

# Laboratory Advisor

University of Louisville School of Medicine

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## Clinicopathologic Diagnosis of Heparin Induced Thrombocytopenia

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### **Introduction**

On February 15, 2010, the laboratory began testing for heparin-induced thrombocytopenia (HIT) using an Enzyme Immunoassay (ELISA) that is specific for IgG platelet factor 4/heparin complex antibodies. The previous assay detected antibodies that were of IgA and Ig M type, as well as IgG. Since HIT antibodies of the IgG class are considered to be the most clinically significant, the new assay will increase the specificity of results, by eliminating weakly positive and equivocal results with low positive initial screening optical density (OD).<sup>1</sup>

This Laboratory Advisor will review the pathophysiology of HIT, clinical findings in HIT, and the technical aspects of the PF4 IgG (HIT) antibody assay recently implemented in the microbiology/serology laboratory.

### **Pathophysiology of HIT**

HIT is a syndrome of immune-mediated thrombocytopenia and potentially life-threatening thrombosis in patients treated with heparin. Therapy with unfractionated heparin (UFH) has a higher association with HIT than low molecular weight heparin (LMWH). However, most antibodies formed in response to UFH will cross-react with LMWH.<sup>2</sup> Patients developing the syndrome should have heparin stopped, and be anticoagulated with a direct thrombin inhibitor. Surgery is a risk factor for development of HIT, as surgery results in platelet activation and PF4 release from platelet alpha granules, which becomes antigenic when bound as a complex to heparin. In general, HIT antibodies will develop in up to 15% of orthopedic surgical patients and up to 50% of cardiopulmonary bypass patients, with clinical HIT developing in 2-3% of the orthopedic group, and 1% of the cardiopulmonary bypass group.<sup>2</sup>

HIT antibodies classically form 5-10 days after initiation of heparin therapy, but may develop sooner if the patient has a previous exposure to heparin. The amount of heparin exposure does not correlate directly with risk of HIT, since antibodies may develop after exposure to low amounts of heparin, such as in heparin flushes of indwelling lines. Additional sources of heparin exposure include bone marrow stem cell preparations, dialysis, extracorporeal circulation, and phlebotomy for heparinized samples.

### **Steps for the formation of platelet factor 4/heparin complex antibodies and thrombosis:**<sup>2,3</sup>

- Patient is started on UFH or LMWH
- Heparin binds to PF4 on platelet surfaces, forming a neoantigen, and inducing the HIT antibody.
- The tail of the antibody binds to platelets through their FcγR1a receptors. Heparin/PF4 antibodies of IgA or IgM class are not able to bind to this receptor. This results in platelet activation with aggregation, and release of platelet microparticles, which initiate formation of a blood clot. The platelet count falls as a result, leading to thrombocytopenia. Release of inflammatory cytokines and tissue factor from activated endothelium and white cells may also contribute to the thrombotic tendency.
- In most cases, the antibody/platelet complexes are removed by the spleen, with development of transient thrombocytopenia only, without associated thrombosis.

The platelet count is determined before institution of anti-coagulant therapy with heparin, giving a baseline value. Platelet counts are usually decreased by 50% or more in true HIT, resulting in moderate thrombocytopenia. Severe thrombocytopenia (less than 10,000) is not typical of HIT, and likely represents another etiology of decreasing platelet count, such as disseminated intravascular coagulation.

Thrombosis is preceded by a fall in the platelet count, and this observation allows the discontinuation of heparin in a patient with clinical suspicion of HIT. The clinical impression of HIT can be scored using a pretest clinical scoring system (4 Ts) as reported by Lo et al.<sup>4</sup>

**Maximum points: 8, low ≤ 3, intermediate 4-5, high 6-8**

**Thrombocytopenia**

- 0: less than 30% fall from baseline platelet count, less than 10,000 nadir (lowest measured platelet count)
- 1: 30-50% fall, 10,000 nadir
- 2: >50% fall, 20-100,000 nadir

**Timing**

- 0: < four days from exposure
- 1: day 5-10 (but not clinically clear), or >10 days, or <1day
- 2: day 5-10, or <1day if recent heparin (within previous 30 days)

**Thrombosis**

- 0 none
- 1 progressive, recurrent or silent
- 2 proven thrombosis, skin necrosis, acute systemic reaction with heparin bolus

**Other causes of Thrombocytopenia**

- 0 definite
- 1 possible
- 2 none evident

**Role of Laboratory Testing in the Diagnosis of HIT**

The serotonin release assay (SRA) is the laboratory gold standard for the diagnosis of HIT. The test involves radioactive materials and thus is performed in reference laboratories only. Platelet aggregation studies using low and high concentrations of heparin have also been used as a specific test for HIT, but routine laboratory sensitivity of approximately 30-50% limits the usefulness of the test. SRA and heparin induced platelet aggregations studies allow assessment of the activity of the PF4/heparin antibodies in regards to platelet activation, and are called “*functional*” assays. The most commonly performed assays are the ELISA tests, as used by our laboratory at University Hospital. The ELISA method differs from the functional assays in that the test recognizes antibody using an antigen/antibody detections system, but the function of the antibody is not studied. Patients with strong clinical suspicion of HIT with a negative ELISA result may benefit from additional testing using the SRA.

The ELISA assay begins with a screening step to detect antibody that is measured in optical density (OD). A screening OD value >0.4 is considered an initial positive test. Heparin is then added to the system to increase the specificity of the assay, and heparin inhibition of the antibody by 50% or more confirms the positive result. Recently, initial screening OD has been shown to have diagnostic value, in that OD >1.4 correlate with positive SRA.<sup>5</sup> Also screening OD of >2.0, indicating a strong antibody, may be insufficiently neutralized by the heparin step, resulting in an equivocal test result for a clinically important antibody.<sup>6</sup> The laboratory at University Hospital reports screening OD, percent inhibition by heparin, and interpretation as to negative, equivocal, or positive, and interpretive comments to aid clinical evaluation of the results. Positive ELISA test results in a patient with intermediate or high clinical score is very good laboratory support for HIT, and a negative ELISA result in a patient with low clinical score may suffice to exclude HIT.

A clinical caveat is that HIT antibodies may not always be detectable by the laboratory when the process is in the acute phase, and platelet count is at nadir. Perhaps in this situation, the antibody is bound to platelet aggregates with insufficient circulating antibody to be detected in patient serum. For this reason, a negative HIT result in a patient with clinical suspicion of HIT should be repeated at weekly intervals for one month. In one study antibodies were not detected for up to 33 days following clinical signs of HIT.<sup>7</sup>

A thrombotic tendency may not manifest itself while a patient is on heparin, but may develop weeks after institution of heparin, known as “delayed HIT.” Because of this, any patient with thrombosis who has received heparin 30 days previously should be tested for HIT. This diagnosis could explain some cases of “coumadin failure”, when patients develop clotting while being stabilized on coumadin. In 2006, the US food and Drug Administration and Baxter Healthcare Corp warned healthcare professionals regarding the risk for delayed-onset HIT in patients receiving heparin sodium, emphasizing that the risk for thrombosis persists even after discontinuation of heparin therapy.<sup>8</sup>

**Technical Aspects of Laboratory Testing for HIT**

It is now known that antibodies associated with HIT recognize sites on a platelet protein designated “platelet factor 4” (PF4) that are created when PF4 is complexed with heparin or another linear polyanionic compound such as polyvinyl sulfonate (PVS). PF4 IgG™ Solid Phase ELISA (GTI Diagnostics) microwells provide immobilized PF4:PVS complexes as a target for the detection of IgG antibodies associated with HIT.

Patient serum is added to microwells coated with platelet factor 4 (PF4) complexed to polyvinyl sulfonate (PVS). If an antibody recognizing a site on PF4:PVS is present, binding will occur. Unbound antibodies are then washed away. An alkaline phosphatase labeled anti-human globulin reagent (Anti-IgG) is added to the wells and incubated. The unbound Anti-IgG is

washed away and the substrate PNPP (p-nitrophenyl phosphate) is added. After a 30-minute incubation period, the reaction is stopped by a sodium hydroxide solution. The optical density of the color that develops is measured in a spectrophotometer.<sup>9</sup>

**Initial Experience with the IgG specific HIT ELISA assay**

Introduction of a HIT ELISA assay specific for IgG PF4/heparin complex antibodies was expected to decrease the number of positive tests, greatly increasing the specificity of the assay to aid in the diagnosis of HIT, although the use of the assay is currently somewhat controversial. Driving the impetus to introduce more specific testing for HIT is the potential risk for considerable over-diagnosis of HIT by laboratories that utilize only an ELISA anti-PF4/heparin assay that detects IgG, IgM, and IgA.<sup>1</sup> Per manufacturer's information, laboratories adopting the IgG specific test have seen a 30-40% decrease in number of positive tests. Our initial experience with the assay indicates that the positivity rate will be around 1.7%. False negatives can occur and also occurred with the older assay which detected all immunoglobulin classes of HIT antibodies. The laboratory will continue to recommend that patients with clinical suspicion of HIT, and a negative initial HIT assay, have the test repeated at weekly intervals for a month, as antibody detection may occur up to 33 days following clinical diagnosis of HIT.<sup>7</sup> The serotonin release assay is considered the gold standard laboratory assay for HIT, and is available as a send out test for cases where the diagnosis of HIT is especially difficult.

**Conclusion**

HIT is an antibody mediated phenomena of clotting instigated by the anticoagulant heparin, and the diagnosis of HIT requires clinicopathologic correlation. Thrombocytopenia is an important symptom of HIT, but nonspecific, as thrombocytopenia has many causes. Area clinicians have appreciated our offering a HIT test, despite the difficulty of detecting and evaluating antibodies for the diagnosis. Our interpretive comments hopefully guide clinicians according to our best current understanding of the value of laboratory testing. The availability and implementation of an IgG specific ELISA HIT test will hopefully improve specificity of the assay, without significantly decreasing sensitivity. We welcome feedback regarding your experience with the new assay, and are willing to provide consultation when needed.

Testing will be performed three times weekly: Monday, Wednesday and Friday. Patients are no longer requested to be off heparin for 24 hours for test performance. Patients may be on heparin at the time of testing, however, this option is not recommended, as all sources of heparin should be discontinued when clinical suspicion of HIT is present. Results of HIT testing may be found under the coagulation section of NetAccess.

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**About the Authors**



Phyllis started in 1979 at Louisville General in the Microbiology Department. Her responsibilities included, reading culture plates (bacterial, fungal and AFB) and performing serological testing. Phyllis performed much of the initial work evaluating and validating several molecular assays including: HIV, GC/Chlamydia, CMV, HCV, etc. Phyllis is currently the Lead Technologist in Serology and Virology sections.



After attending U of L School of Medicine, Sandra C. Hollensead, M.D. completed residency training at UofL in AP/CP, and then performed a heme-pathology fellowship at Ball Memorial Hospital in Muncie Indiana. Dr. Hollensead has been practicing pathology with an interest in fluid hematology since 1988. She is the medical director of the hematology and coagulation laboratory at the University of Louisville Hospital, and provides clinical pathology consultations for the laboratory diagnosis of bleeding and clotting disorders.

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