Heparin induced thrombocytopenia in the critically ill: How to interpret anti-PF4 antibody test results

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Case Study

- 57-year-old female admitted with pneumonia and respiratory failure
- Admission platelet count was 230,000
  - Prophylactic UFH 5000 BID
- On the 7th ICU day, the patient arrested
  - Platelet count 110,000
- Pulmonary angiogram
- Thrombolytic therapy initiated
- HIT was confirmed by ELISA-PF4 assay
- Patient expired
Heparin-Induced Thrombocytopenia (HIT)

- Immune-mediated allergic reaction to heparin/platelet factor 4 complex
- Thrombocytopenia
  - Platelet count <100,000 or a 50% drop from baseline
    - <150,000 and or 33% drop used in more recently
  - Onset 5 to 14 days after heparin
  - With or without thrombotic complications
- Diagnosis is clinical
- Any type of heparin or route of administration can lead to HIT

Warkentin TE. Chest. 2004; 126:311S-337S
PF4 Tetramer

- At a neutral pH, a ring of positive charges is displayed on the PF4 tetramer.
- Negative charges on the linear heparin polysaccharide enable it to interact with the PF4.
- Heparin of sufficient polysaccharide size, spans the circumference of the PF4 molecule.

Warkentin TE. Br J Hemat. 2003; 535-555
## Factors Influencing the Frequency of HIT

<table>
<thead>
<tr>
<th>Factor</th>
<th>Influence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of heparin</td>
<td>Bovine UFH &gt; Porcine UFH &gt; LMWH</td>
</tr>
<tr>
<td></td>
<td>Intravenous &gt; subcutaneous</td>
</tr>
<tr>
<td></td>
<td>Therapeutic-dose &gt; prophylactic-dose</td>
</tr>
<tr>
<td>Patient population</td>
<td>Post surgery &gt; medical &gt; obstetrical</td>
</tr>
<tr>
<td>Duration of heparin</td>
<td>Each day of heparin use beyond day 5 increase risk of HIT</td>
</tr>
<tr>
<td>Sex</td>
<td>Female &gt; male</td>
</tr>
</tbody>
</table>

Adapted from Warkentin TE. British Journal Hematology. 2003; 121:535-555
Frequency of HIT in Critical Care

- Incidence of thrombocytopenia in ICUs is 20 - 45%
- 15% (n=40) of 748 consecutive patients in combined ICU/CCU met clinical criteria for HIT:
  - ≥ 2 consecutive platelet counts below 150,000 or a ≥ 33% decrease in platelet counts 5 or more days after heparin exposure
- Samples for diagnostic testing available for 32 of these 40 patients
  - Among these 32 patients meeting clinical criteria, and ELISA (+) anti-PF4 AB (frequency of HIT = 3.1%)
  - One patient tested positive by SRA, (frequency of 0.4%)

The 4Ts for HIT in ICU Patients

- Combined results of 3 prospective studies enrolling critically ill patients who were investigated for HIT if platelets fell to less than $50 \times 10^9/L$ or if platelet counts decreased to less than 50% of the value upon intensive care unit admission.
  - Of 528 patients: 50 (9.5%) were investigated for HIT
  - 39 (78%) of 50 (64%-88%) of these patients were scored as “low probability” by 4Ts score and none had a positive SRA.
  - Of 49 patients who underwent SRA testing because of thrombocytopenia, only 2 (4.1%; 0.5-14.0) had a positive SRA
    - 1 with a moderate 4Ts score and 1 with a high 4Ts score.
- Therefore, the overall incidence of HIT confirmed by SRA was 2 (0.4%) of 528 (0.04%-1.4%).

Severity of Thrombocytopenia and Thrombotic Complications in HIT

Adapted from Warkentin TE et al. Br J Hematol. 2003; 121:535-555
HITTS: Heparin-induced thrombocytopenia with thrombosis syndrome

- 30%-70% of all HIT patients develop thrombotic complications (HITTS)
  - Amputation: ~20%
  - Death: ~20% to 50%
Additional Clinical Issues Associated With HIT

- Deep venous thrombosis (50%)
- Pulmonary embolism (25%)
- Skin lesions at injection site (10%-20%)
- Acute limb ischemia (5%-10%)
- Warfarin-associated venous limb gangrene (5%-10%)
- Acute thrombotic stroke or myocardial infarction (3%-5%)

Surgery
- Venous thrombosis: more common in orthopedic surgery
- Arterial thrombosis: more common in cardiac procedures

Patients with thrombocytopenia have approximately a 50% risk of developing a thrombotic event within the first 30 days following diagnosis.

Time to a Negative Test for HIT

- 144 patients initially had positive tests for heparin-dependent antibodies and underwent subsequent testing within 180 days
  - Activation assay (144 patients)
  - Antigen assay (93 of the 144 patients)

## Estimating the Pretest Probability of HIT

<table>
<thead>
<tr>
<th>Points</th>
<th>2</th>
<th>1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thrombocytopenia</strong></td>
<td>&gt; 50% fall or platelet nadir 20,000 to 100,000</td>
<td>30-50% fall or platelet nadir 10,000 to 19,000</td>
<td>Fall &lt; 30% or platelet nadir &lt; 10,000</td>
</tr>
<tr>
<td>Timing of platelet count or other sequelae</td>
<td>Onset between 5 and 10 days or &lt; 1 day if heparin exposure within 100 days</td>
<td>Onset of thrombocytopenia after day 10</td>
<td>Platelet fall &lt; 5 days without prior heparin exposure</td>
</tr>
<tr>
<td>Thrombosis or other sequelae</td>
<td>New thrombosis, skin necrosis, post heparin acute systemic reaction</td>
<td>Progressive or recurrent thrombosis, erythematous skin lesions, suspected thrombosis</td>
<td>None</td>
</tr>
<tr>
<td>Other causes of thrombocytopenia</td>
<td>No other causes</td>
<td>Possible other causes</td>
<td>Definite other causes</td>
</tr>
</tbody>
</table>

**Pretest probability score:** 6-8 = high  4-5 = intermediate  0-3 = low

Adapted from Warkentin TE et al. Current Hematology Reports. 2003; 2:148 - 157
# Laboratory Assays to Confirm HIT Following Initiation of Therapy

<table>
<thead>
<tr>
<th>Assay</th>
<th>Component measured</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin-release assay (SRA)(^1)-(^3)</td>
<td>(14^C)-serotonin released from platelets</td>
<td>Highly sensitive and specific</td>
<td>Costly, time-consuming, and technically demanding (involves radioisotopes)</td>
</tr>
<tr>
<td>Heparin-induced platelet aggregation assay (HIPA)(^1)-(^3)</td>
<td>Platelet aggregation</td>
<td>Highly specific, easy</td>
<td>Low sensitivity and technique-dependent</td>
</tr>
<tr>
<td>Enzyme-linked immunosorbent assay (ELISA)(^1)-(^3)</td>
<td>Presence of heparin-dependent antibodies</td>
<td>Highly sensitive, easy, rapid turnaround time</td>
<td>Low specificity (false positives)</td>
</tr>
<tr>
<td>PIFA(^\circledR) Heparin/PF4 Rapid Assay (^4)</td>
<td>Presence of PF4 antibodies</td>
<td>Highly sensitive and specific, easy and rapid turnaround time</td>
<td>Fairly new with limited clinical history, positive and negative controls not provided</td>
</tr>
</tbody>
</table>

ELISA testing
Discordant Results from Two Widely Used Anti-PF4/Heparin ELISA Tests

- 142 archived patient sera were tested in the same freeze-thaw cycle with GTI and STAGO platforms.
- Manufacturer recommended positive breakpoints differ between platforms.
  - GTI positive test = optical density (OD) $\geq 0.400$.
  - STAGO positive test = OD range = 0.41 - 0.48).
- In a 65 patient subset, medical records were reviewed

<table>
<thead>
<tr>
<th>Optical Density (OD)</th>
<th>n</th>
<th>Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$&lt;0.4$</td>
<td>66</td>
<td>95%</td>
</tr>
<tr>
<td>$0.4 - &lt; 1.1$</td>
<td>35</td>
<td>31%</td>
</tr>
<tr>
<td>$\geq 1.1$</td>
<td>41</td>
<td>89%</td>
</tr>
</tbody>
</table>

Quantitative interpretation of optical density

- The risk of a strong-positive SRA result for five categories of OD reactivity (<0.40, 0.40–<1.00, 1.00–<1.40, 1.40–<2.00, and ≥2.00 OD units) using the two commercially available ELISAs.
  - A weak-positive result (0.40–<1.00 OD units) by ELISA indicated a low probability (≤5%) of a strong-positive SRA.
  - For every increase of 0.50 OD units in the ELISA–IgG, the risk of a strong-positive SRA result increased by OR = 6.39 [95% confidence interval (CI), 5.13, 7.95; P < 0.0001].
  - The probability of HIT antibodies being present reached ≥50% only when the OD level was ≥1.40 units.

Particle Immunofiltration Anti-Platelet Factor 4 (PIFA) Rapid Assay

- A negative (-) PIFA result: HIT unlikely in conjunction w/ clinical suspicion (eg. 4Ts score)
- A positive (+) PIFA result: need for further clinical and/or laboratory evaluation to make the diagnosis of HIT.

### 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.
Evaluation of the Particle Immunofiltration Anti-Platelet Factor 4 (PIFA) Rapid Assay

- Patients in the MICU were screened daily for thrombocytopenia
  - Platelet count that has decreased by at least 30% from baseline or is less than 150,000/uL.
- 143 consecutive patients had anti-PF4 laboratory testing.
- 100/143 patients had exposure to heparin.
- A clinical probability score for HIT known as the “the 4Ts” score was determined.
- Clinical data was collected for: age, sex, presence or absence of any DVT or PE, length of ICU stay, total hospital stay, vital status at hospital discharge.

Andrews DM et al. ACC. 2010 (abstract)
# Demographics, Baseline Characteristics and Outcomes

<table>
<thead>
<tr>
<th></th>
<th>PIFA</th>
<th>GTI ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>(n=65)</td>
<td>(n=33)</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>56±14</td>
<td>50±12</td>
</tr>
<tr>
<td>Platelet count</td>
<td>103±50</td>
<td>86±45</td>
</tr>
<tr>
<td>4Ts score, mean (SD)</td>
<td>3.9±1.1</td>
<td>3.2±1.0</td>
</tr>
<tr>
<td>Any VTE (n, %)</td>
<td>8 (12%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>LOS ICU (days)*</td>
<td>13±17</td>
<td>12±13</td>
</tr>
<tr>
<td>LOS Hospital (days)*</td>
<td>22±25</td>
<td>24±25</td>
</tr>
<tr>
<td>Hospital Mortality</td>
<td>16 (25%)</td>
<td>5 (15%)</td>
</tr>
</tbody>
</table>

Andrews DM et al. ACC. 2010 (abstract)
PIFA – GTI correlations*

<table>
<thead>
<tr>
<th></th>
<th>GTI ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n=87)</td>
</tr>
<tr>
<td>PIFA Positive (n=33)</td>
<td>7</td>
</tr>
<tr>
<td>PIFA Negative (n=65)</td>
<td>4</td>
</tr>
</tbody>
</table>

- Of the 65 patients with negative PIFA results, 61 also were negative by GTI ELISA. This represents a 93.8% concordance.
- Of the 4 discordant samples (PIFA negative and GTI ELISA positive) the average GTI ELISA optical density was 0.62±0.21

Andrews DM et al. ACC. 2010 (abstract)

* Since the abstract we ran SRA on all samples.
  - Of the 4 ELISA (+)/PIFA (-) results, all were SRA (-)
  - Of the 26 ELISA (-)/PIFA (+) results, 3 were SRA (+)
Similarities between evaluation of patients for HIT and DVT/PE

Clinical suspicion of DVT/PE in ambulatory patient

Chest pain
Leg Swelling

Heparin + Thrombocytopenia

Clinical suspicion of HIT in ICU patient

“Rule out” Lab test

D-Dimer

Anti-PF4

“Rule out” Lab test

D-Dimer Negative

D-Dimer Positive

Anti-PF4 Negative

Anti-PF4 Positive

DVT/PE unlikely in in conjunction w/ clinical suspicion (eg. Wells Score² or others)

DVT/PE unlikely in in conjunction w/ clinical suspicion (eg. 4Ts score³ or others)

Positive D-Dimer = further evaluation required.

Positive D-Dimer is not a “rule in” lab result for DVT/PE.

Positive PIFA result = need for further clinical and/or laboratory evaluation.

Positive PIFA results is not a “rule in” lab result for HIT.

Positive GTI ELISA anti-PF4 results = does not “rule in” clinical HIT.

Positive anti-PF4 ELISA results with OD less than 1.0 are infrequently positive by a SRA.

Andrews DM et al. ACC. 2010 (abstract)
Flow Chart: Suspected HIT in the ICU

Heparin exposure and Thombocytopenia

Send an Anti-PF4 test PIFA and ELISA

Clinical suspicion of HIT

“Rule Out” test

Anti-PF4 Positive:

PIFA Positive
Need for further clinical and/or laboratory evaluation. A positive PIFA results is not a “rule in” result for HIT.

ELISA Positive
Does not “rule in” clinical HIT. A Positive ELISA results with OD less than 1.0-1.5 are infrequently positive by a SRA.

Anti-PF4 (PIFA) negative: HIT unlikely, especially with a low or intermediate clinical suspicion (Warkentin’s 4Ts score ≤5)

SRA Positive
Best correlation with clinical HIT. The SRA is considered the “Gold Standard” or “Rule In” test.
New Point of Care Test

- True point of care testing
  - Requires 2-3 drops of whole blood
  - Same color scheme as the rapid PIFA test
  - Recently approved for clinical use.
  - Clinical trials being planned
Direct Thrombin Inhibitors: FDA Indications and Usage

- Argatroban
  - Indicated as an anticoagulant for prophylaxis or treatment of thrombosis in patients with HIT
  - Indicated as an anticoagulant in patients with or at risk for HIT undergoing PCI

- Lepirudin
  - Indicated for anticoagulation in patients with HIT and associated thromboembolic disease to prevent further thromboembolic complications

- Bivalirudin
  - Indicated as an anticoagulant in patients undergoing percutaneous transluminal coronary angioplasty (PTCA)
## Direct Thrombin Inhibitors: Pharmacologic and Clinical Parameters

<table>
<thead>
<tr>
<th></th>
<th>Argatroban</th>
<th>Lepirudin</th>
<th>Bivalirudin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition</strong></td>
<td>Synthetic L-arginine derivative</td>
<td>Recombinant hirudin</td>
<td>Synthetic hirulog</td>
</tr>
<tr>
<td><strong>Half-life in healthy subjects</strong></td>
<td>40-50 min</td>
<td>1.3 hrs</td>
<td>25 min</td>
</tr>
<tr>
<td><strong>Elimination</strong></td>
<td>Hepatic</td>
<td>Renal</td>
<td>Renal</td>
</tr>
<tr>
<td><strong>Monitoring needed</strong></td>
<td>aPTT, ACT</td>
<td>aPTT</td>
<td>aPTT, ACT</td>
</tr>
<tr>
<td><strong>Thrombin-binding</strong>*</td>
<td>Reversible</td>
<td>Irreversible</td>
<td>Reversible</td>
</tr>
<tr>
<td><strong>Clot-bound thrombin</strong>*</td>
<td>+++ Inhibition</td>
<td>+ Inhibition</td>
<td>++ Inhibition</td>
</tr>
</tbody>
</table>

aPTT=activated partial thromboplastin time; ACT=activated clotting time; ECT=ecarin clotting time.