Heparin-Induced Thrombocytopenia and Vaccine-Induced Immune Thrombotic Thrombocytopenia Antibodies: Fraternal—Not Identical—Twins

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A major development during the fight against the global pandemic caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) has been the extraordinarily rapid emergence of effective vaccines that elicit an immune response against the "spike" protein of this novel coronavirus.[1,2] Indeed, vaccines have been central to management of this pandemic.[3] Remarkably, a small proportion of otherwise (mostly) healthy vaccine recipients develop a prothrombotic thrombocytopenic syndrome—vaccine-induced immune thrombotic thrombocytopenia (VITT)—that strongly mimics immune heparin-induced thrombocytopenia (HIT) on clinical, laboratory, and serological grounds.[4–7]

As shown in a multi-laboratory study headed by Professor Ulrich Sachs, and reported in this issue of *Thrombosis and Haemostasis*,[8] while there are many serological similarities between VITT and HIT antibodies, they are not identical. In essence, VITT and HIT antibodies (particularly, "autoimmune" HIT antibodies[9]) are fraternal, not identical, twins that despite sharing the key unifying property of anti-platelet factor 4 (PF4) reactivity nonetheless bind to different regions on this target protein.

What insights gained from 50 years of research into HIT can be applied to 5 months of experience evaluating VITT? ►**Table 1** lists three fundamental tenets of HIT serology.[10–15]

Many clinicians do not recognize how special these first two fundamental tenets of HIT serology are. After all, other platelet immunology disorders—(auto)immune thrombocytopenia (ITP), drug-induced ITP, neonatal alloimmune thrombocytopenia—often do not have readily detectable antibodies in patient serum/plasma (indeed, poor sensitivity of platelet antibody assays in ITP makes this disorder a diagnosis of exclusion). In contrast, HIT is defined as a "clinical–pathological" disorder because the key pathological criterion—detectability of serum/plasma HIT antibodies—is fundamental to diagnosis.[16] The fact that patient serum/plasma contains detectable HIT antibodies is probably crucial to explaining HIT pathogenesis, namely that free plasma antibodies are required to assemble over time the in situ multimolecular complexes (multivalent PF4/polyanion/immunoglobulin G [IgG]) on platelet surfaces required to engage the platelet FcγIIa receptors, and thus to trigger the platelet activation response that underlies HIT.[10]

A striking feature of VITT is its triggering by one of two adenoviral vector vaccines (ChAdOx1 CoV-19 [AstraZeneca] and Ad26.COV2.S [Johnson & Johnson/Janssen]),[4–7,17] neither of which contains heparin. In as many as 1 or 2 individuals among 100,000 so vaccinated,[18] approximately 1 week later there can emerge this disorder characterized by one or more unusual and oftentimes severe thrombotic events with marked thrombocytopenia, hypercoagulability (elevated D-dimers, reduced fibrinogen), and—bizarrely—positive testing in standard PF4-dependent enzyme-linked

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Table 1 Fundamental tenets of HIT serology

Law #1	Patient serum/plasma always contain antibodies that recognize PF4 in standard solid-phase PF4/polyanion immunoassays, i.e., ELISAs (sensitivity >99%[a]).
Law #2	Pathogenic anti-PF4 antibodies that cause HIT have platelet-activating properties (i.e., are detectable in platelet activation assays); PF4 supplementation may be required for optimal sensitivity (overall sensitivity >99%).
Law #3	Rapid PF4-dependent immunoassays have high sensitivity (at least 85–90%) but generally not >99% sensitivity as seen with ELISAs.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; PF4, platelet factor 4; HIT, heparin-induced thrombocytopenia.
[a]Although occasional HIT sera will test negative in a particular ELISA, they will generally test positive in another PF4-dependent ELISA.

immunosorbent assays (ELISAs) and in functional (platelet activation) assays.[4,5,8] Thus, VITT strongly resembles HIT—particularly the severe variant known as "autoimmune HIT" (where antibodies strongly activate platelets even in the absence of heparin).[9] Indeed, it was this close clinical resemblance to HIT that led to the speedy identification of pathogenic anti-PF4 antibodies playing a fundamental pathogenic role in VITT. However, as Sachs et al have shown, VITT antibodies do not precisely mimic HIT antibodies in their reactivity profile.

To perform their studies, Sachs and colleagues identified sera from 12 patients with clinical VITT where two laboratories

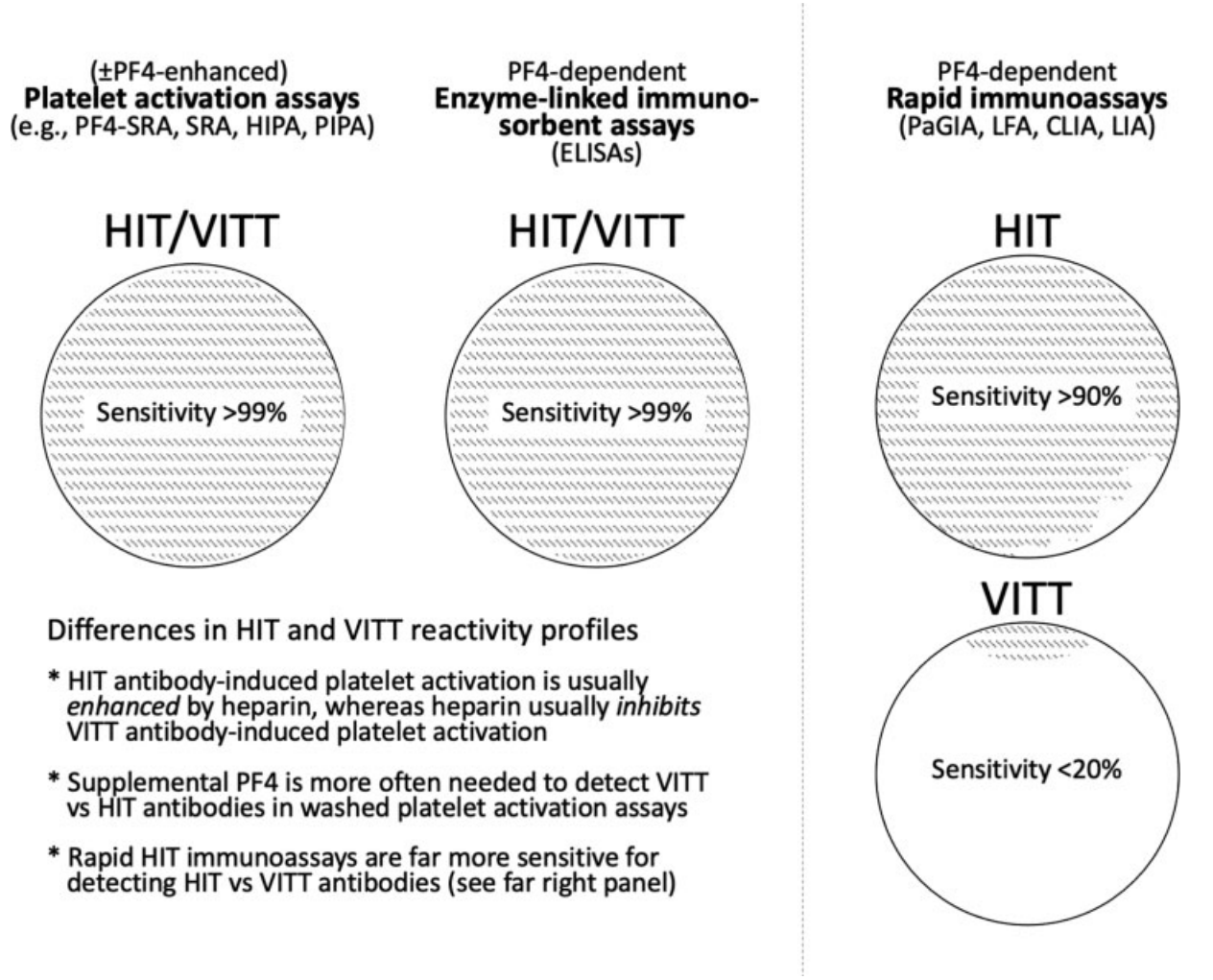

Fig. 1 Serological similarities and differences between HIT and VITT antibodies. The concept is that whereas (PF4-supplemented) washed platelet activation assays and PF4-dependent ELISAs show high sensitivity for both HIT and VITT antibodies, markedly different reaction profiles are seen with rapid HIT assays. PIPA here refers to the classic HIPA test performed with supplemental PF4. CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; HIPA, heparin-induced platelet activation assay; HIT, heparin-induced thrombocytopenia; LFA, lateral flow assay; LIA, latex-enhanced immunoassay (latex immunoturbidimetric assay); PaGIA, particle gel immunoassay; PF4, platelet factor 4; PF4-SRA, PF4-dependent serotonin-release assay; PIPA, PF4-induced platelet activation assay; SRA, serotonin-release assay; VITT, vaccine-induced immune thrombotic thrombocytopenia.

independently demonstrated PF4-dependent platelet-activating properties in patient serum. Thus, they had high confidence that these sera did indeed contain VITT antibodies. They then had four independent laboratories perform various immunoassays—two commercial ELISAs as well as three different rapid assays—to determine their reactivity profiles. The three rapid assays were the particle gel immunoassay (PaGIA; Bio-Rad, Hercules, California, United States), the lateral flow assay (LFA; Milenia Biotec; Giessen, Germany), and the chemiluminescence immunoassay (CLIA; Instrumentation Laboratory, Bedford, Massachusetts, United States). These three immunoassays are all known to have high sensitivity for detecting pathologic HIT antibodies (at least 90%).[12–14,19,20]

What did they find? Both commercial ELISAs showed high sensitivity for VITT antibodies: the IgG-specific PF4/polyvinylsulfonate ELISA (Immucor, Waukesha, United States) tested positive with all 12 sera, with generally strong-positive results (mean optical density: 2.7 units [reference range: <0.40]). A second IgG-specific ELISA (Zymutest HIA, Hyphen Biomed, Neuville-sur-Oise, France), which uses heparin to bind proteins (including PF4) contained within platelet lysate, thereby forming PF4/heparin (and, theoretically, other heparin-dependent antigens), yielded positive results in 11/12 sera tested. (A third ELISA, the IgG-specific PF4/heparin ELISA from Stago—not reported in their paper, but subsequently studied, with data presented on July 17, 2021 at the Scientific and Standardization Committee meeting of the International Society on Thrombosis and Haemostasis—also was positive in 10/11 patient sera tested.) Thus, as is known to be the case with HIT, VITT antibodies are detected with high sensitivity in solid-phase ELISAs that utilize standard plastic wells.

In contrast, all three rapid assays evaluated showed poor sensitivity for detecting VITT antibodies. The PaGIA yielded positive results in only 3/12 (25%) samples tested, the LFA in only 1/12 (8%) samples studied, and most strikingly, the CLIA was positive in none (0%) of 10 samples tested. Given their aforementioned high sensitivity in HIT, this insensitivity of the rapid HIT assays for VITT antibody detection is striking. Indeed, Sachs and colleagues even proposed that an ELISA-positive/CLIA-negative reaction profile, in the appropriate clinical context, could be viewed as laboratory "confirmation" of VITT! Although the authors did not evaluate a fourth rapid assay, the latex immunoturbidimetric assay, others have noted its lack of sensitivity too for detecting VITT antibodies.[5,17,21,22]

Thus, while HIT and VITT share remarkably similar clinical, laboratory, and serological features, they are not identical (►**Fig. 1**). HIT and VITT can be conceptualized as fraternal, not identical, twins; they belong to the same family of platelet-activating anti-PF4 immune responses, but with different target antigen regions on PF4. Indeed, a recent study[23] of PF4 epitope mapping has identified a distinct region recognized by VITT antibodies that corresponds to the heparin-binding site on PF4; in contrast, HIT antibodies recognize separate and distinct sites on PF4, although tellingly autoimmune HIT sera with heparin-independent platelet-activating properties also recognize the VITT epitope site. Presumably, rapid assays for HIT are designed to detect antibodies that recognize heparin-dependent epitopes on PF4, and so they fail to detect VITT antibodies directed at a highly localized epitope on PF4 which supports heparin-independent platelet activation by these highly pathogenic antibodies.

Conflict of Interest

Theodore (Ted) E. Warkentin, MD, has received lecture honoraria from Alexion and Instrumentation Laboratory, and royalties from Informa (Taylor & Francis); has provided consulting services to Aspen Canada, Aspen Global, Bayer, CSL Behring, Ergomed, and Octapharma; has received research funding from Instrumentation Laboratory; and has provided expert witness testimony relating to HIT and non-HIT thrombocytopenic and coagulopathic disorders.

References

1 Krammer F. SARS-CoV-2 vaccines in development. Nature 2020; 586(7830):516–527
2 Voysey M, Clemens SAC, Madhi SA, et al; Oxford COVID Vaccine Trial Group. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet 2021;397(10269):99–111
3 Gerotziafas GT, Catalano M, Theodorou Y, et al; Scientific Reviewer Committee. The COVID-19 pandemic and the need for an integrated and equitable approach: an international expert consensus paper. Thromb Haemost 2021;121(08):992–1007
4 Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. N Engl J Med 2021;384(22):2092–2101
5 Bourguignon A, Arnold DM, Warkentin TE, et al. Adjunct immune globulin for vaccine-induced immune thrombotic thrombocytopenia. N Engl J Med 2021;385(08):720–728
6 Elalamy I, Gerotziafas G, Alamowitch S, et al; Scientific Reviewer Committee. SARS-CoV-2 vaccine and thrombosis: an expert consensus on vaccine-induced immune thrombotic thrombocytopenia. Thromb Haemost 2021;121(08):982–991
7 von Hundelshausen P, Lorenz R, Siess W, Weber C. Vaccine-induced immune thrombotic thrombocytopenia (VITT): targeting pathomechanisms with Bruton tyrosine kinase inhibitors. Thromb Haemost 2021. Doi: 10.1055/a-1481-3039
8 Sachs UJ, Cooper N, Czwalinna A, et al. PF4-dependent immunoassays in patients with vaccine-induced immune thrombotic thrombocytopenia: results of an interlaboratory comparison. Thromb Haemost 2021
9 Greinacher A, Selleng K, Warkentin TE. Autoimmune heparin-induced thrombocytopenia. J Thromb Haemost 2017;15(11): 2099–2114
10 Warkentin TE. Laboratory diagnosis of heparin-induced thrombocytopenia. Int J Lab Hematol 2019;41(Suppl 1):15–25
11 Warkentin TE, Nazy I, Sheppard JI, Smith JW, Kelton JG, Arnold DM. Serotonin-release assay-negative heparin-induced thrombocytopenia. Am J Hematol 2020;95(01):38–47
12 Sun L, Gimotty PA, Lakshmanan S, Cuker A. Diagnostic accuracy of rapid immunoassays for heparin-induced thrombocytopenia. A systematic review and meta-analysis. Thromb Haemost 2016;115 (05):1044–1055
13 Bankova A, Andres Y, Horn MP, Alberio L, Nagler M. Rapid immunoassays for diagnosis of heparin-induced thrombocytopenia: comparison of diagnostic accuracy, reproducibility, and costs in clinical practice. PLoS One 2017;12(06):e0178289

14 Warkentin TE, Sheppard JI, Linkins LA, Arnold DM, Nazy I. Performance characteristics of an automated latex immunoturbidimetric assay [HemosIL® HIT-$Ab_{(PF4-H)}$] for the diagnosis of immune heparin-induced thrombocytopenia. Thromb Res 2017; 153:108–117
15 Warkentin TE, Smythe MA, Ali MA, et al. Serotonin-release assay-positive but platelet factor 4-dependent enzyme-immunoassay negative: HIT or not HIT? Am J Hematol 2021;96(03):320–329
16 Warkentin TE, Greinacher A, Gruel Y, Aster RH, Chong BHscientific and standardization committee of the international society on thrombosis and haemostasis. Laboratory testing for heparin-induced thrombocytopenia: a conceptual framework and implications for diagnosis. J Thromb Haemost 2011;9(12):2498–2500
17 Muir KL, Kallam A, Koepsell SA, Gundabolu K. Thrombotic thrombocytopenia after Ad26.COV2.S vaccination. N Engl J Med 2021; 384(20):1964–1965
18 Pai M, Chan B, Stall NM, et al. Vaccine-induced immune thrombotic thrombocytopenia (VITT) following adenovirus vector COVID-19 vaccination. Science Briefs of the Ontario COVID-19 Science Advisory Table 2021;2(17). Accessed August 29, 2021 at: https://covid19-sciencetable.ca/sciencebrief/vaccine-induced-immune-thrombotic-thrombocytopenia-vitt-following-adenovirus-vector-covid-19-vaccination/
19 Linkins LA, Bates SM, Lee AYY, Heddle NM, Wang G, Warkentin TE. Combination of 4Ts score and PF4/H-PaGIA for diagnosis and management of heparin-induced thrombocytopenia: prospective cohort study. Blood 2015;126(05):597–603
20 Warkentin TE, Sheppard JI, Linkins LA, Arnold DM, Nazy I. High sensitivity and specificity of an automated IgG-specific chemiluminescence immunoassay for diagnosis of HIT. Blood 2018;132 (12):1345–1349
21 Platton S, Bartlett A, MacCallum P, et al. Evaluation of laboratory assays for anti-platelet factor 4 antibodies after ChAdOx1 nCOV-19 vaccination. J Thromb Haemost 2021;19(08):2007–2013
22 Reilly-Stitt C, Kitchen S, Jennings I, et al. Anti-PF4 testing for vaccine-induced immune thrombocytopenia and thrombosis and heparin induced thrombocytopenia: results from a UK National External Quality Assessment Scheme exercise April 2021. J Thromb Haemost 2021;19(09):2263–2267
23 Huynh A, Kelton JG, Arnold DM, Daka M, Nazy I. Antibody epitopes in vaccine-induced immune thrombotic thrombocytopaenia. Nature 2021;596(7873):565–569