantee neutralizing activity and effective immunity against infection [11].

Data on immunity status after two vaccine doses are limited. Moreover, the correlation of post-vaccination antibody titers with the actual protection against severe disease is yet to be defined. One critical question therefore, is whether a serology immunoassay can detect neutralizing antibodies and thus give insight into a person's protective immunity against reinfection.

The gold standard in assessing antibodies effectiveness is virus neutralization, conventionally done by Plaque Reduction Neutralization Tests (PRNTs). Though accurate, these tests require live virus handling and thus biosafety level 3 (BSL3) and specialized personnel while being laborious and time consuming. Hence, more practical, easily accessible and high-throughput assays are needed in order to obtain reliable results and better understanding of both the immunogenicity conferred by vaccines on a large scale as well as the seroprevalence in the population. Furthermore, since RBD-directed antibodies are more potent to neutralize the virus [12], many serology assays use measurement of anti-RBD antibodies in order to evaluate neutralizing activity as it has been shown that anti-RBD immunoglobulin class G (IgG) antibodies rise early in the course of infection and that they could be used as a surrogate of neutralization activity against SARS-CoV-2 infection since they correlate robustly [13, 14].

Thus far, there are a total of 328 CE-IVD (Common Era In Vitro Diagnostic) marked serology immunoassays (lateral flow assays, enzyme-linked immunosorbent assays and chemiluminescent immunoassays) whereas 34 have received Emergency Use Authorization (EUA) by the United States Food and Drug Administration (FDA) [15]. Among these, chemiluminescent immunoassays can offer high-throughput capacity with high sensitivity and thus can be easily integrated in a clinical laboratory's workflow.

The aim of our study was to evaluate the immunogenicity induced 14 days after the administration of the second dose of the BNT162b2 vaccine, assessed by measuring the titers of anti-S SARS-CoV-2 antibodies, anti-RBD SARS-CoV-2 antibodies and neutralizing antibodies, using a surrogate neutralization assay, in accordance to sex, age and prior infection status and also to correlate the 3 assays with one another.

2. Materials and methods

2.1. Clinical sample

We conducted a prospective cohort study of HCWs vaccinated from January to February 2021 at the American Hellenic Educational Progressive Association (AHEPA) University Hospital, a tertiary care Hospital in Thessaloniki, Greece and for the past year one of the reference Covid-19 hospitals in northern Greece, where the vaccination program begun on January 27 and is on-going.

All HCWs at the time of the second dose administration, 21 days after the first dose as suggested by the manufacturer, were given a notice to provide blood samples 14 days, 3 and six months later.

Here we present data on the antibody status 14–18 days after the second dose. We measured 3 types of antibodies; SARS-CoV-2 S IgG, SARS-CoV-2 S-RBD IgG and SARS-CoV-2 Neutralizing.

When giving blood samples, all participants were asked to sign a written consent to participate in the study and declare whether they have been previously infected by SARS-CoV-2. The study was approved by the AHEPA University Hospital Scientific Board which stands as the ethical committee of the hospital and complies with all regulations. Written informed consent was obtained by all individuals included in the study.

The SARS-CoV-2 IgG testing was offered to all HCWs by the hospital's administration regardless of consenting.

Provided that the HCWs were not screened for SARS-CoV-2 infection by serology or Polymerase Chain Reaction (PCR) tests prior to the vaccination and in order to spot any cases of asymptomatic disease that were undetected by the participants, we chose to measure anti-Nucleocapside (anti-N) IgG antibodies to further evaluate prior infection, since the anti-S were expected to rise due to vaccination regardless of infection. Thus, the infection status was presumed by considering a positive PCR test, the presence of anti-N antibodies and/or self-reported previous infection.

Participants were stratified into 5 age groups; <30, 30–39, 40–49, 50–59, >60 years old.

Samples after been left to clot for a minimum of 3 h, were centrifuged for 10 min at 4,000 rpm and serums obtained were processed on the instruments on the same day and then stored at -70 °C until further analysis.

This report followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cross-sectional studies.

2.2. SARS-CoV-2 anti-S IgG antibodies

For the detection of anti-S IgG antibodies against SARS-CoV-2 we used the SARS-CoV-2 IgG II Quant assay, a chemiluminescent microparticle immunoassay (CMIA) performed on the Alinity i system, designed to detect immunoglobulin class G (IgG) antibodies, including neutralizing antibodies, to the RBD of the S1 subunit of the spike protein of SARS-CoV-2 in serum and plasma. The cut-off value of the assay is 50 Arbitrary Units/milliliter (AU/mL), while the Analytical Measuring Interval is 21.0–40,000.0 AU/mL. Values that exceeded 40,000 AU/mL were diluted 1:2 as suggested by the manufacturer. Values above 80,000 AU/mL were considered as equal to 80,000 in order not to affect linearity. According to the IFU, the assay demonstrated 100% positive agreement when compared to a plaque reduction neutralization test (PRNT).

2.3. SARS-CoV-2 S-RBD IgG and surrogate neutralization assay

SARS-CoV-2 S-RBD IgG and SARS-CoV-2 Neutralizing Antibodies were measured by an indirect and a competitive chemiluminescence immunoassay respectively using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyser. The cut-off of the SARS-CoV-2 S-RBD IgG assay is 1 AU/mL and the Linear Range 0.180–100 AU/mL, while for the SARS-CoV-2 Neutralizing Antibody assay the respective values are 0.300 μg/mL and 0.050–30 μg/mL.

Samples that were over the upper limit for each assay were reanalysed after being subjected to the recommended by the manufacturer 1:9 dilution.

2.4. ANTI-N antibodies

The assay is an automated, two step immunoassay for the detection of IgG antibodies to SARS-CoV-2 in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA). The antigen used in the assay is the SARS-CoV-2 nucleocapsid.

The Alinity i system, calculates the calibrator mean chemiluminescent signal from 3 calibrator replicates and stores the result. Results are then reported by dividing the sample result by the stored calibrator result. The default result unit for the SARS-CoV-2 IgG assay is Index (S/C). The cut off is 1.4 Index (S/C).

2.5. WHO International Standard for anti-SARS-Cov-2 immunoglobulin

All values were transformed to WHO Units (IU/mL for neutralizing antibodies and Binding Arbitrary Units per mL (BAU/mL) for binding assay formats) [16]. All 3 assays were calibrated using the WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human)" (NIBSC code 20/136) resulting in the following equations: for SARS-CoV-2 IgG II Quant reagent, 1 AU/mL is equivalent to 0.142 BAU/mL, for SARS-CoV-2 S-RBD IgG reagent, 1 AU/mL is equivalent to

Table 1. Median concentrations of antibody titres of vaccinated HCWs measured by 3 assays and p values of intragroup comparisons.

Characteristics		SARS-CoV-2 IgG II Quant Median concentration (BAU/mL) (N = 955)	p values	SARS-CoV-2 S-RBD IgG Median concentration (BAU/mL) (N = 884)	p values	SARS-CoV-2 Neutralizing Antibodies Median concentration (IU/mL) (N = 884)	p values
Age (years)	<30	2492.55 (n = 58)	<0.001	2794.15 (n = 51)	<0.001	3439.26 (n = 51)	<0.001
	30–39	2455.47 (n = 140)		2535.22 (n = 131)		3036.29 (n = 131)	
	40–49	1997.90 (n = 253)		2084.90 (n = 230)		2358.92 (n = 230)	
	50–59	1727.67 (n = 395)		1839.82 (n = 373)		2221.83 (n = 373)	
	>60	1503.62 (n = 109)		1645.40 (n = 99)		2485.89 (n = 99)	
Sex	Females	1991.35 (n = 620)	0.247	2023.41 (n = 571)	0.801	2435.67 (n = 571)	0.450
	Males	1891.14 (n = 335)		2198.77 (n = 313)		2498.85 (n = 313)	
Infection status	Previously infected	2926.89 (n = 47)	<0.001	3806.07 (n = 36)	<0.001	16029.90 (n = 36)	<0.001
	Infection naive	1899.90 (n = 908)		2019.30 (n = 848)		2373.91 (n = 848)	

4.33 BAU/mL and for SARS-CoV-2 Neutralizing Antibody reagent, 1 μg/mL is equivalent to 405 IU/mL, according to the manufacturers.

The sample size was not calculated beforehand, since anti-S antibodies testing was available by the hospital's administration to all HCWs. Consequently, we included all anti-S antibodies measurements in the study, provided that written consent was given by the participants, while due to logistical and funding reasons, we randomly chose 884 serums to measure anti-RBD SARS- CoV-2 and neutralizing antibodies and 841 for the ANTI-N IgG antibodies.

2.6. Statistical analysis

Descriptive statistics were used to analyze the data. Antibody levels were reported using medians and were stratified by age-groups, gender and former infection status. The Mann-Whitney U test was used for comparisons of antibody titers by gender and infection status. The Kruskal–Wallis test was used for age groups comparison. Correlations between all 3 assays were performed using the Spearman's correlation coefficient. A 2-tailed $P < 0.05$ was considered statistically significant. Values that were greater than the upper limit of quantification (ULOQ) were converted to the ULOQ. Box plots were used in order to illustrate the distribution of antibody levels and the differences between groups. Analyses were performed using the SPSS version 21.

3. Results

Between February 3, 2021 and March 3, 2021, a total of 1173 HCWs were fully vaccinated with two doses of the BNT162b2 and eligible to be included in the analysis. Among them, 1005 provided blood samples for SARS-CoV-2 antibodies analysis, but 50 did not consent to participate in the study.

During the study period, we have identified as eligible to participate only those HCWs whose second dose of vaccination was delivered at least 14 days prior March 3 with only additional exclusion criterion the unwillingness to provide a written informed consent (IC).

Finally, 955 samples where included in the analysis; all of them were tested with the SARS-CoV-2 IgG II Quant assay while 884 with the SARS-CoV-2 S-RBD IgG and SARS-CoV-2 Neutralizing Antibodies assays, as mentioned above.

The 955 participants [620 (64.92%) females] had a median age of 50 years and 47 (4.92%) were infected by SARS-CoV-2 prior to vaccination. Out of these 47, 22 were positive for anti-N IgG antibodies, 31 had a positive PCR test registered in the hospital's files and 5 declared prior infections without documentation of a PCR test.

The median values for each of the 3 assays were 1947.27 BAU/mL (SARS-CoV-2 IgG antibodies), 2064.98 BAU/mL (SARS-CoV-2 S-RBD IgG) and 2464.63 IU/mL (SARS-CoV-2 Neutralizing Antibodies). All samples rendered values above the assays' cut-off for SARS-CoV-2 IgG and SARS-CoV-2 S-RBD IgG, and only 7 out of 884 (0.79%) were negative for the presence of SARS-CoV-2 Neutralizing Antibodies (values <0.300 μg/mL i.e. <121.5 IU/mL). Out of these, 5 were female, their median age was 57 years (age range: 40–61 years) and the yielded Neutralizing Antibodies values range was 14.18–115.02 IU/mL. The antibody titers rendered by the other 2 assays in these participants, ranged from 27.36 to 64.08 BAU/mL when measured by the Abbott SARS-CoV-2 IgG II Quant assay and 25.63 to 65.38 BAU/mL when measured by the MAGLUMI SARS-CoV-2 S-RBD IgG assay.

Antibodies elucidated after vaccination in previously infected individuals were significantly higher than those of not infected, as expected. Median values of antibodies measured in previously infected individuals by SARS-CoV-2 IgG II Quant, SARS-CoV-2 S-RBD IgG and SARS-CoV-2 Neutralizing Antibodies assays were 2926.89 BAU/mL,

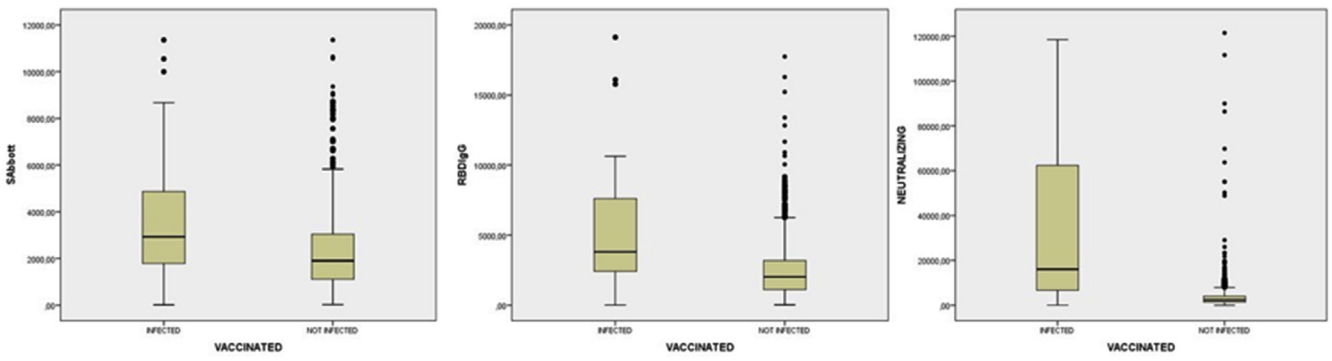

Figure 1. Box plots of the distribution of antibody titres in previously infected and infection naive individuals, measured by all three methods.

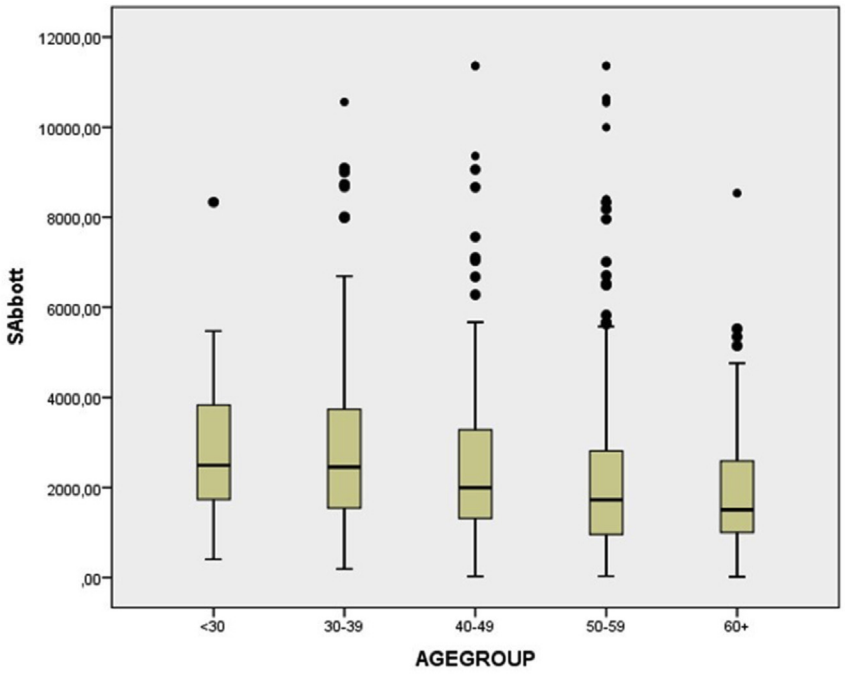

Figure 2. Box plots of the distribution of anti-S antibody titres by age groups, measured by the Abbott's SARS-CoV-2 IgG II Quant assay, in BAU/mL.

3806.07 BAU/mL and 16029.90 IU/mL respectively, while those elucidated by not infected individuals were 1899.90 BAU/mL, 2019.30 BAU/mL and 2373.91 IU/mL (Table 1).

Remarkably, the median of neutralizing antibodies was almost 7 times higher to previously infected participants compared to infection naive individuals although this was not the case when measured by the other 2 assays (Figure 1).

There was no significant difference of antibody titres between genders by any of the 3 assays (p values: 0.247, 0.801 and 0.450) (Table 1).

On the other hand, antibody titres appeared to decrease over age with differences between age groups being statistically significant (p values $<$ 0.001) (Table 1) (Figures 2, 3, and 4).

There was a positive and statistically significant ($p < 0.001$) very strong correlation among all 3 assays (Abbott SARS-CoV-2 IgG II Quant and MAGLUMI SARS-CoV-2 S-RBD IgG rho = 0.969, Abbott SARS-CoV-2 IgG II Quant and surrogate neutralization assay rho = 0.903, MAGLUMI SARS-CoV-2 S-RBD and surrogate neutralization assay rho = 0.903) (Figure 5).

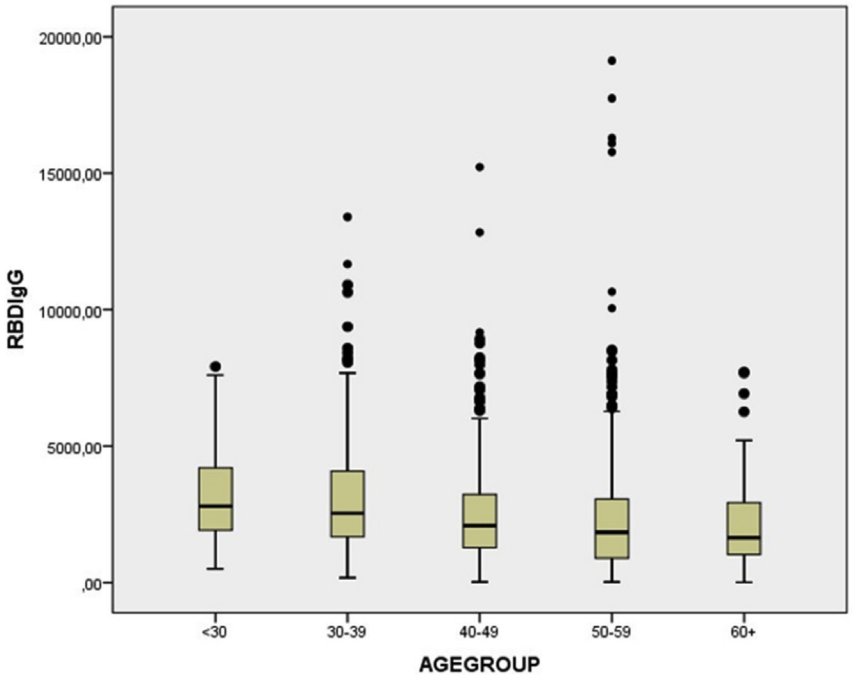

Figure 3. Box plots of the distribution of SARS-CoV-2 S-RBD IgG antibody titres by age groups, measured by the MAGLUMI's SARS-CoV-2 S-RBD IgG assay, in BAU/mL.

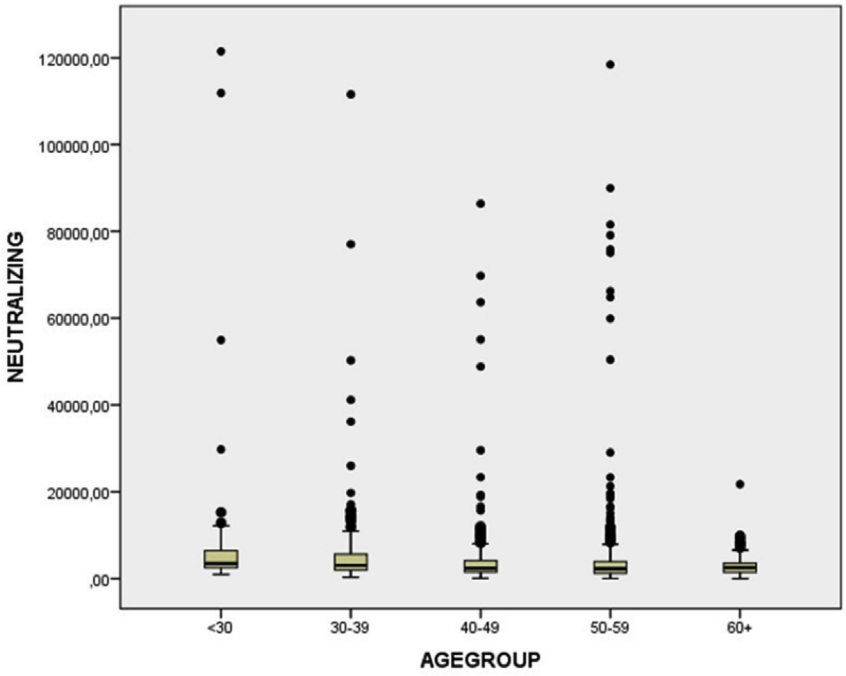

Figure 4. Box plots of the distribution of neutralising antibody titres by age groups, measured by the MAGLUMI's SARS-CoV-2 Neutralizing Antibody assay, in IU/mL.

4. Discussion

Our findings suggest that the BNT162b2 is highly immunogenic since antibodies were elicited in all individuals tested in our study, 14 days after having received a full course of vaccination. Anti-S and anti-RBD antibodies were positive in all individuals while neutralizing antibodies were negative in 7 out of 884 participants that were tested with the surrogate neutralization assay. Our results are in concordance with those rendered at the phase 1 trial of the vaccine where BNT162b2 elicited humoral responses to all participants as well as with results from real world settings [17].

Also, our results show that antibody titres are affected by prior infection status and age but not sex as it has already been demonstrated.

We observed that previously infected individuals had statistically significant higher antibody responses than infection naive ones as it was demonstrated by all three assays we used in our study. This fact has now been established by numerous publications, while it is also evident that immune responses after one vaccine dose in those with prior SARS-CoV-2 infection are similar to the ones elicited by two doses in infection naive ones [18, 19, 20, 21]. Furthermore, we noticed that the difference of antibody titres between the two groups was substantially higher for neutralising antibodies compared to the other two assays as it has already been observed by Anichini et al in a correspondence letter published on New England Journal of Medicine, where the researchers found neutralising antibodies being remarkably lower after two doses of vaccine to those without prior infection compared to previously infected individuals after one vaccine dose [19]. In our study, the median of neutralizing antibodies was almost 7 times higher to previously infected participants compared to infection naive individuals which was not the case for the other two assays. This might suggest that protection against COVID-19 is actually much stronger for those infected prior to vaccination. More precisely, the level of protection seems to be higher than the one suggested by measuring binding antibodies such as anti-S and anti-RBD. To the best of our knowledge, this was not established by the literature thus far.

Additionally, in our study there was no difference in antibody titres between males and females as it has been already demonstrated by Jabal et al. [22] and unlike data published by other researchers like Terpos et al. and Salvagno et al. according to which females had higher anti--Spike-RBD IgGs and total anti-SARS-CoV-2 RBD antibodies respectively [23, 24].

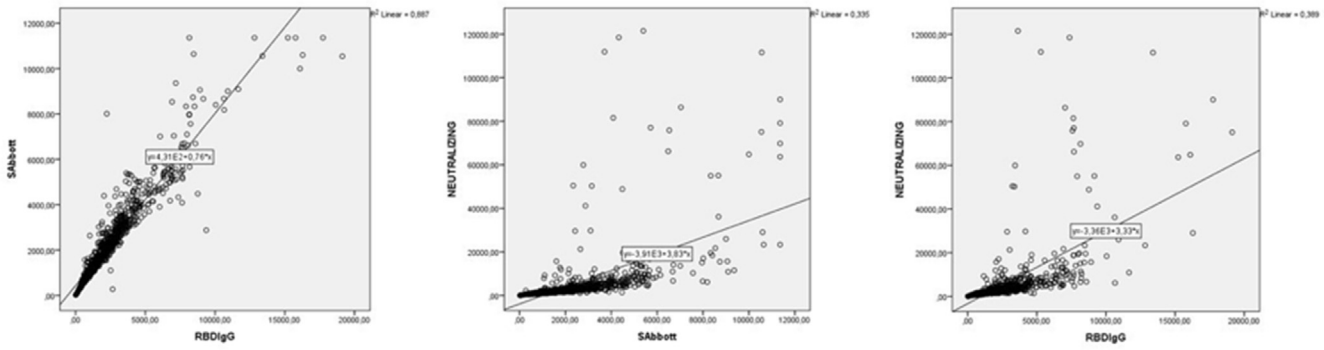

Figure 5. Correlations between Abbott SARS-CoV-2 IgG II Quant and MAGLUMI SARS-CoV-2 S-RBD IgG; Abbott SARS-CoV-2 IgG II Quant and surrogate neutralization assay; MAGLUMI SARS-CoV-2 S-RBD and surrogate neutralization assay.

Age appears to affect vaccine's immunogenicity as expected due to immunosenescence that occurs as age goes by. Indeed, our results showed statistically significant reduction in antibody titres at each age group and they are supported by observations that have already been published by other researchers [22, 25]. For example, Grupper et al. have shown, after examining antibody responses after vaccination by the BNT162b2 in patients undergoing haemodialysis, that age is a crucial factor in humoral immunity regardless of underlying diseases [25]. What is more, Collier et al observed lower serum neutralisation activity and binding IgG and IgA levels in older individuals [26].

Our study had some limitations. We included mostly healthy adults whereas only 25 out of 955 participants were older than 65 years. Additionally, data on participants' medical history and immunity status were not available.

What is important in assessing immunological responses after vaccination is the use of serological assays which can accurately estimate serum's neutralisation activity as well as being easily applicable in a clinical laboratory. We found that anti-S and anti-RBD correlate well with neutralising antibodies and thus could potentially substitute the laborious and time-consuming gold standard neutralising assays.

Longitudinal studies are required to evaluate the persistence of antibodies after vaccination and their relevance to cellular immunity and disease protection. More investigation is necessary to establish protective titre values after vaccination and vaccine immunogenicity in different populations such as children, seniors, pregnant women and individuals with various coexisting medical conditions.

Declarations

Author contribution statement

Areti Tychala: Conceived and designed the experiments; Wrote the paper.

Lemonia Skoura: Conceived and designed the experiments.

Anastasia Boura-Theodorou, Chrysa Chantzi and Maria Koutri: Performed the experiments.

Georgios Meletis, Ioanna Gkeka and Andreas Athanasiadis: Analyzed and interpreted the data.

Eleni Sidiropoulou, Sofia Dionysopoulou, Kali Makedou: Contributed reagents, materials, analysis tools or data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We would like to thank all the technical staff of the Microbiology Department of AHEPA University Hospital of Thessaloniki for their assistance in blood collection and laboratory procedures. Additionally, we would like to thank MAGIRAS DIAGNOSTICS S.A for their donation of reagents used at the MAGLUMI SARS-CoV-2 S-RBD IgG and SARS-CoV-2 Neutralizing Antibodies.

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