

Editorial Focus

Confirmatory procedure and other maneuvers to assess pathogenicity of platelet factor 4 (PF4)-dependent antibodies – distinguishing "signal" from "noise"

Theodore E. Warkentin

Department of Pathology and Molecular Medicine, and Department of Medicine,

Michael G. DeGroot School of Medicine, McMaster University, Hamilton, Ontario, Canada

Heparin-induced thrombocytopenia (HIT) is caused by antibodies that recognize complexes of platelet factor 4 (PF4) bound to heparin (1). Not all such antibodies, however, cause clinically-manifest HIT. Serosurveillance studies (2, 3) show that only a small subset (2% to 15%) of patients who develop PF4/heparin-reactive antibodies evince platelet count declines and/or thrombotic events indicating HIT. One serological feature pointing to a higher risk of HIT is the ability of patient serum to activate platelets strongly *in vitro* (4), but this requires use of technically-challenging assays, such as the platelet serotonin-release assay (SRA). The most common assays for HIT are PF4-dependent enzyme-immunoassays (EIA), which are standardized and commercially-available. Are there any features of EIA reactivity that predict for a greater risk of HIT?

The manufacturer (GTI Inc., Waukesha, WI, USA) of a widely-used EIA, the *PF4 Enhanced*[®], recommends use of a "Procedure for Confirmation of Heparin-associated Antibodies" (5). According to GTI, inhibition of a positive reaction by 50% or more in the presence of excess heparin (100 U/ml) is considered confirmatory for heparin-dependent antibodies characteristic of immune HIT (5). Even if useful, however, the application of this procedure has drawbacks. As noted elsewhere (6), the routine inclusion of this test maneuver will either double test costs (by requiring that each assay be performed both in the absence and presence of high heparin concentrations) or will delay the reporting of a positive test result (if an algorithm is used in which a tentative positive result is subsequently confirmed by repeating the test in the absence and presence of high heparin). But there is an even bigger issue: does this procedure really enhance diagnostic specificity for HIT without compromising test sensitivity?

This is not an easy question to answer. After all, most patients who form heparin-induced antibodies do *not* develop clinical

HIT (2–4). So, two interrelated questions could be: (i) does the confirmatory procedure distinguish anti-PF4/heparin antibodies from false-positive EIA reactions? (ii) does the confirmatory procedure distinguish truly pathogenic HIT antibodies ("signal") from non-pathogenic antibodies ("noise")?

In this issue of *Thrombosis and Haemostasis*, Whitlatch, Perry, and Ortel (7) from Duke University School of Medicine have systematically evaluated the EIA confirmatory procedure. Their assessment gave mixed results. In support of this procedure, they observed a correlation between a positive confirmatory step and a greater likelihood of HIT, as judged by a clinical scoring system for HIT. However, the authors also identified three patients with strong clinical features of HIT and a strong-positive EIA test result (as judged by optical density [od] measurements), and yet these patients' blood did not yield a positive confirmatory test result. These observations suggest that while the confirmatory procedure could eliminate some background assay "noise", it might also occasionally fail to confirm a true HIT antibody "signal", an outcome that would obviate a major strength of the EIA, namely its high diagnostic sensitivity.

Certain strongly reactive HIT sera could be at special risk of failing the confirmatory procedure, perhaps those with high-titer antibodies. Such a phenomenon has been reported with the SRA. Moore and colleagues (8) found that certain putative HIT sera demonstrating otherwise typical heparin-dependent platelet activation were not inhibited by an Fc receptor-blocking monoclonal antibody used by some laboratories as a confirmatory procedure (HIT antibodies activate platelets through platelet Fcγ receptors). However, Moore et al. showed that these non-Fc receptor-inhibitable sera not only reacted strongly in the EIA, they also showed a more typical platelet activation profile – including inhibition by Fc receptor-blocking monoclonal antibody – if the

Correspondence to:

Dr. T. Warkentin
Hamilton Regional Laboratory Medicine Program
Room 1–180A, Hamilton Health Sciences (Hamilton General Site)
237 Barton St. E., Hamilton, Ontario, L8L 2X2 Canada
Tel.: +1 905 527 0271 (ext. 46139), Fax: +1 905 577 1421
E-mail: twarken@mcmaster.ca

Financial support:

Some of the studies described in this editorial were supported by the Heart and Stroke Foundation of Ontario (T5207, T6157 [TEVJ]).

Received August 19, 2008
Accepted August 19, 2008

Prepublished online September 5, 2008
doi:10.1160/TH08-08-0533

Thromb Haemost 2008; 100: 523-524

serum was tested at greater dilutions in the SRA. Together with the studies of Whitlatch et al. (7), it seems that some highly-reactive and/or high-titer HIT antibodies may fail a confirmatory procedure, whether that maneuver is high heparin neutralization (EIA) or Fc receptor blockade (SRA).

So, how does a laboratory distinguish anti-PF4/heparin antibodies from mimicking reaction profiles, and – even more to the point – distinguish pathogenic HIT antibodies from non-pathogenic ones? The study by Whitlatch et al. also found that greater antibody reactivity – as judged by higher OD levels – was predic-

tive for a greater likelihood of HIT being present. This finding is consistent with other studies (9, 10), and suggests that higher reactivity in a PF4-dependent EIA is likely to be a far better predictor of pathogenicity than the confirmatory procedure. Perhaps, by combining an assessment of OD levels together with determination of the effects of sample dilution, the incorporation of a confirmatory procedure may still improve predictivity of the EIA for pathogenic antibodies without an offsetting loss of diagnostic sensitivity.

References

1. Amiral J, Bridey F, Dreyfus M, et al. Platelet factor 4 complexed to heparin is the target for antibodies generated in heparin-induced thrombocytopenia. *Thromb Haemost* 1992; 68: 95–96.
2. Warkentin TE, Sheppard JI, Horsewood P, et al. Impact of the patient population on the risk for heparin-induced thrombocytopenia. *Blood* 2000; 96: 1703–1708.
3. Warkentin TE, Sheppard JI, Moore JC, et al. Laboratory testing for the antibodies that cause heparin-induced thrombocytopenia: how much class do we need? *J Lab Clin Med* 2005; 146: 341–346.
4. Lo GK, Sigouin CS, Warkentin TE. What is the potential for overdiagnosis of heparin-induced thrombocytopenia? *Am J Hematol* 2007; 82: 1037–1043.
5. Package insert, 'PF4 Enhanced®', GTI, Waukesha, WI, January 13, 2005.
6. Warkentin TE, Sheppard JI. No significant improvement in diagnostic specificity of an anti-PF4/polyanion immunoassay with use of high heparin confirmatory procedure. *J Thromb Haemost* 2006; 4: 281–282.
7. Whitlatch NL, Perry SL, Ortel TL. Anti-heparin/platelet factor 4 antibody optical density values and the confirmatory procedure in the diagnosis of heparin induced thrombocytopenia. *Thromb Haemost* 2008; 100: 678–684.
8. Moore JC, Arnold DM, Warkentin TE, et al. An algorithm for resolving 'indeterminate' test results in the platelet serotonin release assay for investigation of heparin-induced thrombocytopenia. *J Thromb Haemost* 2008; 6: 1595–1597.
9. Zwicker JI, Uhl L, Huang WY, et al. Thrombosis and ELISA optical density values in hospitalized patients with heparin-induced thrombocytopenia. *J Thromb Haemost* 2004; 2: 2133–2137.
10. Warkentin TE, Sheppard JI, Moore JC, et al. Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays. *J Thromb Haemost* 2008; 6: 1304–1312.



Thrombosis

ESC Working Group

EUROPEAN
SOCIETY OF
CARDIOLOGY®

www.escardio.org/thrombosis