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Original Article

Comparison of IgG -Predominant Antibody Responses between Conventional Inactivated and mRNA SARS CoV-2 Vaccines Administrated in Jordan

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ABSTRACT

Introduction: Different COVID-19 vaccines can affect various body systems, including the immune system, leading to distinctions in immune responses like natural killer cell cytotoxic activity, complement system function, and B-cell antibody production among vaccinated and non-vaccinated individuals. In Jordan, so far, there is no study exploring these immune response variations for four COVID-19 vaccines, along with the factors influencing the response and post-vaccination clinical outcomes.
Methods: This study focuses on the humoral responses of 350 adult participants. Through participant interviews, one blood sample was collected for subsequent laboratory testing, utilizing the Enzyme-Linked Immunosorbent Assay (ELISA) method to examine the presence of COVID-19 antibodies in participants' plasma. A Chi-square test was performed to test for the statistical significance.
Results: Our results indicate significant differences in positive antibody response efficiency (p value > 0.001) among participants who received two or three doses of COVID-19 inactivated and mRNA vaccines. The order of higher positive antibody frequency is as follows: Pfizer (90.5%), Sputnik V (88.1%), Sinopharm (78.8%), and AstraZeneca (65.8%). There is a noticeable inclination towards the efficacy of the Pfizer-BioNTech vaccine, evidenced by the highest levels of antibodies in recipients of this vaccine. Our study revealed that various factors, including gender, age, and smoking status prior to infection, play a role in significant contributors affecting immune response and influencing the effectiveness of these vaccines.
Conclusion: All the four types of COVID-19 vaccines administered in Jordan demonstrated effectiveness. We recommend studying the ability of B lymphocytes present in the peripheral blood and the effect of T-regulatory cells by flow cytometry in recipients who have high positive titers.

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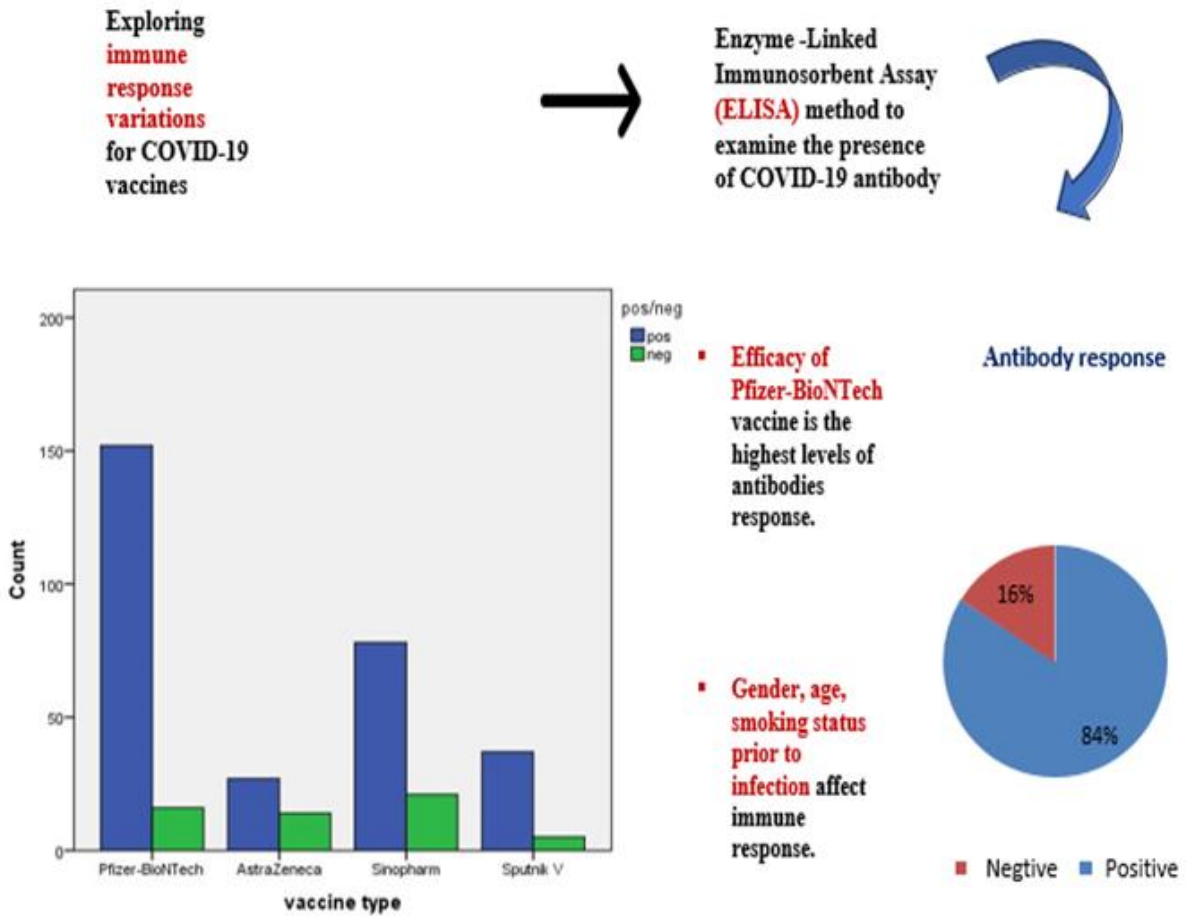
GRAPHICAL ABSTRACT



Introduction

Since its emergence in late 2019, COVID-19, a member of the Coronaviridae family identified as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has left an indelible mark on the world [1]. Begin from outbreak of pneumonia of unknown etiology in Wuhan, China with different symptoms [2, 3]. The World Health Organization (WHO) declared it a pandemic on March 11, 2020, triggering urgent global endeavors that led to vaccine development. As of June 14, 2023, the WHO documented 767,984,989 confirmed COVID-19 cases, 6,943,390 deaths, and 13,397,153,690 administered vaccine doses [4]. Preventive measures, including mask-wearing, social distancing, hand hygiene, and vaccination have been crucial [5]. Initially not anticipated within eighteen months, the rapid global spread prompted the development of various vaccines. Approval dates spanned from June 24, 2020, in China to January 3, 2021, in the UK [6, 7]. By August 19, 2022, Jordan had recorded 1,731,549 positive cases, 14,105 deaths, and 45,538,733 fully vaccinated individuals [8]. The ongoing endorsement and utilization of diverse vaccine brands by the Jordan Food and Drug Administration since February 2021 underscores global collaborative efforts in combating the pandemic [9].

Vaccines trigger immune responses through Toll-like receptor 7 (TLR7) in endosomes. TLR7 activation induces IFN-α and TNF-alpha, along with IL-12 and IL-6 secretion. Viral particles are presented by MHC-I proteins, generating CD8+ T-cytotoxic cells that undergo clonal expansion. This leads to the production of virus-specific effector and memory T cells. Antigen-presenting cells, including dendritic cells and macrophages,

recognize virions via MHC-II proteins, activating CD4+ helper T cells. This activation prompts B-cell activation, resulting in COVID-19 antibody production-initially IgM, followed by virus-specific IgG. The adaptive immune response, crucial for infection control and clinical recovery, is detectable through serological tests like IgG titers and T-cell assessments [10-12].

This study examined the presence of antibodies in recipients who received at least two doses of vaccines: BNT162b2 (Pfizer, New York, NY, USA), BBIBP-CorV (Sinopharm, Beijing, China), and Sputnik V (Gamaleya Research Institute, Moscow, Russia), followed by ChAdOx1 nCoV-19 (AstraZeneca, Cambridge, UK). The investigation focused on assessing immunological responses, identifying factors influencing these responses, and evaluating the corresponding clinical outcomes.

Materials and Methods

Study sample and ethical consideration

In this cross-sectional study carried out in Jordan from May 1st to August 30th, 2023, a cohort of 350 individuals who had received a minimum of two doses of COVID-19 vaccines actively participated. Prior to their involvement, participants provided informed consent. A comprehensive questionnaire, devised for this study, solicited information on demographics, including age, gender, and place of residence. Furthermore, participants provided details about their clinical background, encompassing concomitant diseases, medication history, and any prior occurrences of COVID-19 infection. Pertinent data on the type and number of vaccine doses administered, as well as associated side effects, were systematically collected. The study also compiled information on additional factors such as smoking habits, history of pregnancy during the COVID-19 pandemic, and body mass index (refer to Appendix A).

The study enrolled 350 adult participants, predominantly comprising students and employees of Balqa Applied University, as well as individuals residing in close proximity to the university. Inclusion criteria mandated participants to be Jordanian adults with a national ID, having completed two or three doses of vaccination at least two weeks prior, and having received one of the specified vaccines: Pfizer-BioNTech, AstraZeneca-Oxford, Sinopharm, or Sputnik V. In addition, all vaccine recipients were required to have received their doses in Jordan and maintained their eligibility throughout the entire duration of the research.

The study assessed antibody cell levels in two groups: those who received the mRNA SARS CoV-2 Pfizer vaccine and those who received the inactivated non-Pfizer vaccines. Furthermore, the analysis explored the variations in antibody levels concerning the number of vaccine doses administered.

Ethical approval

The Ethical Review Committee approval at Al-Balqa Applied University and College of Medicine was granted on February 2, 2022 (Reference No. 125) along with an institutional review board (IRB) at Al-Balqa Applied University (BAU).

Blood collection and follow up

350 participants completed the registration and data collection phase. However, after preparing the samples for reading by the ELISA. The study aimed to identify the presence or absence of COVID-19 IgG against the spike antigen (S antigen) in serum samples using the IgG Human ELISA Kit from Vircell, Spain. Blood was drawn into a plain tube and refrigerated for one to seven days for IgG antibody isolation. All procedures were conducted under aseptic conditions in a first-class I biological safety cabinet. Serum samples from 350 patients, along with controls, were thawed, and a self-prepared questionnaire gathered demographic, clinical, and vaccine-related data. The samples were diluted, incubated, and processed through washing steps. IgG conjugate and substrate solutions were added, followed by incubation and the addition of stopping solution. Optical density (OD) results were then read using a microplate ELISA reader within an hour of completing the assay, set at 450/650 nm.

For laboratory-confirmed COVID-19 infection, participants were contacted by phone, three months after sampling.

Data analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 26. Categorical parameters were presented as absolute numbers with percentages and continuous parameters as medians with interquartile ranges. A Chi-square test was performed to test for statistical significance.

Results and Discussion

The objective of the study was to calculate vaccine effectiveness against infection by measuring the levels of specific immunologic markers (Antibody specific to COVID-19) among a sample of healthy vaccinated individuals in ELISA and side effects after receiving at least two doses of any vaccine. Among the responses (N = 350), just over half of the participants were females (51.7%), and the most common age group was 21-30 years old (43.1%). The participants received the vaccines in this order; (48%) Pfizer- BioNTech, (28.3%) Sinopharm, (12%) Sputnik V and (11.7%) AstraZeneca-Oxford. Furthermore, 50% of participants had a history of previous COVID-19 infection with (35.4%) had no history, and (14.6%) did not know.

When asked about history of infection, (50%) half of the participants had no previous infection, (18%) sustained an infection before the first dose, (4.3%) got one after it, (21.7%) after the second dose, and (2.3%) after the third dose while (3.7%) had no idea, as presented in Table 1.

Comparing SARS-CoV-2 antibody response to different COVID-19 vaccines

Plasma samples from participants were assessed for the presence of IgG antibodies against the S antigen. The findings are qualitatively depicted, revealing an initial robust response among vaccine recipients to various COVID-19 vaccines, as listed in Table 2. However, there are discernible distinctions in the positive antibody response efficiency (geometric mean titer 'GMT' 350, 250-450) among participants who received two or three doses of COVID-19 vaccines, with a p-value >0.001. In the case of two doses, the COVID-19 vaccines ranked in the following order based on higher positive antibody frequencies: Pfizer-BioNTech (90.5%), Sputnik V (88.1%), Sinopharm (71.2%), and AstraZeneca-Oxford (65.8%), as outlined in Table 10.

Conversely, among recipients of three doses, primarily vaccinated with Pfizer-BioNTech, the ranking shifted, with the higher positive antibody frequencies observed in Sputnik V (100%) and Sinopharm (100%), followed by Pfizer-BioNTech (90.5%), and AstraZeneca-Oxford (60%), as indicated in Table 2.

Table1: Baseline characteristics of participants

Baseline Characteristics		N (%)
Sex	Female	181 (51.7%)
	Male	169 (48.3%)
Age years (groups)	18-30	219 (62.5%)
	31-50	98 (28%)
	>50	33 (9.4%)
Educational level	>High school	58 (16.6%)
	College	56 (16%)
	University	193 (55.1%)
	High-postgraduate	43 (12.3%)
Family income	Low>500 JD	194 (55.4%)
	500-1000	114 (32.6%)
	High>1000	42 (12%)
Place of residence	Urban areas	230 (65.7%)
	Rural areas	120 (34.3%)
Smoking	Yes	41.4% (145)

	No	58.6% (205)
Alcohol	Yes	8% (28)
	No	92% (322)
Blood group	A	122 (34.9%)
	B	57 (16.3%)
	AB	27 (7.7%)
	O	144 (41.1%)
Body mass index	<18.5	33 (9.4%)
	18.5-25	150 (42.9%)
	25.1-30	97 (27.7%)
	>30	70 (20%)
Chronic diseases	* NO disease	269 (76.9%)
	* Yes	81 (23.1%)
	Endocrine (including Diabetes Mellitus)	19 (5.4%)
	CVD (including Hypertension)	12 (3.4%)
	Hyper-Sensitive disease (including asthma)	18 (5.1%)
	Other diseases (including stomach disease)	17 (4.9%)
	Multiple diseases (two diseases and more)	15 (4.3%)
Have you taken influenza vaccine previously (regular)	Yes	86 (24.6%)
	No	264 (75.4%)
Pregnancy during COVID-19 pandemic?	Yes	17 (4.9%)
	No	164 (46.9%)
Previous COVID-19 infection	Yes	175 (50%)
	No	124 (35.4%)
	I don't know	51 (14.6%)
Hospital admission	Yes	17 (4.9%)
	No	158 (45.1%)
Oxygen used during treatment at home.	Yes	14 (4%)
	No	161 (46%)
Has anyone in your family been infected by COVID 19?	Yes	249 (71.1%)
	No	101 (28.9%)
Type of vaccine for just take two doses (297)	Pfizer-BioNTech	147 (49.5%)
	Sinopharm	73 (24.6%)
	AstraZeneca-Oxford	36 (12.1%)
	Sputnik V	41 (13.8%)
Type of vaccine for third doses (53)	Pfizer-BioNTech	49 (92.4%)
	Sinopharm	4 (7.6%)

Table 2: Vaccines antibody response efficiency

Yes	No
Frequency N (%)	Frequency N (%)
294 (84%)	56 (16%)

All (N=350)

	Yes N (%)	No N (%)	*P*-value
Pfizer-BioNTech	152 (90.5%)	16 (9.5%)	
Sputnik V	37 (88.1%)	5 (11.9%)	0.001
Sinopharm	78 (78.8%)	21 (21.2%)	
AstraZeneca-Oxford	27 (65.9%)	14 (34.1%)	

All (N=350)

Pfizer-BioNTech	152 (90.5%)	16 (9.5%)	*P*-value 0.001
Non-Pfizer-BioNTech	142 (78%)	40 (22%)	

Two doses (N=297)

Pfizer-BioNTech	133 (90.5%)	14 (9.5%)	*P*-value 0.001
Sputnik V	36 (87.8%)	5 (12.2%)	
Sinopharm	52 (71.2%)	21 (28.8%)	
AstraZeneca-Oxford	24 (66.7%)	12 (33.3%)	

Three doses (N=53)

Pfizer-BioNTech	19 (90.5%)	2 (9.5%)	*P*-value 0.02
Sputnik V	1 (100%)	0 (0%)	
Sinopharm	26 (100%)	0 (0%)	
AstraZeneca-Oxford	3 (60%)	2 (40%)	

Factors Affecting antibody response among COVID-19

Several factors influencing the antibody response were identified in this study. Sex played a significant role, with 181 females receiving different vaccines showing an 88.4% positive response, surpassing the 79.3% positive response in 169 males, indicating a significant correlation with a p-value of 0.02. Age was another influential factor, with the highest positive antibody levels found in the 21-30 age group, demonstrating a significant correlation with a p-value of 0.03. The correlation between age and vaccine type was also significant (P > 0.001), revealing the highest antibody levels in participants between 10-20 years for those taking Pfizer-BioNTech (79.6%), 21-30 years for Pfizer-BioNTech (48.3%), and above 50 years for Sputnik V (33.3%). Smoking status showed a significant correlation with antibody response, as 115 smokers receiving different vaccines had a 39.1% positive response compared to 60.9% in non-smokers (p=0.04). The number of vaccine doses impacted antibody levels, with participants who received three doses of Sinopharm or Pfizer vaccines exhibiting higher positivity. Notably, individuals with a Body Mass Index (BMI) between 18.5-25 and 25-30 had the highest antibody levels, whereas those with a BMI less than 18.5 had the lowest (p=0.05).

In addition, individuals previously infected with COVID-19 (52.7%) had higher antibody levels than the uninfected (33%) (p=0.04). Vaccination with the influenza vaccine and non-pregnant females showed higher antibody levels compared to non-vaccinated individuals and pregnant females, respectively (Table 3).

Follow up

After contacting participants by phone, all participants assured that they had no laboratory-confirmed COVID-19 infection within three months of sample collection. In response to the urgent need to produce vaccines for the critical management of the deadly COVID-19 pandemic, various vaccines utilizing different manufacturing methods emerged. However, debates arose regarding the effectiveness of these vaccines and their potential side effects on recipients' health. This study directed its attention to the immune response elicited by diverse COVID-19 vaccines administered in Jordan. Specifically, the investigation centered on understanding the variations in immune responses among 350 vaccinated recipients, taking into account different variables that could influence the efficacy of vaccination. Among the 350 participants in this study, 168 individuals received the Pfizer-BioNTech vaccine, with ages ranging from 18 to above, comprising 50.5% women, and a prior SARS-CoV-2 infection rate of 49.1%. Moreover, 99 participants received the Sinopharm vaccine, with ages between 18 and above 51 years, including 48.5% women, and a prior SARS-CoV-2 infection rate of 31.4%.

Table 3: Factors affecting antibody response among participants

		Positive	Negative	*P*-value
Sex	Female	160 (88.4%)	21 (11.6%)	
	Male	134 (79.3%)	35 (20.7%)	0.02
Age groups	18-30	194 (86.6%)	30 (13.4%)	0.03
	31-50	75 (80.7%)	18 (19.4%)	
	>50	25 (75.8%)	8 (24.2%)	
Educational level	High school	51 (87.9%)	7 (12.1%)	
	Collage	48 (85.7%)	8 (14.3%)	0.16
	University	162 (83.9%)	31 (16.1%)	
	High-postgraduate	33 (76.7%)	10 (23.3%)	
Family income	>500 JD	165 (85.1%)	29 (14.9%)	
	500-1000JD	96 (84.2%)	18 (15.8%)	0.06
	>1000JD	33 (78.6%)	9 (21.4%)	
Place of residence	Urban areas	195 (84.8%)	35 (15.2%)	0.05
	Rural areas	99 (82.5%)	21 (17.5%)	
Smoking	Yes	115 (79.3%)	30 (20.7%)	
	No	179 (87.3%)	26 (12.7%)	0.04
Blood Group	A	98 (80.3%)	24 (19.7%)	
	B	48 (84.2%)	9 (15.8%)	0.05
	AB	24 (88.9%)	3 (11.1%)	
	O	124 (86.1%)	20 (13.9%)	
Body mass index	> 18.5	27 (81.8%)	6 (18.2%)	
	18.5-25	130 (86.7%)	20 (13.3%)	0.05
	25.1-30	78 (80.4%)	19 (19.6%)	
	> 30	59 (84.3%)	11 (15.7%)	
Chronic Diseases	Endocrine	16 (84.2%)	3 (15.8%)	
	CVD	9 (75%)	3 (25%)	
	Hyper-Sensitive	15 (83.3%)	3 (16.7%)	0.92
	Other diseases	15 (88.2%)	2 (11.8%)	
	Multiple diseases	12 (80%)	3 (20%)	
Influenza Vaccine	Yes	75 (87.2%)	11 (12.8%)	0.03
	No	219 (82.95%)	45 (17.05%)	
Pregnancy	Yes	13 (76.5%)	4 (23.5%)	0.06
	No	138 (84.15%)	26 (15.85%)	
Previous COVID19	Yes	155 (88.6%)	20 (11.4%)	
	No	97 (78.2%)	27 (21.8%)	0.05
	I don't know	42 (82.4%)	9 (17.6%)	
Date of Infection	Before vaccination	68 (90.7%)	7 (9.3%)	0.06
	After vaccination	86 (86.9%)	13 (13.1%)	
Hospital Admission	Yes	14 (82.4%)	3 (17.6%)	0.92
	No	280 (84.1%)	53(15.9%)	
Oxygen Support	Yes	12 (85.7%)	2 (14.3%)	0.91
	No	282 (84.9%)	54 (16.1%)	
Family Infection	Yes	216 (86.7%)	33 (13.3%)	0.02
	No	78 (77.2%)	23 (22.8%)	

Furthermore, 41 participants received the Oxford-AstraZeneca vaccine, with ages ranging from 25 to above 50 years, consisting of 43.9% women, and a prior SARS-CoV-2 infection rate of 10.3%. Finally, 42 participants received the Sputnik V vaccine, with ages spanning 25 to above 51 years, comprising 66.6% women, and a prior SARS-CoV-2 infection rate of 9.1%. These findings align with previous research conducted in Iran [13]. In our study, every factor that might impact the immune response was considered to comprehensively understand the short and long-term effects of different vaccines. The primary focus was on adaptive immunity and the external factors influencing it, including B cells. For participants who received two doses of COVID-19 vaccines, there was a variation in positive antibody response (efficiency) with a geometric mean titer (GMT) of 350, ranging from 250-450, and a p-value of > 0.001. The ranking of higher positive antibody frequencies was observed in the order of Pfizer-BioNTech (90.5%), Sputnik V (88.1%), Sinopharm (78.8%), and AstraZeneca-Oxford (65.8%). This percentage was slightly lower than previous studies, possibly due to the gradual decline in the immune system response over time [14-17].

Efficiency differences were evident when comparing recipients of just two doses of COVID-19 vaccines, with Pfizer-BioNTech (90.5%), Sputnik V (88.1%), Sinopharm (71.2%), and AstraZeneca-Oxford (65.8%) exhibiting higher positive antibody frequencies. This aligns with other studies highlighting differences in efficiency between the second and third doses [18]. Individual responses to vaccinations varied based on external and internal factors, such as gender and age. Previous studies also confirmed that females and younger individuals tended to generate a stronger immune response after vaccination [19]. And their Menstrual changes after covid-19 vaccination [20]. Notably, smokers exhibited a lower level of antibodies compared to non-smokers, consistent with a systematic review demonstrating the impact of various smoking methods on antibody titers after vaccination [21]. The association between BMI and antibody levels was close to significant, suggesting that individuals with a weight close to normal exhibited higher antibody levels. This correlation aligns with previous research emphasizing the connection between weight, antibody titers, and immune dysfunction [22].

The number of vaccine doses significantly influenced the immune response, with participants who received three doses of Sinopharm or Pfizer-BioNTech vaccines showing higher positive antibodies compared to those who received only two doses [23]. Upon conducting statistical and laboratory tests to explore the relationship between blood group type and COVID-19 vaccines, no significant association was found ($p > 0.05$). This result is consistent with prior studies that did not identify any connection between vaccine response and blood group [24].

No instances of reinfection were observed among the participants post-vaccine administration. Several factors contribute to this outcome, including the reduced count in laboratory tests for COVID-19. In addition, the robust immune response elicited in individuals resulted in milder COVID-19 symptoms, making it challenging to distinguish between COVID-19 infection and seasonal influenza. The emphasis on raising awareness among individuals about maintaining overall health aligns with a prior study demonstrating a reduction in reinfection rates after COVID-19 vaccination [25].

Conclusion

All four types of COVID-19 vaccines administered in Jordan demonstrated effectiveness. However, there is a noticeable inclination towards the efficacy of the mRNA SARS CoV-2 Pfizer-BioNTech vaccine, evidenced by the highest levels of antibodies in recipients of this particular vaccine. The study further revealed that various factors, including sex, age, smoking status, and prior infection, play a role in influencing the effectiveness of these vaccines.

Limitations

Convenient study sample is an obvious limitation. Unknown time or interval between vaccination and collection of samples could affect results. We did not perform the ELISA prior to vaccination, so

it is impossible to evaluate whether we have detected long lasting immune changes in Antibody IgG Cells or if they were induced by the vaccination.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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