ASH evidence-based guidelines: is the IgG-specific anti-PF4/heparin ELISA superior to the polyspecific ELISA in the laboratory diagnosis of HIT?

Adam Cuker[1] and Thomas L. Ortel[2]

[1]Department of Medicine, University of Pennsylvania, Philadelphia, PA; [2]Departments of Medicine and Pathology, Duke University Medical Center, Durham, NC

You are asked to consult on a 76-year-old man admitted to the hospital with pneumonia and thrombocytopenia. Ten days before the current admission, he had undergone surgery to repair a small bowel obstruction. A preoperative platelet count had been normal. Following surgery, he received subcutaneous unfractionated heparin thromboprophylaxis until his discharge on post-operative day 5. In your differential diagnosis for the patient's thrombocytopenia, you consider heparin-induced thrombocytopenia (HIT) and wish to order laboratory testing. In addition to a polyspecific anti-PF4/heparin ELISA for the diagnosis of HIT, your laboratory has recently begun to offer an IgG-specific ELISA. You wonder which of these assays performs better in the diagnosis of HIT.

HIT is mediated by antibodies that target multimolecular complexes of platelet factor 4 (PF4) and heparin.[1] Conventional ELISAs for the diagnosis of HIT detect anti-PF4/heparin IgG, IgA, and IgM. These polyspecific ELISAs are highly sensitive, but suffer from limited specificity and a high false-positive rate.[2] A growing body of evidence suggests that antibodies of the IgG class have the primary, if not sole, potential to cause HIT.[3-5] Recently, ELISAs that detect only anti-PF4/heparin IgG have become commercially available. It is hoped that these IgG-specific ELISAs will offer equally high sensitivity and superior specificity as compared with the polyspecific assay. To evaluate the operating characteristics of the polyspecific and IgG-specific ELISAs, we performed a comprehensive literature review of all studies in which these two assays were compared.

A literature search of the PubMed database was performed by combining the MeSH term "heparin," subheadings "adverse effects" or "toxicity," with the keyword "heparin-induced thrombocytopenia" (no restrictions, 6720 hits); the MeSH term "immunoassay" with the keyword "immunoassay" (no restrictions, 351,102 hits); and the MeSH term "research" with the keywords "research" and "study" (no restrictions, 8,110,184 hits) between 1950 and 21 May 2009. This strategy yielded 110 citations. Excluded were 91 studies in which one or both of the assays of interest was not performed, 4 case reports, 6 reviews, and 1 editorial. Of the remaining 8 references, 4 did not include the data necessary for determination of test operating characteristics, leaving 4 eligible studies. A review of these studies' bibliographies identified a fifth eligible study (n = 3366). In a pooled analysis of these studies (**Table 1**), the IgG-specific ELISA was associated with greater specificity (93.5% vs 89.4%), but lower sensitivity (95.8% vs 98.1%) than the polyspecific ELISA. **Table 2** shows the operating characteristics of the two assays.

The validity of these results is limited by differences in the study populations, gold standard definitions of HIT, and immunoassays utilized in the 5 studies. Nonetheless, we conclude that the IgG-specific ELISA yields fewer false-positive results than the polyspecific ELISA, but at the

Table 2. Operating characteristics of the polyspecific ELISA and IgG-specific ELISA.

	Sensitivity, %	Specificity, %	PPV, %	NPV, %	LR^+	LR^-
Polyspecific ELISA	98.1	89.4	38.7	99.9	9.29	0.021
IgG-specific ELISA	95.8	93.5	49.6	99.7	14.64	0.045

PPV indicates positive predictive value; NPV, negative predictive value; LR^+, positive likelihood ratio; and LR^-, negative likelihood ratio.

Table 1. Studies comparing the polyspecific ELISA and IgG-specific ELISA in the diagnosis of heparin-induced thrombocytopenia (HIT).

			Polyspecific ELISA					IgG-specific ELISA				
Ref	Study population (n)	Definition of HIT	Assay	TP	TN	FP	FN	Assay	TP	TN	FP	FN
6	Patients with suspected HIT (500)	Positive HIPA and intermediate to high clinical pre-test probability*	GTI†	35	376	89	0	In-house	35	414	51	0
7	Patients with suspected HIT (100)	Positive SRA and intermediate to high clinical pre-test probability*	GTI†	16	68	16	0	In-house	16	69	15	0
8	Patients with suspected HIT (1582)‡	Positive HIPA	In-house	95	1386	100	1	In-house	92	1412§	76	2§
9	Patients with suspected HIT (736)‡	Positive HIPA	In-house	50	632	51	3	In-house	46	655§	28	7§
10	Patients receiving thrombo-prophylaxis after total hip arthroplasty (448)	Clinical¶	GTI†	14	357	77	0	In-house	14	393	36	0
	Pooled total 3366			210	2819	333	4		203	2943	206	9

*Clinical probability estimated using the 4T's scoring system.
†Genetics Testing Institute (Waukesha, WI, USA)
‡Patients with an indeterminate HIPA result in the original study were excluded from the current analysis
§Patients with a negative polyspecific ELISA were not tested with the IgG-specific ELISA and are assumed to be negative with respect to this assay for the current analysis
¶Fall of ≥ 50% in platelet count beginning on day ≥ 5 of heparin therapy without other apparent cause and platelet count recovery upon cessation of heparin
TP indicates true positive; TN, true negative; FP, false positive; FN, false negative; HIPA, heparin-induced platelet activation assay; and SRA, carbon-14 labeled serotonin release assay.

possible expense of missing a small proportion of patients with true HIT who are captured by the polyspecific assay. Clinical economic analyses that define the costs of false-positive and false-negative results are required to determine whether this is an acceptable trade-off. Until such analyses are available, we recommend use of the more sensitive polyspecific ELISA as a screening test for HIT (Grade 2C).

Disclosures

Conflict-of-interest disclosure: The authors declare no competing financial interests.
Off-label drug use: None disclosed.

Correspondence

Adam Cuker, MD, Penn Comprehensive Hemophilia and Thrombosis Program, Medical Arts Bldg, Ste 106, 51 N 39th St, Philadelphia, PA 19104; e-mail: adam.cuker@uphs.upenn.edu

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. Arepally GM, Ortel TL. Clinical practice: heparin-induced thrombocytopenia. N Engl J Med. 2006:355;809-817.
3. Suh JS, Malik MI, Aster RH, Visentin GP. Characterization of the humoral immune response in heparin-induced thrombocytopenia. Am J Hematol. 1997:54;196-201.
4. Vun CM, Evans S, Chesterman CN. Anti-PF4-heparin immunoglobulin G is the major class of heparin-induced thrombocytopenia antibody: findings of an enzyme-linked immunofiltration assay using membrane-bound hPF4-heparin. Br J Haematol. 2001:112;69-75.
5. Lindhoff-Last E, Gerdsen F, Ackermann H, Bauersachs R. Determination of heparin-platelet factor 4-IgG antibodies improves diagnosis of heparin-induced thrombocytopenia. Br J Haematol. 2001:113;886-890.
6. Bakchoul T, Giptner A, Najaoui A, Bein G, Santoso S, Sachs UJH. Prospective evaluation of PF4/heparin immunoassays for the diagnosis of heparin-induced thrombocytopenia. J Thromb Haemost. Prepublished on April 30, 2009, as DOI 10.1111/j.
7. Lo GK, Sigouin CS, Warkentin TE. What is the potential for overdiagnosis of heparin-induced thrombocytopenia? Am J Hematol. 2007;82:1037-1043.
8. Greinacher A, Juhl D, Strobel U, et al. Heparin-induced thrombocytopenia: a prospective study on the incidence, platelet-activating capacity, and clinical significance of antiplatelet factor 4/heparin antibodies of the IgG, IgM, and IgA classes. J Thromb Haemost. 2007;5:1666-1673.
9. Juhl D, Eichler P, Lubenow N, Strobel U, Wessel A,

Greinacher A. Incidence and clinical significance of anti-PF4/heparin antibodies of the IgG, IgM, and IgA class in 755 consecutive patient samples referred for diagnostic testing for heparin-induced thrombocytopenia. Eur J Haematol. 2006;76:420-426.

10. Warkentin TE, Sheppard JI, Moore JC, Moore KM, Sigouin CS, Kelton JG. Laboratory testing for the antibodies that cause heparin-induced thrombocytopenia: How much class do we need? J Lab Clin Med. 2005;46:341-346.