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Letter to the Editors-in-Chief

Covid-19 vaccination, adverse events, and detection of antibodies

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Vaccination against the SARS-CoV-2 virus will hopefully terminate the pandemic of Covid-19 and several vaccines are now available. In March 2021 a rare, but severe adverse event after the ChAdOx1 nCOV-19 vaccine (AstraZeneca) was identified, now referred to as vaccine-induced immune thrombotic thrombocytopenia (VITT) [1–4], presenting with high levels of anti-platelet factor 4 (anti-PF4) antibodies, low platelet count and thromboses, often at unusual sites [1,2,4]. Anti-PF4 antibodies are usually seen in patients diagnosed with heparin induced thrombocytopenia (HIT) or autoimmune HIT (aHIT) [5]. It is still unknown what triggers the formation of these antibodies after vaccination. While VITT is a rare event, adverse events as malaise, headache and fever for a few days after the vaccination are common. However, in our experience some persons do also have prolonged manifestations of the adverse events, and bleedings such as ecchymoses and nose bleedings may occur for weeks, although they do not develop thrombosis.

In HIT an iceberg model of presence of antibodies and symptoms has been described where several persons develop HIT antibodies after heparin treatment of which only a minor part develops thrombocytopenia and only a minority get thromboses [5]. It is not known whether the same is the case with antibodies after Covid-19 vaccinations. Thiele et al. very recently described that 8.0% of 138 investigated persons had anti-PF4 antibodies seven days after ChAdOx1 nCOV-19 vaccination, although a considerable part were positive before the vaccination, and none of these induced platelet activation [6]. Sørvoll et al. reported that 1.2% of the vaccinated population had non-platelet activating anti-PF4 antibodies 10–35 days after the vaccination, all having normal platelet count and side effects similar to the persons with no anti-PF4 antibodies [7].

The aim of this small study was to investigate whether persons who had more severe adverse events after vaccination with ChAdOx1 nCOV-19 vaccine had anti-PF4 antibodies. Initially, we used a rather common rapid test for HIT-IgG (PF4-H), Hemosil Acustar (Werfen Ltd., Warrington, UK). However, due to negative results using this assay testing sera from a VITT-patient we included anti-PF4 testing by ELISA.

In our region (North Denmark Region) 3603 persons received the ChAdOx1 nCOV-19 vaccine with one case of confirmed VITT as reported previously [3] (use of ChAdOx1 nCOV-19 vaccine was terminated on March 11th 2021 in Denmark). Further, we included testing of a follow-up sample one month after the acute event from a surviving VITT patient from Norway [2]. In addition, several individuals presented rather severe adverse events, of which 9 were investigated for the presence of anti-PF4 antibodies. The testing was offered to vaccinated persons with adverse events who contacted us because of this. Thus, this testing was part of their examination, and all gave informed consent for publication (no need for approval from the ethical committee (Statement from the North Denmark Region Committee on Health Research Ethics is attached as a supplementum).

Anti-PF4 antibodies were analyzed with the Hemosil AcuStar HIT-IgG kit (Werfen Ltd., Warrington, UK) (reference values according to the company: 0.03–0.39 U/mL, but only results ≥1.0 U/mL are considered positive for HIT antibodies), and by Lifecodes PF4 IgG ELISA immunoassay (Immucor, Waukesha, WI) using a cut-off (OD) at ≥0.40 according to the manufacturer's instructions as also described in [2] (without addition of PF-4). Furthermore, a functional test of heparin-induced multiple electrode aggregometry (HIMEA) was performed on Multiplate as previously described [7].

Table 1 describes the patients. Patients 1 and 2 were VITT patients with strongly positive ELISA and positive functional HIMEA tests at diagnosis. However, the results of Hemosil Acustar were within the reference interval for healthy individuals in both these patients. Patients 3 to 11 had adverse events lasting more than a week after the vaccination, mainly as bleeding and ecchymoses, and some also protracted headache. Patient 3–5 and 9 got ecchymoses shortly after the vaccination (same or the second day) and the bleeding tendency only lasted for 1–2 weeks. Patient 7 got ecchymoses 11–12 days after the vaccination and the tendency lasted until 4 weeks after the vaccination. Patient 8 got nose bleeding a few days after the vaccination and several ecchymoses in weeks 3–6 after the vaccination. Patient 11 had nose bleeding, ecchymoses and macroscopic hematuria starting some days after the vaccination and lasting until the end of week 2. The samples were taken some time after vaccination, and the platelet number was normal in all but two: Patient 4 was clearly thrombocytopenic (platelet count 55,000 pr μl), and patient 11 had a reduced platelet count (150,000 pr μl) compared with the level measured two months earlier (266,000 pr μl). One patient had an ischemic stroke, but the patient had a normal platelet

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Table 1
Describing the participants.

	Sex	Age (y)	Sampling post-vaccination	Vaccine	Adv. react. time[a]	Adverse reactions	Hemosil Hit-IgG	ELISA Hit-IgG	HIMEA	Platelet count (μl^{-1})
1	F	60	12 d	AstraZeneca	1 w	VITT - died	0.20	Strongly pos – OD 3.19	Pos	5000
2	M	32	40 d	AstraZeneca	7 d	VITT - survived	0.18	Strongly pos – OD 2.10	Pos[b]	(Within nor- mal range)
3	F	58	30 d	AstraZeneca	1–2 w	Large ecchymoses	0.01	Neg – OD 0.11	ND	280,000
4	F	52	39 d	AstraZeneca	1–2 w	Large ecchymoses	0.02	Neg – OD 0.22	Neg	55,000
5	F	59	34 d	AstraZeneca	1–2 w	Ecchymoses	0.00	Neg – OD 0.11	ND	219,000
6	F	62	28 d	AstraZeneca	26 d	Ischemic stroke	0.04	Neg – OD 0.11	Neg	257,000
7	F	55	35 d	AstraZeneca	Up to 4 w	Headache, ecchymoses	0.02	Neg – OD 0.04	Neg	369,000
8	F	53	42 d	AstraZeneca	Up to 6 w	Nosebleeding, ecchymoses	0.03	Neg – OD 0.04	Neg	201,000
9	F	50	56 d	AstraZeneca	1–2 w	Ecchymoses	0.00	Neg – OD 0.05	Neg	297,000
10	M	60	47 d	AstraZeneca	2 w	Headache	0.01	Neg – OD 0.04	Neg	209,000
11	F	57	54 d	AstraZeneca	1–2 w	Hematuria, Nose bleeding, ecchymoses	0.02	Neg – OD 0.04	ND	150,000

Abbreviations: d: days; w: weeks; OD: optical density; ND: not determined.
[a] Time for adverse reactions after vaccination.
[b] HIMEA was positive at the time of VITT, but was negative in this sample (1 month after VITT). Platelet number also normalized at this time.

count and tests for anti-PF4 antibodies were negative, and therefore not considered to have VITT. Anti-PF4 antibody testing was negative for all these patients (patients 3–11) with both methods.

These results show that the two patients with VITT were clearly positive with the ELISA test, but in spite of this, the results from Hemosil HIT-IgG Acustar were negative. The patients with adverse events were negative with both tests although two of them actually had a lowered platelet count compared with their usual level.

A comparison of different assays for measuring anti-PF 4 antibodies has very recently been reported [8], and these results showed important differences of their ability to detect the antibodies associated with VITT. In accordance with our results, the Hemosil HIT-IgG AcuStar method was not able to demonstrate the presence of antibodies in almost all the patients. None of the methods detected all the cases of VITT, but the ELISA tests were clearly superior to the chemiluminescent assays. The authors recommend, therefore, that a platelet activation assay should be performed if there is a strong clinical suspicion. SSC Subcommittee on Platelet Immunology also recommend that an ELISA PF4/heparin-antibodies and a functional assay as HIMEA or serotonin release assay etc. should be used for diagnosis [9] in accordance with a guidance from The German Society of Thrombosis and Haemostasis [10]. Scully et al. describing 22 patients with VITT also showed that Hemosil Hit-IgG AcuStar was negative in 10 patients where this assay had been used [4], so although this test reliably can detect anti-PF4 antibodies in HIT patients it is not valid to detect VITT patients.

Except for the two VITT-cases, we did not find positivity for anti-PF4 antibodies in any of the patients with more severe adverse events although two of them actually had a lowered platelet count. A limitation of this finding is that blood was sampled 1–2 months after vaccination and after the adverse events had ceased for most of the participants. However, the survived patient with VITT (patient 2) still had a strongly positive antibody level (a very high OD-level) one month after the event indicating that the half-life is quite long. In HIT it has been described that antibodies can be detected 40–80 days after a HIT event [5]. We cannot exclude, therefore, that the antibody levels had been lowered from a previous positive level, but it should be noted that the OD values were very low and far below the cut-off value at 0.4. These findings do not indicate that the distribution of antibodies is like the iceberg model described in HIT [5]. None of the patients had alternative explanations for the bleeding tendency but it is a limitation of the study that we have not made any other diagnostic investigations. We cannot exclude that the lowered platelet number in two of the individuals had other causes than the vaccination. Obviously, the bleeding tendency in most of the individuals were not caused by thrombocytopenia, but we have no explanations of the mechanisms.

Thus, although this is a small investigation and firm conclusions cannot be made, it indicates after all two important issues: 1) The results support previous important findings that care should be taken to ensure use of appropriate tests that are able to detect the anti-PF4 antibodies which occur in VITT; 2) although some persons get a bleeding tendency with or without thrombocytopenia after ChAdOx1 nCOV-19 vaccination, this is apparently not related to anti-PF4 antibodies in contrast to the patients developing VITT.

CRediT authorship contribution statement

SR Kristensen and AB Hansen conceived the idea. J Nybo collected samples and information and made analyses. SL Ernstsen made analyses of ELISA tests and HIMEA and interpreted data. SR Kristensen wrote the manuscript and all authors read and critically revised the manuscript and approved the final version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] A. Greinacher, T. Thiele, T.E. Warkentin, K. Weisser, P.A. Kyrle, S. Eichinger, Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination, N. Engl. J. Med. 384 (22) (2021) 2092–2101.
[2] N.H. Schultz, I.H. Sørvoll, A.E. Michelsen, L.A. Munthe, F. Lund-Johansen, M. T. Ahlen, et al., Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination, N. Engl. J. Med. 384 (22) (2021) 2124–2130.
[3] R.A. Blauenfeldt, S.R. Kristensen, S.L. Ernstsen, C.C.H. Kristensen, C.Z. Simonsen, A.M. Hvas, Thrombocytopenia with acute ischemic stroke and bleeding in a patient newly vaccinated with an adenoviral vector-based COVID-19 vaccine, J. Thromb. Haemost. 19 (7) (2021) 1771–1775.
[4] M. Scully, D. Singh, R. Lown, A. Poles, T. Solomon, M. Levi, et al., Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination, N. Engl. J. Med. 384 (23) (2021) 2202–2211.
[5] T.E. Warkentin, Laboratory diagnosis of heparin-induced thrombocytopenia, Int. J. Lab. Hematol. 41 (Suppl 1) (2019) 15–25.
[6] T. Thiele, L. Ulm, S. Holtfreter, L. Schönborn, S.O. Kuhn, C. Scheer, et al., Frequency of positive anti-PF4/polyanion antibody tests after COVID-19 vaccination with ChAdOx1 nCoV-19 and BNT162b2, Blood 138 (4) (2021) 299–303.
[7] I.H. Sørvoll, K.D. Horvei, S.L. Ernstsen, I.J. Laegreid, S. Lund, R.H. Grønli, 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. 19 (7) (2021) 1813–1818.
[8] S. Platton, A. Bartlett, P. MacCallum, M. Makris, V. McDonald, D. Singh, et al., Evaluation of laboratory assays for anti-platelet factor 4 antibodies after ChAdOx1 nCOV-19 vaccination, J. Thromb. Haemost. 19 (8) (2021) 2007–2013.
[9] I. Nazy, U.J. Sachs, D.M. Arnold, S.E. McKenzie, P. Choi, K. Althaus, et al., Recommendations for the clinical and laboratory diagnosis of VITT against COVID-19:

communication from the ISTH SSC Subcommittee on Platelet Immunology, J. Thromb. Haemost. 19 (6) (2021) 1585–1588.
[10] J. Oldenburg, R. Klamroth, F. Langer, M. Albisetti, C. von Auer, C. Ay, et al., Diagnosis and management of vaccine-related thrombosis following AstraZeneca COVID-19 vaccination: guidance statement from the GTH, Hamostaseologie 41 (3) (2021) 184–189.

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