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*Original article*

# BNT162b2 VACCINE ANTIBODY RESPONSE USING THREE ANTIBODY ASSAYS

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**Abstract**

**Aim:** The aim of our study was to compare the durability of BNT162b2 antibody response between male and female healthcare workers (HCWs), before vaccination and at 3, 9 and 12 months after administration of the second dose of the Pfizer/BioNTech BNT162b2 vaccine, using three antibody assays: Maglumi® SARS-CoV-2 Neutralizing Antibody (CLIA), Maglumi® SARS-CoV-2 S-RBD IgG (CLIA) and VIDAS® SARS-CoV-2 IgG (ELFA).

**Material and methods:** This study included 200 HCWs and the gender structure of the participants consisted of 82 (41%) males and 118 (59%) females. We utilized blood samples collected from HCWs who had not previously been infected with SARS-CoV-2. All procedures strictly followed the manufacturer's instructions.

**Results:** Male and female HCWs had similar serum anti-S-RBD IgG concentrations before vaccination. Our findings showed the highest concentration of antibodies three months after vaccination in both genders, where females had non-significantly higher serum anti-S-RBD IgG antibodies with all three methods. Nine months after vaccination, females had significantly lower serum anti-S-RBD IgG measured with Maglumi Neutralizing Antibody (median 161 *vs*. 167 BAU/mL, p=0.017). However, at this time point, the difference between males and females was statistically insignificant regarding the serum values of anti-S-RBD IgG measured with Maglumi RBD (median 171.54 *vs.* 165.22 BAU/mL, p=0.38) and VIDAS RBD (68.16 *vs*. 103.3 BAU/mL, p=0.75). The level of anti-spike-RBD antibodies significantly decreased during 12 months after vaccination in males and females, ($p<0.0001$) determined by all three methods.

**Conclusion:** Our results demonstrated that there were no significant differences in SARS-CoV-2 IgG antibody concentrations between male and female HCWs.

***Keywords:*** SARS-CoV-2, RBD IgG, Maglumi - 800, neutralizing antibodies, healthcare workers

## Introduction

A newly discovered virus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in December 2019 in multiple cases in Wuhan, China[1]. Subsequently, the World Health Organization officially declared the SARS-CoV-2 outbreak a pandemic on March 11th, 2020. SARS-CoV-2 has an enveloped, positive-sense single-stranded RNA genome. Its structure resembles a crown, characterized by spike-like proteins known as "S proteins" which coat its surface and bind to the host cell receptor angiotensin-converting enzyme 2 (ACE2), facilitating the entry of the virus into the host cell[2]. The S

protein represents a critical target for inducing antibodies, especially neutralizing antibodies (NAb's) that are directed specifically against SARS-CoV-2[3]. NAb's are essential components of the body's humoral immune response, which protects cells from SARS-CoV-2 infection by inhibiting the virus entry into cells and neutralizing its biological activity[4]. Detection of SARS-CoV-2 antibodies serves as a valuable indicator of prior COVID-19 infection.

As the COVID-19 pandemic evolves, maintaining sensitivity over an extended period is a crucial characteristic for an antibody assay, intended for population seroprevalence studies. HCWs are identified as a group with an elevated risk, susceptible to exposure and possible transmission of SARS-CoV-2 infection, particularly within patient care environments[5]. Due to the limited availability of COVID-19 vaccines, they were a priority group for vaccination[6]. Therefore, it is essential to assess the coverage of COVID-19 vaccines due to various factors influencing the antibody response[7]. Different immune responses are observed in men and women in relation to SARS-CoV-2 infection, characterized by variations in prevalence, intensity, and outcomes[8]. This contrast is evident in both cases of vaccination and natural infection[9]. Similar differences have been previously documented for other viral infections[10] and, more broadly, for immune response[11]. Several studies indicated that male patients in China were prone to experiencing more severe symptoms and exhibited a higher mortality rate compared to their female counterparts[12-14]. It is now evident that gender is correlated with the severity of COVID-19, manifesting in males with pronounced symptoms and a heightened mortality rate. A cohort study in England, encompassing 17 million adults, revealed a significant association between male sex and the risk of death from SARS-CoV-2[15]. Approximately 60% of global COVID-19-related fatalities were observed among male individuals, as reported by Gebhard *et al.*[16]. The increased mortality rate of COVID-19 in males compared to females can be attributed to variations in biological factors, encompassing differences in DNA, steroid hormones and reproductive organs. Additionally, gender-related factors, including adherence to traditional and social norms, contribute to these differences[16]. It was observed that males exhibit higher age-adjusted rates of coexisting diseases such as chronic obstructive pulmonary disease and cardiovascular disease. Both of these conditions are associated with a weak prognosis of COVID-19[14,17]. Moreover, even when accounting for age, a stratified study reveals that the impact of comorbidities on COVID-19 mortality is more pronounced in males than in females[18]. In contrast, women exhibit greater immune responses to vaccines, displaying a more robust reaction to vaccination, but with increased side effects compared to men[19,20]. These findings suggest that females may have enhanced resistance and immunity against infectious agents. However, the precise mechanism by which SARS-CoV-2 leads to severe outcomes in males compared to females in the development of COVID-19 remains unclear. The identification and detection of antibodies against SARS-CoV-2 have been implemented as crucial components of COVID-19 prevention and management[21]. Monitoring the long-term effectiveness of the vaccine is very important. Numerous studies have shown a decline in protection against infection with SARS-CoV-2[22-24].

The aim of this study was to compare the durability of BNT162b2 antibody response between male and female HCWs after the second dose of the Pfizer/BioNTech (BNT162b2) vaccine, using three different commercially available antibody assays: Maglumi® SARS-CoV-2 Neutralizing Antibody (CLIA), Maglumi® SARS-CoV-2 S-RBD IgG (CLIA) and VIDAS® SARS-CoV-2 IgG (ELFA).

## Material and methods

This study included 200 HCWs from the Republic of North Macedonia, considering the impact of gender on the durability of BNT162b2 antibody response using three different assays, as described below, starting from February 2021 to the middle of June 2022. The gender

structure of the participants consisted of 82(41%) males and 118(59%) females, with age ranging from 26 to 53 years. In the context of this study, we utilized blood samples collected from HCWs before vaccination and at 3, 9, and 12 months after administration of the second dose of the Pfizer/BioNTech BNT162b2 vaccine. The research protocol was reviewed and received approval from the Ethics Committee at the Faculty of Medicine, Ss. Cyril and Methodius University (Approval No. 03-2529/2). HCWs were informed about the study procedures and were requested to visit the laboratory to obtain peripheral blood for the assessment of antibody levels. The serum was collected and stored at a temperature range of 4 to 8°C. All analytical procedures were performed at the Department of Medical and Experimental Biochemistry, Faculty of Medicine in Skopje. We utilized the following CE-marked binding assays:

Maglumi SARS-CoV-2 S-RBD IgG assay, which is an indirect chemiluminescence immunoassay (CLIA) and detects antibodies directed against the RBD of the viral spike protein, using the Maglumi 800 instrument from Snibe Diagnostic, Shenzhen, China. As declared by the manufacturer, the cut-off value in arbitrary units (AU) per milliliter, the conversion factor to obtain BAU/mL, the cut-off value in BAU/mL, and the linearity range in AU/mL are: 1, 4.33, 4.33, and 0.18-100, respectively. Samples with values over 100 AU/mL (433 BAU/mL) were diluted and measured 1:10 or 1:20. This procedure allowed an extension of the analysis dynamic range to 2000 AU/mL (8660 BAU/mL).

NAb's were measured by competitive CLIA method. Within the sample, SARS-CoV-2 NAb's compete with ACE2 antigen immobilized on magnetic microbeads for binding recombinant SARS-CoV-2-RBD antigen labeled with N-(4-aminobutyl)-N-ethyl-isoluminol (ABEI). Regarding the SARS-CoV-2 neutralizing antibody reagent, a concentration of 1 μg/mL is equivalent to 405 IU/mL. As defined by the limit of detection and the maximum of the master curve, the linear range is between 0.050-30 μg/mL, and 0.300 μg/mL was used as a cut-off for positivity that is based on the conversion of 0.3 μg/mL equivalent to 121.5 IU/mL with the SARS-CoV-2 neutralizing antibody assay.

VIDAS Anti-SARS CoV-2 IgG which is a two-step sandwich ELFA (Enzyme-linked fluorescent assay) performed on Vidas instrumentation from Biomerieux, France, strictly adhering to the manufacturer's instructions. This assay enables detection of SARS-CoV-2-IgG, from 100 µl serum or plasma. In this assay, SARS-CoV-2 IgG is initially captured by recombinant SARS-CoV-2 antigen coated on a solid phase. Subsequently, anti-human IgG labeled with alkaline phosphatase specifically detects the IgG. The conjugate enzyme then catalyzes the hydrolysis of substrate into a fluorescent product (4-Methyl-umbelliferone), with fluorescence measured at 450 nm. The fluorescence intensity is directly proportional to the antibody level in the sample. The assay was conducted with a standard (S1) and a positive control (C1) that contains humanized recombinant anti-SARS-CoV-2 antibody, and a negative control (C2) was also supplied. The results were automatically calculated by the instrument, according to S1 standard, and an index value (*i*) was obtained (where $i = RFV_{sample}/RFV_{S1}$). The assay is considered negative when i<1.00 and positive when i ≥1.00. Assay sensitivity is 96.6% at ≥16 days after positive rRT-PCR confirmation.

***Statistical analysis***

Statistical analysis was carried out using the statistical software SPSS (version 23.0; IBM, SPSS, USA). Categorical data is presented in absolute and relative numbers. Quantitative variables are given as means, standard deviation, minimum-maximum values, median and interquartile rank. Comparison of values measured with the three assays was made using the FRIEDMAN ANOVA Chi-square test and Wilcoxon Matched pairs test. McNemar test was used to test the difference in positive and negative results. The agreement of the methods was analyzed with Kappa index and Spearman's rank correlation coefficient. The comparison of

parameters between male and female group was done by Mann-Whitney U test. Statistical significance was assumed if P values were below 0.05.

## Results

Male and female HCWs had similar serum anti-S-RBD IgG concentrations before vaccination, independent of the method used (p=0.3, p=0.2 and p=0.9, respectively for Maglumi RBD, Maglumi NAb's and VIDAS RBD) (Table 1).

**Table 1.** Anti-S-RBD IgG values before vaccination with the three methods depending on gender

| **Before vaccination** | | | | | | |
|---|---|---|---|---|---|---|
| **Statistical parameter** | **SARS-CoV-2 antibody test** | | | | | |
| | **Maglumi RBD** | | **Maglumi NAb's** | | **VIDAS RBD** | |
| **gender** | **male** | **female** | **male** | **female** | **male** | **female** |
| mean ± SD | 1.50±1.1 | 1.35±0.97 | 61.25±44.8 | 55.32±37.3 | 7.73±5.4 | 13.88±70.3 |
| median | 1.47 | 1.44 | 40.09 | 38.47 | 7.1 | 6.1 |
| (IQR) | 0.39-2.17 | 0.29-1.96 | 32.4-85.05 | 28.35-85.05 | 2.78-12 | 4-12 |
| min- max | 0.06-3.69 | 0.03-3.95 | 0.05-264.06 | 21.87-283.5 | 0.23-20 | 0.36-769 |
| p-level | Z=0.9 p=0.3 | | Z=1.3 p=0.2 | | Z=0.15 p=0.9 | |

Z (Mann-Whitney U test)

Three months after vaccination, females had non-significantly higher serum anti-S-RBD IgG antibodies with all three methods (p=0.41 for Maglumi RBD, p=0.15 for Maglumi NAb's and p=0.75 for VIDAS RBD) (Table 2).

**Table 2.** Anti-S-RBD IgG values 3 months after vaccination with the three methods depending on gender

| **3 months after vaccination** | | | | | | |
|---|---|---|---|---|---|---|
| **Statistical parameter** | **SARS-CoV-2 antibody test:** | | | | | |
| | **Maglumi RBD** | | **Maglumi NAb's** | | **VIDAS RBD** | |
| **gender** | **male** | **female** | **male** | **female** | **male** | **female** |
| mean ± SD | 638.10±452.2 | 584.19±420.2 | 281.52±107.9 | 268.34±97.9 | 823.02±1417.1 | 654.89±699.4 |
| median | 609.34 | 627.15 | 251.86 | 245.0 | 340.81 | 516.5 |
| (IQR) | 168.74-898.1 | 129.37-847.4 | 225-301.32 | 223-281.88 | 86-1039.2 | 88-995.9 |
| min- max | 26.25-1597.4 | 12.19-1709.9 | 158-810 | 138-729 | 6.24-7361 | 2.72-5196 |
| p-level | Z=0.8 p=0.41 | | Z=1.4 p=0.15 | | Z=0.3 p=0.75 | |

Z (Mann-Whitney U test)

The difference between male and female HCWs nine months after vaccination was statistically insignificant regarding the serum values of anti-S-RBD IgG measured with Maglumi RBD (median 171.54 *vs.* 165.22 BAU/mL, p=0.38) and VIDAS RBD (68.16 *vs.* 103.3 BAU/mL, p=0.75). Females at this time point had significantly lower serum anti-S-RBD IgG measured with Maglumi NAb's (median 161 *vs.* 167 BAU/mL, p=0.017) (Table 3).

**Table 3**. Anti-S-RBD IgG values 9 months after vaccination with the three methods depending on gender

| **9 months after vaccination** | | | | | | |
|---|---|---|---|---|---|---|
| **Statistical parameter** | **Statistical parameter** | | | | | |
| | **Maglumi RBD** | | **Maglumi NAb's** | | **VIDAS RBD** | |
| **gender** | **male** | **female** | **male** | **female** | **male** | **female** |
| mean±SD | 208.39±228.7 | 170.0±155.3 | 165.31±20.6 | 157.68±22.66 | 164.61±283.4 | 130.98±139.9 |
| median | 171.54 | 165.22 | 167 | 161 | 68.16 | 103.3 |
| (IQR) | 44.53-269.0 | 32.34-239.0 | 157-179 | 151-175 | 17.2-207.84 | 17.6-199.18 |
| min- max | 6.56-1300 | 3.05-1100 | 106-220 | 100-190 | 1.25-1472.2 | 0.54-1039.2 |
| p-level | Z=0.9 p=0.38 | | Z=2.4 *p=0.017 | | Z=0.3 p=0.75 | |

Z (Mann-Whitney U test); *sig p<0.05

Twelve months after vaccination, female HCWs had non-significantly lower level of serum anti-S-RBD IgG measured by Maglumi RBD (p=0.38) and Maglumi NAb's (p=0.11) and non-significantly higher levels as measured by VIDAS RBD (p=0.75) (Table 4).

**Table 4.** Anti-S-RBD IgG values 12 months after vaccination with the three methods depending on gender

| | **12 months after vaccination** | | | | | |
|---|---|---|---|---|---|---|
| **Statistical parameter** | **Statistical parameter** | | | | | |
| **gender** | **Maglumi RBD** | | **Maglumi NAb's** | | **VIDAS RBD** | |
| | **male** | **female** | **male** | **female** | **male** | **female** |
| mean ± SD | 52.09±57.2 | 42.51±38.8 | 100.52±22.6 | 95.78±24.9 | 54.87±94.5 | 43.40±46.1 |
| median | 42.88 | 41.31 | 96.5 | 88.25 | 22.72 | 34.43 |
| (IQR) | 11.13-67.25 | 8.08-59.75 | 81.5-118 | 79-115 | 5.73-69.28 | 5.87-66.39 |
| min- max | 1.64-325 | 0.76-275 | 53-152 | 53-160 | 0.42-490.73 | 0.18-346.4 |
| p-level | Z=0.9 p=0.38 | | Z=1.6 p=0.11 | | Z=0.3 p=0.75 | |

Z (Mann-Whitney U test)

Before vaccination, anti-S-RBD IgG were detected in 7(8.54%) males and 4(3.39%) females with Maglumi NAb's method. The seropositivity for Maglumi NAb's test changed from 100% after 3 months of vaccination in both men and women, to 17.07% in men and 18.64% in women after 12 months of vaccination. The percentage of positive Maglumi NAb's test was non-significantly higher in males 9 months after vaccination (92.68% *vs.* 87.68%, p=0.22) and non-significantly higher in females 12 months after vaccination (18.64% *vs.* 17.07%; p=0.78) (Table 5).

**Table 5.** Distribution of positive and negative results of Maglumi NAb's by gender

| | **Maglumi NAb's** | | | |
|---|---|---|---|---|
| | ***Male*** | | | |
| **SARS-CoV-2 antibody test** | **before vaccination (%)** | **3 months n (%)** | **9 months n (%)** | **12 months n (%)** |
| Negative | 75(91.46) | 0 | 6(7.32) | 68(82.93) |
| Positive | 7(8.54) | 82(100) | 76(92.68) | 14(17.07) |
| | ***Female*** | | | |
| Negative | 114(96.61) | 0 | 15(12.71) | 96(81.36) |
| Positive | 4(3.39) | 118(100) | 103(87.29) | 22(18.64) |
| difference test p-level (male *vs.* female) | p=0.12 | | p=0.22 | p=0.78 |

With the VIDAS RBD method anti-S-RBD IgG were detected in one female before vaccination. The seropositivity rates for the VIDAS RBD test among males and females also changed significantly over time. Three months after vaccination, the rates were 96.34% for males and 97.46% for females, whereas twelve months post-vaccination, they decreased to 52.44% for males and 61.02% for females (Table 6). No statistically significant difference was found in the percentage of positive males and females for the VIDAS RBD test at the three time points analyzed after vaccination (p=0.65, p=0.64 and p=0.23, respectively 3, 9 and 12 months after vaccination).

Prior to vaccination, the Maglumi RBD method did not detect anti-S-RBD IgG in male and female HCWs. Seropositivity for the Maglumi RBD test varied from 100% 3 months after vaccination in both men and women, to 89.03% in men and 87.29% in women 12 months after vaccination. The Maglumi RBD test showed a slightly higher rate of positivity among male HCWs at 9 and 12 months post-vaccination compared to female HCWs. However, these differences were not statistically significant (100% *vs.* 99.15%, p=0.4) and (89.03% *vs.* 87.29%, p=0.71), respectively (Table 7).

**Table 6.** Distribution of positive and negative VIDAS RBD results by gender

| | Vidas RBD | | | |
|---|---|---|---|---|
| | ***Male*** | | | |
| **SARS-CoV-2 antibody test** | **before vaccination n (%)** | **3 months n (%)** | **9 months n (%)** | **12 months n (%)** |
| Negative | 82(100) | 3(3.66) | 24(29.27) | 39(47.56) |
| Positive | 0 | 79(96.34) | 58(70.73) | 43(52.44) |
| | ***Female*** | | | |
| Negative | 117(99.15) | 3(2.54) | 31(26.27) | 46(38.98) |
| Positive | 1(0.85) | 115(97.46) | 87(73.73) | 72(61.02) |
| difference test p-level (male *vs.* female) | | p=0.65 | p=0.64 | p=0.23 |

**Table 7.** Distribution of positive and negative results from Maglumi RBD by gender

| | Maglumi RBD | | | |
|---|---|---|---|---|
| | ***Male*** | | | |
| **SARS-CoV-2 antibody test** | **Before vaccination n (%)** | **3 months n (%)** | **9 months n (%)** | **12 months n (%)** |
| Negative | 82(100) | 0 | 0 | 9(10.98) |
| Positive | 0 | 82(100) | 82(100) | 73(89.03) |
| | ***Female*** | | | |
| Negative | 118(100) | 0 | 1(0.85) | 15(12.71) |
| Positive | 0 | 118(100) | 117(99.15) | 103(87.29) |
| difference test p-level (male *vs.* female) | | | p=0.4 | p=0.71 |

The level of anti-spike-RBD antibodies determined by Maglumi NAb's method significantly decreased during 12 months after vaccination in males (Friedman $\chi 2 = 216.4$, $p<0.0001$) and females (Friedman $\chi 2 = 324.2$, $p<0.0001$) (Figure 1).

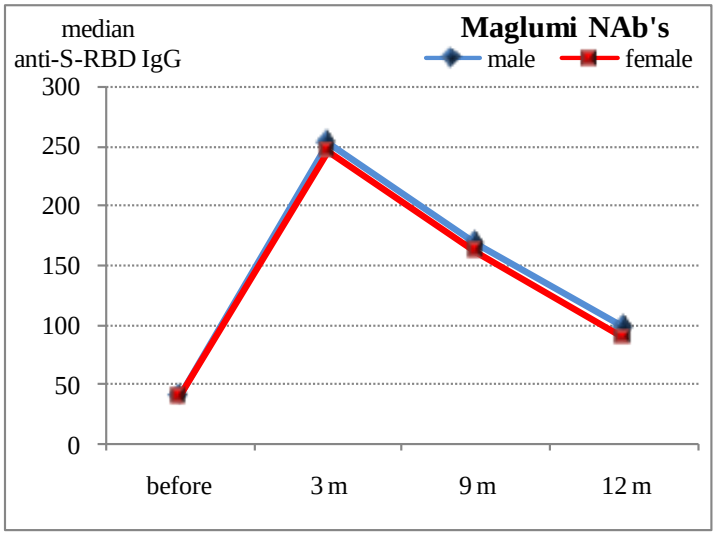


**Fig. 1.** Kinetics of anti-S-RBD IgG with Maglumi NAb's in both sexes

The VIDAS RBD method revealed a significant decline in the levels of anti-spike-RBD antibodies over the 12-month period postvaccination for both males (Friedman $\chi 2 = 212.2$, $p<0.0001$) and females (Friedman $\chi 2=295.8$, $p<0.0001$) (Figure 2).

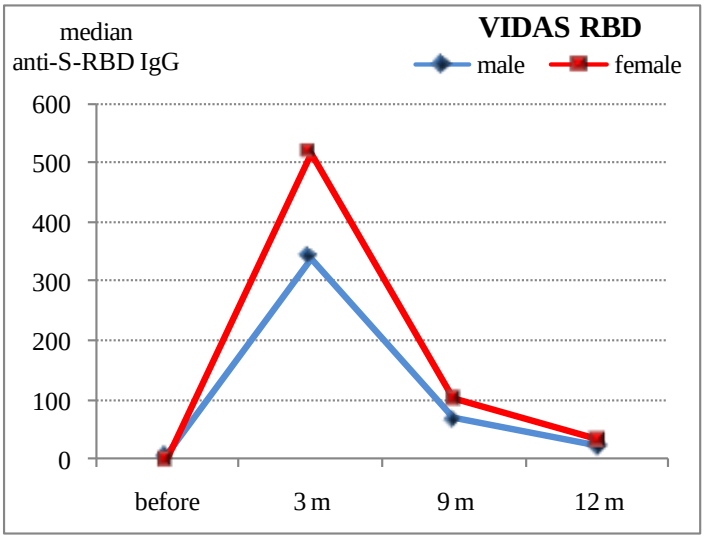


**Fig. 2.** Kinetics of anti-S-RBD IgG with VIDAS RBD in both sexes

Also, the concentration of anti-spike-RBD antibodies detected using the Maglumi RBD method showed a significant decline over the 12-month period following vaccination, both in males (Friedman $\chi 2=244.8$, $p<0.0001$) and females (Friedman $\chi 2=352.8$, $p<0.0001$) (Figure 3).

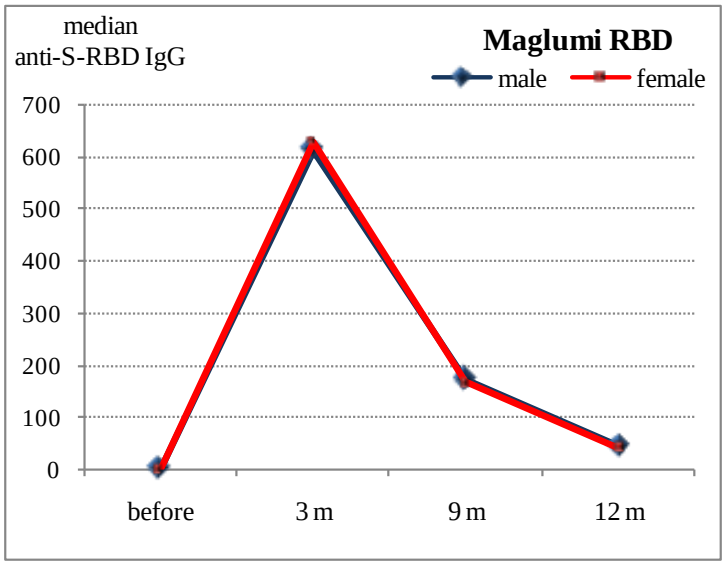


**Fig. 3.** Kinetics of anti-S-RBD IgG with Maglumi RBD in both sexes

**Discussion**

Different strategies have been suggested to enhance awareness about the COVID-19 disease and vaccine effectiveness[25]. A novel area of research has emerged, focused on the examination of the correlation between antibody titers and the degree of protection against SARS-CoV-2 infection[26]. SARS-CoV-2 antibody assays play a crucial role in assessing the percentage of infected individuals. Incorporating gender considerations into clinical practice can positively impact the enhancement of diagnosis and treatment approaches, leading to increased effectiveness in health services. This is particularly significant because influences on both the physiological aspects and the pathological progression of diseases affecting individuals of both genders[27,28].

The results of our study showed that male and female HCWs had similar serum anti-S-RBD IgG concentrations before vaccination, independent of the method used (p=0.3 for

Maglumi RBD, p=0.2 for Maglumi NAb's and p=0.9 for VIDAS RBD). Our findings demonstrated the highest concentration of antibodies three months after vaccination in both genders, where females had non-significantly higher serum anti-S-RBD IgG antibodies with all three methods. The median value of anti-spike-RBD antibodies measured by Maglumi RBD was 609.34 and 627.15 BAU/mL; by Maglumi NAb's it was 251.86 and 245 BAU/mL, and by VIDAS RBD 340.81 and 516.5 BAU/mL for males and females, respectively. The level of anti-spike-RBD antibodies significantly decreased in all HCWs during the 12 months post-vaccination using the three commercial methods ($p<0.0001$). This is in agreement with several studies that have demonstrated a decline in the efficacy of BNT162b2 against symptomatic infections with SARS-CoV-2 over time[22,29,30]. Unlike the recent studies of Salvagno *et al*., who demonstrated that females had a significantly higher response to total anti-SARS-CoV-2 RBD antibodies[31], and Terpos *et al*., who also observed that among octogenarian vaccine recipients, females had higher levels of total anti-SARS-CoV-2 RBD antibodies in comparison to males[32], we found significantly lower serum anti-S-RBD IgG measured with Maglumi NAb's (median 161 *vs*. 167 BAU/mL, p=0.017) in females nine months after vaccination. But, at this time point, the difference between male and female HCWs was statistically insignificant regarding the serum values of anti-S-RBD IgG measured with Maglumi RBD (median 171.54 *vs.* 165.22 BAU/mL, p=0.38) and VIDAS RBD (68.16 *vs.* 103.3 BAU/mL, p=0.75). This is in accordance with the study by Dörschug *et al*., who used a spike protein-based IgG serological immunoassay to monitor the humoral response to the COVID-19 mRNA BNT162b2 vaccine[33]. Similarly, they did not identify significant differences between genders. Interestingly, infected women demonstrate elevated IgG levels compared to men, suggesting a potential association with the higher survival rates and improved prognosis observed in females[34,35]. Furthermore, the study by Kutsuna *et al*. demonstrated that the antibody levels in males were higher than in females[36]. Unlike this study, Robbiani *et al*. showed that antibody levels in males were lower than in females[37]. Nonetheless, the results of our study showed that there were no significant differences in IgG level in both genders. Despite the potential influence of genetic and non-genetic factors on this variation, the specific cellular and molecular mechanisms underlying the antibody outcomes remain unknown and necessitate further research.

## Conclusion

Our results demonstrated no significant differences in SARS-CoV-2 IgG antibody concentrations against spike protein over a period of 3, 9 and 12 months after two doses vaccine regimen with BNT162b2 between male and female HCWs in the Republic of North Macedonia. This discovery clarifies that a distinct therapeutic approach for males and females is not necessary. Additional research is needed to clarify the relationship between serological response and functional immunity against SARS-CoV-2 infection in both genders.

*Conflict of interest statement.* None declared.

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