COLLEGE of AMERICAN PATHOLOGISTS

Heparin-Induced Thrombocytopenia Testing

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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 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 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 module 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.

OBJECTIVES

As a result of participating in this activity, participants will be able to:

1. Recognize clinical information that affects HIT pre-test probability.
2. Recognize the pitfalls of performing HIT testing in patients with a low pre-test probability 4Ts score.
3. Employ strategies to prevent unnecessary HIT IA or SRA testing in patients with a low 4Ts score.
4. Apply laboratory utilization principles to reduce excess testing and avoid alternative anticoagulant therapy.
5. Explain how institution-specific protocols can be developed for HIT testing, including proper use of HIT immunoassays.
6. Perform post-intervention analysis of HIT IA testing to demonstrate improvement in appropriate tests and avoid spurious results.

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 (**Appendix C**).[5] The reported negative predictive value of a low probability 4Ts score ($\leq$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 ≥ 1.0 to < 2.0 is associated with a greater probability of HIT, approximately 50 percent, and an OD of ≥ 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]

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 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.

INTERVENTIONS

1. Publish a HIT order set that includes a mandatory 4Ts score with drop-down support for correct calculation. Order sets should also identify categories of patients where 4Ts scores have not been validated or may not work such as in cancer patients.
2. A pharmacist or health care practitioner calculates the 4T score for all IA orders and contacts the ordering provider to suggest test cancellation for low probability scores.
3. Scrutinize orders with low and miscalculated 4T scores and contact the provider to cancel the HIT IA order.
4. A pathologist is available to provide consultation, if needed, in order to facilitate appropriate test ordering.
5. IA testing for HIT is performed only for orders with correctly added 4T scores of 4 or more.
6. Perform continued monitoring of 4T scores and HIT IA test volumes for trends.
7. Educate clinicians on appropriate HIT and SRA test ordering.

Other interventions, if needed, should be considered that offer the best opportunity to improve testing practices in your practice setting.

INTERVENTION ANALYSIS

Data collection to measure the impact is accomplished during a defined time period using the steps in **Appendix A**.

Once interventions occur, the opportunity rate (Number of patients for which HIT IA is ordered in patients without a 4Ts score or a low 4Ts score divided by (÷) the number of unique patients for whom HIT IA tests are ordered) can be restudied at an appropriate interval in order to determine the effectiveness of the intervention(s).

Data collection will depend on your computer system. For inpatients, it may be possible to compare laboratory orders with pharmacy information regarding each patient's heparin usage. This could also happen directly from the hospital information system/EHR or, for outpatients, the outpatient EHR.

APPENDICES

APPENDIX A: OPPORTUNITY RATES DURING DEFINED TIME PERIOD

Time period for data collection in months	A1
Number of patients evaluated for heparin-induced thrombocytopenia by IA without a 4T score.	A2
Number of patients evaluated for heparin-induced thrombocytopenia by IA with a 4T score of less than or equal to 3.	A3
Annual number of patients evaluated for heparin-induced thrombocytopenia by IA without a 4T score.	(12 ÷ A1) *A2 = A4
Annual number of patients evaluated for heparin-induced thrombocytopenia by IA with a 4T score of less than 4.	(12 ÷ A1) *A3 = A5

APPENDIX B: POST-INTERVENTION IMPACT ANALYSIS

Annual number of HIT IA tests performed pre-intervention	B1
Total number of improperly ordered HIT IAs pre-intervention	A4 + A5 = B2
Percent of appropriate HIT IA testing: pre-intervention	(B1-B2)/B1 x 100% = B3%
Annual number of HIT IA tests performed post-intervention	B4
Total number of improperly ordered HIT IAs post-intervention	B5
Percent of appropriate HIT IA testing: post-intervention	(B4-B5)/B4 x 100% = B6%
Change in percent appropriate HIT IA testing post-intervention	B3%-B6% = B7%

APPENDIX C: 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.
Adapted with permission from Warkentin *et al.* Hematology/The Education Program of the American Society of Hematology. Copyright 2003, American Society of Hematology.[16]

For "Thrombocytopenia Timing" category, it is important to consider the possibility of variant forms of HIT. These include immediate onset HIT that can occur when a patient has had recent heparin therapy and may have pre-existing HIT antibodies or late onset HIT.

2= Clear onset between days 5–10 OR platelet fall ≤1 day (prior heparin exposure within 30 days)
1= Consistent with days 5–10 fall, but not clear (such as missing platelet count) OR onset after day 10 OR fall ≤1 day (prior heparin exposure 30–100 days ago)

0= Platelet count fall <4 days after heparin exposure without recent prior exposure

QUESTIONS AND ANSWERS

QUESTION 1 OBJECTIVE
Recognize clinical information that affects HIT pre-test probability.

QUESTION 1
Which clinical information yields a 4Ts score indicating a high risk of HIT?

A. A patient with a past history of thrombosis who received their last dose of LMWH a month ago and develops thrombocytopenia with a platelet count of 30,000/µL, with no new thrombosis.
B. A patient develops a platelet count decrease of 30 - 50%, but onset is not clear; there is no thrombosis and other causes of thrombocytopenia are possible.
C. A patient experiences a platelet count decrease of less than 30%, without thrombosis, with other possible causes which occurs two weeks after heparin exposure.
D. A patient develops a platelet count decrease of greater than 50% with onset one week after heparin exposure and develops new thrombosis with no other cause.
E. A patient develops recurrent thrombosis of unknown cause with thrombocytopenia and platelet count nadir of 15000/µL with no recent heparin exposure.

The correct answer is D. A patient develops a platelet count decrease of greater than 50% with onset one week after heparin exposure and develops new thrombosis with no other cause. 4Ts score: 8.
A is incorrect. A patient with a past history of thrombosis received last dose of LMWH a month ago and develops thrombocytopenia with platelet count 30,000/µL with other possible causes and no new thrombosis. 4Ts score: 2.
B is incorrect. A patient develops a platelet count decrease of 30 - 50%, but onset is not clear; there is no thrombosis and other causes of thrombocytopenia are possible. 4Ts score: 3.
C is incorrect. A patient experiences a platelet count decrease of less than 30%, without thrombosis, with other possible causes which occurs two weeks after heparin exposure. 4Ts score: 2.
E is incorrect. A patient develops recurrent thrombosis of unknown cause with thrombocytopenia and platelet count nadir of 15000/µL with no recent heparin exposure. 4Ts score: 3.

REFERENCE

1. 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.
2. Cuker A, Arepally GM, Chong BH, *et al*. American Society of Hematology 2018 guidelines for management of venous thromboembolism: heparin-induced thrombocytopenia. *Blood Adv.* 2018;2(22):3360-3392.

QUESTION 2 OBJECTIVE

Understand the correct application of HIT IA and SRA functional testing to reduce excess testing and avoid alternative anticoagulant therapy.

QUESTION 2

Which of the following statements regarding laboratory testing for HIT is most accurate?

A. The HIT IA frequently yields false-negative results.
B. The HIT IA has high sensitivity and has a high negative predictive value.
C. The HIT IA has high diagnostic specificity.
D. The SRA is easy to perform and has high diagnostic sensitivity.
E. The heparin-induced platelet aggregation assay (HIPAA) is easy to perform, reproducible and has high sensitivity.

The correct answer is B. The HIT IA has high sensitivity and has a high negative predictive value.
A is incorrect. The HIT IA can yield false positive results in a low prevalence population, leading to follow-up and treatment.
C is incorrect. The IA has high diagnostic sensitivity, but lower specificity.
D is incorrect. The SRA is a complex test with high diagnostic specificity, but sensitivity is < 100%.
E is incorrect. The heparin-induced platelet aggregation assay (HIPAA) has high specificity, but sensitivity is < 100%.

REFERENCES

1. Linkins LA, Bates SM, Lee AY, 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. Warkentin TE, Sheppard JI, Linkins LA, Arnold DM, Nazy I. Performance characteristics of an automated latex immunoturbidimetric assay [HemosIL((R)) HIT-Ab(PF4-H)] for the diagnosis of immune heparin-induced thrombocytopenia.*Thromb Res.* 2017;153:108-117.

QUESTION 3 OBJECTIVE

Recognize the pitfalls of performing HIT testing in patients with a low pre-test probability 4Ts score.

QUESTION 3

What are the potential consequences of an incorrect diagnosis of heparin-induced thrombocytopenia (HIT)?

A. Withholding heparin from patients who need anticoagulant therapy, with a resulting increased thromboembolic risk.
B. Misdiagnosis of other thrombocytopenic disorders, causing a missed opportunity for correct therapy.
C. Implementation of alternate anticoagulants that may be difficult to manage and can result in increased bleeding risk.
D. Need for follow-up testing (eg, SRA).
E. All of the above

The correct answer is E, all of the above
All of the above answers are consequences of incorrectly classifying HIT.

REFERENCES

1. Linkins LA, Bates SM, Lee AY, 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. Hicks LK, Bering H, Carson KR, et al. Five hematologic tests and treatments to question. *Blood.* 2014;124(24):3524-3528.

QUESTION 4 OBJECTIVE

Understand the application of pre-test probability determination in HIT IA testing.

QUESTION 4

Which result indicates a correct HIT testing decision based on the result of the calculated 4Ts score?

A. The calculated 4Ts score is uncertain and the IA is positive; perform repeat IA.
B. The calculated 4Ts score is low (0-3) and the score is certain; HIT is unlikely.
C. The calculated 4Ts score is intermediate (4-5) and the IA is positive; HIT is likely, SRA not needed.
D. The calculated 4Ts score is high (6-8); HIT is likely; IA/SRA not needed.
E. Calculated 4Ts score is Intermediate (4-5) and the IA is weakly positive, SRA is negative; continue heparin.

The correct answer is B, The calculated 4Ts score is low (0-3) and the score is certain; HIT is unlikely.
A is incorrect. A positive functional SRA assay indicates HIT is likely, requiring appropriate HIT anticoagulation management with heparin discontinuation. When the SRA is negative, HIT is unlikely and may be managed accordingly. Repeat immunoassay (IA) is not likely to be informative in this situation.
C is incorrect. The SRA is needed to differentiate between 'HIT possible' and 'HIT likely' categories.
D is incorrect. HIT is likely; however, if the IA and SRA are negative, the patient may have a worse prognosis due to the presence of non-HIT causes.
E is incorrect. HIT is possible; discontinue heparin, consider an alternate anticoagulant, repeat IA/SRA.

REFERENCES

1. Linkins LA, Bates SM, Lee AY, 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. Linkins LA, Warkentin TE. The approach to heparin-induced thrombocytopenia. *Semin Respir Crit Care Med.* 2008;29(1):66-74.
3. Greinacher A. Heparin-Induced Thrombocytopenia. *N Engl J Med.* 2015;373(3):252-261.

MODULE 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. https://doi.org/10.1182/asheducation-2003.1.497