Understanding the Misunderstood Disease of Heparin Induced Thrombocytopenia (HIT)

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Q&A
Steve Simpson
Molecular and Special Diagnostics Business Manager
Immucor
“Understanding the Misunderstood Disease of HIT”

Faculty Name: Oksana Volod, M.D.
Faculty Institution: Cedars Sinai Medical Center, Los Angeles
Speaker Disclosure: None
Objectives

• Describe the features of Heparin Induced Thrombocytopenia (HIT) including:
  ➢ Mechanism
  ➢ Risk Factors
  ➢ Diagnosis
  ➢ Clinical implications
  ➢ Treatment
• Review HIT Case Studies
• Discuss Workup Protocols used at Cedars Sinai to diagnose and treat HIT
**Heparins (H)**

- Consist of heterogeneous polysaccharide chains that have been extracted and partially purified from either beef lung or pork intestinal mucosa.
- Heparin converts AT III from a slow to a very rapid inhibitor.
- Hep AT III complex inactivates XIIa, XIa, IXa, Xa, and IIa (most sensitive) *
- Only 1/3 of administered H binds to AT III and is responsible for most of its anticoagulant effect.
- Binds to platelets (Inhibits platelet function and contributes to the hemorrhagic effects of heparin).

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Mechanism of Action
Heparin vs. LMWH vs. Fondaparinux

Unfractionated Heparin
- Pentasaccharide sequence
- Antithrombin
- Inhibition of Factor Xa
- Inhibition of Factor IIa

Low Molecular Weight Heparin (Enoxaparin, Reviparin)
- Synthetic pentasaccharide sequence
- Inhibition of Factor Xa
- Minimal inhibition of Factor IIa

Fondaparinux (Arixtra)
- Synthetic pentasaccharide sequence
- Inhibition of Factor Xa
- No inhibition of Factor IIa
Heparin-Induced Thrombocytopenia
HIT & HITT *

- **HIT/HITT** is an antibody-mediated adverse reaction to heparin that can result in venous or arterial thrombosis.
- Diagnosis of HIT is based on both clinical and serological features.

**VS**

- **HAT** caused by nonimmunologic mechanisms (mild direct platelet activation by heparin)

- Onset within 4 days
- Incidence 5%-30%
- Recovery 1-3 days
HIT a Historical Perspective

• Heparin discovery (1916, McLean)
• Routine platelet count measurements were not routinely performed until the 1970’s
• In 1957 at the International Society of Angiology 10 patients who developed arterial embolism during systemic heparin therapy were presented
• In 1969, the term “heparin induced thrombocytopenia” (HIT) was used by Natelson
• In 1973 Dr. Rhodes first identified the central features of the HIT syndrome-thrombocytopenia, thrombosis and its immune pathogenesis
Mechanism of HIT

1. PF4/Heparin Complex
2. Anti-PF4/Heparin Antibody
3. PF4/Heparin/Ab Immune Complex
4. IgG receptors
5. Anti-PF4/Heparin IgG Ab
6. Activated Platelet
7. Thrombogenic Microparticles
8. PF4
9. Heparin-like molecules
10. Tissue Factor

- Damaged endothelial cells
- Activated Monocyte
- Thrombin
Mechanism of HIT

1. Platelet factor-4 (PF4) from platelet $\alpha$-granules binds to heparin chain to form PF4/Heparin complex

2. Anti-PF4/Heparin antibody (Ab) may form in up to 8% of patients receiving heparin

3. Ab binds to form PF4/Heparin/Ab immune complex -- detected by PF4 ELISA screening test

4. IgG receptors are found on the platelet surface

5. Pathogenic IgG class of anti-PF4/Heparin Ab forms in subset (5-30%) of these patients – this is called the HIT Ab

6. PF4/Heparin/HIT Ab complex binds to IgG receptor leading to platelet activation – detected by Serotonin Release Assay (SRA)

7. Activated platelet releases thrombogenic microparticles and PF4

8. Released PF4 leads to further formation of PF4/Heparin complexes and platelet activation, as well as neutralization of the anticoagulant effects of heparin

9. HIT Ab also reacts with PF4 bound to heparin-like molecules on endothelial cells and monocytes – releases tissue factor (TF)

10. TF initiates the coagulation cascade
What is Platelet Factor 4 (PF4)?

- Platelet factor-4 is a 70-amino acid protein that is released from the alpha-granules of activated platelets and binds with high affinity to heparin.
- Its major physiologic role appears to be neutralization of heparin-like molecules on the endothelial surface of blood vessels, thereby inhibiting local antithrombin III activity and promoting coagulation.
HIT Antibodies : Risk Factors

- Duration and type of heparin exposure (UH > LMWH)
  Immunogenicity is influenced by relative size (1000 Da), amount, and stability of the PF4/heparin complexes
- Patient population (cardiac/orthopedic > medical/obstetric)
- Severity of trauma
- Gender (W > M)
- Only PF4/heparin IGG antibodies can bind and activate the platelet Fc receptor

- At most 5% to 30% of patients who form HIT –IgG, will develop clinical HIT depending upon the patient population
# Incidence of HIT According to Patient Population and Type of Heparin Exposure

<table>
<thead>
<tr>
<th>Patient Population (Minimum of 4-d Exposure)</th>
<th>Incidence of HIT, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Postoperative patients</strong></td>
<td></td>
</tr>
<tr>
<td>Heparin, prophylactic dose(^1)</td>
<td>1-5</td>
</tr>
<tr>
<td>Heparin, therapeutic dose(^2)</td>
<td>1-5</td>
</tr>
<tr>
<td>Heparin, flushes(^a)</td>
<td>0.1-1</td>
</tr>
<tr>
<td>LMWH, prophylactic or therapeutic dose(^1,2)</td>
<td>0.1-1</td>
</tr>
<tr>
<td>Cardiac surgery patients(^1,2,7,28,29)</td>
<td>1-3</td>
</tr>
<tr>
<td><strong>Medical</strong></td>
<td></td>
</tr>
<tr>
<td>Patients with cancer(^24,30,31)</td>
<td>1</td>
</tr>
<tr>
<td>Heparin, prophylactic or therapeutic dose(^24)</td>
<td>0.1-1</td>
</tr>
<tr>
<td>LMWH, prophylactic or therapeutic dose(^26,30)</td>
<td>0.6</td>
</tr>
<tr>
<td>Intensive care patients(^32)</td>
<td>0.4</td>
</tr>
<tr>
<td>Heparin, flushes(^33)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Obstetrics patients(^21,22,34,35)</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>
HIT/HITT : Diagnosis

Clinical

• Moderate Thrombocytopenia
• Thrombosis
  ➢ Venous (DVT, PE, adrenal)
  ➢ Arterial (Limb, CVA, MI)
• Appropriate timing
• Other cause for Thrombocytopenia are excluded

Pathological

• Heparin – dependent, platelet activating IgG
  ➢ Detected by Antigen assay (PF4 ELISA)
  ➢ Confirmed by platelet activation assay (e.g. serotonin release assay (SRA))

HIT/HITT: Thrombocytopenia

- A fall in the platelet count of >50% occurs in >95% of patients diagnosed with HIT

- Important features of the thrombocytopenia include:
  - Timing of the onset of the thrombocytopenia (5-10 days of heparin therapy)
  - Severity of the thrombocytopenia (moderate, median platelet count 50 000-60 000/ml)
  - Course of the platelet count after stopping heparin

- Consider other etiologic processes if thrombocytopenia is severe (<10,000/mL).
Thrombocytopenia Following Cardiac Surgery

- 50% of patients will develop HIT antibodies
- 1-2% will develop HIT
- Consider HIT if:
  1. Fall begins > 4 days postoperatively
  2. Thrombocytopenia that persists for > 4 days after surgery

*CHEST 2012; 141(2)(Suppl):e495S–e530S*
HIT: Thrombosis (HITT)

- **Thrombosis** occurs in the majority of patients with HIT
- 17% to 55% of untreated patients with HIT develop DVT & PE
- In up to 25% of patients thrombosis precedes the development of thrombocytopenia
- Venous thrombosis is most common type of thromboembolic complication (4:1)
- Unusual location for venous thrombosis (cerebral and adrenal venous thrombosis (less common))
- 5% to 10% of patients with HIT die of thrombotic complications
- Hemorrhage is uncommon despite low platelet counts
# Warkentin 4T Score

<table>
<thead>
<tr>
<th>Score = 2</th>
<th>Score = 1</th>
<th>Score = 0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thrombocytopenia</strong>&lt;br&gt;Compare the highest platelet count within the sequence of declining platelet counts with the lowest count to determine the % of platelet fall.&lt;br&gt;(Select only 1 option)</td>
<td>o &gt; 50% platelet fall AND nadir of ≥ 20 AND no surgery within preceding 3 days</td>
<td>o &gt; 50% platelet fall BUT surgery within preceding 3 days OR any combination of platelet fall and nadir that does not fit criteria for Score 2 or Score 0 (eg, 30-50% platelet fall or nadir 10-19)</td>
</tr>
<tr>
<td>*<em>Timing (of platelet count fall or thrombosis</em>)**&lt;br&gt;Day 0 = first day of most recent heparin exposure&lt;br&gt;(Select only 1 option)</td>
<td>o platelet fall day 5-10 after start of heparin&lt;br&gt;o platelet fall within 1 day of start of heparin AND exposure to heparin within past 5-30 days</td>
<td>o consistent with platelet fall days 5-10 but not clear (eg, missing counts)&lt;br&gt;o platelet fall within 1 day of start of heparin AND exposure to heparin in past 31-100 days&lt;br&gt;o platelet fall after day 10</td>
</tr>
<tr>
<td><strong>Thrombosis (or other clinical sequelae)</strong>&lt;br&gt;(Select only 1 option)</td>
<td>o confirmed new thrombosis (venous or arterial)&lt;br&gt;o skin necrosis at injection site&lt;br&gt;o anaphylactoid reaction to IV heparin bolus&lt;br&gt;o adrenal hemorrhage</td>
<td>o recurrent venous thrombosis in a patient receiving therapeutic anticoagulants&lt;br&gt;o suspected thrombosis (awaiting confirmation with imaging)&lt;br&gt;o erythematous skin lesions at heparin injection sites</td>
</tr>
<tr>
<td><strong>Other cause for Thrombocytopenia</strong>&lt;br&gt;(Select only 1 option)</td>
<td>o no alternative explanation for platelet fall is evident</td>
<td><strong>Possible other cause is evident:</strong>&lt;br&gt;o sepsis without proven microbial source&lt;br&gt;o thrombocytopenia associated with initiation of ventilator&lt;br&gt;o other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs implicated in drug-induced immune thrombocytopenia (D-I TP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relatively Common: glycoprotein llb/llla antagonists (abciximab, epftibatide, tirofiban); quinine, quinidine, sulfa antibiotics, carbamazepine, vancomycin</td>
</tr>
<tr>
<td>Less Common: actinomycin, amitriptyline, amoxicillin/piperacillin/nafcillin, cephalosporins (cefazolin, cefazidime, ceftriaxone), cefepime, ciprofloxacin, esomeprazole, fexofenadine, fentanyl, fucidic acid, furosemide, gold salts, levofloxacin, metronidazole, naproxen, oxaliplatin, phenytoin, propranolol, propoxyphene, ranitidine, rifampin, suramin, trimethoprim. Note: This is a partial list.</td>
</tr>
</tbody>
</table>

**Figure 1.** 4T’s score. *Timing of clinical sequelae, such as thrombocytopenia, thrombosis, or skin lesions. **Two points if necrotizing heparin-induced skin lesions even if thrombocytopenia not present. (Modified with permission from Warkentin and Linkins.*)
4T Score and HIT Probability

- Low 4T score = low HIT probability (0%-3%)*

- High 4T score - 24% -61% of patients prove not to have HIT *

- Isolated HIT antibodies are both frequent and not diagnostic of HIT


Conclusion: The HEP Score is the first pre-test clinical scoring model for HIT based on broad expert opinion, exhibited favorable operating characteristics and may permit clinicians to confidently reduce use of alternative anticoagulants. Prospective multicenter validation is warranted.
<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Magnitude of fall in platelet count (measured from peak platelet count to nadir platelet count since heparin exposure)</td>
<td></td>
</tr>
<tr>
<td>a. &lt; 30%</td>
<td>-1</td>
</tr>
<tr>
<td>b. 30%-50%</td>
<td>1</td>
</tr>
<tr>
<td>c. &gt; 50%</td>
<td>3</td>
</tr>
<tr>
<td>2. Timing of fall in platelet count</td>
<td></td>
</tr>
<tr>
<td>For patients in whom typical onset HIT is suspected</td>
<td></td>
</tr>
<tr>
<td>a. Fall begins &lt; 4 days after heparin exposure</td>
<td>-2</td>
</tr>
<tr>
<td>b. Fall begins 4 days after heparin exposure</td>
<td>2</td>
</tr>
<tr>
<td>c. Fall begins 5-10 days after heparin exposure</td>
<td>3</td>
</tr>
<tr>
<td>d. Fall begins 11-14 days after heparin exposure</td>
<td>2</td>
</tr>
<tr>
<td>e. Fall begins &gt; 14 days after heparin exposure</td>
<td>-1</td>
</tr>
<tr>
<td>For patients with previous heparin exposure in last 100 days in whom rapid onset HIT is suspected</td>
<td></td>
</tr>
<tr>
<td>f. Fall begins &lt; 48 h after heparin re-exposure</td>
<td>2</td>
</tr>
<tr>
<td>g. Fall begins &gt; 48 h after heparin re-exposure</td>
<td>-1</td>
</tr>
<tr>
<td>3. Nadir platelet count</td>
<td></td>
</tr>
<tr>
<td>a. $\leq 20 \times 10^9$ L$^{-1}$</td>
<td>-2</td>
</tr>
<tr>
<td>b. $&gt; 20 \times 10^9$ L$^{-1}$</td>
<td>2</td>
</tr>
<tr>
<td>4. Thrombosis (Select no more than one)</td>
<td></td>
</tr>
<tr>
<td>For patients in whom typical onset HIT is suspected</td>
<td></td>
</tr>
<tr>
<td>a. New VTE or ATE $\geq$ 4 days after heparin exposure</td>
<td>3</td>
</tr>
<tr>
<td>b. Progression of pre-existing VTE or ATE while receiving heparin</td>
<td>2</td>
</tr>
<tr>
<td>For patients in whom rapid onset HIT is suspected</td>
<td></td>
</tr>
<tr>
<td>c. New VTE or ATE after heparin exposure</td>
<td>3</td>
</tr>
<tr>
<td>d. Progression of pre-existing VTE or ATE while receiving heparin</td>
<td>2</td>
</tr>
<tr>
<td>5. Skin necrosis</td>
<td></td>
</tr>
<tr>
<td>a. Skin necrosis at subcutaneous heparin injection sites</td>
<td>3</td>
</tr>
<tr>
<td>6. Acute systemic reaction</td>
<td></td>
</tr>
<tr>
<td>a. Acute systemic reaction after intravenous heparin bolus</td>
<td>2</td>
</tr>
<tr>
<td>7. Bleeding</td>
<td></td>
</tr>
<tr>
<td>a. Presence of bleeding, petechiae or extensive bruising</td>
<td>-1</td>
</tr>
<tr>
<td>8. Other causes of thrombocytopenia (Select all that apply)</td>
<td></td>
</tr>
<tr>
<td>a. Presence of a chronic thrombocytopenic disorder</td>
<td>-1</td>
</tr>
<tr>
<td>b. Newly initiated non-heparin medication known to cause thrombocytopenia</td>
<td>-2</td>
</tr>
<tr>
<td>c. Severe infection</td>
<td>-2</td>
</tr>
<tr>
<td>d. Severe DIC (defined as fibrinogen $&lt; 100$ mg dL$^{-1}$ and D-dimer $&gt; 5.0$ $\mu$g mL$^{-1}$)</td>
<td>-2</td>
</tr>
<tr>
<td>e. Indwelling intra-arterial device (e.g., IABP, VAD, ECMO)</td>
<td>-2</td>
</tr>
<tr>
<td>f. Cardiopulmonary bypass within previous 96 h</td>
<td>-1</td>
</tr>
<tr>
<td>g. No other apparent cause</td>
<td>3</td>
</tr>
</tbody>
</table>
Prospective Comparison Of The HIT Expert Probability (HEP) Score Versus The Warkentin’s 4T’s Score In A Quaternary Care Center

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Department of Pathology, Cedars-Sinai Medical Center, Los Angeles, California – U.S.A.

Introduction

Currently, clinicians routinely employ the Warkentin’s 4T’s score to assess and quantify the pre-test probability of actual HIT. More recently, the novel HEP Expert Probability (HEP) score has been developed in hopes of supplanting the 4T’s score as a model that is easier to use and more predictive of HIT.

Aim

We sought to prospectively evaluate and compare the Warkentin’s 4T’s score versus the HEP score and their correlation with:
1) Platelet factor 4 (PF4) optical density ELISA assay
2) Serotonin release assay (SRA)

Design

• Sera from 453 consecutive surgical/medical patients who received unfractionated or low-molecular-weight heparin, and in whom HIT was suspected or to be ruled out, were evaluated.

• The patient samples were referred for diagnostic HIT testing to our laboratory between May 2011 and February 2012.

• Of the original 453 specimens, 36 workups from 49 different patient samples (corresponding to poly-ELISA optical densities > 0.40) were selectively chosen for the additional testing for the purposes of this study; all of these patients were exposed to unfractionated heparin.

• Confirmatory testing in the form of send-out SRAs were run on all of these selected specimens. Additionally, all workups included pre-test scoring using the Warkentin’s 4T’s and HEP scoring models.

• Residents rotating on the coagulation service determined the 4T’s/HEP scores, which were later confirmed by one of two coagulation hematopathology consultants.

Results

Graph 1: HEP vs 4T Scores (n=45)

Discussion

• For PF4 OD=0.41-0.99, the mean and 95% confidence interval (CI) for 4T’s/HEP scores were 3.2±0.7 and 2.6±1.1, respectively.

• For PF4 OD=1.0-1.99, the mean and 95% CI for 4T’s/HEP scores were 3.5±1.2 and 2.5±1.2, respectively.

• For PF4 OD=2.0-2.99, the mean and 95% CI for 4T’s/HEP scores were 4.3±2.0 and 4.6±4.7, respectively.

• For SRA-negative patients, the average 4T’s/HEP scores were 3.3 and 2.2, respectively.

• For SRA-positive patients, the average 4T’s/HEP scores were 4.4 and 5.0, respectively.

• The p-values for the comparison of the mean score between SRA-positive and SRA-negative individuals for 4T’s/HEP scores were 0.13 and 0.008, respectively.

Conclusions

• The average 4T’s score demonstrated an incremental increase with increasing PF4 optical densities. In contrast, the average HEP scores did not demonstrate an incremental increase with increasing PF4 optical densities (i.e. the average HEP score was lower for OD 1.0-1.99 than for OD 0.4-0.99 and did not demonstrate a positive correlation).

• The HEP score did not distinguish between SRA-positive and SRA-negative patients (mean HEP score of 5.0 vs. 2.6, p=0.08). Therefore, the HEP score is not more predictive of HIT than the 4T’s score. This is in contrast to a previously published, retrospective study comparing HEP vs. 4T’s as they relate to SRA results.

• Since no differences were found between the HEP and 4T’s score in their ability to correlate with SRA and because 4T is better correlated with OD, we advocate for the use of the 4T’s score. Furthermore, the 4T score is easier to recall and relatively straightforward than the HEP score which can be complex and cumbersome.

Table 1: P values of variables analyzed

<table>
<thead>
<tr>
<th></th>
<th>SRA-negative</th>
<th>SRA-positive</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average HEP</td>
<td>2.6</td>
<td>5.0</td>
<td>0.088</td>
</tr>
<tr>
<td>Average 4T</td>
<td>3.3</td>
<td>4.4</td>
<td>0.13</td>
</tr>
</tbody>
</table>
HIT Laboratory Testing

Antigen Assays
- Detect HIT antibodies
  - ELISA

Functional Assays
- Detect presence of platelet activation by HIT antibodies
  - SRA
  - HIPA
# Heparin Antibody Assays

- Survey of Coag labs in North America ID 8 different assays and wide discrepancies in practice between centers using the same assay *

<table>
<thead>
<tr>
<th>Test</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| Particle Immuno Filtration Assay (PIFA) | • POC assay  
• Quick TAT (15 min) | • Sensitivity 94-95%  
• Specificity 88-92%  
• Results NOT “quantifiable” |
| Immunoassays (PF4 ELISA)    | • Sensitivity 100%  
• Technically easy  
• TAT 3.5 hours | Specificity 82-85% |
| Serotonin Release (SRA)     | • High Sensitivity and Specificity  
• FP rare | • Technically demanding  
• Require radioisotopes  
• Performed in specialized laboratories |
| Platelet Aggregation        | High Specificity                                 | • Low Sensitivity  
• Technique dependant |

**Solid-phase Anti-PF4/heparin-ELISA “Immunoassay” PF4 ELISA**

**PF4 ELISA**
- Recognize binding of antibodies to PF4/polyanion complexes
- Detects antibodies presence

![Diagram of PF4 ELISA process](image-url)

- Patient Plasma or Serum Sample
- PF4 coated ELISA plate
- Tagged goat anti-human Ig
- Add substrate
- Detect absorbance
## Heparin-Induced Thrombocytopenia

<table>
<thead>
<tr>
<th>Method/Qualitative Result</th>
<th>Participants No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTI Lifecodes PF4 IgG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>50</td>
<td>98.0</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Equivocal</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GTI Lifecodes PF4 Enhanced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>111</td>
<td>97.4</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>Equivocal</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>Other</td>
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<td></td>
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<tr>
<td>Positive</td>
<td>13</td>
<td>81.3</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>18.8</td>
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<tr>
<td>Equivocal</td>
<td>-</td>
<td>-</td>
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</table>

<table>
<thead>
<tr>
<th>Method/Quantitative Result</th>
<th>No. Labs</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V.</th>
<th>Median</th>
<th>Low Value</th>
<th>High Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTI Lifecodes PF4 IgG OD</td>
<td>46</td>
<td>1.0827</td>
<td>0.2446</td>
<td>22.6</td>
<td>1.075</td>
<td>0.407</td>
<td>1.530</td>
</tr>
<tr>
<td>Percent Inhibition</td>
<td>28</td>
<td>100.6</td>
<td>5.4</td>
<td>5.4</td>
<td>100</td>
<td>89</td>
<td>110</td>
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<tr>
<td>GTI Lifecodes PF4 Enhanced</td>
<td>93</td>
<td>1.1671</td>
<td>0.3006</td>
<td>25.8</td>
<td>1.154</td>
<td>0.532</td>
<td>1.993</td>
</tr>
<tr>
<td>OD</td>
<td>49</td>
<td>99.8</td>
<td>4.2</td>
<td>4.2</td>
<td>100</td>
<td>90</td>
<td>110</td>
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<tr>
<td>Percent Inhibition</td>
<td>10</td>
<td>0.6999</td>
<td>0.4032</td>
<td>57.6</td>
<td>0.609</td>
<td>0.183</td>
<td>1.564</td>
</tr>
</tbody>
</table>

No data is presented since too few laboratories reported results at the time of data summarization.
**14C - Serotonin Release Assay**

Platelet Activation Assay

- **Load Dense Granules With 14C-Serotonin**
- **Platelet** + **Serum**
  - **Heparin - High**
  - **Heparin - Low**
  - **No Release**
  - **Measure 14C-Serotonin Released**
Treatment and Prevention of Heparin-Induced Thrombocytopenia

Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians
Evidence-Based Clinical Practice Guidelines

Lori-Ann Linkins, MD; Antonio L. Dans, MD; COL Lisa K. Moores, MC, USA, FCCP;
Robert Bona, MD; Bruce L. Davidson, MD, MPH, FCCP; Sam Schulman, MD, PhD;
and Mark Crowther, MD
Treatment of Suspected HIT/HITT

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

• **Confirm diagnosis** of HIT with the appropriate laboratory test (screening and confirmatory)

• **Consider alternative anticoagulation** (DTI)
  - If left untreated, the overall risk of thrombosis is 38% to 76% *

• **Monitor carefully for thrombosis**

  Thrombotic complications:
  - Amputation up to 20%
  - Death up to 30-50%

• Monitor platelet counts until recovery

• **Avoid prophylactic platelet transfusions**

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 percutaneous coronary intervention (PCI)

**Bivalirudin***
✓ Indicated as an anticoagulant in patients undergoing percutaneous transluminal coronary angioplasty (PTCA)

*Approved for with or at risk HIT patients undergoing PCI. (Dec. 2005)

Fondaparinux (Arixtra)?

- Five saccharide molecule that is functionally and structurally like heparin
- One multicenter in vitro study demonstrated a lack of cross-reactivity between fondaparinux and HIT antibodies.
- 2 case reports of thrombocytopenia without thromboembolic complications while receiving fondaparinux have been described. *
- At this time, the American College of Chest Physicians continues to recommend the use of direct thrombin inhibitors as the first-line agents in the setting of HIT.


Therapy Duration

- DTI are recommended for both HIT & HITT
- DTI until platelet count is at least 150 000
- DTI Overlap with VKA for a minimum of 5 days and until the INR is within the target range
- VKA should be initially given in low doses (5 mg)
- HITT should be treated for a minimum of 3 months (provoked risk factor)
- Isolated HIT should be treated for up to 4 weeks
- If VKA has already been started when a patient is diagnosed with HIT, Vit K should be administered

CHEST 2012; 141(2)(Suppl):e495S–e530S
Point to remember

• **Warfarin** initiated prior to sustained and adequate platelet recovery in the presence of DTI therapy is associated with a greater risk of venous limb gangrene

• It has a distinct features: a supratherapeutic INR that coincides with progression of DVT to distal limb necrosis

• > INR is caused by severe reduction in FVII that parallels a severe reduction in PC (should be treated with Vit K and FFP)
Role of Plasma Exchange in HIT Treatment

THROMBOSIS AND HEMOSTASIS

Plasma exchange to remove HIT antibodies: dissociation between enzyme-immunoassay and platelet activation test reactivities

Theodore E. Warkentin,¹,² Jo-Ann I. Sheppard,¹ F. Victor Chu,³ Anil Kapoor,³ Mark A. Crowther,¹,² and Azim Gangji²

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Key Points

• Repeated plasma exchange removes sufficient HIT-IgG to achieve negative SRA despite ongoing strong-positive EIA.
• Serially-diluted HIT sera tested in both SRA and EIA show that SRA negativity can be achieved with minimal decrease in EIA reactivity.

Repeated therapeutic plasma exchange (TPE) has been advocated to remove heparin-induced thrombocytopenia (HIT) IgG antibodies before cardiac/vascular surgery in patients who have serologically-confirmed acute or subacute HIT; for this situation, a negative platelet activation assay (e.g., platelet serotonin-release assay [SRA]) has been recommended as the target serological end point to permit safe surgery. We compared reactivities in the SRA and an anti-PF4/heparin IgG-specific enzyme immunoassay (EIA), testing serial serum samples in a patient with recent (subacute) HIT who underwent serial TPE precordial surgery, as well as for 15 other serially-diluted HIT sera. We observed that post-TPE/diluted HIT sera—when first testing SRA-negative—continue to test strongly positive by EIA-IgG. This dissociation between the platelet activation assay and a PF4-dependent immunoassay for HIT antibodies indicates that patients with subacute HIT undergoing repeated TPE before heparin reexposure should be tested by serial platelet activation assays even when their EIAs remain strongly positive. (Blood. 2015;125(1):195-198)
Natural History of HIT Antibodies

- Anti-heparin/PF4 antibodies are frequently not detectable at a median of 50-80 days after HIT initially documented.
- Recurrent HIT does not necessarily recur upon re-exposure to heparin.
HIT WORK UP AT CSMC
Historical Perspective

DTI Expenditures FY03-FY07

- Total DTI Expenditures
- Cost Avoided

Assume ~50% Average Annual Increase

- $84,400 (FY '03)
- $189,500 (FY '04)
- $283,300 (FY '05)
- $224,300 (FY '06)
- $464,325 (FY '07)
- $424,950 (FY '06)

- $173,100 (FY '07)
- $637,425

- ✓ June 2004: Pharmacist managed protocol implemented
- ✓ April 2005: HIT/HITTS Task Force
- ✓ April 2005: PF4 assay ordered per pharmacy on Day 1 of all DTI therapy
- ✓ December 2005: HIT/HITTS 'Prescription' Drug Bulletin article published
- ✓ February 2006: Pharmacist Inservice
- ✓ June 2006: Pharmacist CE Lecture
- ✓ August 2006: P&T/CIC approval for pharmacists to order 'HIT Panel with Pathologist Consult' to aid in earlier discontinuation of unnecessary therapy.
CS Medicine Initiative

• Our 2011 audit (6 months, 493 cases) showed that we had:
  • Significant number of borderline/low positive/positive results that were not HIT (91/103 cases)
  • Too many SRA were sent out for confirmation, even on very negative PF4 ELISA results (176 /493)
  • During that period of time we had only 12 confirmed HIT PF4 ELISA results 2.4% , which were confirmed by SRA.
  • The results of the 2011 audit lead to a 2012 Cedars-Sinai Medicine Initiative project
Overdiagnosis of Heparin-Induced Thrombocytopenia in Surgical ICU Patients

Cherisse Berry, MD, Oxana Tcherniachtchouk, MD, Eric J Ley, MD, Ali Salim, MD, FACS, James Mirocha, MS, Sylvia Martin-Stone, PharmD, BCPS, Dennis Stolpner, MD, Daniel R Margulies, MD, FACS

**BACKGROUND:** Heparin use in surgical patients places them at increased risk for developing heparin-induced thrombocytopenia (HIT). The false positive rate of HIT using the current standard criteria is unknown in surgical ICU patients, who often have confounding factors that cause thrombocytopenia.

**STUDY DESIGN:** Surgical ICU patients, admitted over a 2-year period with a positive screening test for HIT (platelet factor [PF] 4 ≥ 0.4 optical density [OD]), were reviewed retrospectively at a single institution. Correlation of the Warkentin 4-T score and commercial heparin PF4 ELISA with serotonin releasing assay (SRA) was performed. Logistic regression was used to determine independent risk factors associated with the development of HIT.

**RESULTS:** PF4 tests were requested in 643 patients based on a clinical suspicion of HIT. Of these, 104 patients had a PF4 result, an SRA value (%), and a 4-T score available. Twenty patients (19%) had true positive HIT, defined as a positive PF4 and positive SRA (SRA ≥ 20%). Eighty-four patients (81%) were false positive, defined as a positive PF4 and negative SRA. Five of 58 patients with Warkentin score of 0 to 3, and 6 of 14 patients with Warkentin score of 6 to 8 were HIT positive by SRA.

**CONCLUSIONS:** In surgical ICU patients, clinical suspicion for HIT necessitates PF4 and SRA analysis. Testing for HIT or treatment with a direct thrombin inhibitor should not depend on the Warkentin 4-T score alone. Although a PF4 ≥ 0.4 OD is considered a positive screening test for HIT, a PF4 ≥ 2.0 OD is preferable in surgical ICU patients. (J Am Coll Surg 2011;213:10–18. © 2011 by the Western Surgical Association.
Cost-effective HIT diagnosis: utilizing IgG-specific PF4 immunoassays reduces the number of confirmatory Serotonin Release Assays without missing true HIT.

Study Aim:

- Compare polyspecific vs. IgG-specific PF4 ELISA assays
- To determine whether the IgG specific assay can reduce confirmatory SRA testing (expensive send-out with long turn-around-time)
- Without missing true HIT and/or unnecessary treatment with DTIs

Methods:

453 HIT work-ups (4T’s score, polyspecific PF4) were reviewed (05/2011-02/2012), including 86 work-ups on 49 patients with polyspecific OD ≥ 0.4
Study Conclusion

22 of 29 “borderline” poly-ELISAs were negative by the IgG-ELISA

- IgG-ELISA reduced the number of “borderline” cases by 75%
- Positive IgG ELISA results need to be confirmed by platelet activation assay (SRA)
Cost-Effective HIT Diagnosis: Utilizing IgG-Specific PF4 Immunoassays Reduces the Number of Confirmatory Serotonin Release Assays without Missing True HIT

Abstract

In the United States, the most commonly employed screening assay for heparin-induced thrombocytopenia (HIT) is the PF4 ELISA, of which both polyclonal (poly-ELISA) and IgG-specific (IgG-ELISA) assays are commercially available. We compared the IgG-ELISA versus the poly-ELISA to determine whether the IgG-ELISA is more sensitive and specific for the diagnosis of HIT. 453 HIT work-ups were reviewed. Patients with poly-ELISA optical density (OD) values greater than or equal to 0.40 (n=49) were selected for further analysis, including serotonin-release assays (SRAs). IgG-ELISAs, and pre-test probability scoring (4T’s score). Both the poly-ELISA and IgG-ELISA identified PF4/heparin antibodies in all true HIT patients (n=8). IgG-ELISA reduced the number of “borderline” cases by 75%. IgG-ELISA is more specific than poly-ELISA and can reduce number of confirmatory SRA required.

Keywords: HIT; IgG-ELISA; PF4 ELISA; Poly-ELISA; Serotonin Release assay (SRA); False positive
Negative IgG PF4 ELISA and SRA will miss IgA and IgM Antibodies
Cases
Case 1 – Borderline PF4 IgG

- 43 yo African American male with acute on chronic CHF
- Heparin exposure on 7/4/2015 - 163 K
- Thrombocytopenia on 7/5/2015 - 88 K
- There was prior heparin Exposure in May
- Switched to Argatroban
- No thrombosis

Interpretation: Rapid HIT onset in a patient with prior heparin exposure

W4T score – 3 points
PF4 IgG – 0.64
SRA – 69%
Case 2 – Strongly Positive PF4 IgG, Initial Neg SRA

- 55 YO male admitted with massive MI, VSD
- Heparin exposure 12/21/2015 – 227K
- Thrombocytopenia 12/28/2-15 – 119K...74K
- Thrombosis - Yes (12/31)
- 1/1 taken to OR for VSD repair
- Treated with Argatroban

HIT score - 7 points
12/29 - PF4 IgG – 2.57
SRA - 1%
1/4 - PF4 IgG – 2.33
SRA - 87%

Interpretation: HIT with delayed SRA reactivity
Case 3 – Stroke During Hemodialysis

- 53 yo Female with ESRD, history of multiple chronic clots
- s/p TPA
- ? Prior history of HIT
- Started on Argatroban

PF4 IgG 0.03 (CSMC)
PF4 IgM 0.05 (BCW)
PF4 IgA – Neg
Case 3: 2009 - Positive Poly PF4- Neg SRA

- Multiple medical problems
- Received Heparin during 07/14 - 07/24 admission (Platelets 314K)
- Readmitted on 7/31 with Platelets 39K
- 8/8 - Acute DVT
- Started on Argatroban

Polyclonal PF4 8/14 – 2.36
BCW:
- SRA- 0%
- Strong IgG (not inhibited by heparin)
- Strong IgM – reacting with heparin

Polyclonal PF4 8/22 - 1.34
BCW:
- SRA - 0%
- Strong IgG (not inhibited by heparin)
- Absent IgM

Interpretation: IgM antibodies mediated HIT
HIT: Take Home Points

• HIT is a clinico-pathologic diagnosis

• Advantages and limitations of each assay performed are necessary to be aware of:
  ➢ IgG-ELISA reduces the number of “borderline” cases
  ➢ Positive IgG ELISA needs confirmation with functional assay (SRA)
  ➢ Both will detect IgG antibodies only

• If there is a strong clinical probability of HIT, repeat testing is recommended in 2-3 days
• If Thrombocytopenia persists > 4 days following cardiac surgery, HIT work up is recommended
• Dedicated multidisciplinary team (pathology, pharmacy, blood bank, hemonc) is needed for appropriate diagnosis and management of HIT
Continuing Education

• Each attendee must register for CE at:
  – https://www.surveymonkey.com/r/6STH7FV

• Registration deadline is February 15, 2016

• Certificates will be sent via email only to those who have registered by February 29, 2016

• No registration for CE will be accepted after February 15, 2016

Thank you!