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Coronavirus Pandemic

Evaluation of neutrophil-to-lymphocyte ratio and immune response in patients vaccinated with Pfizer-Biontech vaccine

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Abstract

Introduction: Research on SARS-CoV-2 virus has focused on aspects such as treatment, virology, epidemiology and vaccine development. The efficacy of vaccines against SARS-CoV-2 is important for controlling the pandemic. This study assessed how the immune response is affected by age and gender, and its role in causing inflammation as measured by neutrophil-to-lymphocyte ratio (NLR) in vaccinated patients versus non-vaccinated COVID-19 negative patients.

Methodology: A case-control study was done involving 187 randomly selected patients who had undergone laboratory examinations to evaluate the SARS-CoV-2 IgG antibody titer and hematological parameters at 21 to 31 days after the second dose of vaccination. Patients were divided into case and control groups according to their vaccination status.

Results: The average age among the cases was 51 ± 13 years whereas the average age among the control group was 47 ± 15 years. In cases where the response to immunization was measured by SARS-CoV-2 IgG antibody, results had a median of 7.7 U/mL characterized by a large variation ($p < 0.0001$).

There was no significant difference based on age ($p = 0.451$) and gender ($p = 0.622$) in SARS-CoV-2 IgG antibody titers in patients vaccinated with two doses of Pfizer–BioNTech COVID-19 vaccine. In addition, there was no significant difference in NLR ratio between cases and controls ($p = 0.117$).

Conclusions: Our data showed that there is no inflammation at 21 to 31 days post vaccination with Pfizer-BioNTech COVID-19 vaccine, regardless of age and gender, based on the hematological parameters.

Key words: COVID-19, vaccine, immune response, Neutrophil, Lymphocyte.

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Introduction

COVID-19 is defined as an illness caused by a novel coronavirus designated as severe acute respiratory syndrome coronavirus 2, SARS-COV-2. Evaluation of the disease is of importance in countries where the epidemic spread of SARS-COV-2 is affected by migration [1]. COVID-19 is caused by a beta corona virus, which belongs to a family of viruses that are common in animals worldwide and have the potential to spread to humans, as was likely the case with the SARS corona virus [2]. SARS-CoV-2 RNA encodes for four structural proteins including spike (S), membrane (M), envelope (E), and nucleocapsid (N), with the S protein comprised of two subunits S1 and S2 [3]. The receptor binding domain (RBD) is included within the S1 subunit and has a high affinity for the angiotensin converting enzyme 2 (ACE2) receptor on the cell surface membrane. Infection is mediated by interaction of the SARS-CoV-2 RBD with the ACE2 viral receptor on host cells [4]. In the case of SARS-CoV-2 (and SARS-CoV), the spike protein binds to angiotensin-converting enzyme 2 (ACE2) on host cells and is then endocytosed [5-6]. This step is followed by fusion of viral and endosomal membranes and release of the viral genome into the cytoplasm [7]. Antibodies that bind to the spike protein, especially to its receptor-binding domain (RBD), prevent its attachment to the host cell and neutralize the virus. It has been shown that serum and plasma antibodies typically produce structural proteins (RBD, S, and N), with antibodies appearing as early as a few days to a few weeks after the onset of symptoms and often after the detection of viral ribonucleic acid (RNA) declines or is no longer detectable [8–10].The persistence of IgG antibodies allows for identification of subjects who have been infected in the past and recovered from the illness and is useful in serological surveys to assess the prevalence of SARS-CoV-2 infection in selected groups or broader populations.

Based on the data obtained from preclinical studies with SARS-CoV and Middle East respiratory syndrome corona virus MERS-CoV [11], the spike protein was identified as an antigenic target for the development of a vaccine against SARS-CoV-2 at a very early stage [12].The World Health Organization (WHO) maintains a working document that includes most of the vaccines in development. The main platforms used for COVID-19 vaccine development can be divided into 'traditional' approaches (inactivated or live-virus vaccines), and RNA and DNA vaccines. All COVID-19 vaccines act by activating the innate and adaptive immune responses and are designed to elicit spike protein-specific antibodies, as these have been reported to be effective in combating the disease. Further, anti-spike protein receptor-binding domain (anti S-RBD), immunoglobulin G (IgG) levels play a role in determining immunity to SARS-CoV-2 [13].

The Pfizer-BioNTech vaccine, is based on a technology called messenger RNA, which uses a key genetic messenger found in cells to create protein that the immune system then learns to attack [14]. The vaccine is administered in two doses three weeks apart. [15-16]. The antibody titers elicited by COVID-19 vaccines can be measured using serological diagnostic tests. The introduction of the vaccination against SARS-CoV-2 infection creates the need for precise tools for quality control of vaccination procedures, detection of poor humoral response, and estimation of achieved protection against the disease [17]. In addition, there has been a growing concern regarding heart-inflammation in younger male patients which may be caused by Pfizer/BioNTech COVID-19 vaccine administration [18]. Such inflammation may be modulated by testosterone and interleukin-1β alteration following a viral infection [19]. Although there are certain criteria to diagnose and evaluate the severity of myocarditis [20,21] it has been shown that neutrophil to lymphocyte ratio correlates well with hospital stay as well as degree of severity [22]. Thus, this study is aimed at assessing the immune response as affected by age and gender and its role in causing inflammation as measured by neutrophil-to-lymphocyte ratio (NLR) in vaccinated patients versus non-vaccinated, COVID-19 negative patients.

Methodology

Patient selection

Our study was a case-control study involving 187 randomly selected patients who had performed laboratory examinations at Labor Dr. Limbach-Laboratory in Albania. All patients provided written consent for data collection and extrapolation. In addition, patients were divided into cases group involving 119 patients with positive SARS-CoV-2 IgG antibodies who had received the second dose of Pfizer-BioNTech vaccination 21 to 31 days prior to having their blood drawn and control group involving 68 patients who were negative for SARS-CoV-2 IgG antibodies and no prior medical history indicating SARS-CoV-2 infection. Both groups, had similar age and gender, and had no medical history of hematological, inflammatory or chronic disease prior to blood collection.

Sample collection and processing

Venous blood samples were collected following overnight fasting in compliance with standardized procedure using Sarstedt Monovette® (Nümbrecht, Germany) serum and S-Monovette EDTA K2 (Nümbrecht, Germany) for SARS-CoV-2 IgG antibodies determination and complete blood count differential respectively. All laboratory analysis were performed within two hours of sample collection. SARS-CoV-2 IgG antibody titer was determined by Siemens Atellica® IM Analyzer (Dublin, Ireland) using quantitative SARS-CoV-2 IgG (sCOVG) a 2-step sandwich immunoassay assay detecting IgG antibodies, including neutralizing antibodies to SARS-CoV-2 in human serum. Following manufacturer's guidelines, samples with IgG values ≥ 1.0 U/mL were considered positive for SARS-CoV-2 IgG antibodies whereas samples with values < 1.0 U/mL were considered negative for presence of SARS-CoV-2 IgG antibodies.

Complete blood count differential providing data on absolute lymphocyte and neutrophile count was performed on Siemens ADVIA® 2120i Hematology (Tarrytown, USA) System using light scatter and the peroxidase/lobularity/nuclear density methods to count and distinguish between peroxidase-positive cells, such as neutrophils, eosinophils, and monocytes, and peroxidase-negative cells, which include lymphocytes and basophils

Statistical Analysis

Statistical analysis was conducted using MedCalc Statistical Software version 14.8.1 (MedCalc Software, Ostend, Belgium; http://www.medcalc.org; 2014). Continuous variables were presented as medians with interquartile ranges and means ± standard deviation. Data were further analyzed using Mann-Whitney test for independent samples. Categorical data were presented as percentages (%) and compared using Fischer exact test. In addition, D'Agostino-Pearson test

was used to evaluate whether the data set followed normal distribution. Statistical significance was considered for $p < 0.05$

Results

Patient demographics

Summary of patient demographics is shown in Table 1. The average age among cases was 51 ± 13 years (median = 49 years) whereas in control group the average age was 47 ± 15 (median = 43.5 years) indicating that there was no significant difference in age between vaccinated and un-vaccinated patients (p = 0.0687). In addition, the numbers of female patients among cases and controls were 72 (60.5%) and 46 (67.75%) respectively. On the other hand, there were 47 (39.5%) and 22 (32.3%) male patients among case and control groups. There was no significant difference in gender between groups (p = 0.349).

The response to immunization measured by SARS-CoV-2 IgG antibody was variable among the cases and had a mean of 48.69 ± 167.83 (U/mL) (Table 2). Further computation using D'Agostino-Pearson test which takes into account a combination of the coefficients of Skewness and Kurtosis indicated that SARS-CoV-2 IgG antibody titers among cases were abnormally distributed ($p < 0.0001$). Similarly, SARS-CoV-2 IgG antibody results among controls had a mean value of 0.30 ± 0.32 (U/mL) indicating a large variance ($p < 0.0001$) with no clinical significance.

Further statistical analysis was performed with the data on the cases to evaluate the distribution of SARS-CoV-2 IgG titers according to age. Patients in the cases group < 55 years of age, had a mean antibody titer of 59.02 ± 205 U/mL and a median of 7.38 U/mL whereas those ≥ 55 years of age had a mean antibody titer of 29.77 ± 50.99 U/mL and a median of 10.29 U/mL indicating no statistically significant difference (p = 0.451) (Table 3)

Due to disproportionate number of females and males within each group as well as the abnormal distribution of SARS-CoV-2 IgG results further statistical evaluation was performed in order to evaluate possible differences with regards to immunization response after inoculation with Pfizer-BioNTech vaccine among cases. Males and females in the cases group had a mean SARS-CoV-2 IgG antibody titer of 58.5 ± 221U/mL (Median of 9.18 U/mL) and 42.3 ± 122 U/mL (median of 7.46 U/mL) respectively indicating no statistical significance according to gender (p = 0.622) (Table 4).

Further statistical evaluation was performed in order to evaluate immune response with regards to IgG titer in patients having received the Pfizer-BioNTech COVID-19 vaccine with the control group (Tables 4-5).

As shown in the median, mean lymphocyte and neutrophils count among the cases was 2.32 ± 0.64 and 3.85 ± 1.30 (10^9/L) respectively whereas the neutrophil/lymphocyte ratio was 1.79 ± 0.89 (Table 5). Similarly, mean lymphocyte and neutrophils count among the controls was 2.72 ± 2.67 and 4.14 ± 1.71 (10^9/L) respectively whereas the neutrophil to lymphocyte ratio was 1.75 ± 0.48 (Table 5). Respective medians and interquartile ranges are also shown for neutrophils, lymphocytes and their ratio among cases and controls. Further statistical analysis revealed no statistical difference of NLR ratios between cases and controls (p = 0.117).

Discussion

Our data shows that there is a non-normal SARS-CoV-2 IgG titer distribution among vaccinated patients. This is similar to findings by other researchers indicating that there is high variability of antibody response depending on multiple factors including time of infection or vaccination [23,24]. In addition, it has been shown that SARS-CoV-2 antibody response following Pfizer-BioNTech COVID-19 vaccine is lower in the elderly compared to young individuals [25-26]. On the contrary, SARS-CoV-2 IgG titers, among patients with natural immunity to COVID-19 show a negative correlation with age in pediatric patients and a moderately positive correlation in adults [27]. However our data reveals no statistically significant variation of SARS-CoV-2 IgG titers between patients above and below 55 years of age following Pfizer-BioNTech COVID-19 vaccination (p = 0.451).

Gender was also an important factor in the immune response induced by vaccination. Females in particular had stronger antibody response to certain vaccines and faster waning of antibody titer compared to men. Nevertheless there were a few circumstances when males exhibit stronger immune response [28]. In addition, previous data has demonstrated the role of estrogen in women as an important down-regulator of angiotensin-converting enzyme 2 which is a known SARS-CoV-2 receptor [29]. In fact female patients infected with COVID-19 exhibit stronger T cell activation when compared to males [30]. Gene expression of X and Y chromosomes also affect different immunological responses to vaccination [31]. However, our data indicated no statistically significant difference (p = 0.622) between median antibody titers among males and females following the second dose of Pfizer-Biotech vaccination.

Table 1. Patient demographics of case and control groups.

	Cases	Controls	*p* value
Nr. patients	119	68	
Mean age ± Standard deviation	51 ± 13	47 ± 15	0.0687[1]
Median	49	43.5	
Females: number (%)	72 (60.5)	46 (67.7)	0.349[2]
Males: number (%)	47(39.5)	22 (32.3)	

[1] Statistical *p* value calculated by employing Mann-Whitney U test; [2] Statistical *p* value calculated by Fischer's exact test.

Table 2. SARS-CoV-2 IgG results among cases and controls.

SARS-CoV-2 *IgG status*	Nr patients	mean	Std Dev	variance	min	median	max	skewness	kurtosis	*p* value[1]
Cases										
IgG Positive	119	48.69	167.83	28167.4	1.06	7.70	1500	7.06	54.95	< 0.0001
Controls										
IgG Negative	68	0.30	0.32	0.10	0.0	0.18	0.86	0.52	-1.38	< 0.0001

[1] Statistical *p* value calculated by D'Agostino-Pearson test; St Dev: Standard deviation.

Table 3. SARS-CoV-2 IgG antibody titer according to age among cases.

Cases	Nr. patients	IgG titer (U/mL) Mean ± SD	IgG titer (U/mL) Median	*p* value
< 55 years old	77 (64.7%)	59.02 ± 205	7.38	0.451[1]
≥ 55 years old	42 (35.3%)	29.77 ± 50.99	10.29	

[1] Statistical *p* value calculated through Mann-Whitney U test.

Table 4. SARS-CoV-2 IgG antibody titer between males and females in case group.

Cases	Nr. patients	IgG titer (U/mL) Mean ± SD	IgG titer (U/mL) Median	*p* value
Male	47 (39.5%)	58.5 ± 221	9.18	0.622[1]
Female	72 (60.5%)	42.3 ± 122	7.46	

[1] Statistical *p* value calculated through Mann-Whitney U test.

Table 5. White blood cell differential profile; neutrophile to lymphocyte ratio in cases and controls.

	Lymphocytes 10^9/L	Neutrophils 10^9/L	Neutrophil/Lymphocyte	*p* value
Cases				**0.117[1]**
Mean ± SD (10^9/L)	2.32 ± 0.64	3.85 ± 1.30	1.79 ± 0.89	
Median (10^9/L)	2.26	3.75	1.61	
IQR (10^9/L)	0.73	1.61	0.78	
Controls				
Mean ± SD (10^9/L)	2.72 ± 2.67	4.14 ± 1.71	1.75 ± 0.48	
Median (10^9/L)	2.3	3.8	1.78	
IQR (10^9/L)	0.80	1.72	0.54	

[1]Statistical *p* value calculated through Mann-Whitney U test.

A similar finding was reported in a Korean population in which SARS-CoV-2 IgG antibody titer among male and female subjects had no significant difference following the administration of the second dose of the Pfizer-BioNTech vaccine [32]. In addition, data from large scale studies to understand the effectiveness as well as negative side effects of Pfizer-BioNTech COVID-19 vaccine have indicated that the increased cellular protective immunity following vaccination is characterized by an increase in cytokine and chemokine induction which in influenza and small pox vaccination have been shown to cause rare cases of myocarditis [33-34]. Nevertheless, there have been isolated cases of multisystem inflammation and organ dysfunction during the post vaccination period [35]. Following the inflammatory response caused by administration of Pfizer-BioNTech COVID-19 vaccine there is often an alteration of neutrophile and lymphocyte absolute counts which can be used to further investigate the degree of inflammation [22-36]. Our study, based on carefully selected patient groups of specific age and gender has shown that there is no significant difference in NLR ratio between cases and controls indicating that 21 to 31 days post vaccination period following administration of Pfizer-BioNTech COVID-19 vaccine is not marked by an increased inflammation based on hematological parameters. In line with our finding, an inflammatory response marked by high cytokine release has been observed only as a transitory phase following the first 8 days from last vaccination returning to normal after this time interval [33]. Nevertheless, further studies including larger patient groups as well as detailed laboratory data pertaining to specific markers of inflammation are needed to draw comprehensive conclusions regarding the aggravated inflammatory response following Pfizer-BioNTech COVID-19 vaccination.

Conclusions

This study demonstrates that patients vaccinated with two doses of Pfizer-BioNTech COVID-19 vaccine have different immune responses as measured by SARS-CoV-2 IgG antibody titer, and this does not appear to be correlated with gender or age. In addition, there is no indication of increased inflammatory response 21 to 31 days after the second dose of vaccine, as indicated comparison of NLR in vaccinated and un-vaccinated patients with known immune status to SARS-CoV-2 antibody. This study is in line with other publications in which the frequency of negative side effects related to administration of Pfizer-BioNTech COVID-19 vaccine is low. Further investigations, involving larger patient data evaluated in specific time periods are required to elucidate the long-term effects of such vaccine.

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