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Humoral response to the SARS-CoV-2 BNT162b2 mRNA vaccine: Real-world data from a large cohort of healthcare workers

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ABSTRACT

Background: The SARS-CoV-2 pandemic was responsible for the death of millions of people around the world, which accelerated the study of vaccines. The BNT162b2 mRNA COVID-19 is a messenger RNA vaccine that encodes the spike protein of the virus. However, the duration of the protection conferred by this vaccine and factors associated with immune responses require validation in large cohorts.

Methods: Here, we present data of humoral immune response to vaccination in 4264 healthcare workers, tested before (T0) and 15 and 90 days (T1 and T2, respectively) following vaccination. Peripheral blood was collected for immunological analysis using the Quant SARS-CoV-2 IgG II Chemiluminescent Microparticle Immunoassay (CMIA) to determine anti-spike IgG, receptor binding domain (RBD), S1 subunit of SARS-CoV-2.

Findings: At T0, 96·8% (n = 4129) of participants had IgG antibodies non-reactive to anti-SARS-CoV-2. Fifteen days after completing the vaccination, the IgG overall median titer was significantly elevated ($21{\cdot}7x10^3$ AU/mL). Both for uni- and multivariate logistic regression analyses women presented higher antibody levels than men, independent of age. Titers were significantly altered among age groups, decreasing by each increase in 10-year of age. At 3 months after completing the vaccination, anti-SARS-CoV-2 IgG titers were 6·3-fold diminished.

This real-world post-vaccination data confirmed production of a frequent and elevated anti-SARS-CoV-2 IgG titers, associated with high protection rates. Females and younger participants had higher titer 15 days after vaccination, and despite the significant reduction from 15-to-90 days, those with higher pre-vaccination titers maintained higher levels throughout the remaining timepoints.

Interpretation: These findings support the need to track humoral immunity kinetics to uncover viral susceptibility and eventually implement re-vaccination, particularly in groups prone to lower humoral immune response.

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1. Introduction

In the late 2019, coronavirus disease (COVID-19) spread all over the word declaring a new pandemic. At that time, the immunology of coronavirus infections was not at the forefront of research in most laboratories. However, over the past 12 months, we have gained novel insights into the innate and adaptive immune responses against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and vaccines against the virus have since been developed [1].

Messenger RNA (mRNA) vaccines against severe acute respiratory syndrome (SARS-CoV-2), the causative agent of COVID-19, offer a great promise to control the spread of infection. One of the available vaccines is BNT162b2 mRNA COVID-19 vaccine (Pfizer/BioNTech), a lipid nanoparticle-formulated, nucleoside-modified RNA encoding SARS-CoV-2 full length spike, modified by two proline mutations to lock in the prefusion confirmation [2]. Data from clinical trials and noncontrolled reports demonstrated efficacy above 90% for preventing disease [3].

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Humoral immune responses to SARS-CoV-2 are mediated by antibodies directed to viral surface glycoproteins, mainly the spike and nucleocapsid proteins. These antibodies neutralize viral infection of human cells and tissues expressing angiotensin-converting enzyme 2 (ACE2). The 180 kDa spike glycoprotein contains the S1 subunit, which holds a receptor-binding domain (RBD; residues 331–524) that mediate viral binding to ACE2 receptors on susceptible cells and is the main target for SARS-CoV-2 neutralizing antibodies [4]. Therefore, antibody titer might be a good biomarker for the protective efficacy of antibodies and successful humoral immune responses after SARS-CoV-2 exposure. Indeed, antibody response against spike, nucleocapsid and RBD proteins were well correlated with plaque reduction neutralization test in patients with PCR-confirmed COVID-19 [5].

The duration and kinetics of humoral immunity from Sars-CoV-2 vaccine developed by Pfizer, remains unknown, particularly lacking data from large, real-world studies. It was recently shown that after the first contact with the virus, B cells produce antibodies, which however decrease after a few weeks [6]. Notwithstanding, mRNA vaccines seem to induce a persistent immune response in germinal centers that remain active producing B cells, which are producing antibodies to fight infection [7].

In Portugal, the vaccination campaign started in late December 2020, with the first available doses of BNT162b2 mRNA COVID-19 vaccine (Pfizer/BioNTech) being delivered to healthcare workers (HCW). In this study, we report humoral immunity data of the first 3 months follow up post-vaccination.

2. Methods

2.1. Study design

This study began in December 2020, with peripheral blood collection for immunological analysis at 5 key points: pre-vaccine baseline (T0), and then two weeks (T1), three months (T2), six months (T3) and a year (T4) after second dose. Here, we present findings from timepoints T0-to-T2. The study was approved by the CHUC ethics committee (OBS.SF.106–2021). The requirement for informed consent was waived by the Ethics Committee.

2.2. Setting and participants

Healthcare workers from Centro Hospitalar e Universitário de Coimbra (CHUC) were tested for anti-spike IgG antibody, before taking the first dose of vaccine (up to 72 h) (T0), and then 15 days (T1) and 3 months (T2) after taking the second dose. Patients with prior SARS-CoV-2 diagnosis were excluded from the first phase of vaccination and from the analysis. Fig. 1 depicts the workflow with included and excluded subjects. Data of HCW who attended T0 and T1 were first analyzed to evaluate the peak response after vaccination. To analyze the kinetics of antibody titers over time, only health care worker who attended T0, T1 and T2 were included. All data was analyzed anonymously.

2.3. Laboratory assays

Serum was obtained and processed within 4 h after blood collection. A chemiluminescent microparticle immunoassay (CMIA) SARS-CoV-2 IgG II Quant was used to determine IgG anti-spike, receptor-binding domain (RBD), S1 subunit of SARS-CoV-2, following manufacturer's instructions (Abbott Laboratories), on Alinity *i* (Abbott Laboratories). As per manufacturer recommendations, antibody titers above 50 AU/mL were considered reactive. All measurements were undertaken following appropriate quality control procedures, daily performed for routine clinical assessment of SARS-CoV-2 IgG.

2.4. Statistical methods

Departure from normality was tested using Shapiro-Wilk. Data was presented as median (M) and interquartile range (IQR). SARS-CoV-2 IgG titers were compared among groups using Mann-Withney U and Kruskal-Wallis tests, with Bonferroni correction for multicomparison. Antibody levels were compared between timepoints using Wilcoxon Signed rank tests.

The neutralizing activity, able to efficiently block virus entry in human cells, was measured by Abbott Laboratories using the Broad Institute Plaque Reduction Neutralization Test (PRNT) as inhibition at a sample dilution of 1:250 or greater. The 95% probability (95% C.I.: 78% – 99%) of being at or above that PRNT dilution (1:250) for SARS-CoV-2 IgG II Quant AU/mL values, yielded a value of 4160. Thus, for logistic regression analyses we used IgG antibodies titers > 4160 AU/mL as an indicator of strong neutralizing activity, as previously reported [8]. Regression analyses with stepwise were conducted including as covariates gender and age. Spearman correlation coefficients were calculated to assess the causal association among continuous variables. The level of significance was established at $P < 0{\cdot}05$. Statistical analyses were conducted using R.

3. Results

Participants (n = 4264) provided samples before and 15 days after vaccination with Pfizer BioNTech vaccine, whereas 3417 also donated a third blood sample 3 months later. The median age was 44 years (IQR 34 – 54 years) and 3221 were women (75·7%).

Before vaccination, 96.8% (n = 4129) had anti-SARS-CoV-2 spike IgG antibody levels below 50 AU/mL, with a median value under 6·8 AU/mL and none presenting titers above 4160 AU/mL.

At T1, we found a significantly elevated overall median titer of $21{\cdot}7x10^3$ AU/mL ($13{\cdot}1–32{\cdot}2x10^3$ AU/mL), compared to T0 ($p < 0{\cdot}0001$). At the T1 timepoint, subjects with titers < 50 AU/mL on T0 had significantly lower titers than those with titers > 50 AU/mL (20·8, 13·0-31·8 $x10^3$ and 30·4, 19·1-41·6 $x10^3$ AU/mL, respectively) ($P < 0{\cdot}0001$). Fifteen days following vaccination (T1) only 6 subjects (0·1%) presented with values below 50 AU/mL, 95 (2·2%) between 50 and 4160 AU/mL and 4163 (97·6%) above 4160 UA/mL. At this time point, females had a median titer significantly higher than males ($22{\cdot}1$ $x10^3$, $13{\cdot}9–33{\cdot}4$ $x10^3$ and $18{\cdot}0$ $x10^3$, $11{\cdot}5-28{\cdot}2$ $x10^3$ AU/mL, respectively) ($P < 0{\cdot}0001$), whereas significant differences were observed between age groups titers ($P < 0{\cdot}0001$), decreasing as age increased (Table 1). Logistic regression analysis including gender and age to predict the development of titers above 4160 AU/mL in T1, revealed women are at increased odds for developing robust humoral immune response (OR, 2·33; CI 95%, 1·6-3·5). Furthermore, for each 10 year-increase of age there was a lower probability for a humoral immune response above 4610 AU/mL (OR, 0·5; CI 95%, 0·4-0·6) (Fig. 2). Interaction factors were not significant and were excluded in stepwise analysis.

A significant decrease in IgG titers was observed between T1 and T2 ($P < 0{\cdot}0001$), with an overall median of $3{\cdot}2$ $x10^3$ AU/mL ($2{\cdot}0-5{\cdot}2$, $p < 0{\cdot}0001$) at T2. At 3 months following vaccination differences among genders and between age groups remained significant, (both at $P < 0{\cdot}0001$). Titers decreased by 6·3 times (IQR 4·8-8·3) from T1-to-T2 (Table 2). Logistic regression analysis evidenced that female gender (OR, 1·4; CI 95%, 1·6-2·3) and earlier age (OR, 0·8; CI 95%, 0·7-0·8) were significantly associated with antibody levels above 4160 UA/mL, still after 3 months of vaccination.

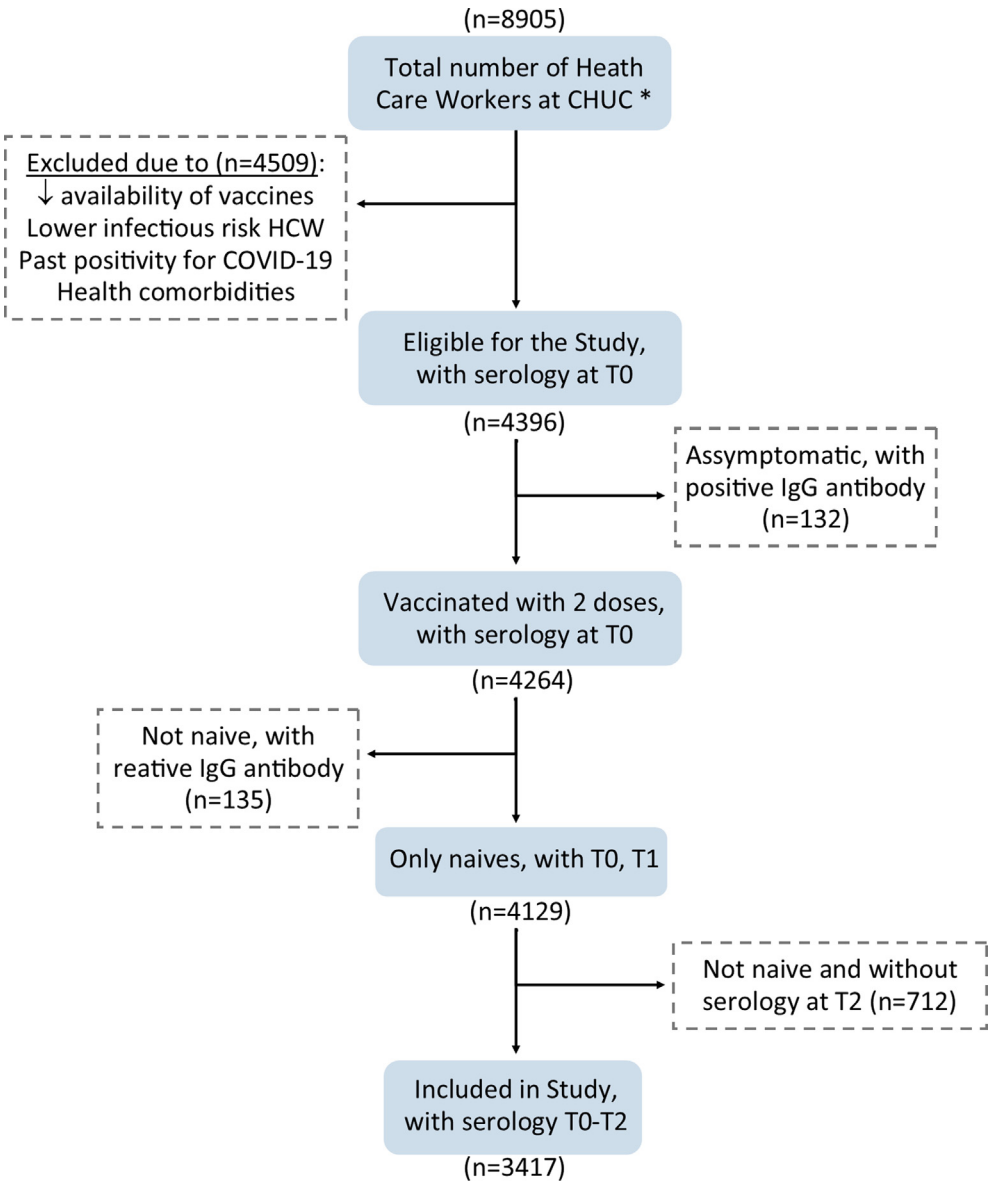

Fig. 1. Workflow with procedures for subject inclusion and exclusion· CHUC, Centro Hospitalar e Universitário de Coimbra; HCW, health care worker; T0, timepoint of blood collection before vaccine; T1, timepoint of blood collection 15 days after the second dose of vaccine; T2, timepoint of blood collection 3 months after the second dose of vaccine.

4. Discussion

This real-world COVID-19 vaccination study yielded strong immune response, with 97·7% subjects with antibody levels above 4160 UA/mL 15 days after the second dose of the BNT162b2 mRNA vaccine. This observation matches evidence regarding efficacy so far [3].

Despite consistent post-vaccine immune response, inter-individual heterogeneity has been noted in specific populations, particularly elderly and immunosuppressed patients [6,9]. Notably, comorbidities and immunosuppression have been reported in association with worse outcome from SARS-CoV-2 infection [10], and as significant predictors of failure to mount a humoral response after SARS-CoV-2 vaccination [11–13]. Here, the 6 participants that have not developed IgG antibodies in response to vaccination in T1, were on immunosuppressive anti-TNF therapy. In agreement, the response rate to HBV vaccination, even with a double-dose schedule, was exceptionally low for patients receiving anti-TNF [14].

Our data from univariate and multivariate analyses in a large set of vaccinated subjects, demonstrate that as age increase it becomes less probable to develop a robust immune response, both at 15 days and 3 months following vaccination. It is consensual, that the immune system suffers from the effects of biological aging, exhibiting a progressive decline in function, collectively resulting in diminished humoral and cellular immune responses [15,16]. This has been associated with reduced antibody responses after COVID-19 vaccination [17–20].

The association between total anti-SARS-CoV-2 antibody levels and sex has been controverse in response to COVID-19 mRNA vaccine. Some studies failed to find significant differences between sexes [20], while other observed higher levels in women compared to men [12,17,20,21]. In our real-world study that included a large sample of HCW, women presented higher SARS-CoV-2 IgG titers at

Table 1
HCW characteristics and SARS-CoV-2 IgG antibody titers after vaccination for HCW without previous immune response [Median (IQR) x10^3 AU/mL].

	Post-vaccination (T1)		
	n (%)	SARS-CoV-2 IgG Ab	P
Overall	4264(100)	21·7 (13·1-32·2)	–
Gender			
Male	1047 (24·6)	18·0 (11·5-28·1)	
Female	3217 (75·4)	22·1 (13·9-33·4)	< 0·0001
Age group, years			
18–30	695 (16·3)	25·7 (17·3-36·4)	
30–40	1064 (25·0)	22·5 (15·5-33·7)	
40–50	1085 (25·4)	19·6 (12·3-30·9)	
50–60	1057 (24·8)	19·37 (11·3-30·7)	
>60	363 (8·5)	15·7 (9·8-26·8)	< 0·0001*
SARS-CoV-2 reactivity, AU/mL			
< 50	4129 (96·8)	20·8 (13·0-31·8)	
> 50	135 (3·2)	30·4 (19·1-41·6)	<0·0001

* Kruskal Wallis test; pairwise comparisons revealed significant differences between all age groups (P < 0·001- 0·006), except for 50–60 compared with 40–50 (P = 1·0) (P-values were adjusted using Bonferroni multicomparison correction). AU, arbitrary units.

15 days and at 3 months after mRNA vaccine, with this difference remaining significant within all age groups. Despite limitations from reduced sample size or distinct IgG serological immunoassays might partially explain the lack of consistence, biological differences associated with sex also represent an important source of variation that impact the immune response to vaccination [22]. Gonadal hormones and stronger T cell activation are hypothesized as women immunological advantages in response to vaccination [22,23]. Sex differences in antibody responses have been well established in influenza A and B viruses [24].

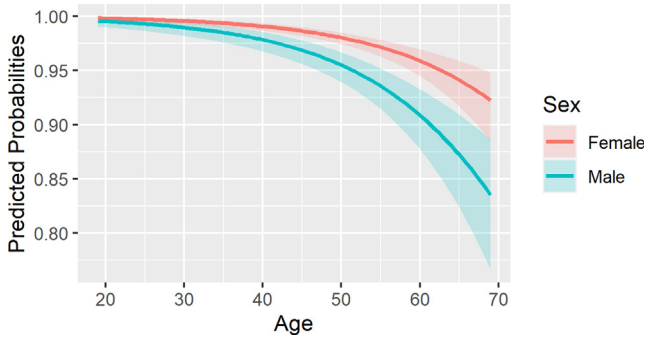

Fig. 2. Estimated probabilities for an immune response above 4160 AU/mL, by age and gender before vaccination.

Even though from over 4000 participants, only 135 presented with seropositive SARS-CoV-2 IgG levels before vaccination. We first analyzed the response to vaccination in naïve subjects, and then included seropositive (possible previous contact with the virus, asymptomatic HCW) as a small independent group. Individuals who naturally contacted with SARS-CoV-2 are expected to develop a more rapid and sustained response to COVID-19 vaccines than naïve individuals [25,26]. In our cohort, the antibody response was maintained at higher titers in seropositive compared to seronegative participants for the timepoints T1 and T2, which is supported by others [13,27–30]. To the best of our knowledge, only one report showed no difference between vaccinated subjects, with and without previous SARS-CoV-2 infection [21]. As observed

Table 2
HCW characteristics and SARS-CoV-2 IgG antibody titers after vaccination for naïve HCW.

		SARS-CoV-2 IgG Ab			
		Pre-vaccine (AU/ml)	**Post-vaccine** (x10^3 AU/ml)		
	N (%)	**T0**	**T1**	**T2**	**Fold Decrease (T1-T2)**
Overall	3417 (100)	<6·8 (-)	21·2 (13·2-32·5)	3·2 (2·0-5·2)	6·3 (4·8-8·3)
Gender					
Male	818 (23·9)	<6·8 (-)	18·1 (11·2-28·6)	2·9 (1·7-4·8)	6·0 (4·5-7·8)
Female	2599(76·1)	<6·8 (-)	22·1 (14·1- 33·7)	3·4 (2·1-5·3)	6·4 (4·9-8·4)
P-value		0·32	<0·0001	<0·0001	<0·0001
Age group, yrs					
18–30	520 (15·2)	<6·8 (-)	26·1 (17·6-37·7)	4·3 (2·9-6·1)	6·1 (4·7-7·8)
30–40	827 (24·2)	<6·8 (-)	22·7 (15·7-33·8)	3·5 (2·3-5·4)	6·3 (4·9-8·3)
40–50	900 (26·3)	<6·8 (-)	19·9 (12·4-31·6)	2·9 (1·8-4·6)	6·7 (5·1-8·8)
50–60	864 (25·3)	<6·8 (-)	19·4 (11·4-30·7)	2·9 (1·7-5·0)	6·0 (4·7-8·0)
>60	306 (9·0)	<6·8 (-)	16·7 (10·7-28·8)	2·8 (1·7-4·2)	5·9 (4·2-7·5)
P-value		0·72*	<0·0001**	<0·0001***	<0·0001****
Baseline, AU/mL					
T0 < 50	81 (2·4)	< 6·8(-)	21·1 (13·2-32·1)	3·3 (2·0-5·1)	6·3 (4·9-8·3)
T0 > 50	3417(97·6)	156 (96–371)	31·6 (17·9-42·2)	7·8 (3·5-13·8)	3·7 (2·5-5·5)
P-value		0·72	<0·0001	<0·0001	<0·0001

* Kruskal Wallis test, pirwise comparisons did not reveal significant differences between groups;
** Kruskal Wallis test, pairwise comparisons revealed significant differences all groups, except for 40–50 vs· 50–60 years and 50–60 vs· >60 years· (p < 0·001-0·02);
*** Kruskal Wallis test, pairwise comparisons revealed significant differences all groups, except for 40–50. vs 50–60 years, 40–50 vs. > 60 years and 50–60 vs. > 60 years (p < 0·001);
**** Kruskal Wallis test, pairwise comparisons revealed significant differences between > 60 vs. 30–40 and 40–50, <30 vs. 40–50, 30–40 vs. 50–60 and 40–50 vs. 50–60 years (p < 0·0001-0·013) (P-values were adjusted using Bonferroni multicomparison correction). AU, arbitrary units.

in other studies [7,20], both seropositive and seronegative subjects had significant antibody decline at 3 months versus 15 days following vaccination.

Although we observed a significant increase in SARS-CoV-2 IgG titer from T0 to T1 (15 days after second dose vaccination), only 2.5 months after T1 (at T2) we found a significant 6·3-fold decrease in antibody levels. In agreement, significant declines were also observed in other cohorts at 3 months post-COVID-19 mRNA vaccination [20,21]. Long-term antibody kinetics in vaccinated subjects remain largely unknown, however a peak of anti-S IgA and IgG titers has been observed 5 weeks after immunization with a decline by 15 weeks after the second dose of vaccine [7]. Cumulatively, in other human seasonal corona virus and MERS infections, similar declines on antibody response were reported, with a short-lasting protective immunity [31].

Emerging information on COVID-19 mRNA vaccine, mostly from small studies with short follow up, are in line with data presented here. We designed a cohort study to assess humoral immunity response (SARS-CoV-2 IgG levels) in over 4000 subjects, at pre-vaccine, and two weeks, three months, six months, and a year after the second dose. This real-world post-vaccination data revealed significant titters of anti–SP1-RBD, confirming high protection rates. After 3 months follow up, findings suggest that being female, seropositive for SARS-CoV-2 or younger before vaccination are important predictors for developing a robust humoral immune response to BNT162b2 mRNA vaccine. Despite early significantly elevated antibody levels 15 days post-vaccination, we noticed a 6·3-fold decrease at 3 months follow-up, pinpointing the need to track humoral immunity kinetics to uncover viral susceptibility and re-vaccinate. Serological analyses might add knowledge to refine testing strategies for those requiring closer monitoring or earlier vaccination, to ensure effective immunity and protection against infection.

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Declaration of Competing Interest

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.

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Authors contribution

JOS, TReis, CL and RB contributed to conceptualization, methodology, investigation, writing the original draft and to final review and editing of the manuscript. RR contributed to conceptualization, methodology, investigation, writing the original draft, supervision, and to final review and editing of the manuscript. GM contributed to data curation and to writing - review and editing. VP, TRodrigues, AA, VP, contributed to resources, investigation and final review and editing of the manuscript. LA, FR, IA contributed to conceptualization, supervision, and to final review and editing of the manuscript.

References

[1] Carvalho T, Krammer F, Iwasaki A. The first 12 months of COVID-19: a timeline of immunological insights. Nat Rev Immunol. 2021;21:245–56. Available from: 10.1038/s41577-021-00522-1.
[2] Kitchin N, Absalon J, Gurtman A, Lockhart S, Neuzil K, Mulligan MJ, et al. Safety and Immunogenicity of Two RNA-Based Covid-19. Vaccine Candidates. 2021:2439–50.
[3] Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020;383 (27):2603–15.
[4] Sternberg A, Naujokat C. Structural features of coronavirus SARS-CoV-2 spike protein: Targets for vaccination. Life Sci. 2020;257(118056):1–7.
[5] Poland GA, Ovsyannikova IG, Kennedy RB. SARS-CoV-2 immunity: review and applications to phase 3 vaccine candidates. Lancet [Internet]. 2020;396 (10262):1595–606. Available from: http://dx.doi.org/10.1016/S0140-6736 (20)32137-1
[6] Geisen UM, Berner DK, Tran F, Sümbül M, Vullriede L, Ciripoi M, et al. Immunogenicity and safety of anti-SARS-CoV-2 mRNA vaccines in patients with chronic inflammatory conditions and immunosuppressive therapy in a monocentric cohort. Ann Rheum Dis Epub ahead print [Jully 7 2021]. 2021; (Cid):1–6.
[7] Turner JS, O'Halloran JA, Kalaidina E, Kim W, Schmitz AJ, Zhou JQ, et al. SARS-CoV-2 mRNA vaccines induce persistent human germinal centre responses. Nature 2021.
[8] Ebinger JE, Fert-Bober J, Printsev I, Wu M, Sun N, Prostko JC, et al. Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2. Nat Med [Internet]. 2021;27:981–4. Available from: 10.1038/s41591-021-01325-6.
[9] Chavarot N, Ouedrani A, Marion O, Leruez-Ville M, Villain E, Baaziz M, et al. Poor Anti-SARS-CoV-2 Humoral and T-cell Responses After 2 Injections of mRNA Vaccine in Kidney Transplant Recipients Treated with Belatacept. Transplantation 2021.
[10] Cravedi P, Mothi SS, Azzi Y, Haverly M, Farouk SS, Pérez-Sáez MJ, et al. COVID-19 and kidney transplantation: Results from the TANGO International Transplant Consortium. Am J Transpl. 2020;00:1–9.
[11] Grupper A, Rabinowich L, Schwartz D, Schwartz IF, Ben-Yehoyada M, Shashar M, et al. Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus. Am J Transpl. 2021;00:1–8.
[12] Kageyama T, Ikeda K, Tanaka S, Taniguchi T, Igari H, Onouchi Y, et al. Antibody responses to BNT162b2 mRNA COVID-19 vaccine in 2015 healthcare workers in a single tertiary referral hospital in Japan. medRxiv [Preprint] [Internet]. 2021;1–16. Available from: https://medrxiv.org/cgi/content/short/2021.06.01.21258188.
[13] Boyarsky BJ, Werbel WA, Avery RK, Tobian AAR, Massie AB, Segev DL, et al. Immunogenicity of a Single Dose of SARS-CoV-2 Messenger RNA Vaccine in Solid Organ Transplant Recipients. JAMA 2021;325:1784–6.
[14] JP G, JR V, A R-N, M C. No TitleEfficacy of hepatitis B vaccination and revaccination and factors impacting on response in patients with inflammatory bowel disease. Am J Gastroenterol. 2012;107(10):1460–6.
[15] Gustafson CE, Kim C, Weyand CM, Goronzy JJ. Influence of immune aging on vaccine responses. J Allergy Clin Immunol. 2020;145(5):1309–21.
[16] Bajaj V, Gadi N, Spihlman AP, Wu SC. Aging, Immunity, and COVID-19: How Age Influences the Host Immune Response to Coronavirus Infections ? Front Physiol. 2021;11(January):1–23.
[17] Terpos E, Trougakos IP, Apostolakou F, Charitaki I, Sklirou AD, Mavrianou N, et al. Age-dependent and gender-dependent antibody responses against SARS-CoV-2 in health workers and octogenarians after vaccination with the BNT162b2 mRNA vaccine. Am J Hematol. 2021;96(7):E257–9.
[18] Mülle L, Andrée M, Moskorz W, Drexler I, Walotka L, Grothmann R. Age-dependent immune response to the Biontech/Pfizer BNT162b2 COVID-19 vaccination. Clin Infect Dis. 2021;ciab381:1–18.
[19] Anderson EJ, Rouphael NG, Widge AT, Jackson LA, Roberts PC, Makhene M, et al. Safety and Immunogenicity of SARS-CoV-2 mRNA-1273 Vaccine in Older Adults. N Engl J Med. 2020;383(25):2427–38.
[20] Salvagno GL, Henry BM, Piazza G, Pighi L, De NS, Bragantini D, et al. Anti-SARS-CoV-2 Receptor-Binding Domain Total Antibodies Response in Seropositive and Seronegative Healthcare Workers Undergoing COVID-19 mRNA BNT162b2 Vaccination. Diagnostics. 2021;11(832).
[21] Sasso B Lo, Giglio RV, Vidali M, Scazzone C, Bivona G, Gambino CM, et al. Evaluation of Anti-SARS-Cov-2 S-RBD IgG Antibodies after COVID-19 mRNA BNT162b2 Vaccine. Diagnostics [Internet]. 2021;11(1135). Available from: 10.3390/diagnostics11071135.
[22] Klein SL, Jedlicka A, Pekosz A. The Xs and Y of immune responses to viral vaccines. Lancet Infect Dis 2010;10(5):338–49.
[23] Cai Y, Kim DJ, Takahashi T, Broadhurst DI, Yan H, Ma S, et al. Kynurenic acid may underlie sex-specific immune responses to COVID-19. Sci Signal 2021;14 (eabf8483).
[24] Fink AL, Klein SL, Hopkins TJ. The evolution of greater humoral immunity in females than males: implications for vaccine efficacy. Curr Opin Physiol 2018;6:16–20.
[25] Gobbi F, Buonfrate D, Moro L, Rodari P, Piubelli C, Caldrer S, et al. Antibody Response to the BNT162b2 mRNA COVID-19 Vaccine in Subjects with Prior SARS-CoV-2 Infection. Viruses. 2021;13(3):422.

[26] Anderson M, Stec M, Rewane A, Landay A, Cloherty G, Moy J. SARS-CoV-2 Antibody Responses in Infection-Naive or Previously Infected Individuals After 1 and 2 Doses of the BNT162b2 Vaccine. JAMA 2021;4(8):8–10.
[27] Manisty C, Otter AD, Treibel TA, McKnight Á, Altmann DM, Brooks T, et al. Antibody response to first BNT162b2 dose in previously SARS-CoV-2-infected individuals. Lancet 2021;397(10279):1057–8.
[28] Jabal KA, Ben-amram H, Beiruti K, Batheesh Y, Sussan C, Zarka S. Impact of age, ethnicity, sex and prior infection status on immunogenicity following a single dose of the BNT162b2 mRNA COVID-19 vaccine : real-world evidence from healthcare workers, Israel, December 2020 to January 2021. Euro Surveill. 2021;26(6):2100096.
[29] Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science (80-). 2021;371(587):1–13.
[30] Salvagno GL, Henry BM, Pighi L, De Nitto S, Gianfilippi GL, Lippi G. Three-month analysis of total humoral response to Pfizer BNT162b2 mRNA COVID-19 vaccination in healthcare workers. J Infect [Internet]. 2021;88(2):10–1. Available from: https://doi.org/10.1016/j.jinf.2021.06.024.
[31] Carrillo J, Izquierdo-Useros N, Avila-Nieto C, Pradenas E, Clotet B, Blanco J. Humoral immune responses and neutralizing antibodies against SARS-CoV-2; implications in pathogenesis and protective immunity. Biochem Biophys Res Commun. 2021;538:187–91.