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Received: 3 April 2021 | Accepted: 23 April 2021

DOI: 10.1111/jth.15352

BRIEF REPORT

An observational study to identify the prevalence of thrombocytopenia and anti-PF4/polyanion antibodies in Norwegian health care workers after COVID-19 vaccination

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Funding information
We have received external funding from the Northern Norway Regional Health Authority (Helse Nord).

Abstract

Background: The COVID-19 vaccine from AstraZeneca (AZD1222) is one of several vaccines introduced to provide immunity against SARS-CoV-2. Recently, more than 50 cases have been reported presenting a combination of thrombosis, thrombocytopenia, and remarkably high levels of anti-platelet factor 4 (PF4)/polyanion antibodies post-AZD1222 vaccination. Now linked to the vaccine, the condition is referred to as vaccine-induced immune thrombotic thrombocytopenia. The European Medicines Agency still recommends vaccination with AZD1222, but several European countries have temporally paused and/or restricted its use because of the perceived risk of this severe side effect. Because there is no description of PF4/polyanion antibody testing in the clinical trials, knowledge about the prevalence of such antibodies in a vaccinated cohort is needed.

Objectives: To investigate prevalence of thrombocytopenia and anti-PF4/polyanion antibodies in a population recently vaccinated with AZD1222.

Patients/Methods: Four hundred and ninety-two health care workers recently vaccinated with the first dose of AZD1222 were recruited from two hospitals in Norway. Study individuals were screened for thrombocytopenia and the presence of anti-PF4/polyanion antibodies with a PF4/PVS immunoassay. Side effects after vaccination were registered.

Results: The majority of study participants had normal platelet counts and negative immunoassay. Anti-PF4/polyanion antibodies without platelet activating properties

Sørvoll and Horvei dual first authorship

Manuscript Handled by: Matthew T. Rondina

Final decision: Matthew T. Rondina, 23 April 2021

were only detected in six individuals (optical density ≥0.4, range 0.58–1.16), all with normal platelet counts. No subjects had severe thrombocytopenia.

Conclusions: We found low prevalence of both thrombocytopenia and antibodies to PF4/polyanion-complexes among Norwegian health care workers after vaccination with AZD1222.

KEYWORDS
COVID-19 vaccines, COVID-19 vaccines/adverse effects, drug related side effects and adverse reactions, platelet factor 4, thrombocytopenia

1 | INTRODUCTION

Massive vaccination campaigns using various anti-COVID vaccines have started around the world to bring COVID-19 pandemic to a halt. Among these, the AZD1222 (ChAdOx nCoV-19) vaccine from AstraZeneca has been shown to be effective and safe in preventing COVID-19.[1] This vaccine was widely administered to health care workers under the age of 65 years in Norway. Recently, a link between the AZD1222 and a rare thrombotic disorder has been reported by The European Medicines Agency (EMA)[2] with more than 50 cases reported in Europe.[3–5] The disorder has a resemblance to heparin-induced thrombocytopenia (HIT); however, it does not require heparin as a trigger, thus mimicking spontaneous autoimmune heparin-induced thrombocytopenia.[6]

We recently reported this condition in five Norwegian health care workers presenting with venous thrombosis at unusual sites, and thrombocytopenia 7–10 days after receiving AZD1222.[4] At the Norwegian National Unit for Platelet Immunology, we revealed remarkably high levels of anti-platelet factor 4 (PF4)/polyanion antibodies in enzyme-linked immunosorbent assay (ELISA) in all five cases. Four of these cases also demonstrated platelet-activating properties in functional testing assay. This tentative vaccine-induced condition has been referred to as vaccine-induced immune thrombotic thrombocytopenia (VITT). Other groups have also reported several cases with very similar clinical presentation after AZD1222 vaccination.[3,5] The pathogenesis of VITT is still unknown.

Across Europe, millions of people aged 18 years or older have been vaccinated with AZD1222. Systemic adverse reactions are common, but rarely severe,[7] in contrast to the devastating VITT development that often has a fatal outcome.

Several countries have put the vaccine on hold after the emergence of cases of these rare thrombotic events. The safety committee of EMA concluded after evaluation in March 2021 that the benefits of the vaccine in combating COVID-19 still outweigh the risk of side effects. Further, according to EMA, there is no evidence of product quality issues related to specific vaccine batches or manufacturing sites.[2] The safety studies report fewer cases of thrombosis than in unvaccinated controls.[7]

In Norway, approximately 135 000 individuals have received AZD1222 since February 2021.[8] The VITT cases have raised concern that vaccinated individuals have a higher risk of development or boosting of anti-PF4/polyanion reactive antibodies. Platelet antibody testing has not been previously described in the intervention group or control group in the clinical phase 1/2/3 studies.[1,9,10]

Essentials

- PF4/polyanion antibodies are associated with vaccine-induced immune thrombotic thrombocytopenia (VITT).
- Four hundred and ninety-two vaccinated individuals were screened for thrombocytopenia and anti-PF4/polyanion antibodies.
- We found low prevalence of thrombocytopenia in the vaccinated individuals.
- There was a low prevalence of anti-PF4/polyanion antibodies 11–35 days post vaccination.

The aim of this study was to investigate the prevalence of thrombocytopenia and anti-PF4/polyanion antibodies in a population recently vaccinated with AZD1222, both to identify individuals that may be at risk of developing VITT, and to investigate the prevalence of apparently subclinical anti-PF4/polyanion antibodies. ELISA has been shown to reliably detect anti-PF4 antibodies associated with VITT,[3–5] and was thus used for antibody screening in this study.

2 | MATERIALS AND METHODS

2.1 | Subjects and materials

Health care workers were in an open invitation recruited from the University Hospital of North Norway (UNN) and Østfold Hospital Trust during March 22–29, 2021, into two identical studies, here reported together. Study participants who had received first dose of AZD1222 vaccine within 10–35 days were invited. All participants gave written informed consent. The number of recruited participants was selected to ensure detection of PF4/polyanion antibodies in the event this is a common occurrence postvaccination. The studies were approved by two ethics committees (REK 257384 and REK 255184). In addition, we included nonvaccinated healthy blood

donors with no history of COVID-19 from the blood donation service (UNN) as a control group. Plasma and serum were also biobanked for further analysis related to the safety of this vaccine.

2.2 | Questionnaire and blood sampling

Side effect data were reported on a questionnaire grading experienced symptoms, as fever, headache, vomiting, fatigue, cutaneous bleeding, malaise, and muscle and joint ache on a scale from 0 to 6. Demographic data were registered. Also, the participants reported use of medication such as paracetamol or ibuprofen in relation to vaccination. Whole blood was collected and processed by standard procedures for hematology and platelet immunology testing.

2.3 | Laboratory analyses

Platelet counts were measured on Sysmex (UNN) and ADVIA (Østfold) hematology analyzers. Severe thrombocytopenia was defined as a platelet count $<50 \times 10^9$/L, and moderate thrombocytopenia as $50–150 \times 10^9$/L. Antibodies to PF4/PVS (polyanionic polyvinyl sulfonate) were screened for by LIFECODES PF4 immunoglobulin G (IgG) ELISA immunoassay (Immucor, Waukesha, WI), according to the manufacturer's instructions, dilution 1:50, and an optical density (OD) cutoff value ≥0.400. Positive samples in ELISA were tested by heparin-induced multiple electrode aggregometry (HIMEA) on the multiplate analyzer (Dynabyte Medical, Germany). In the HIMEA assay,[11] normal blood group O donor platelets were incubated with PF4/PVS-positive sera in the presence of low-dose heparin (unfractionated heparin 0.96 IU/mL), high-dose heparin (unfractionated heparin 96 IU/mL), and saline buffer. A previously confirmed HIT serum was used as a positive control, and normal pooled plasma as negative control. The test was performed according to in-house validated protocol.

2.4 | Statistics

Proportions and confidence intervals (CI) for descriptive data and side effects were calculated in SPSS Statistics 26 with 95% significance level (bootstrap method). GraphPad Prism 8.12 was used to create figures for platelet counts and ELISA OD values. Line represents median value, and whiskers 95% CI.

3 | RESULTS AND DISCUSSION

Anti-PF4/polyanion reactive antibodies in high levels seem to be a defining feature of VITT, the devastating clinical picture recently described after AZD1222 vaccination.[4] The link between vaccination and antibody formation or boosting is unknown and determining the frequency of PF4/PVS antibodies in a vaccinated group compared to unvaccinated is an important contribution to investigate such an association.

TABLE 1 Demographic of the study population

	Vaccinated cohort (*n* = 492)	Controls (*n* = 110)
Age, y[a]	44 (21–69)	43 (21–66)
Female, *n*, %	373, 76%	56, 51%
Time since vaccination, days[a]	20 (11–35)	-

[a]Medians (range).

A total of 502 vaccinated health care workers were included in the study. Because of missing data, 10 individuals were excluded. The remaining 492 study participants were included in final data analysis. In the control group, 110 blood donors were included (Table 1).

The vast majority of study participants had normal platelet counts and low OD values in anti-PF4/PVS IgG ELISA (Figure 1). In the vaccinated cohort, eight subjects had reduced platelet counts, all above 100×10^9/L (1.6%, 95% CI: 0.6–2.8). Anti-PF4/polyanion antibodies with OD values over cutoff ≥0.4 were detected in six subjects (1.2%, 95% CI: 0.4–2.2) in the vaccinated cohort, all having platelet count above 150×10^9/L (Figure 2). None of the PF4/PVS-positive sera induced platelet aggregation in the HIMEA assay. In the control group, there were no ELISA-positive sera.

Noteworthy, the cutoff value ≥0.4 in the PF4 IgG ELISA is set to achieve a specificity appropriate for the detection of clinically relevant antibodies in typical HIT investigations. VITT patients have presented remarkably high OD values in ELISA; however, the antibody level in vaccinated individuals with no clinical signs of VITT is not known. Given this, we note that about 6% (*n* = 28) of the vaccinated cohort had OD values in the range of 0.2–0.4, as opposed to none in the control group. Gender distribution and mean platelet count in this group was not different from individuals with OD values <0.2 (mean 274 vs. 270×10^9/L). These individuals may not be at risk for disease, but they might have mounted an immune response weakly detectable in the ELISA and could in theory trigger a clinically relevant stronger response with a second vaccination or booster in the future.

Whether or not the established cutoff ≥0.4 is suitable in a screening setting to determine the prevalence of low levels of antibodies after COVID-19 vaccination remains to be discussed, but should preferably be supported by studies including a prevaccination baseline sample.

Interestingly, the prevalence of anti-PF4/polyanion antibodies in our study were lower in the vaccinated cohort (1.2%, OD ≥0.4), than reported previously for blood donors. Hursting et al. found low levels of nonpathogenic PF4/polyanion antibodies (IgG/IgM/IgA) detectable in ELISA in 5%–7% of blood donors, of which 50% had the IgG isotype.[12] This discrepancy in reported seroprevalence and our results may be explained by differences between the study populations and/or the immunoassays.

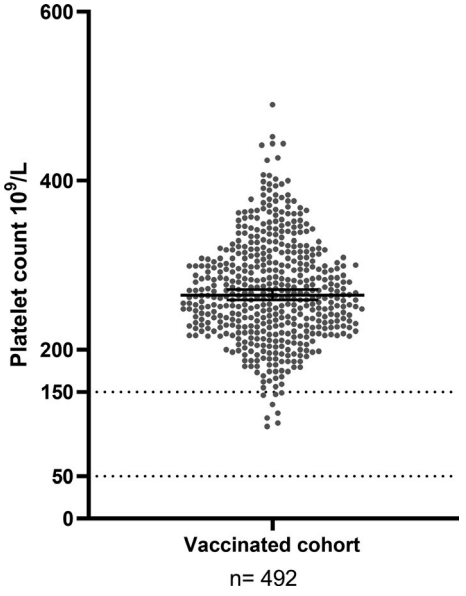

FIGURE 1 In total, only eight of the 492 vaccinated individuals (1.6%) had thrombocytopenia, with platelet count above 100×10^9/L. Line represents median, error bars 95% confidence interval

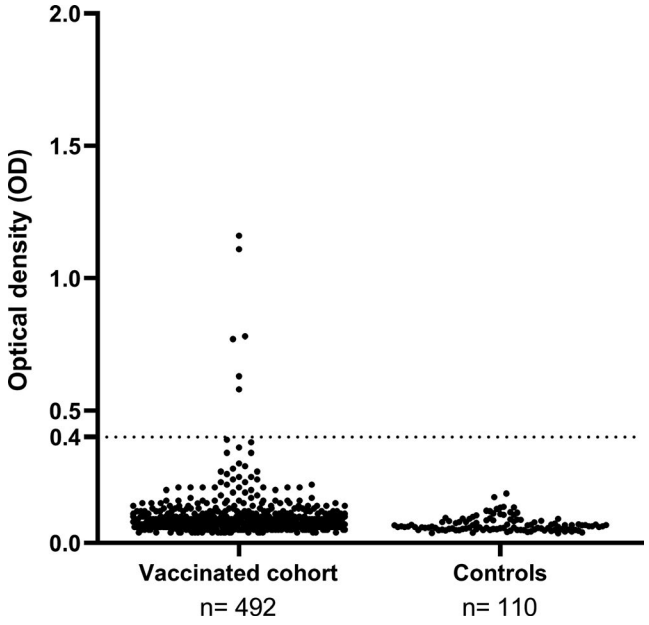

FIGURE 2 Anti-PF4/PVS reactive antibodies were detectable in six individuals (1.2%) in the vaccine cohort by ELISA. The highest OD value was 1.16, OD ≥0.4 indicate presence of antibodies. OD, Optical density; PF4, platelet factor 4

Our narrow inclusion window of 10–35 days postvaccination was selected to be able to detect both an immunological boosting of preformed antibodies, as well as a de novo anti-PF4/polyanion IgG response. There are limited data regarding the time range for antibody detection for autoimmune HIT, but for typical HIT the detection window is relatively short because these antibodies tend to disappear within 50–85 days.[13]

Most participants reported fever, headache, fatigue, malaise, and muscle/joint ache (Table 2). A total of 65% used medication to relieve symptoms in relation to vaccination. To detect potential VITT development at the time of the study, participants were asked whether they experienced recent malaise; 94% denied malaise the last days before blood sampling.

It has been suggested that an inflammatory response to the vaccine triggers the antibody production tentatively causing VITT.[3,4] Therefore, Norwegian health authorities have instructed vaccinated individuals to be aware of persisting symptoms, skin bleeding, or neurological symptoms postvaccination. The side effects in our vaccinated cohort are similar to the reports from the clinical safety study for this vaccine.[7] In summary, more than 60% of the subjects reported side effects as fever, headache, fatigue, malaise, and muscle/joint ache, of which >40% reporting symptoms as moderate or severe. A few individuals reported persisting symptoms; 10% reported skin bleeding/bruising. The individuals with anti-PF4/polyanion antibodies did not report higher rates of malaise before blood sampling, and three of six reported fever and muscle/joint ache the days following vaccination, a frequency not differing from subjects without anti-PF4 antibodies. Taken together, our data indicate that inflammatory symptoms show low pretest probability for anti-PF4/PVS antibody detection and the development of VITT.

Based on reported VITT cases in Norway, the incidence can be estimated to 1 in 25 000 vaccinated individuals.[4] This seems to be higher than in other European countries, possibly because of a more robust national reporting system and short lines of communication within the health care community in a low population country. Also, the demographic group receiving this vaccine might differ from other European countries because it was given to health care workers. Importantly, our study population of vaccinated health care workers reflects a similar cohort as the Norwegian VITT cases. However, because the entity emerged very recently with only a few cases reported worldwide, the condition may be underreported, and its true incidence is yet unknown.

Perceived risk of severe side effects can lead to vaccine hesitancy in the general population, which may delay vaccine coverage. Vaccination is of utter importance in combating the COVID-19 pandemic. To earn public trust, gathering knowledge about serious adverse effects from vaccination is important to be able to make well-informed decisions regarding vaccine strategy.

In conclusion, this is the first study to screen for anti-PF4/polyanion antibodies in a population vaccinated with AZD1222. We did not find anti-PF4/polyanion platelet activating antibodies, nor severe thrombocytopenia in approximately 500 vaccinated participants. This suggests that both the natural occurrence and postvaccine *de novo* generation or boosting of such antibodies are rare. If the incidence of high-level anti-PF4/polyanion antibodies tentatively causing VITT is closer to the observed incidence of VITT, the number of individuals included in our observational study is clearly too low. Determining a low incidence would require

TABLE 2 Self-reported side effects after vaccination

Symptom (n = 492)	None	Mild	Moderate	Severe	Any
	N, % (95% CI)				
Fever	193, 39% (35%–44%)	90, 18% (15%–22%)	111, 23% (19%–26%)	98, 20% (17–24%)	299, 61% (57%–65%)
Headache	140, 28% (24%–32%)	100, 20% (17%–24%)	169, 34% (30%–38%)	83, 17% (14%–20%)	352, 72% (68%–76%)
Vomiting	471, 96% (94%–98%)	15, 3.0% (1.6%–4.7%)	5, 1.0% (0.2%–1.8%)	1, 0.2% (0.0%–0.6%)	21, 4.3% (2.4%–6.1%)
Fatigue	188, 38% (34%–43%)	78, 16% (13%–19%)	144, 29% (25%–34%)	82, 17% (14%–20%)	304, 62% (58%–66%)
Cutaneous bleeding	443, 90% (87%–93%)	32, 6.5% (4.3%–8.7%)	13, 2.6% (1.2%–4.3%)	4, 0.8% (0.2%–1.6%)	49, 10% (7.3%–13%)
Malaise	190, 39% (34%–43%)	56, 11% (8.5%–14%)	139, 28% (24%–32%)	107, 22% (18%–25%)	302, 61% (57%–66%)
Muscle/joint ache	170, 35% (30%–39%)	75, 15% (12%–19%)	142, 29% (25%–33%)	105, 21% (18%–25%)	322, 65% (61%–70%)

Note: Categories of symptoms in questionnaire 0–6: none (0), mild (1–2), moderate (3–4), severe (5–6), any (1–6).

a larger study, preferably with more high-throughput methods for antibody testing.

ACKNOWLEDGMENTS

The authors are grateful to all involved personnel at the Department of Laboratory Medicine, University Hospital of North Norway, the Departments of Research and Laboratory Medicine, and the Research and laboratory departments, and Thrombosis and Hemostasis Research Group, Østfold Hospital. They also thank the blood donors and health care workers for taking part in the study.

CONFLICT OF INTEREST

Dr. Ghanima reports grants from Bayer and BMS/Pfizer, and fees for lectures and consultancy from Novartis, Amgen, Principia, Bayer, Pfizer, Sobi, and Sanofi. Dr. Ahlen reports shares in the biotech/pharmaceutical companies Vaccibody, Photocure, ArcticZymes Technologies, Exact Therapies, and Viramabs Inc. Dr. Sørvoll reports that her spouse is the chief financial officer in ArcticZymes Technologies. The other authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

Ingvild Hausberg Sørvoll, Kjersti Daae Horvei, Siw Leiknes Ernstsen, Ingvild Jenssen Lægreid, Eirik Tjønnfjord, Waleed Ghanima and Maria Therese Ahlen designed the study. Renathe Henriksen Grønli and Svetlana Lund performed antibody analysis. Ingvild Hausberg Sørvoll, Kjersti Daae Horvei, and Maria Therese Ahlen drafted the manuscript. Eirik Tjønnfjord, Waleed Ghanima, Anna Eriksson, Magnus Kringstad Olsen, Hege Karine Jacobsen, and Anne Marie Halstensen collected and discussed data. Ingvild Hausberg Sørvoll, Kjersti Daae Horvei, Siw Leiknes Ernstsen, Ingvild Jenssen Lægreid, Waleed Ghanima, and Maria Therese Ahlen collected, analyzed, and discussed data. All authors revised the manuscript and approved the final version for publication.

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How to cite this article: Sørvoll IH, Horvei KD, Ernstsen SL, et al. An observational study to identify the prevalence of thrombocytopenia and anti-PF4/polyanion antibodies in Norwegian health care workers after COVID-19 vaccination. *J Thromb Haemost*. 2021;19:1813–1818. https://doi.org/10.1111/jth.15352