

Evaluation of hematological parameters and thrombocytopenia following Pfizer-BioNTech (BNT162b2) SARS-CoV-2 vaccination

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ABSTRACT

الأهداف: تقييم العلامات الدموية وملامح الجلطة للأفراد الأصحاء الذين تلقوا لقاحات فايزر بيونتك (BNT162b2) في المملكة العربية السعودية.

المنهجية: تم تقييم العلامات الدموية ونسبة حدوث الأجسام المضادة لمعامل الصفائح مضاد / PF-4 لـ 40 مشاركاً تم استقباليهم في مستشفى جامعة جازان. تم تقييم هذه المعلمة في نقطتين زمنيتين مختلفتين؛ في اليوم الأول (يوم تلقي الجرعة الأولى من اللقاح وقبل التطعيم) و14 إلى 21 يوماً بعد تلقي اللقاح.

النتائج: كان حوالي 38 (80%) من المشاركين في الدراسة من الذكور بمتوسط عمر 27 عاماً. من بين جميع المشاركين، أبلغ 15% من المشاركين عن إصابة سابقة بفيروس كورونا و 3 مرضى لديهم تاريخ مرضي من مرض السكري وارتفاع ضغط الدم. لم تظهر العلامات الدموية أي فروق ذات دلالة إحصائية بين النقطتين الزمنيتين، أي اليوم قبل تلقي التطعيم مباشرة و 14 إلى 21 يوماً بعد التطعيم. علاوة على ذلك، كانت الأجسام المضادة / PF4 سلبية لجميع المشاركين بعد التطعيم.

الخلاصة: أظهرت بياناتنا أن حدوث التشوهات الدموية و/أو الأجسام المضادة / PF4 بعد تطعيم فايزر بيونتك لا يُرى بشكل شائع وهو ما يتوافق مع العديد من التقارير السابقة. ومع ذلك، فإن الدراسات الأكبر مع المزيد من المشاركين الذين تم تقييمهم في نقاط زمنية مختلفة بعد التطعيم لها ما يبررها لاستبعاد التشوهات الدموية العابرة المحتملة.

Objectives: To evaluate hematological parameters and thrombotic profiles of healthy individuals who received Pfizer-BioNTech (BNT162b2) vaccines in Saudi Arabia.

Methods: Hematological parameters and the incidence of anti-platelet factor-4 (anti/PF-4) antibodies were evaluated in 40 participants who were eligible for COVID-19 vaccination in Saudi Arabia (above 18 years old) at Jazan University Hospital. These parameters were assessed at 2 different timepoints; at day 0 (the day of receiving the first dose of Pfizer-BioNTech (BNT162b2) and prior to vaccination) and 14-21 days after receiving the vaccine.

Results: Among the participants, 38 (80%) were men, while 12 (20%) were women, with a mean age of 27 years. A total of 15% of the participants reported previous infection with SARS-CoV-2 and 3 patients had a history of diabetes mellitus and hypertension. Hematological parameters results in those vaccines showed no significant changes between the 2 timepoints, such as, day 0 (just before receiving vaccination) and 14 to 21 days post vaccination. Further, anti/PF4 antibodies were negative for all participants following vaccination.

Conclusion: Our data showed that the incidence of hematological abnormalities or induction of anti/PF4 antibodies following Pfizer-BioNTech (BNT162b2) vaccination is not common, which is consistent with several previous reports. However, larger studies with more participants evaluated at different timepoints following vaccination are warranted to exclude potential transient hematological abnormalities.

Keywords: SARS-CoV-2, COVID-19 pandemic, COVID-19, thrombocytopenia, vaccine, Pfizer-BioNTech, platelets

Saudi Med J 2022; Vol. 43 (6): 567-571
doi: 10.15537/smj.2022.43.6.20220135

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Received 13th February 2022. Accepted 11th May 2022.

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Substantial vaccination campaigns have been initiated all over the world to combat COVID-19 pandemic. Several vaccines have been recently licensed and are now being used in many countries around the world.^{1,2} More than 9 billion doses of COVID-19 vaccines were given globally, with a rate of 39.7 million doses given on a daily basis.³ Pfizer-BioNTech (BNT162b2) messenger ribonucleic acid (mRNA) vaccine is considered one of the most widely administered COVID-19 vaccines globally.⁴ Like other approved COVID-19 vaccines, the Pfizer-BioNTech vaccine which is based on the mRNA technology, has shown high safety profile and efficacy in preventing COVID-19.⁵ However, since mid-March of 2021, several cases of unexpected thrombosis which is associated with thrombocytopenia have been reported in people receiving different forms of COVID-19 vaccines, including the Pfizer-BioNTech (BNT162b2) mRNA vaccine.^{6,7} Notably, a case of intracranial hemorrhage and eventual death was also identified following Pfizer-BioNTech vaccine.⁸ Subsequently, 17 events of thrombocytopenic episodes were also documented among more than 20 million mRNA-vaccinated individuals in North America.⁶ In general, cases of secondary immune thrombocytopenia (ITP) after receiving other types of COVID-19 vaccines were also reported but in inconsistent scenarios.^{9,10} These reports have contributed to increasing the trepidation on receiving COVID-19 vaccines.^{12,13} Robust and validated data concerning the potential side effects following COVID-19 vaccines from countries with extensive vaccination programs are needed which can highlight the burden of possible adverse effects. Therefore, the aim of this study was to investigate the prevalence of thrombocytopenia and anti-platelet factor-4 (anti-PF4)/antibodies in individuals who receive mRNA Pfizer-BioNTech vaccines in Saudi Arabia.

Methods. A total of 40 study participants were recruited among individuals who were eligible for COVID-19 vaccines at Jazan University hospital (JUH), Jazan, Saudi Arabia between March 22 to June 30, 2021. The recruitment process was based on questioning individuals who visited JUH for vaccination whether they agreed to participate in the

study or not. Participants who refused to participate or those who had received vaccines other than Pfizer-BioNTech were excluded from the study. Individuals who are under the age of 18 years old were not eligible for COVID vaccines in Saudi Arabia, thus they were excluded in this study. In addition, participants who did not come for second time blood collection were excluded from the study. Blood samples were collected from participants receiving Pfizer-BioNTech on the day of the first vaccine dose and before receiving the vaccine (day 0 sample, n=40), and 14–22 days after the first vaccine dose. Blood samples were collected into ethylenediaminetetra-acetic acid (EDTA-for hematological studies) and plain tube (to collect sera for anti-PF4 antibodies tests). Clinical data were also reported from all participants during their second visits for the second blood collection. Participants were asked to report all of the following: previous infection with SARS-CoV2, comorbidities such as hypertension, cancer, immune disorders, diabetes, and whether they experienced any of the following side effects: fever, headache, vomiting, fatigue, cutaneous bleeding, malaise, muscle, or joint ache after receiving the vaccines. Samples for hematological studies were processed immediately and serum samples were obtained from plain tubes by centrifugation and maintained at -80°C till the day of analysis. Hematological parameters were assessed using hematology analyzers DxH 500 automated using standard laboratory protocols. The EDTA anticoagulated blood samples were used to prepare slides for peripheral blood smear analysis. Leishman-stained blood smears were independently read by 2 specialized hematologists. Cellular morphologies were examined using light microscopy. Qualitative detection of immunoglobulin G (IgG) antibodies against PF4/polyanion complexes was screened using STic Expert® HIT rapid test (Stago, France), Cat. Nr. 01058, according to the manufacturer's instructions, and results were displayed as either positive or negative. Samples with abnormal results were subjected to further evaluation by a certified hematologists using light microscopy. GraphPad Prism 9 was used to make figures for platelet counts and other hematological parameters.

Ethical approval for this study was obtained from the ethical approval committee at Jazan University (reference number; REC42/1/087, date 22 March 2021). Written consents were obtain from all participants prior to participation in the study.

Statistical analysis. Descriptive statistics were reported for the collected data. Both t-test and Chi-square tests were done for univariate statistical analysis. The data were statistically analyzed using SPSS

Disclosure. This study was funded by the Deanship of Scientific Research, Jazan University, Jazan, Saudi Arabia. Project number: Cov19-56

version 23 (IBM Corp, Armonk, NY, USA) with a significance level of $p \leq 0.05$.

Results. A total of 40 participants were recruited to this study of whom 80% were male. The participants' ages range from 18 to 58 years, with a mean age of 27 years. Approximately 15% of the study participants had been previously infected with SARS-CoV-2 and only 3 participants reported to have comorbidities (2 participants with diabetes mellitus type 2 and hypertension). These data are summarized in **Table 1**. Then we assessed the side effects which in our study's participants following the first dose of Pfizer-BioNTech vaccination (**Table 2**). Almost one-third of the participants reported pain at the site of injection, while 13% of the participants reported muscle and joint pain, and only 10% experienced fever after the vaccine injection. Approximately 8% of our participants experienced fatigue while only 5% of the study participants headache post vaccination. Only one participant reported having bruises after vaccination. Of note, no patients in our cohort reported bleeding of gums or skin or any other signs deemed important for thrombocytopenia evaluation. Platelets count was analyzed for all participants; just before vaccine injection (day 0) as well as at days 14 to 22 post vaccination. Platelets count showed no significant difference in pre-

and post- vaccination analysis (**Figure 1**). The incidence of anti/PF4 antibodies was also measured for all the study participants using STic Expert® HIT rapid test (Stago, France). All the samples were negative for anti/PF4 antibodies when assessed at 14-21 days following BNT162b2 vaccination. Additional hematological evaluation was also performed for all samples (pre- and post-vaccination) using blood films and complete

Table 1 - Demographics of the study participants (N=40).

Variable	n (%)
Age (median), year*	27 (20-58)
Male,	32 (80.0)
Infected before	6 (15.0)
Comorbidities	3 (7.5)
*range	

Table 2 - Side effects after vaccination (N=40).

Side effect	n (%)
Pain	14 (35.0)
Fever	4 (10.0)
Headache	2 (5.0)
Vomiting	1 (2.5)
Fatigue	3 (7.5)
Cutaneous bleeding	0 (0)
Muscle/joint pain	5 (12.5)
Bruises	1 (2.5)
Bleeding gums	0 (0)
Malaise	0 (0)

Percentage of participants who report the different side effects after receiving one dose of Pfizer-BioNTech (BNT162b2) COVID-19 vaccine

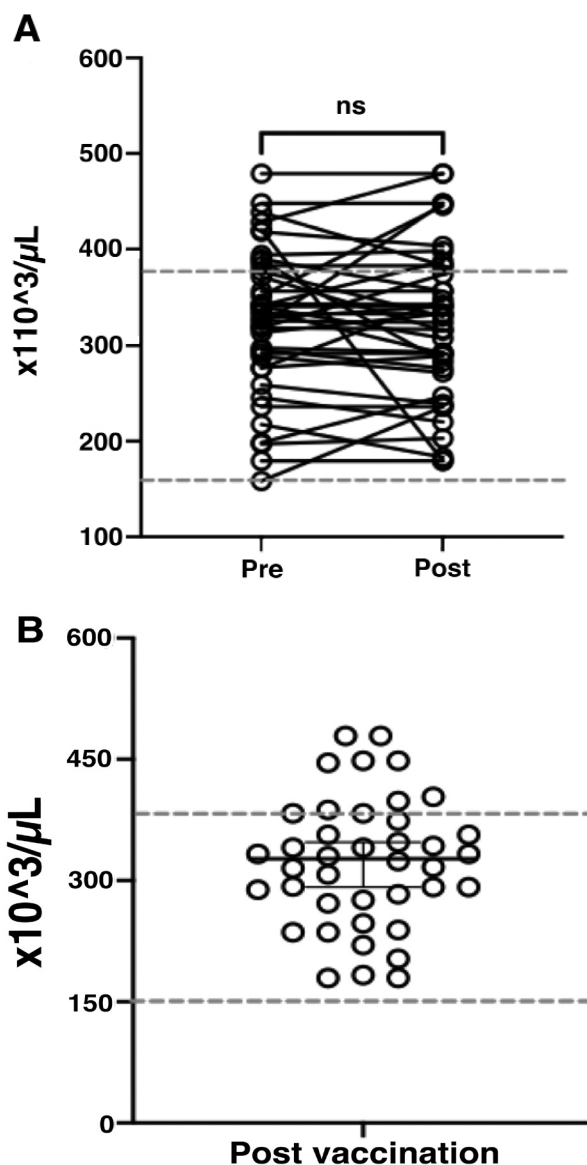


Figure 1 - Pfizer-BioNTech vaccine did not affect platelet counts. A) Platelets were quantified at day 0 (pre vaccine) and 14-22 days post-vaccination. B) Platelets count for study participants at 14-22 days following vaccination. Dashed lines represent upper and lower range values. NS: non-significant, $p > 0.5$. Paired T test was used for the comparison. GraphPad software version 8.1 was used for the statistical analysis.

blood count (CBC) and neither morphological changes nor alarming values were identified in all the study participants.

Discussion. Recently, several reports of unexpected thrombotic events and thrombocytopenia were reported following the administration of different forms of COVID-19 vaccines.^{6,7} Few cases of thrombotic events were also reported in individuals who received Pfizer-BioNTech SARS-CoV-2 vaccines.^{6,7} Similar cases were also reported previously in children following the introduction of MMR vaccines.¹¹ Although such events are rare, perceived risk of these side effects may lead to vaccine hesitancy in the general population.¹³ Consequently, this may contribute to delaying the reach of herd immunity which is the utmost requirement to relieve the ongoing pandemic. Here, we aimed to investigate the potential effects of Pfizer-BioNTech vaccination on different hematological parameters, including thrombocytes in addition to screening for subclinical existence of pathological anti-PF4/polyanion antibodies, a major finding of vaccine-induced thrombocytopenia.¹⁴ To test this, we recruited a total of 40 participants, aging above 18 years old (**Table 1**). Blood samples were collected pre-vaccination and 14-21 days following receiving the vaccine. The study participants were questioned to report any post-vaccination side effects including several definitive symptoms which may indicate thrombocytopenia such as severe headache, unusual neurological symptoms, severe abdominal pain, shortness of breath, leg swelling, petechiae and new or easy bruising.¹⁵ Most of our study participants reported side effects that were similar to the expected side effects reported in previously published studies (**Table 2**).¹⁶ However, only one participant reported an indicative symptom, bruises. We further assessed platelets counts in all participants and we showed that the platelet counts were not significantly different post-vaccination compared to the baseline/pre-vaccine levels in all participants including the participant with the bruises (**Figure 1A**). Platelet counts at day 14-22 post-vaccines fall within the normal range for the majority of the participants, except for a few cases which showed borderline thrombocytosis (**Figure 1B**). Our chosen time window for the second samples post-vaccination (14-22 days) was based on the fact that previously reported cases of thrombocytopenia were identified at this timeframe.⁶ Furthermore, detection of anti-PF4/polyanion IgG response is more appropriate at these timepoints as these antibodies tend to disappear within 50-85 days.¹⁷ Make sure that they appear consecutively in Hence, we further screened our

participants for the existence of subclinical anti-PF4 IgG antibodies using a rapid test and we did not observe positive results in all of the study participants following vaccination (data not shown). These data are consistent with several published reports where very low prevalence of both thrombocytopenia and antibodies to PF4/polyanion-complexes after vaccination were identified. For example, In Norway, a study carried out by Sørvoll et al¹⁸ on 492 healthcare workers following the administration of single doses of AstraZeneca (AZD1222) vaccine showed that only 6 of the study participants had positive anti-PF4/polyanion antibodies with normal platelets counts reported in all participants. Further, Thiele et al¹⁹ assessed the rate of anti-PF4/polyanion antibodies in 281 healthy individuals following vaccination with either Pfizer-BioNTech or AstraZeneca vaccines. The study reported the incidence of PF4/polyanion antibodies in 8% of individuals following AstraZeneca vaccine, however, only 5% of Pfizer-BioNTech vaccinees were positive for the PF4/polyanion antibodies.¹⁹ Of note, the study revealed that the positivity of PF4/polyanion antibodies did not result in platelets activation. This study concluded that there is very minor clinical relevance for the anti/PF4 induced-platelets activation following vaccination with COVID19 vaccines.¹⁹ We further extended our analysis to include other hematological parameters including red blood cells, white blood cells, lymphocytes, monocytes, neutrophils, eosinophils, basophils, hemoglobin, and hematocrits. We did not find significant differences in these parameters post-vaccination compared to the baseline values/pre-vaccines (data not shown). Microscopic examination of cell morphologies was also assessed and similarly, the vaccines did not seem to alter cellular structures and morphologies (data not shown). Microscopic examination of thrombocytes showed normal morphologies, with no megathrombocytes or stress platelets observed (data not shown). It is noteworthy that so far globally there is very few cases reported positive for antiPF4 and thrombotic changes after COVID-19 vaccination. Apparently, these cases are rare and most likely to be related to other immunological conditions rather than COVID-19 vaccines, which were not reported yet to induce platelets activation.^{20,21} This study is one of the few studies which evaluated hematological changes and thrombocytopenia following Pfizer-BioNTech SARS-CoV-2 vaccines in Saudi Arabia. However, our study cannot totally exclude the possibility that this vaccine may still induce transient hematological abnormalities as this study has several weaknesses. First, the total number of participants included in our study is low, hence, a future

large-scale study with more population is warranted. Additionally, the screening for anti-PF4/ polyanion IgG response was performed using a rapid chromatographic immunoassay, a test that has its known limitations in the clinical setting. Although this rapid test has high sensitivity which was reported in several publications, using a more confirmatory and quantitative assay such as ELISA would be of interest.^{22,23}

In conclusion, this study evaluated the hematological parameters, morphological characteristics, and prevalence of de novo anti-PF4/ polyanion IgG response in a population vaccinated with Pfizer-BioNTech SARS-CoV-2 in Saudi Arabia. We did not find major changes in hematological parameters, and morphological characteristics following vaccination with Pfizer-BioNTech SARS-CoV-2. Additionally, the vaccine did not induce anti-PF4/polyanion antibody responses or platelets activation. As it was previously reported in several reports, these data confirmed that hematological changes, generation, or reactivation of preexisting anti-PF4/polyanion platelet antibodies due to Pfizer-BioNTech SARS CoV-2 are rare.

Acknowledgment. *The authors extend their appreciation to the Deputyship for Research and Innovation, Ministry of Education, Saudi Arabia for funding this research work through project number ISP20-2. We are also immensely thankful to the participants of this study. We also would like to acknowledge the support of the Medical Research Center and the University Hospital staff, Jazan University, Jazan, Kingdom of Saudi Arabia for their great help and support. We would like to acknowledge MDPI for the English language editing.*

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