

PF4 ELISA Results in the Clinical Diagnosis of Heparin-Induced Thrombocytopenia (HIT)

Background

Heparin-induced thrombocytopenia (HIT), without or with thrombosis, is an important cause of morbidity and mortality in patients treated with this otherwise extremely useful anticoagulant.^{1,2} Work done in several laboratories³⁻⁶ has shown that patients with HIT almost invariably have antibodies specific for a platelet derived CXC chemokine, platelet factor 4 (PF4, CXCL4), modified by an external agent, heparin. In the past two decades, much has been learned about the pathogenesis of HIT. However, knowledge of the molecular nature of the immune-response is far from complete and it is unknown why a high percentage of some patient populations treated with heparin form antibodies, yet only a minority experience thrombocytopenia and/or thrombosis.

The immune response in HIT has several unusual features:

1. Thrombocytopenia, usually characterized by a transient fall in platelet count 5-10 days (typical onset) after initiation of treatment or, more rarely, fall in platelet counts beginning soon after heparin is started (rapid onset) in patients previously and recently (generally within the past 2-3 weeks) exposed to heparin;⁷
2. Patients with HIT almost invariably have antibodies specific for complexes consisting of two normal body constituents: heparin and PF4;³⁻⁶ in addition a significant minority of patients have antibodies that recognize PF4 alone in the absence of heparin;^{3,5,8}
3. Platelet activation by anti-PF4:heparin immune complexes (IC), engaging with the Fc gamma RIIA receptor on platelets, is integral to the pathogenesis of HIT;^{3,9}
4. Antibodies appear to recognize conformational determinants on PF4 created when the PF4 binds to heparin;¹⁰⁻¹²
5. The humoral immune response consists of a primary IgM reaction usually followed by secondary formation of IgG and/or IgA antibodies suggesting involvement of helper T cells.^{13,14}

Clinical Presentation

As noted, heparin-induced thrombocytopenia is characterized by an unexpected fall in the platelet count occurring five days or more after initiation of treatment with unfractionated heparin.⁷ However, in patients with previous heparin exposure, HIT antibodies may be present initially or may increase rapidly in titer, often within 48 hours of heparin administration. This may lead to an acute drop in platelets soon after heparin is started.^{7,15} It has been claimed that, rarely, HIT antibodies may be present in patients never before exposed to heparin and that these patients are also at risk for “acute HIT”.¹⁶ The thrombocytopenia, associated with HIT itself, is rarely severe enough to provoke bleeding. However, a subset of patients develop arterial and/or venous thrombosis and thromboembolism which can be debilitating or fatal.²

HIT usually occurs in patients given standard doses of unfractionated heparin, especially of bovine origin,^{17,18} but has been reported in patients treated with low dose, subcutaneous heparin¹⁹ and in patients exposed to heparin “flushes” used to maintain the patency of intravenous lines.²⁰ Even minute quantities of heparin released from heparin-bonded catheters appear to be capable of causing the disorder in previously sensitized patients.²¹ Recently, several reports of HIT apparently induced by the pentasaccharide fondaparinux (Arixtra) have appeared.^{22,23}

Functional Tests

For many years, it was known that serum from patients with HIT contains immunoglobulins (Ig) that activate platelets in the presence of pharmacologic doses of heparin (0.1-1 units/ml).^{1,24,25} However, attempts to demonstrate heparin-dependent binding of these Ig to normal platelets were generally unsuccessful. Because the heparin-dependent platelet activation triggered by the antibodies could be blocked by monoclonal antibodies specific for the platelet Fc receptor (FcγRIIA),⁹ it was thought for some time that antibodies associated with HIT were specific for heparin and reacted with the anticoagulant to form platelet-activating immune complexes. However, various investigators were unable to demonstrate such complexes.^{25,26} Despite lack of knowledge about the specificity of HIT-associated antibodies, recognition of their platelet-activating properties led to the development of two useful diagnostic procedures – the platelet aggregation test^{27,28} and the serotonin release test (SRA).²⁹ The latter test is somewhat more sensitive than the former, but it is generally agreed that it is incapable of diagnosing all cases of HIT. Moreover, both of these assays have the disadvantage of requiring fresh platelets.

In the early 1990s, an important clue to the etiology of HIT was provided by the finding in several laboratories³⁻⁶ that the antibodies in patients with this condition are specific for complexes containing heparin and PF4 leading to the development of PF4-dependent enzyme immunoassays (PF4-H ELISA).

Clinical Relevance of the Presence of PF4-Heparin Specific Antibodies

The diagnosis of HIT is made clinically with the support from the laboratory. It is important to recognize that a positive PF4-H ELISA test only indicates the presence of antibodies specific for PF4-heparin complexes and is not intended for diagnosis of the *clinical entity*, HIT. It is important to make a distinction between specificity and sensitivity for “antibody detection” and for “disease diagnosis”. Some reports have claimed that the PF4-H ELISA is “insensitive” and “non-specific” for HIT diagnosis because many patients positive for antibody do not have clinical HIT. However, the assay is intended only for antibody detection and it is very sensitive and specific for that purpose. The high sensitivity of the PF4-H ELISA for antibody detection is not necessarily a drawback because from the clinical standpoint, a missed diagnosis of HIT can be catastrophic for an individual patient. Furthermore, the presence of antibodies specific for PF4-heparin complexes (even if “not clinically significant” at the time a specimen was collected) could prompt a physician to limit exposure to heparin since there is no way to know how many of these patients might develop HIT if heparin treatment is continued. Lastly, it should be noted there are reported cases of patients who were positive in PF4-H ELISA but negative in SRA who had clinical HIT, some with thrombosis (unpublished observations).

It is also important to recognize that there is a close relationship between the optical density (OD) value in the PF4-H ELISA and the likelihood that a patient has HIT and is at risk for thrombosis.^{30,31} To paraphrase Warkentin et al,³¹ laboratories reporting EIA test results (should) consider informing clinicians about the actual OD value of the PF4-H ELISA (rather than just reporting “positive” or “negative”) and should emphasize the importance of a strong positive test result.

Moreover, in a retrospective, case-control study conducted on cardiac surgery patients with postoperative thrombocytopenia, Karendi et al. found serial monitoring of antibody strength, by ELISA tests, to be useful in making treatment decisions.³² In this study, patients with borderline or negative initial assay who had a subsequent second, third, or fourth assay, 28% ultimately had a positive result on follow-up tests. Furthermore, 91% of these positive results occurred on

the second or third assay, suggesting the usefulness of investigating for the presence of PF4-heparin dependent antibodies at multiple time points if the clinical suspicion for HIT exists.³² The latter approach could provide clinicians with a useful tool to monitor heparin therapy prior to switching to a different anticoagulant treatment; therefore, possibly preventing development of HIT had heparin been continued.

References

1. Edson, J. R. 1992. Heparin-induced thrombocytopenia. *J Lab Clin.Med.* 120:355-356.
2. Warkentin, T. E. 1999. Heparin-induced thrombocytopenia: a ten-year retrospective. *Annu.Rev.Med.* 50:129-147.
3. Visentin, G. P., S. E. Ford, J. P. Scott, and R. H. Aster. 1994. Antibodies from patients with heparin-induced thrombocytopenia/thrombosis are specific for platelet factor 4 complexed with heparin or bound to endothelial cells. *J Clin.Invest* 93:81-88.
4. Amiral, J., F. Bridey, M. Dreyfus, A. M. Vissoc, E. Fressinaud, M. Wolf, and D. Meyer. 1992. Platelet factor 4 complexed to heparin is the target for antibodies generated in heparin-induced thrombocytopenia. *Thromb Haemost.* 68:95-96.
5. Greinacher, A., B. Potzsch, J. Amiral, V. Dummel, A. Eichner, and C. Mueller-Eckhardt. 1994. Heparin-associated thrombocytopenia: isolation of the antibody and characterization of a multimolecular PF4-heparin complex as the major antigen. *Thromb Haemost.* 71:247-251.
6. Kelton, J. G., J. W. Smith, T. E. Warkentin, C. P. Hayward, G. A. Denomme, and P. Horsewood. 1994. Immunoglobulin G from patients with heparin-induced thrombocytopenia binds to a complex of heparin and platelet factor 4. *Blood* 83:3232-3239.
7. Warkentin, T. E. and J. G. Kelton. 2001. Temporal aspects of heparin-induced thrombocytopenia. *N.Engl.J Med.* 344:1286-1292.
8. Amiral, J., F. Bridey, M. Wolf, C. Boyer-Neumann, E. Fressinaud, A. M. Vissac, E. Peynaud-Debayle, M. Dreyfus, and D. Meyer. 1995. Antibodies to macromolecular platelet factor 4-heparin complexes in heparin-induced thrombocytopenia: a study of 44 cases. *Thromb Haemost.* 73:21-28.
9. Kelton, J. G., D. Sheridan, A. Santos, J. Smith, K. Steeves, C. Smith, C. Brown, and W. G. Murphy. 1988. Heparin-induced thrombocytopenia: laboratory studies. *Blood* 72:925-930.
10. Suh, J. S., R. H. Aster, and G. P. Visentin. 1998. Antibodies from patients with heparin-induced thrombocytopenia/thrombosis recognize different epitopes on heparin: platelet factor 4. *Blood* 91:916-922.
11. Ziporen, L., Z. Q. Li, K. S. Park, P. Sabnekar, W. Y. Liu, G. Arepally, Y. Shoenfeld, T. Kieber-Emmons, D. B. Cines, and M. Poncz. 1998. Defining an antigenic epitope on platelet factor 4 associated with heparin-induced thrombocytopenia. *Blood* 92:3250-3259.

12. Visentin, G. P., M. Moghaddam, S. E. Beery, J. G. McFarland, and R. H. Aster. 2001. Heparin is not required for detection of antibodies associated with heparin-induced thrombocytopenia/thrombosis. *J Lab Clin.Med.* 138:22-31.
13. Suh, J. S., M. I. Malik, R. H. Aster, and G. P. Visentin. 1997. Characterization of the humoral immune response in heparin-induced thrombocytopenia. *Am.J.Hematol.* 54:196-201.
14. Bauer, T. L., G. Arepally, B. A. Konkle, B. Mestichelli, S. S. Shapiro, D. B. Cines, M. Poncz, S. McNulty, J. Amiral, W. W. Hauck, R. N. Edie, and J. D. Mannion. 1997. Prevalence of heparin-associated antibodies without thrombosis in patients undergoing cardiopulmonary bypass surgery. *Circulation* 95:1242-1246.
15. Pappalardo, F., G. Crescenzi, A. Franco, and A. Zangrillo. 2006. Early heparin-induced thrombocytopenia (HIT) after cardiac surgery. *Eur.J.Anaesthesiol.* 23:806-808.
16. Warkentin, T. E., M. Makris, R. M. Jay, and J. G. Kelton. 2008. A spontaneous prothrombotic disorder resembling heparin-induced thrombocytopenia. *Am.J.Med.* 121:632-636.
17. Bell, W. R. and R. M. Royall. 1980. Heparin-associated thrombocytopenia: a comparison of three heparin preparations. *N.Engl.J Med.* 303:902-907.
18. Lee, D. H. and T. E. Warkentin. 2003. Frequency of Heparin-Induced Thrombocytopenia. In *Heparin-Induced Thrombocytopenia*, Vol. 47. T. E. Warkentin and A. Greinacher, eds. Marcel Dekker, New York, pp. 107-148.
19. Hrushesky, W. J. 1978. Subcutaneous heparin-induced thrombocytopenia. *Arch.Intern.Med.* 138:1489-1491.
20. Heeger, P. S. and J. T. Backstrom. 1986. Heparin flushes and thrombocytopenia. *Ann Intern.Med.* 105:143.
21. Laster, J. L., W. K. Nichols, and D. Silver. 1989. Thrombocytopenia associated with heparin-coated catheters in patients with heparin-associated antiplatelet antibodies. *Arch.Intern.Med.* 149:2285-2287.
22. Warkentin, T. E., B. T. Maurer, and R. H. Aster. 2007. Heparin-induced thrombocytopenia associated with fondaparinux. *N.Engl.J.Med.* 356:2653-2655.
23. Rota, E., M. Bazzan, and G. Fantino. 2008. Fondaparinux-related thrombocytopenia in a previous low-molecular-weight heparin (LMWH)-induced heparin-induced thrombocytopenia (HIT). *Thromb.Haemost.* 99:779-781.
24. Chong, B. H., W. R. Pitney, and P. A. Castaldi. 1982. Heparin-induced thrombocytopenia: association of thrombotic complications with heparin-dependent IgG antibody that induces thromboxane synthesis in platelet aggregation. *Lancet* 2:1246-1249.
25. Warkentin, T. E. and J. G. Kelton. 1991. Heparin-induced thrombocytopenia. *Prog.Hemost.Thromb.* 10:1-34.

26. Greinacher, A., I. Michels, and C. Mueller-Eckhardt. 1992. Heparin-associated thrombocytopenia: the antibody is not heparin specific. *Thromb.Haemost.* 67:545-549.
27. Kelton, J. G., D. Sheridan, H. Brain, P. J. Powers, A. G. Turpie, and C. J. Carter. 1984. Clinical usefulness of testing for a heparin-dependent platelet-aggregating factor in patients with suspected heparin-associated thrombocytopenia. *J Lab Clin Med.* 103:606-612.
28. Greinacher, A., I. Michels, V. Kiefel, and C. Mueller-Eckhardt. 1991. A rapid and sensitive test for diagnosing heparin-associated thrombocytopenia. *Thrombosis & Haemostasis* 66:734-736.
29. Sheridan, D., C. Carter, and J. G. Kelton. 1986. A diagnostic test for heparin-induced thrombocytopenia. *Blood* 67:27-30.
30. Zwicker, J. I., L. Uhl, W. Y. Huang, B. H. Shaz, and K. A. Bauer. 2004. Thrombosis and ELISA optical density values in hospitalized patients with heparin-induced thrombocytopenia. *J.Thromb.Haemost.* 2:2133-2137.
31. Warkentin, T. E., J. I. Sheppard, J. C. Moore, C. S. Sigouin, and J. G. Kelton. 2008. Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays. *J.Thromb.Haemost.* 6:1304-1312.
32. Kerendi, F., V. H. Thourani, J. D. Puskas, P. D. Kilgo, M. Osgood, R. A. Guyton, and O. M. Lattouf. 2007. Impact of heparin-induced thrombocytopenia on postoperative outcomes after cardiac surgery. *Ann.Thorac.Surg.* 84:1548-1553.