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LETTERS TO THE EDITOR

Laboratory testing for post ChAdOx1 nCOV-19 vaccination VITT: A challenge. Comment on: Recommendations for the clinical and laboratory diagnosis of VITT against COVID-19: Communication from the ISTH SSC Subcommittee on Platelet Immunology

Recent publications reveal the presence of pathogenic antibodies against platelet factor 4 (PF4) in patients with suspected vaccine-induced immune thrombotic thrombocytopenia (VITT), after administration of the ChAdOx1 nCoV-19 vaccine.[1–5] There is similarity with heparin-induced thrombocytopenia (HIT); however, the causality and clinical implications of the PF4 antibodies in VITT are still unclear. The recommendation of the ISTH SSC Subcommittee on Platelet Immunology concerning the clinical and laboratory diagnosis of VITT against COVID-19 provides case definitions and includes a test strategy for laboratories. The authors propose a methodology similar to HIT testing, in which the platelet-activating properties of PF4 antibodies detected by a PF4/heparin ELISA need to be confirmed with a functional HIT assay.[6] Thereby, the HemosIL AcuStar HIT IgG chemiluminescence immunoassay and other rapid immunoassays are not recommended to detect PF4/heparin antibodies because of a high rate of false-negative results. Indeed, Platton et al. concluded that the HemosIL AcuStar HIT IgG may not be of use in VITT testing because 31 of the 33 tested sera of suspected patients were negative on this platform, with the two remaining sera only showing borderline reaction.[3]

Positive PF4/heparin ELISA sera or plasma samples may be confirmed by different functional assays, in which a positive result strongly suggests VITT.[6] However, the circumstances or patterns at which a sample may be confirmed as VITT are not stated. Multiple groups have shown that PF4 antibody-related platelet activation in VITT occurs in a heparin-independent manner, with platelet activation observed both in presence and absence of heparin (e.g., buffer solution).[1,2,4] Moreover, functional test methods are being adapted to include platelet incubation with PF4, which seem to enhance platelet activation in some sera.[2,5] Still, it is known that VITT sera are quite heterogeneous with respect to their activation profile. Another aspect is that laboratory features of VITT patients resemble those of patients previously described with autoimmune HIT. The term "autoimmune HIT" comprises several distinct disorders, including some triggered by heparin (e.g., delayed-onset HIT, refractory HIT) but also some not triggered by heparin (e.g., post-knee replacement HIT syndrome) called "spontaneous HIT."[7] These syndromes might pass the proposed test strategy and lead to wrong recognition and diagnosis of VITT, and could contribute to an overestimation of the vaccine-related severe side effect of thrombocytopenia/thrombosis. We have to take care that reporting "false" VITT does not contribute to public anxiety against vaccination and we have to avoid unnecessary therapeutic action.

To illustrate the resemblances between VITT and autoimmune/spontaneous HIT, we selected two cases suspected for VITT, as well as two autoimmune HIT and two confirmed HIT patients from the pre-SARS-CoV-2 era. Both VITT patients, one woman and one man, presented with thrombocytopenia and altered coagulation parameters (e.g., low fibrinogen, high D-dimers), although thrombosis was only objectified in the male patient at the moment of testing. Citrated plasma samples were obtained shortly after hospital admission, 10 and 8 days after receiving a first dose of the ChAdOx1 nCoV-19 vaccine, and before any exposure to heparin, respectively. The autoimmune HIT cases were selected based on their atypical HIT pattern. Although the second patient had recently received intra-articular infiltration in treatment of his frozen shoulder, therapeutic conditions of the first putative autoimmune HIT patient were not obtained. The HIT cases were based on positive immunoassay, positive functional assay, and high 4T score.

Following the ISTH-SSC recommendation, all selected cases tested positive with the Zymutest HIA IgG ELISA (Hyphen Biomed, Neuville-sur-Oise, France). Results are shown in Figure 1. Two patients with clinical suspected HIT with negative ELISA were selected as negative controls. Platelet-activating properties of the PF4/heparin antibodies were determined by the flow cytometric functional HIT assay as previously described by Denys et al. and compatible with the ISTH SSC recommendation.[6,8] Evolved from current literature, HIT test conditions consisting of 100 U per milliliter unfractionated heparin (UFH, B. Braun Medical SA, Diegem, Belgium),

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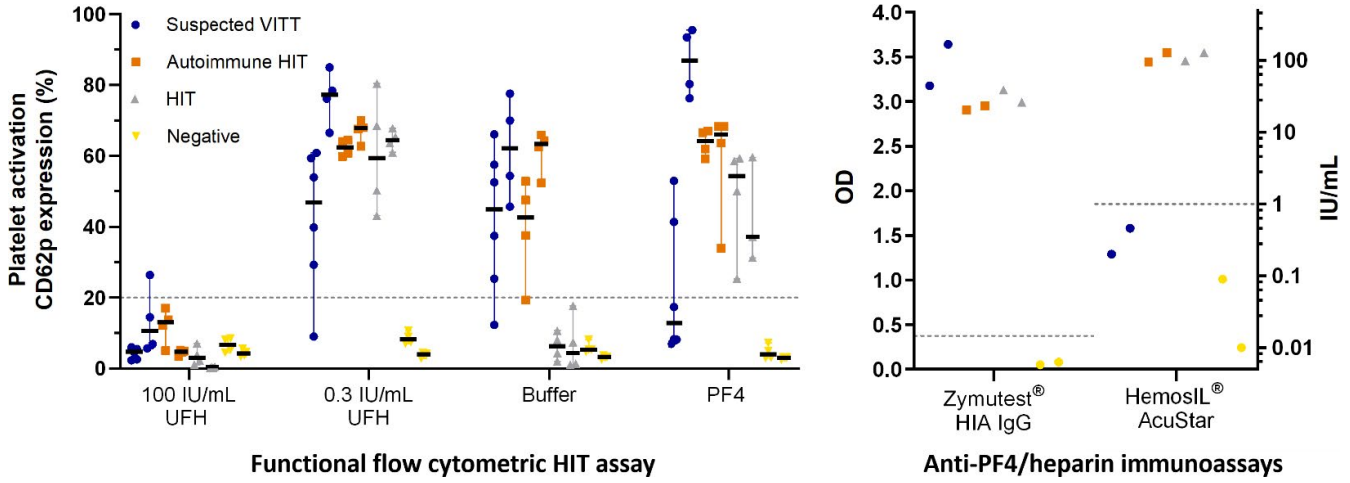

FIGURE 1 Reactivity of different patient samples with the flow cytometric platelet-activation assay and anti-PF4/heparin immunoassays. Each case is presented individually, using four colors to indicate their respective clinical condition. Platelet activation, as of percentage CD62p expression, is presented for the incubation conditions on the left part of the figure. Each dot represents the result obtained with a single donor, whereas the horizontal line represents the median value. Immunoassay results are presented by the dots on the right side of the figure. Horizontal dotted lines represent positivity cutoffs. Flow cytometric results of the suspected VITT samples show heparin-independent platelet activation because a high percentage CD62p expression was found both with and without presence of 0.3 U/ml UFH. This pattern is also obtained with both autoimmune HIT samples. Addition of PF4 had variable effects on platelet activation in VITT cases. High titers of UFH inhibited platelet activation in all samples. Besides negative controls, all samples showed a strongly positive result on the Zymutest HIA IgG. In contrast to HIT and autoimmune HIT samples, VITT samples did not react on the HemosIL AcuStar platform. HIT, heparin-induced thrombocytopenia; PF4, platelet factor 4; UFH, unfractionated heparin; VITT, vaccine-induced immune thrombotic thrombocytopenia

0.3 U per milliliter UFH and saline buffer, were expanded by platelet incubation with 10 μg/ml PF4 (Chromatec, Greifswald, DE), as this was found to enhance platelet activation.[2,5] The functional assay is based on platelet-rich plasma (PRP) methodology, with the activation end point being percent CD62p expression (>20% cutoff), as well as the 0.3 U per milliliter UFH to buffer and 100 U per milliliter UFH ratios of CD62p activation being used for interpretation in HIT testing. Each patient sample is tested using thrombocytes from at least four healthy donors. Samples were also tested on HemosIL AcuStar HIT IgG assay (Werfen, Bedford, MA), defining 1 U/ml as positivity cutoff.[9]

Our data illustrate the different flow cytometric platelet-activation patterns observed in both HIT and VITT samples. Classical HIT samples show a heparin-dependent platelet activation, with a high percentage of platelets expressing activation marker CD62p in presence of 0.3 U per milliliter UFH but not with high doses of heparin and buffer, whereas both VITT and autoimmune HIT samples show a heparin-independent platelet activation, depicted by the additional reaction in buffer. Addition of PF4 had variable results on VITT sera, as illustrated previously,[2] and does not seem to allow proper differentiation between autoimmune HIT and VITT. Instead, the Hemosil Acustar HIT assay, which has shown to be insensitive to PF4 antibodies in VITT and therefore not recommended in VITT testing, reveals high antibody titers in both HIT and autoimmune HIT patients, allowing clear differentiation of VITT. In case heparin-independent platelet activation is observed in the functional assay, the HemosIL AcuStar HIT IgG assay can therefore be used to differentiate between VITT and autoimmune HIT because only the latter show strongly positive results.

The ISTH-SSC recommendation does not define positivity thresholds or patterns for VITT testing, neither does it state test conditions. Many different assay formats and conditions are currently being used. This lack of standardization combined with the heterogeneity of the activation profile in VITT samples makes that the diagnosis of VITT remains a challenge for laboratories. Moreover, because clinical and ELISA laboratory characteristics of VITT seem similar to HIT, a functional assay is mandatory to differentiate both syndromes. In this short correspondence with selected cases by way of example, we illustrate that the laboratory findings in VITT might resemble those of patients previously described with spontaneous or autoimmune HIT, which also are syndromes that can be provoked independent of heparin. Although it is challenging to be able to reach strong conclusions based on a limited number of samples, we want to raise clinical awareness of the existence of these heparin-independent platelet-activating syndromes, and highlighted the resemblance with VITT. We suggest a possible diagnostic tool for differentiation that might be of relevance for future updates on laboratory diagnosis of VITT.

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CONFLICT OF INTEREST

Both authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Katrien M. J. Devreese designed the study. Kobe Vercruysse and Katrien M. J. Devreese selected the patients. Kobe Vercruysse analyzed the data. Katrien M. J. Devreese and Kobe Vercruysse discussed the results. Kobe Vercruysse and Katrien M. J. Devreese wrote the manuscript.

Kobe Vercruysse[1]
Katrien M. J. Devreese[1,2]

[1]*Coagulation Laboratory, Department of Laboratory Medicine, University Hospital Ghent, Ghent, Belgium*
[2]*Department of Diagnostic Sciences, Ghent University, Ghent, Belgium*

Correspondence
Katrien M. J. Devreese, Coagulation Laboratory, Ghent University Hospital, Corneel Heymanslaan 10, 9000 Gent, Belgium.
Email: Katrien.devreese@ugent.be

ORCID

Kobe Vercruysse https://orcid.org/0000-0002-4201-4158
Katrien M. J. Devreese https://orcid.org/0000-0002-7559-2579

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Comment on: Reduced dose direct oral anticoagulants (DOACs) in the extended treatment of venous thromboembolism: a systematic review and meta-analysis. Journal of Thrombosis and Haemostasis: JTH 2018 Jul; 16(7): 1288–95

Several current guidelines[1] mention that a reduced dose of apixaban or rivaroxaban should be considered for extended anticoagulation after the first 6 months of treatment of pulmonary embolism and/or thrombosis.

Therefore, the systematic review and meta-analysis of reduced-dose direct oral anticoagulants (DOACs) in the extended treatment of venous thromboembolism, published in *JTH*,[2] is of significant importance in many clinical settings. This information is necessary to evaluate benefit and risk individually of prolongation of DOAC administration.

We noticed, however, that in this article the numbers of the primary outcome events (Figure 1) recurrent venous thromboembolism events in the reduced-dose DOAC group) were incorrectly mapped for the rivaroxaban results from EINSTEIN CHOICE.[3] Instead of this, the event counts for full-dose anticoagulation with

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