Heparin-Induced Thrombocytopenia Pathophysiology and Diagnosis

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Objectives

- Pathophysiology of HIT
- Diagnosis of HIT
  - Clinical
  - Laboratory
- Laboratory monitoring patients treated for HIT
1916 discovered heparin as a 2nd year medical student at Johns Hopkins
1935 first to use heparin clinically (University of Toronto)
Heparin

- 12 million patients exposed annually in the USA

- 1 TRILLION units given annually in the USA
Heparin Production

- Found to be an oversulfated chondroitin sulfate contaminant
Heparins

- **Unfractionated**
  - > 17 saccharide units

- **Low Molecular Weight**
  - 6-17 saccharide units

- **Pentasccharides**
  - 5 saccharide units
Member of heterogeneous family of glycosaminoglycans; MW=3,000–40,000 daltons

Platelet Factor 4 (Front View)

Antigen Site 1
(Pro 37)

Antigen Site 2
(Asp7-Gln, Pro34)

Lys (K) residues of C-Terminus

Other Lys(K) and Arg (R) residues

Ring of Positive Charge

http://www.asheducationbook.org/content/vol2003/issue1/images/large/Warkentin_fig4color.jpeg
PLATELET ACTIVATION & THROMBIN GENERATION
Any clinical event explained by “HIT” antibodies (platelet Factor 4 (PF4)/heparin reactive antibodies) in a patient who is receiving or recently received heparin.

Most patients show a 50% fall in platelet count which may or may not bring them into thrombocytopenia range

Clinical importance stems from paradoxical association with thrombosis in 35 –70% of patients. (HITT)
Relative Risk of Thrombosis

HIT compared to other Inherited Hypercoagulable States

Warkentin TE. Can J Cardio, May 1995; 11 Suppl C: 29C-34C
HIT-Medical-Legal Issues

Average Settlement for HIT in US $2 million

- Informed consent
- Platelet count surveillance
- Diagnosis
- Treatment

Honoré Daumier. Three Lawyers c. 1862-65.
The Phillips Collection, Washington, DC
Diagnosis of HIT

- **Clinical assessment**
  - high index of suspicion
  - awareness of clinical events associated with PF4/heparin antibodies

- **Laboratory assessment**
Thromboembolic and other clinical manifestations associated with HIT

- Venous thrombosis
- Arterial thrombosis
- Other complications
  - Adrenal hemorrhagic infarction
  - Heparin-induced skin lesions (at injection sites)
  - Acute systemic reactions (post IV heparin bolus)
  - Disseminated intravascular coagulation
Skin Necrosis
Causes of Thrombocytopenia in Adults

1. Increased Platelet Destruction
   - Non-immune
     - Septicemia/Inflammation
     - Disseminated intravascular coagulation
   - Immune
     - Autoimmune: Idiopathic or secondary immune thrombocytopenia, TTP
     - Alloimmune: Post-transfusion purpura,
     - Drug-induced: Heparin, gold, quinine, quinidine, sulfa antibiotics, rifampin, vancomycin, nonsteroidal antiinflammatory drugs, many others

2. Decreased Platelet Production
   - Alcohol, cytotoxic drugs
   - Aplastic anemia
   - Leukemia, myelodysplasia
   - Metastatic invasion of marrow
   - Certain infections

3. Hypersplenism
4. Hemodilution
   - Infusion of blood products, colloids, or crystalloids
Platelet Count Nadirs in HIT Patients w/ and w/o Thrombosis

![Graph showing platelet count nadirs in HIT patients with and without thrombosis.](chart.png)

- **Median platelet count nadir, 55 x 10⁹/L**
- **Platelet fall <50%**

Warkentin & Greinacher, *Heparin Induced Thrombocytopenia*, 3rd Ed, 2004
Clinical Diagnosis of HIT: The 4 T’s

<table>
<thead>
<tr>
<th>Suspicion of HIT based upon the “4 T’s”</th>
<th>Pre-test Probability Score Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td><strong>Thrombocytopenia</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>nadir 20-100, or &gt;50% platelet fall</td>
</tr>
<tr>
<td><strong>Timing of onset of platelet fall</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>day 5-10, or ≤day 1 with recent heparin*</td>
</tr>
<tr>
<td><strong>Thrombosis or other sequelae</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>proven thrombosis, skin necrosis, or ASR†</td>
</tr>
<tr>
<td><strong>Other cause of platelet fall</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>none evident</td>
</tr>
</tbody>
</table>

| Total Pre-test Probability Score       |    |    |    |
|                                        |  □ |    |    |
|                                        | periodic reassessment as new information can change pre-test probability (e.g., positive blood cultures) |

<table>
<thead>
<tr>
<th>Total Pre-test Probability Score</th>
<th>High</th>
<th>Moderate</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

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Stop heparin‡, give alternative non-heparin anticoagulant
argatroban¶ or lepirudin# or danaparoid** (or bivalirudin†† or fondaparinux‡‡)

Physician judgment

Continue (LMW) heparin

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Warkentin ASH Handbook 2003
Heparin Ab.
Thrombosis
Thrombocytopenia

Heparin Ab.
Thrombocytopenia

Heparin Ab.
Occurrence of HIT in Orthopedic Surgery
UFH vs. LMWH

<table>
<thead>
<tr>
<th>Test</th>
<th>UFH (%)</th>
<th>LMWH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive PF-4/H ELISA</td>
<td>15%</td>
<td>8%</td>
</tr>
<tr>
<td>Positive Platelet Activation Assay</td>
<td>8%</td>
<td>3%</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>5%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>3.0%</td>
<td>0.3%</td>
</tr>
</tbody>
</table>

Ref #1
Platelet Activation by Antibodies against the PF4–Heparin Complex.

Serotonin Release Assay

1. Washed Donor Platelets (FcγRIIA 131 His/His or His Arg) + C-14 Serotonin

2. Heparin & inactivated patient serum

3. Incubated Platelets w/patient serum for 1 hour at RT at 600 rpm


5. Add supernatant to scintillation fluid

6. Beta Counter
Confidential – Do not copy or distribute

Platelets + Buffer
- 0%
- 0%
- 0%
- 0%

Platelets + Triton
- 100%
- 100%
- 100%
- 100%

Platelets + Patient serum
- 0.1 Hep.
- 0.5 Hep.
- 100 Hep.
- Buffer

Platelets + Pos control serum
- 0.1 Hep.
- 0.5 Hep.
- 100 Hep.
- Buffer

Platelets + Neg control serum
- 0.1 Hep.
- 0.5 Hep.
- 100 Hep.
- Buffer

Positive SRA = >= 20%

Release w/ 0.1 and 0.5 uHep and inhibition w/ 100 uHep

LMWH

LMWH

LMWH
100% = Platelets + Triton
(all platelets lysed)

0% = Platelets + Buffer
(background)

% Release = \( \frac{\text{(Test sample- Background counts)}}{\text{(100% release – Background)}} \) X 100
Platelet Aggregation Study With Heparin
Specific laboratory tests for HIT

Antigen assays

Platelet activation assays

Enzyme-Linked Immunoassay (ELISA)

Platelet Activation Assays
- c-Platelet Rich Plasma (unwashed)
- HIPA (washed)
- Serotonin Release Assay (SRA)

More sensitive
Less specific
Technically simple
Standardized

Less sensitive
More specific
Technically demanding
Not standardized
PFP4 PF4 Complex

Microtiter Plate

Color

p-nitrophenyl phosphate (PNPP)

Alkaline Phosphatase

Goat anti-human anti-IgG/IgM/IgA

Antibody in patient’s serum

PF4 Complex

Heparin/PF4 ELISA Assay
### Results/Interpretation Guide

<table>
<thead>
<tr>
<th>TEST Window</th>
<th>CONTROL Window</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO Blue</td>
<td>ANY Red</td>
<td>Positive/Reactive</td>
</tr>
<tr>
<td>ANY Blue*</td>
<td>ANY Red</td>
<td>Negative/Non-reactive</td>
</tr>
<tr>
<td>NO Blue</td>
<td>NO Red</td>
<td>Invalid</td>
</tr>
<tr>
<td>ANY Blue*</td>
<td>NO Red</td>
<td>Invalid</td>
</tr>
</tbody>
</table>

*Intensity of BLUE color in TEST window may vary.
Particle Gel Immunoassay: Heparin/PF4 Test

- Incubate serum + red-dyed particles coated with PF4/H
- After centrifugation particles agglutinate
  - remains on top: strong positive
  - disperses within gel: weak positive
  - sediments to bottom: negative

DiaMed
ANTI-PF4 ELISA **Summary**

- Sensitivity Cut-off is 0.4 OD
- Specificity for HIT increases > 1.5 OD
- Patients with negative results just below the cut-off of 0.4 OD have a high probability of having positive results on repeat testing a few days later
- Heparin neutralizing step possible
Comparison of ELISA and SRA in HIT Antibody Detection

<table>
<thead>
<tr>
<th>Diagnostic Assay</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin/PF4 ELISA</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>Platelet SRA</td>
<td>90-98%</td>
</tr>
<tr>
<td>ELISA and SRA</td>
<td>100%</td>
</tr>
</tbody>
</table>

Adapted from Warkentin TE and Greinacher A. Chest, Sep 2004; 126: 311 - 337
Thrombocytopenia in a patient receiving heparin or LMWH

- High or intermediate clinical suspicion of HIT
  - Discontinue heparin or LMWH; initiate alternative anticoagulant treatment
  - Results of immunoassay
- Low clinical suspicion of HIT
  - Heparin or LMWH therapy may be continued
  - Consider alternative diagnosis

Arepally GM NEJM 2006
**Result of Immunoassay**

- **Positive with high suspicion of HIT**
  - HIT confirmed

- **Positive with intermediate suspicion of HIT**
  - Results of functional assay

- **Negative with high suspicion of HIT**
  - Consider alternative diagnosis, HIT intermediate

- **Negative with intermediate suspicion of HIT**
  - Consider alternative diagnosis; can restart heparin

**Positive**
- HIT likely

**Negative**
- HIT indeterminate

*Arepally GM NEJM 2006*
Treatment of Suspected HIT

- Discontinue all heparin immediately
  - Heparin flushes
  - Heparin-coated pulmonary catheters
  - Heparinized dialysate and any other medications or devices containing heparin

- Avoid platelet transfusions

- Alternative anticoagulation

- Monitor carefully for thrombosis

- Confirm diagnosis of HIT with laboratory test

- Monitor platelet counts until recovery
The Coagulation Cascade

# Medications Used for Treatment of Heparin-Induced Thrombocytopenia

<table>
<thead>
<tr>
<th>Agent</th>
<th>Clearance</th>
<th>Half-Life</th>
<th>Antidote</th>
<th>Dosing Regimen</th>
<th>Monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argatroban</td>
<td>Hepatobiliary</td>
<td>40–50 min</td>
<td>No</td>
<td>Intravenous infusion of 2.0 μg per kilogram of body weight per minute (no bolus); decrease initial infusion to 0.5–1.2 μg per kilogram per minute in patients with liver disease or critical illness or after cardiac surgery</td>
<td>Adjust dose to maintain activated partial-thromboplastin time at 1.5–3.0 times baseline value (maximum 10 μg per kilogram per minute)</td>
</tr>
<tr>
<td>Desirudin</td>
<td>Renal</td>
<td>2–3 hr</td>
<td>No</td>
<td>Fixed subcutaneous dose of 15 or 30 mg every 12 hr (most appropriate dose for treatment if heparin-induced thrombocytopenia has not been determined)</td>
<td>None required</td>
</tr>
<tr>
<td>Bivalirudin</td>
<td>Enzymatic and renal</td>
<td>25 min</td>
<td>No</td>
<td>Dose not established; 0.15–2.0 mg per kilogram per hour (no bolus) has been suggested</td>
<td>Adjust dose to maintain activated partial-thromboplastin time at 1.5–2.5 times baseline value</td>
</tr>
<tr>
<td>Danaparoid</td>
<td>Renal</td>
<td>24 hr</td>
<td>No</td>
<td>Intravenous bolus (1500 U if patient &lt;60 kg; 2250 U if 60 to &lt;75 kg; 3000 U if 75 to 90 kg; 3750 U if &gt;90 kg) followed by intravenous infusion of 400 U per hour for 4 hr, 300 U per hour for 4 hr, then 150–200 U per hour</td>
<td>Adjust to anti-Xa activity of 0.5–0.8 U per milliliter (with use of danaparoid standard curve)</td>
</tr>
<tr>
<td>Fondaparinux</td>
<td>Renal</td>
<td>17–20 hr</td>
<td>No</td>
<td>5.0 mg subcutaneously once daily for patients &lt;50 kg; 7.5 mg for 50–100 kg; 10.0 mg for &gt;100 kg</td>
<td>None required</td>
</tr>
</tbody>
</table>

Anticoagulant monitoring in HIT

Lepirudin
- aPTT X 1.5 – 2.0 baseline
- Half life affected by renal function
- Ecarin clotting time for coronary artery by-pass

Bivalirudin
- aPTT X 1.5 –2.0 baseline
- 20% renal excretion 80% enzymatic

Argatroban
- aPTT X 1.5 –3.0 baseline
- Half life affected by liver function

Pentasaccharide (Fondaparinux)
- Anti-Xa
- Half life affected by renal function
In patients with strongly suspected or confirmed HIT, we recommend against starting VKA until platelets have substantially recovered (ie, usually to at least $150 \times 10^9/L$) over starting VKA at a lower platelet count and that the VKA be initially given in low doses (maximum, 5 mg of warfarin or 6 mg phenprocoumon) over using higher doses (Grade 1C).
Cotherapy with Warfarin

- Start warfarin (low dose) after thrombocytopenia resolved
- At least 5 days overlap

**INR**

- Argatroban > Bivalirudin > Lepirudin
- Aim for INR of 4
- Effect on INR is dependent on ISI of reagent
- Consider chromogenic X
Monitoring Coumadin with Chromogenic Factor X

- **INR < 2 Subtherapeutic**
- **INR 2-3 Therapeutic**
- **INR > 3 Supratherapeutic**

11-42% Activity
Case Vignette

- A 57-year-old man remains in the hospital after experiencing complications from knee-replacement surgery 7 days ago.
- Low-molecular-weight heparin prophylaxis is initiated on the first postoperative day.
- Compression ultrasonography performed for left leg swelling noted on day 7 shows a proximal deep-vein thrombosis.
- A complete blood count reveals that his platelet count has decreased from $300 \times 10^9$ per liter to $125 \times 10^9$ per liter, and an enzyme immunoassay for heparin-induced thrombocytopenia shows a high titer of antibodies against platelet factor 4 (PF4)–heparin complexes.
- The patient has normal renal function.
- The physician in the intensive care unit wonders about the best treatment.
Conclusions and Recommendations

- The patient described in the vignette has a high pretest probability of heparin-induced thrombocytopenia.

- We would perform a platelet-activating assay such as the serotonin-release assay to confirm the diagnosis; however, if such a test were not available, a strongly positive test for IgG anti-PF4–heparin antibodies would be sufficient for the diagnosis in this patient.

- Heparin should be immediately discontinued, and a nonheparin anticoagulant should be administered in therapeutic doses.

- Given his normal renal function, we recommend fondaparinux at a dose of 7.5 mg subcutaneously once daily.

- The platelet count should be followed closely, and a vitamin K antagonist should be started when the platelet count has recovered to at least $150 \times 10^9$ per liter.
Conclusions and Recommendations

- The administration of fondaparinux and the vitamin K antagonist should overlap for at least 5 days or until the INR is within the therapeutic range for 2 consecutive days.
- Since this patient had thrombosis with heparin-induced thrombocytopenia, we would recommend continuing the vitamin K antagonist for 3 months.
- The patient should be advised to avoid heparin, especially in the subsequent 3 to 4 months after the diagnosis of heparin-induced thrombocytopenia, and to consult with a specialist if heparin is needed in the future.
# Three presentations of HIT

<table>
<thead>
<tr>
<th>Typical Onset</th>
<th>Rapid-Onset</th>
<th>Delayed-Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thrombocytopenia</strong></td>
<td><strong>Thrombocytopenia</strong></td>
<td>With or without thrombocytopenia</td>
</tr>
<tr>
<td>*50% or more drop in platelet count from baseline or a count &lt; 150k</td>
<td>*Occurs within minutes to day(s)</td>
<td>Previous heparin exposure without complications</td>
</tr>
<tr>
<td>*Onset 5 – 14 days after heparin exposure</td>
<td>*Recent heparin exposure (&lt;100 days) Abrupt onset of platelet activation due to residual circulating HIT antibodies</td>
<td>Patient readmitted:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*9 to 40 days later</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*New thrombosis (usually)</td>
</tr>
<tr>
<td>Exclusion of other causes of thrombocytopenia</td>
<td>25-30% of all HIT cases</td>
<td>3-5% of all HIT patients</td>
</tr>
<tr>
<td>With or without thrombotic complications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66% of all HIT cases</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Questions
Platelet Count Monitoring Combined With the 4Ts Score for Patients Receiving Heparin/LMWH

For patients receiving heparin in whom clinicians consider the risk of HIT to be > 1%, we suggest that platelet count monitoring be performed every 2 or 3 days from day 4 to day 14 (or until heparin is stopped, whichever occurs first) (Grade 2C).
Platelet Transfusions

In patients with HIT and severe thrombocytopenia, we suggest giving platelet transfusions only if bleeding or during the performance of an invasive procedure with a high risk of bleeding (Grade 2C).
In patients with strongly suspected or confirmed HIT, we recommend against starting VKA until platelets have substantially recovered (ie, usually to at least \(150 \times 10^9/L\)) over starting VKA at a lower platelet count and that the VKA be initially given in low doses (maximum, 5 mg of warfarin or 6 mg phenprocoumon) over using higher doses (Grade 1C).
In patients with acute HIT (thrombocytopenic, HIT antibody positive) or subacute HIT (platelets recovered but still HIT antibody positive) who require urgent cardiac surgery, we suggest the use of bivalirudin over other nonheparin anticoagulants and over heparin plus antiplatelet agents (Grade 2C).
Patients Who Require Nonurgent Cardiac Surgery

In patients with acute HIT who require non-urgent cardiac surgery, we recommend delaying the surgery (if possible) until HIT has resolved and HIT antibodies are negative (see section 6.1) (Grade 2C).

Remarks: Other factors not covered by our analysis, such as drug availability, cost, and ability to monitor the anticoagulant effect may influence the choice of agent. For recommendations for patients with a past history of HIT (> 3 months previous) who require cardiac surgery, see section 6.1.
In patients with acute HIT or subacute HIT who require percutaneous coronary interventions, we suggest the use of bivalirudin (Grade 2B) or argatroban (Grade 2C) over other nonheparin anticoagulants.

*Remarks*: Other factors, such as drug availability, cost, and ability to monitor the anticoagulant effect, may influence the choice of agent.