COLLEGE of AMERICAN PATHOLOGISTS

Heparin-Induced Thrombocytopenia Testing

SYNOPSIS AND RELEVANCE

Heparin-induced thrombocytopenia (HIT) is a serious complication of heparin therapy that can cause life-threatening thrombosis. HIT is caused by antibodies directed against heparin-platelet factor 4 (PF4) complexes, which can be detected by enzyme-linked and latex-enhanced immunoassays (IA) that have high diagnostic sensitivity. However, the less specific nature of these assays leads to the need to confirm positive results using less available and expensive confirmatory tests (eg, serotonin release assay [SRA] and the heparin-induced platelet aggregation assay [HIPAA]). A positive IA supports a clinical decision to discontinue heparin and initiate alternate anticoagulants, typically parenteral direct thrombin inhibitors (eg, argatroban and bivalirudin), which are expensive and difficult to manage, with potential increased bleeding risk.[1]

The predictive value of HIT IA tests is improved by limiting testing to those patients with a greater likelihood of HIT, which can be estimated using a pre-test predictive scoring system (4Ts or HIT Expert Probability [HEP] Score). This document will explain how institution-specific protocols can be developed for HIT testing, including proper use of HIT IA and confirmatory assays. Limiting HIT IA testing to patients with higher pre-test probabilities of HIT can ensure optimal utilization of laboratory (ie, less HIT IA and confirmatory testing) and pharmacy (ie, less alternative anticoagulant use) resources.

INSIGHTS

1. The negative predictive value of a low probability 4Ts score is high; therefore, it is generally considered to be a reliable method to exclude HIT.
2. A positive HIT IA test result in a patient with a low 4Ts scores is much more likely to be a false positive for the HIT syndrome.
3. The HIT IA should not be measured in patients with low pre-test probability.
4. An incorrect HIT diagnosis based on misinterpretation of clinical information and a false positive HIT IA can lead to patient harm due to patient exposure to alternate anticoagulants, with increased risk of bleeding, and to deny future heparin therapy.
5. The ASH guideline panel recommends using the 4Ts score to estimate pre-test probability in patients with suspected HIT. The 4Ts score will identify patients with intermediate to high pre-test probability of HIT which should be managed by heparin discontinuation, alternative anticoagulation and HIT immunoassay. For patients with low pre-test probability 4Ts score ≤ 3, HIT is unlikely and should be managed accordingly.
6. Systems can be implemented to educate participants on the correct application of HIT IAs and strategies to limit unnecessary HIT antibody testing.
7. The SRA has high sensitivity and specificity, but it is expensive, has a long turn-around-time and has limited availability, so it should only be used for decision making when the 4Ts score is intermediate and the IA is positive or when the clinical score and test results are mismatched.

BACKGROUND

Heparin induced thrombocytopenia (HIT) is a prothrombotic drug reaction usually caused by antibodies to PF4-heparin complexes. In unusual cases, heparin-independent, autoimmune HIT has also been described.[2] Extremely rarely, sera from patients with venous thromboses and thrombocytopenia after adenoviral vector COVID-19 vaccinations contain antibodies that recognize PF-4 and activate platelets in the absence of heparin. The risk of HIT is as high as 2.6 % in surgical patients treated with unfractionated heparin.[3] HIT is a serious complication of heparin therapy that can cause life-threatening thrombosis.[4] Proper management requires accurate diagnosis, quickly followed by cessation of heparin therapy and initiation of a non-heparin anticoagulant.[5] The diagnosis of HIT depends on appropriate clinical assessment and accurate laboratory testing. An incorrect diagnosis of HIT may cause the clinician to discontinue heparin and use alternate anticoagulants (eg, direct thrombin inhibitors).[5] Even more dangerously, some clinicians discontinue heparin with the development of thrombocytopenia and consideration of HIT, without starting alternative anticoagulation. Using a validated tool to predict pretest probability of HIT improves clinical decision making and test utilization. The 4Ts pretest scoring system incorporates magnitude and timing of thrombocytopenia, concurrent thrombosis, and likelihood of other thrombocytopenia causes (See **Appendix A**).[5] The reported negative predictive value of a low probability 4Ts score (≤3) is high; therefore, it is a reliable method to exclude HIT.[5,6]

Most IA tests for HIT have high false-positive rates for clinical HIT since they are incapable of determining whether the detected antibodies have the platelet activating properties that define the HIT syndrome.[7] A positive HIT IA test result in a patient with a low 4Ts scores is much more likely to represent a false positive for the HIT syndrome. This

can lead to an incorrect diagnosis of HIT, which may lead to starting a thrombocytopenic patient on an alternate anticoagulant that can cause an increased risk of bleeding.[8] The patient may also be denied future heparin based on an incorrect diagnosis. Thus, the American Society of Hematology (ASH) 2014 stewardship campaign recommended not to test or treat for heparin-induced thrombocytopenia if the clinical pretest probability of heparin-induced thrombocytopenia is low.[8] A high proportion of patients tested for HIT have low pre-test probability when the 4Ts score was used. Contact with physicians for patients with low pre-test risk of HIT has demonstrated improved ordering practices.[9] However, clinician education has shown limited impact on improving HIT immunoassay ordering practices.[10] An approach to improve HIT Immunoassay ordering practice employs a HIT order set with mandatory 4Ts calculation.

The 4Ts test is useful to identify patients with low, intermediate or high pre-test probability.[5] If the patient has a low 4Ts score ($\leq$ 3), they are unlikely to have HIT and the IA is less likely to add to the accuracy of the diagnosis.[1,11] In this case, it is not necessary to discontinue heparin. If a patient's 4T score indicates that they should be tested ($\geq$ 4), heparin should always be discontinued with transition to alternative anticoagulant while awaiting test results. If the 4Ts score is intermediate (4-6), then a negative IA identifies patients who are unlikely to have HIT syndrome. The IA may be repeated if data to calculate a 4Ts score are incomplete or uncertain and if the clinical situation changes, such as a new drop in platelet count or appearance of thrombosis.

HIT IA assays with high sensitivity and negative predictive value (NPV) are useful to rule out HIT, especially in patients with low or intermediate pre-test probability.[1,12] However, they have lower specificity for the HIT syndrome (~30-70%), which can lead to false-positive determinations in this patient population. Several commercially available immunoassays vary substantially in regard to their sensitivity, specificity and diagnostic accuracy. A subset of these HIT immunoassays have high sensitivity, specificity and diagnostic accuracy with low optical density thresholds and are recommended for HIT immunoassay screening.[7] The HIT IA intensity or optical density (OD) value is correlated with probability of platelet-activating properties and HIT.[13] An ELISA OD of < 0.4 is negative and has a very low probability of HIT. Similarly, a weakly positive ELISA with OD of < 1.0 has a very low (less than 5 percent) probability of HIT. In contrast, a moderately positive OD of $\geq$ 1.0 to < 2.0 is associated with a greater probability of HIT, approximately 50 percent, and an OD of $\geq$ 2.0 has a high probability (> 80 to 90 percent) of HIT, as compared to the gold standard serotonin release assay, (SRA).[12, 14, 15]

In 2018, the American Society of Hematology (ASH) published an algorithmic approach to evaluation and testing patients with suspected heparin-induced thrombocytopenia (HIT) with guidelines (referred to here as the ASH guideline panel) to manage heparin-induced thrombocytopenia.[14] The ASH guideline panel recommends using the 4Ts score to estimate HIT pre-test probability in patients with suspected HIT. For a low-probability 4Ts score ($\leq$ 3), the panel recommends against HIT laboratory testing and against discontinuing of heparin, unless there is uncertainty about the accuracy of the 4Ts score.[14] In patients with suspected HIT and an intermediate or high probability 4Ts score of greater than or equal to 4, the ASH guideline panel recommends to discontinue heparin and suggests to initiate a non-heparin anticoagulant and to perform an immunoassay.[12] A low threshold, high sensitivity Immunoassay is preferred over a high threshold assay.[7,14] For the patients with intermediate or high probability 4Ts score and negative immunoassay, the ASH guideline panel recommends considering discontinuing the non-heparin anticoagulant and resuming heparin.[14] If the immunoassay is positive, the panel recommends avoiding heparin, administering a non-heparin anticoagulant, and performing a functional HIT test, for example, the SRA.[14] A positive functional assay confirms HIT syndrome. Rarely, a patient with a high 4Ts score and strongly positive IA has a negative functional HIT test. Functional HIT tests, like the SRA, are considered to be specific with high positive predictive value for HIT syndrome. If the SRA is negative, HIT is unlikely; however, false negative SRA results do occur. In such situations, the panel recommends clinical reevaluation and repeat testing of the immunoassay or functional assay to clarify the diagnosis. Refer to the ASH guideline for full details of the panel's HIT treatment and testing recommendations.[14]

APPENDIX A: 4TS PRE-TEST PROBABILITY SCORES

Points	2	1	0
Thrombocytopenia degree	> 50% decrease or nadir > 20 K/uL	30 - 50% decrease or nadir 10-19 K/uL	< 30% decrease or nadir < 10 K/uL
Thrombocytopenia Timing	Onset between days 5 and 10	Missing platelet count; Onset not clear	No recent heparin
Thrombosis or other sequelae	Proven thrombosis, skin necrosis or acute systemic reaction after heparin bolus	Progressive, recurrent or silent thrombosis; erythematous skin lesions	No thrombosis
Thrombocytopenia cause	No other cause	Possible other cause	Definite other cause

< 3 = low probability of HIT. 4-5 = intermediate probability of HIT. > 6 = high probability of HIT.
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REFERENCES
1. Linkins LA, Bates SM, Lee AYY, Heddle NM, Wang G, Warkentin TE. Combination of 4Ts score and PF4/H-PaGIA for diagnosis and management of heparin-induced thrombocytopenia: prospective cohort study. *Blood.* 2015;126(5):597-603.
2. Greinacher A, Selleng K, Warkentin TE. Autoimmune heparin-induced thrombocytopenia. *J Thromb Haemost.* 2017;15(11):2099-2114.
3. Martel N, Lee J, Wells PS. Risk for heparin-induced thrombocytopenia with unfractionated and low-molecular-weight heparin thromboprophylaxis: a meta-analysis. *Blood.* 2005;106(8):2710-2715.
4. Linkins LA, Warkentin TE. The approach to heparin-induced thrombocytopenia. *Semin Resp Critical Care Med.* 2008;29(1):66-74.
5. Cuker A, Gimotty PA, Crowther MA, Warkentin TE. Predictive value of the 4Ts scoring system for heparin-induced thrombocytopenia: a systematic review and meta-analysis. *Blood.* 2012;120(20):4160-4167.
6. Crowther M, Cook D, Guyatt G, et al. Heparin-induced thrombocytopenia in the critically ill: Interpreting the 4Ts test in a randomized trial. *J Crit Care.* 2014;29(3):470.e477-415.
7. Nagler M, Bachmann LM, Ten Cate H, Ten Cate-Hoek A. Diagnostic value of immunoassays for heparin-induced thrombocytopenia: a systematic review and meta-analysis. *Blood.* 2016;127(5):546-557.
8. Hicks LK, Bering H, Carson KR, et al. Five hematologic tests and treatments to question. *Blood.* 2014;124(24):3524-3528.
9. Burnett A, Bowles H, Borrego M, Montoya T, Garcia D, Mahan C. Heparin-induced thrombocytopenia: reducing misdiagnosis via collaboration between an inpatient anticoagulation pharmacy service and hospital reference laboratory. *J Thromb Thrombolysis.* 2016;42(4):471-478.
10. Malalur P, Greenberg C, Lim MY. Limited impact of clinician education on reducing inappropriate PF4 testing for heparin-induced thrombocytopenia. *J Thromb Thrombolysis.* 2019;47(2):287-291.
11. Greinacher A. Heparin-Induced Thrombocytopenia. J Thromb Haemost. 2017;15(8):1640-1645.
12. Warkentin TE, Sheppard J-AI, Linkins L-A, Arnold DM, Nazy I. Performance characteristics of an automated latex immunoturbidimetric assay [HemosIL® HIT-Ab(PF4-H)] for the diagnosis of immune heparin-induced thrombocytopenia. *Thrombosis Research.* 2017;153:108-117.
13. Raschke RA, Gallo T, Curry SC, et al. Clinical effectiveness of a Bayesian algorithm for the diagnosis and management of heparin-induced thrombocytopenia. *J Thromb Haemost.* 2017;15(8):1640-1645.
14. Cuker A, Arepally GM, Chong BH, et al. American Society of Hematology 2018 guidelines for management of venous thromboembolism: heparin-induced thrombocytopenia. *Blood Advances.* 2018;2(22):3360-3392.
15. Warkentin TE, Sheppard JI, Moore JC, Sigouin CS, Kelton JG. Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays. *J Thromb Haemost.* 2008; 6(8):1304-1312.
16. Warkentin TE, Aird WC, Rand JH. Platelet-endothelial interactions: sepsis, HIT, and antiphospholipid syndrome. *Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program.* 2003;2003(1):497-519.