The Laboratory’s Role in the Evaluation of Heparin-Induced Thrombocytopenia

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Instrumentation Laboratory
Objectives

• Review the pathophysiology of Heparin-Induced Thrombocytopenia (HIT)
• Describe the clinical significance of HIT
• Explore current diagnostic lab tests for HIT
• Discuss benefits of rapid, on-demand HIT testing
Definition of Heparin-Induced Thrombocytopenia (HIT)

• HIT is an adverse affect of heparin therapy causing an immune-mediated disorder that increases risk for venous and arterial thrombosis, and can lead to increased morbidity and mortality
• Caused by the development of platelet-activating antibodies directed against Platelet Factor 4/Heparin complexes

Pathogenesis of HIT

Pathophysiology of HIT

Orgaran - International Guidelines for Diagnosis and Treatment. Poster presented at ISTH, 2005.
Antibody Formation in HIT

• PF4 becomes immunogenic when it binds to heparin, eliciting antibody production by circulating B-lymphocytes

• Three classes of antibodies are produced
  - IgG, IgM, IgA

• IgG appears to be the pathogenic platelet-activating antibody
  - Leads to thrombocytopenia via binding to the FcγRIIa receptor on platelets

• IgM and IgA are not considered to be pathogenic as they should not crosslink the FcγRIIa receptor
  - Have been reported to be associated with HIT-related thrombotic complications in a few studies

• Heparin can bind nonspecifically to plasma proteins in some patients, leading to antibody binding to heparin chemokine complexes → HIT without typical heparin-PF4 antibodies

Clinical Overview of HIT

• Syndrome associated with the development of immune-mediated thrombocytopenia
  - 8% of patients receiving heparin develop antibody to PF4-heparin
  - 1-5% will develop HIT with thrombocytopenia

• Results in a prothrombotic diathesis that may lead to significant morbidity and mortality
  - Up to 50% of patients present with complicating venous or arterial thrombosis
  - 10-20% of patients may require limb amputation
  - 20-30% mortality

• Occurs in
  - Up to 5% of patients receiving UFH
  - Up to 1% of patients receiving LMWH
Heparin-Associated Thrombocytopenia (HAT)

- Benign condition, frequency 10-20% of patients
- **Nonimmune-mediated** mechanism resulting in thrombocytopenia
- No heparin-dependent antibodies present
- Heparin therapy continued
Heparin-Induced Thrombocytopenia
HIT Type II

• Serious, life-threatening condition
• **Immune-mediated** mechanism resulting in thrombocytopenia and thrombosis
• Heparin-dependent antibodies present
• Heparin therapy discontinued
## HAT vs HIT Clinical Manifestation

<table>
<thead>
<tr>
<th></th>
<th>HAT</th>
<th>HIT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frequency</strong></td>
<td>10-20%</td>
<td>1-3%</td>
</tr>
<tr>
<td><strong>Time of onset</strong></td>
<td>1-4 days</td>
<td>5-10 days</td>
</tr>
<tr>
<td><strong>Platelet nadir</strong></td>
<td>100,000</td>
<td>30-55,000 (50% drop in platelet count)</td>
</tr>
<tr>
<td><strong>Antibody-mediated</strong></td>
<td>No—thrombocytopenia due to platelet activation</td>
<td>Yes—antibodies bind to PF4</td>
</tr>
<tr>
<td><strong>Thromboembolic sequelae</strong></td>
<td>None</td>
<td>30-80%</td>
</tr>
<tr>
<td><strong>Hemorrhagic sequelae</strong></td>
<td>None</td>
<td>Rarely</td>
</tr>
<tr>
<td><strong>Management</strong></td>
<td><strong>Observe</strong>—patient may be maintained on Heparin if indicated</td>
<td><strong>Immediate cessation</strong> of Heparin—alternate anticoagulation if indicated</td>
</tr>
</tbody>
</table>

## Factors Associated with HIT

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Highest Risk</th>
<th>Moderate Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administration</td>
<td>IV (high dose)</td>
<td>SC (low dose)</td>
</tr>
<tr>
<td>Type</td>
<td>UFH</td>
<td>LMWH</td>
</tr>
<tr>
<td>Source</td>
<td>Bovine</td>
<td>Porcine</td>
</tr>
<tr>
<td>Patient</td>
<td>Surgical, Coronary Artery Bypass Grafting, Orthopedic</td>
<td>Medical</td>
</tr>
</tbody>
</table>
Clinical Presentation of HIT

- Platelet count fall > 50% from baseline
- Venous and/or arterial thromboses
- Skin necrosis
- Anaphylactic reactions

Medical illustration of Venous Thrombosis and Pulmonary Embolism
Clinical Presentation

• Thrombocytopenia is the most common clinical manifestation of HIT

• Important features of the thrombocytopenia include
  - Timing of the onset of the thrombocytopenia
  - Severity of the thrombocytopenia
  - Course of the platelet count
    • Following withdrawal of heparin
    • While still on heparin
Manifestations of HIT

Skin Necrosis

Venous Limb Gangrene


Typical Onset HIT

• Thrombocytopenia
  - Generally occurs **5-10 days** after the initiation of heparin therapy
  - Platelet count rarely drops to severe levels (<50), unlike other types of immune-mediated thrombocytopenia (*i.e.*, ITP, TTP)

• Thrombosis
  - Major clinical presentation
Rapid-Onset HIT

- Recent prior exposure to heparin
- The following can occur in patients sensitized to heparin within 5–30 minutes:
  - Fever, chills
  - Tachycardia, hypertension
  - Flushing, headache
  - Chest pain, dyspnea
  - Nausea, vomiting, large-volume diarrhea
  - Transient global amnesia
  - Sudden “anaphylactoid” death

Delayed-Onset HIT

- Occurs a **week or more** after heparin cessation
- Patients typically demonstrate a further decrease in platelet count
- Should be suspected in a patient with recent heparin exposure who presents with:
  - thrombosis or
  - thrombocytopenia
- Immune-mediated

Frequency of HIT

• Activation assays are more specific than antigen assays for clinical HIT
• Incidence of HIT varies with clinical setting

Evaluation of HIT
Recommended Procedure for HIT Diagnosis

1. Clinically assess probability for HIT (e.g., 4T score).
   a. Patients with Low probability (≤ 3) do not need further testing and Heparin can be maintained.

2. If clinically indicated, order HIT screening test
   a. If HIT antibodies not present, HIT is unlikely and heparin can be maintained if clinical pre-test probability is not high
   b. If HIT antibodies present, confirmatory testing is needed

# Clinical Probability: The 4Ts

<table>
<thead>
<tr>
<th>Points for each category; maximum score = 8</th>
</tr>
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<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>Timing of platelet count fall or other sequelae</td>
</tr>
<tr>
<td>Thrombosis or other sequelae (ie. skin lesions)</td>
</tr>
<tr>
<td>Other causes for thrombocytopenia are not evident</td>
</tr>
</tbody>
</table>

**Probability of HIT score:**

6-8 = High; 4-5 = Intermediate; 0-3 = Low

Laboratory Testing

- Detection of antibodies against PF4/Heparin complexes
  - ELISA
    - Commercial
    - Home-brew
  - Particle-based Immunoassays
  - HemosIL® assay

- Functional assays
  - Serotonin Release Assay (SRA)
  - Heparin-Induced Platelet Aggregation (HIPA)

- Recommends:
  - 4Ts scoring
  - HIT antibody testing
  - Functional assay testing

Recognition and Treatment of HIT

• Prompt recognition is key because heparin therapy must be immediately suspended
  - Patients should be treated with alternative, non-heparin anticoagulants (*i.e.*, argatroban, bivalirudin, fondaparinux)

• Challenges:
  - Alternative drugs are more expensive than heparin
  - Alternative drugs have an increased bleeding risk
  - Patient management with alternative drugs can be very challenging:
    • No antidote
    • Transition to warfarin (Direct thrombin inhibitors prolong PT)
Laboratory Evaluation of HIT
Immune-Based Assays

- ELISA-based assay
- Particle Immunofiltration or particle immunoagglutination
- Immunoturbidimetry
ELISA-Based Assay

Legend
- Anti-human IgG + AP
- Heparin Antibody
- Heparin:PF4 complex

Patient Plasma Sample

PF4 and Heparin coated ELISA plate

Wash

Tagged goat anti-human Ig+ alkaline phosphatase

Detect absorbance

Substrate
PIFA-4

- **Particle Immunofiltration Assay**
- Dyed microparticles coated with purified PF4
- Sample is moved through a filter medium to react with PF4
- Reactive/non-reactive

http://akersbiosciences.com/pdf/pifa_instruction%20sheet_ce_v3_4_26_05.pdf
HemosIL HIT-Ab\textsubscript{(PF4-H)}

The first, on-demand, qualitative, fully automated assay for the detection of anti-platelet factor 4/heparin (PF4/H) antibodies in human 3.2% or 3.8% citrated plasma on the ACL TOP Family of instruments in a laboratory setting.*

The on-demand solution for fast PF4-H antibody detection

- Simple, Fast
  - Fully automated, liquid, ready-to-use
  - Results available 24 hours/day, 7 days/week
  - Results in minutes

*For adult population. To be used in conjunction with other laboratory and clinical findings.
HemosIL HIT-Ab\textsubscript{(PF4-H)}

- **Analytical excellence**
  - Detects total immunoglobulin against PF4-H complexes
  - Dedicated controls for a complete quality management program
  - Excellent agreement with commercially available ELISAs

- **Cost-effective**
  - Fully automated—no manual processes required
  - Faster response impacts cost and quality of care
HemosIL HIT-Ab\textsubscript{(PF4-H)} Assay Principle

<table>
<thead>
<tr>
<th>HemosIL HIT Kit</th>
<th>+ HIT Sample</th>
<th>No agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex coated with mAb</td>
<td>PF4-H</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>+ Normal Sample</th>
<th>Agglutination</th>
</tr>
</thead>
</table>
Serotonin Release Assay (SRA)

Radio-labeled serotonin uptake by platelet → Binding of PF4-H antibodies → Activation of platelet & serotonin release

SRA and Heparin Dose

Heparin-Induced Platelet Aggregation (HIPA)

- HIPA examines platelet aggregation at 3 different heparin concentrations

- Diagram showing aggregation at 0 U/ml, 0.5 U/mL, and 100 U/mL Heparin, with >25% aggregation indicated.
## Laboratory Testing for HIT

<table>
<thead>
<tr>
<th>Test</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ELISA</strong></td>
<td>HIGH Sensitivity (75-90%)</td>
<td>LOW Specificity</td>
</tr>
<tr>
<td></td>
<td>Technically easy</td>
<td>False positives (for some populations)</td>
</tr>
<tr>
<td></td>
<td>3 hr TAT</td>
<td></td>
</tr>
<tr>
<td><strong>PIFA</strong></td>
<td>HIGH Sensitivity</td>
<td>Limited Clinical History</td>
</tr>
<tr>
<td></td>
<td>HIGH Specificity</td>
<td>Pos/Neg controls not provided</td>
</tr>
<tr>
<td></td>
<td>Technically easy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rapid TAT</td>
<td></td>
</tr>
<tr>
<td><strong>Immunoturbidimetric</strong></td>
<td>On demand 24/7</td>
<td>Dedicated instrumentation</td>
</tr>
<tr>
<td></td>
<td>Technically easy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liquid, ready to use</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rapid TAT</td>
<td></td>
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<tr>
<td></td>
<td>Excellent agreement with ELISA</td>
<td></td>
</tr>
<tr>
<td><strong>SRA</strong></td>
<td>HIGH Sensitivity, HIGH Specificity (~99%)</td>
<td>Technically demanding</td>
</tr>
<tr>
<td></td>
<td>False positives rare</td>
<td>Radioisotopes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not routinely available</td>
</tr>
<tr>
<td><strong>HIPA</strong></td>
<td>HIGH Specificity</td>
<td>LOW Sensitivity (29-82%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Technique dependent</td>
</tr>
</tbody>
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Treatment of HIT
Treatment of HIT

- Discontinue all heparin immediately, including
  - Heparin flushes
  - Heparin-coated pulmonary catheters
  - Heparinized dialysate and any other medications or devices containing heparin
- Initiate alternative anticoagulation therapy
- Confirm diagnosis of HIT with appropriate laboratory tests
- Monitor patient carefully for thrombosis
- Monitor platelet counts until recovery
- Avoid prophylactic platelet transfusions
Direct Thrombin Inhibitors used to treat HIT

• Argatroban
  - Synthetic and reversible, direct thrombin inhibitor from L-arginine
  - Half-life—40 minutes
  - Monitored by aPTT
  - Elevates PT/INR
  - Excreted by liver
  - Approved for the prophylaxis or treatment of thrombosis in patients with HIT
• Bivalirubin and Fondaparinux also used. Less common

HIT Diagnosis in Current Practice

1. In current clinical practice two scenarios may occur:
   • Patients with > 50% drop in platelet count have heparin immediately suspended and alternative anticoagulants are administered with no HIT testing
   • **Result:** Overuse of alternative anticoagulant treatments

2. Patients with >50% drop in platelet count have heparin immediately suspended and alternative anticoagulants are administered with HIT testing
   • HIT antibodies testing is immediately performed in hospital with ELISA methods
     Low efficiency in reagent use, very high testing costs
   • HIT antibodies testing is performed in batch or sent out to reference labs
     Delay in diagnostic response, overuse of alternative drugs, revert back to Heparin if patient is negative

Value of HIT Antibody Assays

- Evaluating patients suspected of HIT
  - Sensitivity: 
    ~99%
  - Specificity: 
    50-75% (IgG/M/A)
    55-90% (IgG)

Determining Risk of HIT

- A rapid, 24/7, fully automated, reliable test can promptly identify patients without HIT antibodies in approximately 90% of suspected patients and provide physicians with highly relevant information before determining anticoagulant alternatives.
- ~10% antibody positive samples will require further functional tests for confirmation.
- Approximately 50% of patients testing positive with HIT IgG tests are negative with functional assays.

Testing Algorithm

Clinical Assessment (The 4Ts score)

Intermediate or High Probability

HIT-Ab Testing

Positive HIT-Ab
~ 10% of patients

Further testing and review of clinical symptoms required to determine appropriate anticoagulant therapy

Low Probability

Negative HIT-Ab
~ 90% of patients

Consult original clinical assessment to determine heparin therapy continuation
Importance of HIT Antibody Testing

- If HIT antibodies are present, the clinicians must be informed immediately

- With this information, they can then decide to continue heparin or suspend it and replace with alternative anticoagulants
Why Can’t They Just Switch to New Anticoagulants?

• Expensive

• More difficult to manage (e.g., bleeding, no antidote, transition to warfarin)

• A HIT diagnosis increases length of stay; thus, an erroneous diagnosis leads to unnecessary hospital costs

• HIT Antibody testing improves patient care and can save costs
When to Suspect HIT and When to Test

Has the patient:
• Been treated with heparin (UFH or LMWH)
• Experienced a platelet-count drop >50% within 5-10 days of heparin exposure
• Presented with thrombosis, skin necrosis, anaphylactic response
• HIT Antibody testing is key in determining the presence or absence of PF4-H antibodies, which are those ultimately responsible for the thrombocytopenia
• IgG HIT antibodies are considered more specific since this isotype can activate platelets.
• However, cases of HIT with IgM and IgA isotypes have also been reported
Which Test?

The value of HIT antibody testing relies mainly on negative predictive value rather than positive predictive value. That is, identifying the patient population that does not have HIT:

- IgG positive samples are not necessarily HIT (≈ 50%)
- 90% of patients suspected of HIT are negative, which makes HIT antibody testing extremely valuable independent of the isotype
Conclusions

• HIT is a serious condition that requires fast results
• A rapid, on-demand fully automated test can:
  - Improve patient safety and the quality of patient care
  - Result in significant hospital cost savings
  - Improve the lab's service to clinicians
Our Passion.
Your Results.