BRIEF REPORT

Plasma exchange to remove HIT antibodies: dissociation between enzyme-immunoassay and platelet activation test reactivities

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Key Points

* Repeated plasma exchange removes sufficient HIT-IgG to achieve negative serotonin-release assay (SRA) despite ongoing strong-positive EIA

* Serially-diluted HIT sera tested in both SRA and EIA show that SRA negativity can be achieved with minimal decrease in EIA reactivity

Abstract

Repeated therapeutic plasma exchange (TPE) has been advocated to remove heparin-induced thrombocytopenia (HIT) IgG antibodies prior to cardiac/vascular surgery in patients who have serologically-confirmed acute or subacute HIT; for this situation, a negative platelet activation assay (e.g., platelet serotonin-release assay [SRA]) has been recommended as the target serological endpoint to permit safe surgery. We compared reactivities in the SRA and an anti-PF4/heparin IgG-specific enzyme-immunoassay (EIA), testing serial serum samples in a patient with recent (subacute) HIT who underwent serial TPE pre-cardiac surgery, as well as for 15 other serially-diluted HIT sera. We observed that post-TPE/diluted HIT sera—when first testing SRA-negative—continue to test strongly positive by EIA-IgG. This dissociation between the platelet activation assay and a PF4-dependent immunoassay for HIT antibodies indicates that patients with subacute HIT undergoing repeated TPE prior to heparin re-exposure should be tested by serial platelet activation assays even when their EIAs remain strongly positive.
Introduction

Heparin-induced thrombocytopenia (HIT) is an adverse drug reaction caused by platelet-activating IgG antibodies that recognize multimolecular PF4/heparin complexes.\(^1\) Therapeutic plasma exchange (TPE) has been recommended as a way to remove HIT antibodies quickly, as might be required to permit administration of heparin for urgent cardiac surgery.\(^2\) However, HIT antibodies (IgG) are not as effectively removed by TPE as IgM.\(^3\) Using serial pre-/post-TPE sera obtained from a patient with subacute HIT (i.e., recent HIT with platelet count recovery but persisting HIT antibodies\(^4\)) who underwent repeated TPE pre-cardiac surgery, we compared antibody reactivity by the \(^{14}\)C-serotonin-release assay (SRA)\(^5\)—a functional (platelet activation) test for HIT antibodies—versus an IgG-specific anti-PF4/heparin enzyme-immunoassay (EIA).\(^6\) We found that while a negative SRA could be achieved quickly post-TPE, corresponding EIA reactivities remained strongly positive. This observation proved to be a general feature of HIT antibody reactivity, as 15 other acute HIT sera showed rapid diminution of SRA reactivity upon serial dilutions, but with major reductions in EIA reactivity requiring much greater sample dilutions.

Case Report

A 76-year-old female with renal carcinoma invading the inferior vena cava (IVC)/right atrium developed HIT without thrombosis (4Ts score,\(^7\) 6 points), with strong-positive SRA and EIA, and with uneventful platelet count recovery during fondaparinux 7.5mg once-daily subcutaneously. Cardiac surgery was scheduled three months post-HIT for coronary artery-bypass grafting (CABG) and removal of IVC/intra-cardiac tumor thrombus. However, as her SRA and EIA remained strongly positive (99% serotonin-release at 0.3 IU/mL UFH; IgG-
specific EIA, 2.58 OD units), TPE was performed on 4 consecutive days (3-liter exchanges with 5% albumin replacement), yielding a persistently negative SRA post-second TPE, despite the EIA-IgG remaining strongly positive (1.85 OD units post-second apheresis versus 2.30 OD units on serum obtained immediately before). Two days post-4th TPE, she received UFH (30,000 units intraoperatively) for cardiopulmonary-bypass, undergoing: quadruple CABG, IVC/intra-cardiac tumor thrombectomy, and radical nephrectomy. Unfortunately, tumor removal was incomplete, and intraoperative splenic injury resulted in major blood loss, requiring splenectomy in massive transfusion setting (16 red cell units, 10 frozen plasma, 10 cryoprecipitate, 3 units platelets). The preoperative platelet count was 141×10^9/L, with an intraoperative nadir of 48×10^9/L. Postoperatively, daily fondaparinux 2.5mg and aspirin 81mg were given. Complications included complex-partial seizures (CT brain scan was negative for thrombotic or hemorrhagic stroke), and ileus/aspiration pneumonitis on postoperative day 6. She was discharged on postoperative day 34.

**Methods**

Testing for HIT antibodies was performed using the SRA and an in-house anti-PF4/heparin IgG-specific EIA (McMaster Platelet Immunology Laboratory), as described.\(^5,6\) Serial serum samples were drawn for HIT antibody testing immediately pre- and post- each TPE session. SRA-positive control sera obtained from 15 different patients previously diagnosed with HIT (each serum yielding >50% serotonin-release at 0.3 U/mL UFH) were tested in four-fold serial dilutions (1/5 to 1/5120). The SRAs were performed over two days, and the EIA-IgG in one day using 3 plates, with internal HIT-positive and -negative control sera producing expected results. For comparison, sera are usually diluted 1/5 (final) in our SRA and 1/50 (final) in our EIA-IgG. Statistical analysis was performed by t-test, paired 2-sample for means (Microsoft Excel 97-
2003). The patient provided written consent to report her case, and permission was obtained from the Hamilton Integrated Research Ethics Board to perform the studies using HIT-positive sera.

Results and Discussion

Fig. 1 shows the percent serotonin-release (at 0.3 IU/mL UFH) induced by the patient’s sera obtained at different time points pre-cardiac surgery (cardiac surgery, day 0; HIT diagnosis, day –87), as well as immediately pre/post each of four TPE sessions (days –5 through –2). We found that the EIA-IgG reactivity—expressed in OD units—abruptly fell after each TPE session (by a mean of 0.94 units; \( P < .001 \)), but by the following day, EIA-IgG reactivity had risen significantly once again (by mean of 0.69 units; \( P = .0025 \)). These observations are consistent with the partial removal of IgG antibodies by TPE, with subsequent redistribution of IgG from the extravascular/interstitial spaces into the intravascular compartment. Nevertheless, TPE was sufficient in our patient to remove IgG antibodies to a meaningful extent, as indicated by the negative SRA.

Fig. 2A shows SRA and EIA-IgG results for 15 serially diluted HIT sera. The data show that samples testing SRA-negative can still have strongly positive EIA-IgG reactivities. For example, at a 1/320 sample dilution, only 2/15 HIT sera tested SRA-positive, whereas all 15 corresponding EIAs tested positive (and mostly strongly positive), with a median OD (interquartile range) of 2.55 (1.77, 2.65). Further, when we compared the corresponding EIAs for the most dilute (but still SRA-positive) sera versus the corresponding four-fold more dilute (but now SRA-negative) sera, the mean OD values were similar (2.75 vs 2.60, respectively; \( P = .157 \)) (Fig. 2B). Interestingly, the maximum OD reactivity (at any dilution) was similar to the OD reactivity of the most diluted, but still SRA-positive sample.
Our observations have implications for managing patients with (sub)acute HIT using TPE prior to planned heparin re-exposure. Previous American College of Chest Physicians (ACCP) consensus conference guidelines on HIT management published in 2008 recommend that patients with previous HIT can receive UFH provided that heparin-dependent, platelet-activating antibodies are no longer detectable by (washed) platelet activation assay, even if the EIA remains positive (a recommendation based on favorable outcomes among EIA-positive/(washed platelet) activation assay-negative patients who were re-exposed to heparin for urgent cardiac surgery), and we followed this approach to manage our patient. (Although the 2012 ACCP guidelines also recommend heparin use with “heparin antibodies… absent” the applicable assays are not specified.) Although SRA-negative status usually occurs within a few weeks post-HIT, when surgery is required urgently yet SRA-positive status persists, TPE becomes an important option. Our studies of serial pre-/post-TPE serum (Fig. 1), as well as corroborative studies using serially-diluted HIT sera (Figs. 2A/B), demonstrate that EIAs usually remain strongly positive, even when a patient is otherwise at acceptable risk for heparin re-exposure (per negative washed platelet activation assay). Although our findings might appear surprising—given the known predictivity for a positive SRA with increasing strength of EIA reactivity—they point to the critical dependence of HIT serum-induced platelet activation to a crucial threshold level of platelet-activating antibodies (a phenomenon which itself helps to explain the EIA-SRA interrelationship), and how quickly platelet-activating properties can be lost in an individual patient with declining antibody levels, either occurring naturally over time or by serum dilution performed experimentally or via TPE. Our observations also help to explain the serological profiles of two previously-reported patients (patient 1/Fig. 1A in reference 9; patient 20/Fig. 3 in reference 10) with recent HIT undergoing serosurveillance to assess readiness for...
heparin re-exposure: consistent with our findings, these patients continued to have strong positive anti-PF4/heparin antibodies by EIA (with values similar to the highest ones obtained at HIT diagnosis) even when their sera had become negative by washed platelet activation assay.9,10

In summary, our observations indicate that diluted HIT serum—whether achieved clinically through serial TPE, or experimentally through serial sample dilutions—demonstrates loss of SRA reactivity well before a decrease in EIA reactivity. These findings point to the importance of performing platelet activation assays in parallel with the EIA, when testing pre- and post-TPE samples, when judging patient suitability for a planned heparin re-exposure.

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Authorship

Contribution: T.E.W. designed and supervised the experiments, analyzed the data, and interpreted the results; J.I.S. designed and performed the experiments, and interpreted the results; T.E.W., F.V.C., A.K., and A.G. helped to manage the patient using some of the data obtained in this report; M.A.C. provided the initial concept for the study; all authors reviewed and approved the final version of the manuscript.

Conflict-of-interest disclosure: T.E.W. has received lecture honoraria from Pfizer Canada and Instrumentation Laboratory, has provided consulting services to, and/or has received research funding from W.L. Gore, and has provided expert witness testimony relating to HIT.

M.A.C. has sat on advisory boards for Janssen, Leo Pharma, Portola, and AKP America. He holds a Career Investigator award from the Heart and Stroke Foundation of Ontario, and the Leo Pharma Chair in Thromboembolism Research at McMaster University. His institution has
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**Figure legend.**

**Figure 1.** Serial SRA and IgG-specific anti-PF4/heparin EIA test results in relation to 4 therapeutic plasma exchange (TPE) sessions performed on 4 consecutive days (last TPE performed 2 days before cardiac surgery utilizing heparin). Note: heparin rechallenge during cardiac surgery did not result in increased levels of anti-PF4/heparin antibodies during the postoperative period (testing until postoperative day 7) (not shown). Although at the time of initial HIT diagnosis the patient also had detectable anti-PF4/heparin antibodies of IgA class (EIA-IgA = 1.20 OD units; normal, <0.45 units) and IgM class (EIA-IgM = 0.46 units; normal, <0.45 units), both the IgA and IgM EIAs were negative 3 months later pre-TPE (0.30 and 0.34 OD units, respectively).
Abbr.: EIA-IgG, enzyme-immunoassay (IgG-specific); OD, optical density; SRA, serotonin-release assay.

**Figure 2.** Comparative studies in the SRA and IgG-specific anti-PF4/heparin EIA using serially-diluted HIT sera. A. Known HIT sera (n = 15) diluted from 1/5 to 1/5120 were tested in the SRA and EIA. (The 1/5 dilution in the SRA—20 μL patient serum to 80 μL washed platelets with heparin added—represents the standard conditions in the SRA.) Typical assay cutoffs are shown with dashed lines. Horizontal lines indicate median values. Compared with standard assay conditions, serial sample dilution generally results in a negative SRA well before a negative EIA result is attained. B. Individual IgG-specific EIA ODs are shown for the same 15 HIT sera, in three groupings: SRA+ showing maximum OD of any SRA+ dilution (leftmost data points); SRA+ showing OD of the most dilute yet still SRA+ serum dilution (middle data points); and SRA– showing OD of the first SRA– dilution (rightmost data points). There is a four-fold serum dilution between the two rightmost data sets. No significant differences were observed between the SRA+ and SRA– data sets. Abbr.: EIA-IgG, enzyme-immunoassay (IgG-specific); OD, optical density; PF4, platelet factor 4; SRA, serotonin-release assay; SRA–, SRA-negative; SRA+, SRA-positive.
Days Before Cardiac Surgery

HIT diagnosis

Therapeutic plasma exchange (TPE) session
#1 #2 #3 #4

Cardiac surgery

Serotonin-release, percent (        )

Anti-PF4/heparin IgG, OD units (        )

SRA cutoff

EIA-IgG cutoff
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