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Evaluation of the Antibody Response Induced by the Pfizer-BioNTech COVID-19 Vaccine and the Effect Prior COVID-19 Infection has on the Response Elicited by the Vaccine.

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Abstract

Introduction

Understanding the immune memory of individuals who have naturally contracted SARS CoV-2 versus naïve individuals might help to optimise the vaccination campaigns. Here we describe the Anti-SARS-CoV-2 IgG response induced by the Pfizer-BioNTech COVID-19 Vaccine in both naive individuals and those with prior confirmed SARS-CoV-2 infection. We also look at the durability of that response over a six-month period.

Methods

This study enrolled a total of 219 participants who had completed the full course of the Pfizer BioNTech BNT162b1 COVID 19 vaccine. SARS CoV-2 IgG levels were measured at two different stages over a period of six months using Abbott Architect SARS-CoV-2 IgG II quantitative assay.

Results

After two doses of the Pfizer BioNTech BNT162b1 COVID 19 vaccine, the median SARS CoV-2 IgG concentration from all participants was 4866 AU/mL (IQR 2738-8424). Median IgG levels in naïve individuals were 4219 AU/mL (IQR 2450-7602). Median SARS CoV-2 IgG levels were significantly higher in those with a previous SARS CoV-2 infection at 8323 AU/mL (IQR 4728-16579 $p<0.001$). Median SARS-CoV-2 IgG levels decreased to 953 AU/mL (IQR 512-1730) after six months post vaccination. This represented a median decrease of 80% between the two testing periods

Conclusion

Our findings suggest that those with natural infection before vaccination produce a higher IgG response than naïve individuals as shown by a nearly 2-fold increase in the mean concentrations between the two groups.SARS-CoV-2 IgG levels showed a median decline of 2% per day.

Introduction

The coronavirus disease (COVID 19) is caused by the novel coronavirus, commonly known as SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2). The disease is thought to have first originated in Wuhan, China, in 2019 and was declared a global pandemic by the World Health Organisation on March 11th, 2020. Globally there have been over 245 million confirmed cases of COVID-19and over 5 million deaths[1].

The causative agent of COVID 19, SARS-CoV-2, is a member of the coronaviridae family, specifically the betacoronaviruses, which is one of seven known to infect humans[2,3].The pandemic's severe health and economic burden meant that there needed to be a global effort to halt the virus's spread through an effective vaccine. Several candidates appeared early in 2020, with different vaccine technologies being employed in the hope of creating an effective and safe vaccine to combat the virus. Early studies are beginning to appear surrounding the immune dynamics from the vaccines. It is essential that we fully understand this immune response to optimise the vaccination campaigns and facilitate decisions regarding booster doses.

The immune response to the invasion of SARS-CoV-2 is complex and shares similarities to its common precursors. Once the virus enters the host, the immune system then recognises epitopes on the virus's surface and activates the innate and adaptive immune response[4].The adaptive immune response plays a crucial role in controlling SARS-CoV-2 infection and inhibiting future re-infection. The adaptive immune response consists mainly of B cells and T cells (CD4+ and CD8+ T cells). These cells play a role in eliminating the invading cells and producing antibodies that help fight re-infection. T and B cells are typically seen one week post-onset of COVID-19 symptoms[5,6]. T cells are detected in almost all SARS-CoV-2 infections with CD4+ cells being more predominant than CD8+ cells[7].CD8+ T cells possess cytotoxic abilities that help to kill infected cells. The presence of CD8+ T cells has been linked to improved prognostic outcome due to their potent abilities to remove the virus[8].It is suggested that T cell response hold the key to long-duration protective immunity. Studies on SARS CoV-1 showed that T cells were present from three months up to six years post-infection[9].

While the correlates of protection have not been defined yet, it is presumed that neutralisation of the virus through NAb is the primary mechanism for viral suppression. Serum IgG concentrations have been known to correlate with circulating NAb concentrations. Measurement of IgG concentrations is a promising diagnostic biomarker for protection post-infection or vaccination[10,11].High levels of IgG concentrations have also been linked to high levels of T cells producing IFN γ. While antibodies levels do decline over time, strong evidence suggests that T cell immunity does persist longer, implying that immunity does persist long after undetectable antibodies. Studies from MERS and SARS-CoV-1 patients have shown to have persistent memory T cell immunity years after infection [12].

This study aims to look at the IgG response following a two-dose campaign of the Pfizer–BioNTech COVID-19 vaccine. Particular attention will be focused on those who have previously tested positive for SARS-CoV-2. The study also aims to evaluate the durability of the IgG response over a period of six months. The study aims to look at how quickly the immune response declines after vaccination and also to measure the level of IgG response to confer protective immunity.

Methods

This study enrolled 215 participants from the Mercy University Hospital in Cork, Ireland. All individuals who partook in the study had to have completed the full course of the Pfizer BioNTech BNT162b1 COVID 19 vaccine (2 doses with an interval of 21-28 days between first and second doses). The study was open to every profession from within the hospital. All participants were to be >18 years of age.

Participants were asked to provide one adult serum sample (2.5 ml) at two different time periods. The initial phase of this study aimed to obtain the first sample from all participants at 14 days post second dose of the Pfizer BioNTech BNT162b1 COVID 19 vaccine. Samples taken from the initial cohort of participants ranged from 14-72 days post second dose. Participants were asked to return six months post vaccine to provide another adult serum sample (2.5 ml).Samples taken at the six-month interval ranged from 166-227 days post second dose of the vaccine.

SARS CoV-2 IgG levels were measured using Abbott Architect SARS-CoV-2 IgG II quantitative assay (Abbott, Abbot Park, US). The assay is a chemiluminescent microparticle immunoassay (CMIA) designed to detect SARS CoV-2 IgG antibodies, including neutralising antibodies to the RBD of the S1 subunit of the spike protein with high specificity and sensitivity.

Results

A total of 219 participants were included in the first phase of this study with the median age of enrolled participants being 40 years of age. Demographic variables and corresponding SARS-CoV-2 IgG levels are displayed in table 1 in the form of median and IQR. Groups were comparable for gender ($p = 0.351$) and profession ($p = 0.161$) but differed significantly for age ($p = 0.018$), close contact ($p = 0.031$) and prior infection status ($p < 0.001$).During GLM analysis, age ($p =0.001$) and prior infection status ($p >0.001$) (figure 1), but not gender ($p = 0.557$), close contact status ($p = 0.249$) and profession ($p = 0.139$), were independent predictors of SARS-CoV-2 IgG levels. Analysis on severity of symptoms during COVID-19 infection showed that those with mild ($p >0.001$) and severe ($p >0.001$), but not moderate ($p = 0.393$) symptoms, were independent predictors of SARS-CoV-2 IgG levels over naïve individuals (figure 2).Of note there was one female participant aged 63 who produced a response of >40,000 AU/ml, which was at the upper limit of detection for this assay. A 1:10 dilution was made of their serum, and a value of 7,132 AU/mL was obtained. This individual had a severe SARS-CoV-2 infection three months before sampling.

Participants were resampled again at 6 months post vaccine. A total of 133 participants were enrolled in the second phase of testing with a median age of 42. Demographic variables and corresponding SARS-CoV-2 IgG levels are displayed in table 2 in the form of median and IQR. Groups were comparable for gender ($p = 0.563$), age ($p = 0.223$) and profession ($p = 0.732$) but differed significantly for close contact ($p = 0.036$), prior infection status ($p < 0.001$) and COVID-19 infection post vaccination ($p = 0.017$).During GLM analysis, age ($p >0.001$), post vaccination infection status ($p > 0.001$), prior infection status pre vaccination ($p > 0.001$) and profession ($p = 0.036$), but not gender ($p = 0.563$), were independent predictors of SARS-CoV-2 IgG levels at 6 months post vaccination.

Of the 133 participants who were sampled at 6 months post vaccination, only two had subsequent positive PCR confirmed COVID-19 infections. Both subjects reported only mild infection. The first individual had an antibody titre of 609.6 AU/mL at 28 days post vaccination. This individual tested positive 136 days post vaccination with a subsequent increase in SARS-CoV-2 IgG level to 16,689 AU/mL. The second individual had an antibody titre of 3597.7 AU/mL at 27 days post vaccination. This individual tested positive 150 days post vaccination with a subsequent increase in SARS-CoV-2 IgG levels to 12,021 AU/mL.

The rate of antibody decline was measured in 133 returning participants. The median decline in SARS-CoV-2 IgG levels was seen at 79% (IQR 68%-88%) over the period between testing, with male and females at 86% (IQR 79%-89%) and 78% (IQR 65%-87%)respectively. The median rate of decline was seen to be 2% per day of SARS-CoV-2 IgG levels.

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Table 1. Median concentration of SARS-CoV-2 IgG antibodies in Healthcare Workers post full course of the Pfizer BioNTech BNT162b1 COVID 19 vaccine (2 doses with an interval of 21-28 days between first and second doses).

Variables		Sample Size n (%)	IgG median concentration (AU/mL[a])	Interquartile ranges (Q1-Q3)
All participants in the study		219 (100)	4866	2738-8424
Sex	Male	47 (21.4)	4078	2685-9577
	Female	172 (78.6)	5029	2680-8423
Age	<30	48 (22.3)	5884	3665-12505
	30-39	57 (26.5)	5281	3020-9026
	40-49	67 (30.7)	3706	2042-6070
	50-59	39 (16.7)	4532	3055-7612
	60+	8 (3.7)	7387	1235-11747
Professional Category	Doctor	37 (16.7)	5972	3464-11062
	Nurse	80 (36.3)	4223	2111-7324
	Medical Scientist	52 (24.2)	5222	2464-8428
	Clerical	11 (5.1)	6697	3868-9286
	Allied Health Professionals [b]	29 (13)	4906	3415-8108
	Household Services [c]	10 (4.7)	3076	1219-9287
Prior Infection Status	Laboratory confirmed PCR positive for SARS-CoV-2	35 (16.3)	8323	4728-16579
	No previous PCR confirmed SARS-CoV-2 Infection	184 (83.7)	4219	2450-7602
The severity of symptoms associated with prior infection status	Mild	19 (8.8)	8323	5396-17979
	Moderate	12 (5.6)	6104	3547-13340
	Severe	4 (1.9)	15902	3805-35126
COVID 19 Symptoms within the last month	Yes	9 (4.2)	5798	2538-15335
	No	210 (95.8)	4849	2675-8458
Been deemed a close contact previously [d]	Yes	50 (23.3)	5892	3920-10137
	No	169 (76.7)	4619	2325-8168

[a] *Arbitrary units per millilitre*

[b] *Allied Health Professionals consisted of: Healthcare Assistants, Physiotherapists, Speech and Language Therapists, Phlebotomists, Dieticians and ECG Technicians.*

[c] *Household Services consisted of: Catering, Porters, Security and Maintenance*

[d] *Close contact definition according to the WHO, "Spending more than 15 minutes of face-to-face contact within 2 metres of someone who has COVID-19, indoors or outdoors. Living in the same house or shared accommodation as someone who has COVID-19. Sitting within 2 seats of someone who has COVID-19 on public transport or an airplane".*

Table 2. Median concentration of SARS-CoV-2 IgG antibodies in Healthcare Workers at **six** months post full course of the Pfizer BioNTech BNT162b1 COVID 19 vaccine (2 doses with an interval of 21-28 days between first and second doses)

Variables		Sample Size n (%)	IgG median concentration (AU/mL[a])	Interquartile ranges (Q1-Q3)
All participants in the study		133 (100)	953	512-1730
Sex	Male	28 (21.1)	1213	433-2079
	Female	105 (78.6)	915	490-1575
Age	<30	26 (19.5)	1081	579-1823
	30-39	29 (21.8)	976	523-2169
	40-49	44 (33.3)	747	193-1168
	50-59	28 (21.1)	1020	457-1772
	60+	6 (4.5)	1625	411-15929
Professional Category	Doctor	15 (11.3)	1077	399-1912
	Nurse	54 (40.6)	873	538-1328
	Medical Scientist	39 (29.3)	1258	533-2546
	Clerical	8 (6)	963	391-1719
	Allied Health Professionals [b]	11 (8.3)	1029	454-1633
	Household Services [c]	6 (4.5)	643	289-11551
Infection Status post-vaccine	Laboratory confirmed PCR positive for SARS-CoV-2	2 (1.5)	14355	12021-16689
	No PCR confirmed SARS-CoV-2 Infection	131 (98.5)	951	490-1673

[a] *Arbitrary units per millilitre*

[b] *Allied Health Professionals consisted of: Healthcare Assistants, Physiotherapists, Speech and Language Therapists, Phlebotomists, Dieticians and ECG Technicians.*

[c] *Household Services consisted of: Catering, Porters, Security and Maintenance*

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Figure 1. SARS-CoV-2 IgG levels post 2 doses of the Pfizer-BioNtech vaccine by prior infection status.

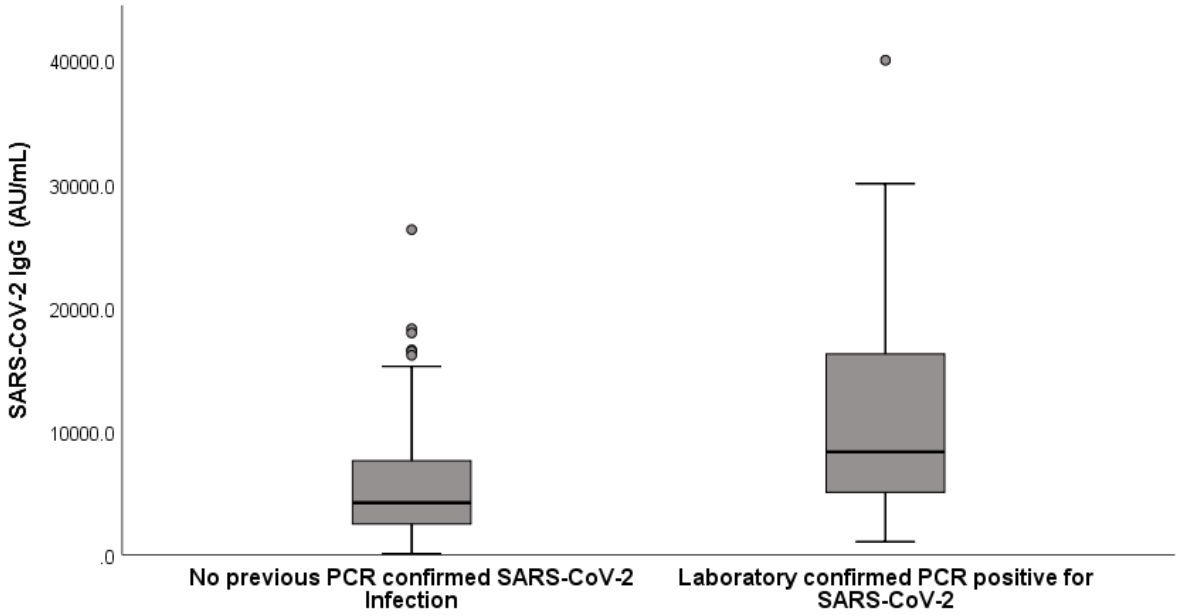
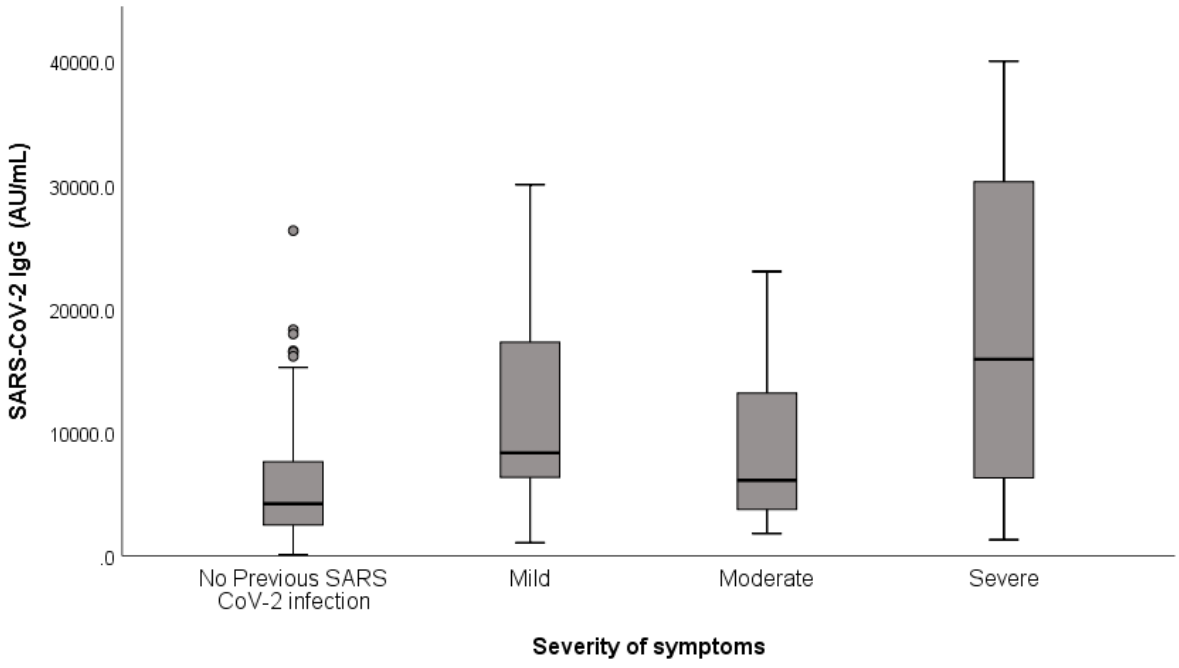


Figure 2. SARS-CoV-2 IgG levels post 2 doses of the Pfizer-BioNtech vaccine by severity of symptoms.



Discussion

With current global vaccinations continuing and some countries adopting new restrictions, the need for a successful and effective vaccine is ever evident. A two-dose campaign 21days apart of the Pfizer BioNTech BNT162b1 COVID 19 vaccine has shown to be 100% immunogenic, with all participants showing evidence of an IgG immune response in this study.

As expected, the biggest determinant of magnitude in SARS-CoV-2 IgG response was prior infection status, with symptom severity also contributing to that response. Our data suggest that those with a natural infection before vaccination have produced a higher IgG response than naïve individuals. There was a near 2-fold increase in the median concentrations between the two groups. Those who reported having severe symptoms had a substantial difference over those with lesser symptoms. A mild infection showed to be more reactive than those with moderate symptoms. There were some limitations in how this question was asked. Criteria should have been set out on how to determine which category of symptoms one fitted in. Some may interpret symptoms in the moderate category as mild and may account for the differences in the two categories. That being said, there is still a marked difference in the immunogenicity of infected subjects. In other studies, evidence has suggested that those with prior infection before vaccination may benefit from a single dose campaign of the vaccine[13–15]. While most westernised countries having a large cohort of their population already vaccinated, lower income countries with very little of the population vaccinated may benefit from this approach.

With regards to age and gender our findings found no significant difference with gender ($p = 0.577$) but showed some significance with age ($p = 0.001$). The data showed that immunogenicity was highest in the 60+ age group with those in the age group 40-49 producing the lowest response. This evidence may be falsely misleading given the small proportion of subjects in the 60+ age group with 3/8 of those having previously testing positive prior to vaccination. The one individual who produced the highest IgG response in the study was also in that category which may have falsely elevated the median. The majority of literature looking at age as a determinant of immunogenicity have quoted a decrease in IgG levels as age goes up[13,16–18]. High seroconversion rates were seen however in the <30 age group compared with the older age groups.

This study followed up with 133/219 participants at a time interval of six-months post vaccination in which 132/133 (99.3%) still had detectable SARS-CoV-2 IgG levels (i.e.>50 AU/mL as set by the manufacturer). The median SARS-CoV-2 IgG level had decreased by80% in the period between the two phases which equated to a median decrease of 2% per day. A similar Italian study with a sample size of 352 subjects found that there was a 1.1% median decrease in IgG levels per day over a period of 72 days[16]. This study could have been further strengthened if we allowed for more time intervals to accurately assess the rate of decline. It is intended to further sample the same population at eight- and ten-months post vaccination.

A correlate of protection (CoP) is urgently needed given the fact that a lot of countries have most of their population vaccinated but infections are still on the rise. It would also be beneficial as we head into the winter months and booster doses will be needed for the most vulnerable.

While the immune response to COVID-19 infection is complex and not solely based on antibody production, measurement of IgG response can be readily performed in routine diagnostic laboratories making it a very attractive target for assessing the response to SARS-CoV-2 vaccination. While virus neutralisation is thought to occur through NAb there is a strong correlation between binding antibody and NAb and therefore measurement of binding antibody is a reliable determinant for protection [19]. Our study reported two positive cases out of 133 participants post vaccination. These individuals had a SARS-CoV-2 IgG range of 609-3597 AU/mL. Our study was insufficiently powered to determine the relationship between SARS-CoV-2 IgG titre and protection.

This paper does present itself with several limitations. All participants are from a healthcare setting which increases their risk of exposure to infected individuals. The average concentration of SARS-CoV-2 IgG levels may be elevated due to this exposure. Some naïve individuals produced similar responses to those who had been previously infected. No participants were under the age of 23 or over the age of 66 years and may not be representative of the wider population. Participants were not asked whether they were on any immunosuppressant or immunomodulatory drugs before vaccination which may have accounted for decreased responses. Nonetheless this paper contributes to the understanding of the degree of immunity afforded by both vaccine and combined vaccine plus natural infection.

Ethics Declaration:

Full approval for this study was granted by the Clinical Ethics Committee of the Cork Teaching Hospitals, University College Cork. CREC Review Reference Number: ECM 4 (b) 09/02/2021 COVID-19 & 3 (ff) 09/02/2021

Declaration Conflicts of Interest:

There is no conflict of interest, on the part of any of the authors that could be perceived as prejudicing the impartiality of the research reported.

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