CHEST

A Platelet Factor 4-Dependent Platelet Activation Assay Facilitates Early Detection of Pathogenic Heparin-Induced Thrombocytopenia Antibodies

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Heparin-induced thrombocytopenia (HIT) is a dangerous complication of heparin therapy. HIT diagnosis is established by recognizing thrombocytopenia and/or thrombosis in an affected patient and from the results of serological tests such as the platelet factor 4 (PF4)/heparin immunoassay (PF4 ELISA) and serotonin release assay (SRA). Recent studies suggest that HIT antibodies activate platelets by recognizing PF4 in a complex with platelet glycosaminoglycans (and/or polyphosphates) and that an assay based on this principle, the PF4-dependent P-selectin expression assay (PEA), may be even more accurate than the SRA for HIT diagnosis. Here, we demonstrate that the PEA detected pathogenic antibodies before the SRA became positive in two patients with HIT studied serially, in one case even before seropositivity in the PF4 ELISA. In one of the patients treated with plasma exchange, persistent dissociation between the PEA and SRA test results was observed. These results support a role for the PEA in early HIT diagnosis. CHEST 2017; 152(4):e77-e80

KEYWORDS: early diagnosis of HIT; heparin; HIT; plasma exchange, plasmapheresis; thrombocytopenia

HIT is characterized by thrombocytopenia, usually beginning 5 to 10 days after starting heparin therapy and poses a high risk of thromboembolic complications.[1,2] Patients with HIT produce antibodies that recognize the chemokine platelet factor 4 (PF4) in complex with heparin, or a similar negatively charged polyanion, and can be rapidly detected in immunoassays that use PF4: heparin (or polyvinylsulfonate) complexes as targets (PF4 enzyme-linked immunosorbent assay [ELISA]).[3-6] Recent studies demonstrate that "pathogenic" (platelet-activating) HIT antibodies

ABBREVIATIONS: ELISA = enzyme-linked immunosorbent assay; HIT = heparin-induced thrombocytopenia; OD = optical density; PEA = PF4-dependent P-selectin expression assay; PF4 = platelet factor 4; SRA = serotonin release assay; TPE = therapeutic plasma exchange; UFH = unfractionated heparin

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Findings contained in this manuscript were presented in part at the American Society of Hematology Annual Meeting and Exposition, December 3-6, 2016, San Diego, CA.

FUNDING/SUPPORT: This study was supported in part by funds from the American Heart Association (A. P.), CTSI of Southeastern Wisconsin (A. P), and the National Institutes of Health [Grant HL-13629] (R. H. A).

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DOI: http://dx.doi.org/10.1016/j.chest.2017.06.001

preferentially recognize PF4 in a complex with endogenous platelet surface glycosaminoglycans[7] and/or polyphosphates.[8] On the basis of these findings, we described a novel diagnostic test, the PF4-dependent P-selectin expression assay (PEA).[9,10] In the PEA, platelets pretreated with PF4 are incubated with samples from patients suspected of having HIT and platelet surface p-selectin expression is measured as a marker of platelet activation. Preliminary findings suggest that the PEA may be even more accurate than the SRA for diagnosis of HIT.[10] In the aforementioned study, stringent criteria were used to define a patient as having HIT (high 4Ts score (thrombocytopenia, timing, thrombosis, other) and PF4 ELISA optical density [OD] ≥ 1.0, or intermediate 4Ts score and PF4 ELISA OD ≥ 2.0), leaving little doubt that classification of patients as "HIT-positive" was accurate.

Notably, HIT-positive patients who were PEA-positive but SRA-negative in that study tended to have lower levels of P-selectin expression than those who were positive in both assays. Mean (1 SD) P-selectin expression was 70% (33%) and 97% (10%) in SRA-/PEA+ and SRA+/PEA+ HIT-positive patients, respectively ($P = .05$ by the Mann-Whitney test; data not shown), and patient antibodies studied produced positive results in the PEA at higher dilutions than in the SRA,[10] suggesting that the former assay may be inherently more sensitive for the detection of pathogenic platelet-activating antibodies. This led us to question whether the PEA may be particularly valuable for identifying patients with HIT early in their clinical course, before the SRA becomes positive. To investigate this possibility, we studied serial blood samples collected from two local patients who developed thrombotic HIT.

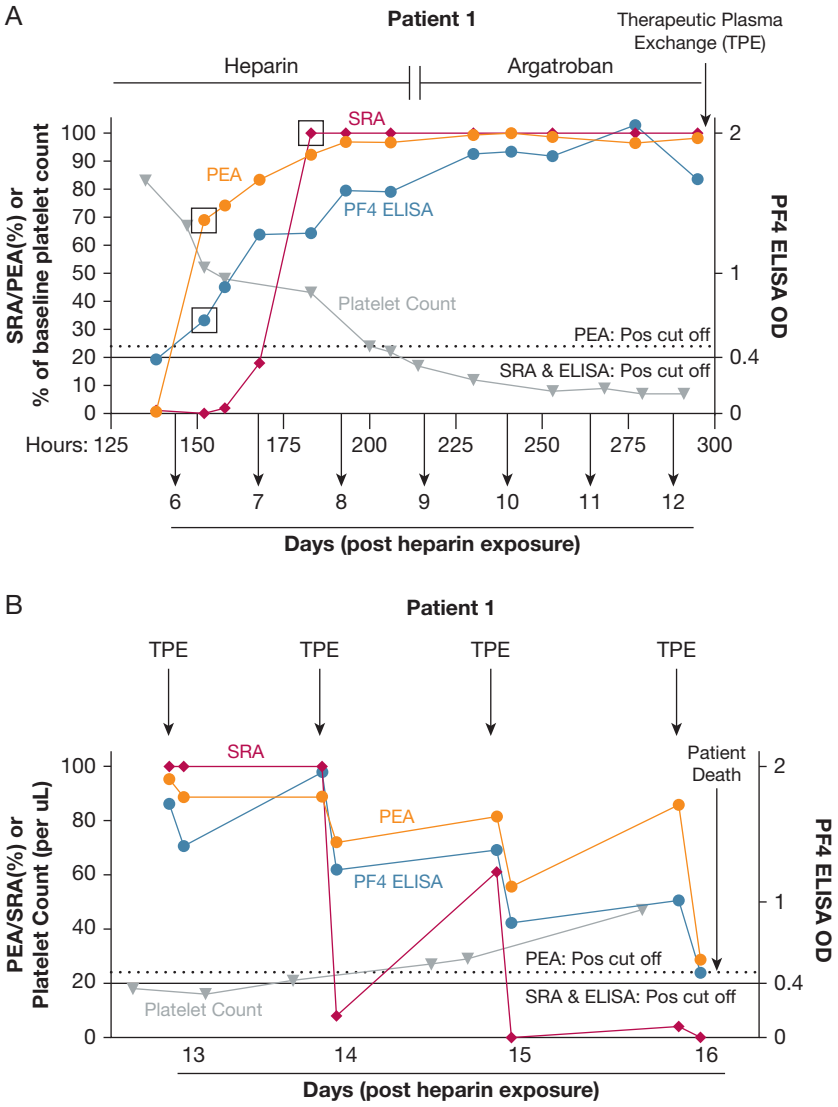

Figure 1 – *PEA detects pathogenic HIT antibodies earlier than the SRA, and results dissociate during TPE treatment. HIT serologic results and platelet counts during the early part of patient 1's hospitalization (A) and during TPE treatment (B) are shown. Time (hours, days after heparin exposure) is depicted on the abscissa; platelet count (percent of baseline [A] or absolute count [B], inverted gray triangle), PEA (% of maximal activation, orange circle), and SRA (% serotonin release, red diamonds) are depicted on the left ordinate; PF4 ELISA (OD, blue circle) is depicted on the right ordinate. The first positive result in each assay is shown in black squares in (A). Anticoagulation used is indicated on top of the horizontal bars shown in the figure. ELISA = enzyme-linked immunosorbent assay; HIT = heparin-induced thrombocytopenia; OD = optical density; PEA = PF4-dependent P-selectin expression assay; Pos = positive; SRA = serotonin release assay; TPE = therapeutic plasma exchange.*