LABORATORY TESTING FOR HEPARIN INDUCED THROMBOCYTOPENIA

Andreas Greinacher
Institut für Immunologie und Transfusionsmedizin,
Ernst-Moritz-Arndt Universität Greifswald, Greifswald, Germany

Heparin-induced thrombocytopenia (HIT) is a clinicopathologic syndrome in which thrombocytopenia, thrombosis, injection site skin lesions occur with platelet-activating antibodies that recognize platelet factor 4 (PF4)/heparin complexes. HIT exhibits striking differences from other immune thrombocytopenias. These include: mild-to-moderate thrombocytopenia, association with thrombosis, and lack of bleeding. Its pathogenesis differs fundamentally from other immune thrombocytopenias. In HIT, IgG antibodies bind to multimolecular PF4/heparin complexes, producing platelet activation via platelet FcγIIa receptors, with resulting hypercoagulability and thrombosis. In contrast, platelet antibodies in autoimmune thrombocytopenia (ITP) recognize platelet glycoproteins and in case of drug dependent antibodies (D-ITP) complexes of the drug and glycoproteins. The opsonized platelets are cleared by phagocytic cells, leading to severe thrombocytopenia and bleeding. Antibodies induced by heparins occur frequently, especially after major surgery in up to 30-70% of patients, but only a few antibody-positive patients develop clinical HIT. In contrast, ITP antibodies and especially D-ITP antibodies are rare, difficult to detect by current laboratory methods, but if positive, these assays have a high diagnostic specificity. Current tests for HIT, however, washed platelet activation assays (e.g., serotonin-release or heparin-induced platelet activation assays) and anti-PF4/polyanion enzyme-linked immunosorbent assays (ELISAs), have high sensitivity for detecting clinically-relevant HIT antibodies, providing in *combination* a sensitivity close to 100%. Both tests are needed because for example, the platelet activation test may give an indeterminate result due to concomitant non-HIT platelet-activating factors or the ELISA yields a false-negative result due to heparin-dependent platelet-activating antibodies against rare non-PF4 antigens. The strength of the laboratory tests is that negative results with both assays essentially rule out HIT. The specificity of these assays and especially of the ELISAs alone is low for diagnosing HIT. Performing only the commercially available antigen tests leads to overdiagnosis of HIT by about 100%.Approaches to increase diagnostic specificity include: detecting antibodies by their platelet-activating properties (use of washed platelet activation assays), restricting ELISAs to detection of IgG antibodies (anti-PF4/heparin IgM/IgA antibodies are of minor clinical relevance), and utilizing higher cutoffs (OD of ELISA, or lag time in activation tests) as reaction strength correlates strongly with risk of HIT, and a confirmatory step in the ELISA showing that high heparin concentrations can decrease the OD. Currently the most specific approach is to interpret lab-test results in the clinical context. A recently developed scoring system is a validated approach to obtain the clinical information systematically.

Recommended reading: Greinacher A, et al.: Heparin-induced thrombocytopenia: a prospective study on the incidence, platelet-activating capacity and clinical significance of anti-PF4/heparin antibodies of the IgG, IgM, and IgA classes. *J Thromb Haemost* 2007;5:1666-73. Pouplard C, et al.: Prospective evaluation of the '4Ts' score and particle gel immunoassay specific to heparin/PF4 for the diagnosis of heparin-induced thrombocytopenia. *J Thromb Haemost* 2007;5:1373-9. Warkentin TE, et al.: Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays. *J Thromb Haemost* 2008;6:1304-12.