Understanding the Misunderstood Disease of Heparin Induced Thrombocytopenia (HIT)

OKSANA VOLOD M.D., FCAP
Director, Pathology and Laboratory Medicine Residency Program
Cedars-Sinai Medical Center
Los Angeles, CA

Handouts

Continuing Education

- PACE, California DHS
  - 437-300-16
- Florida BPR
  - 20518785
- 1.5 Contact Hours
- Each attendee registers at:
  - https://www.surveymonkey.com/r/6STH7FV

Continuing Education

- Each attendee must register
- Registration deadline is February 15, 2016
- Certificates will be sent via email only to those who have registered by February 29, 2016

Other

- Session will be recorded and posted on LEARN
  - No CE issued for participating in recording
- You are all muted
- Q&A following session
  - Type in questions
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 $X_{IIa}, X_{Ia}, X_{a}, X_{Ila}$ (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)

Heparin vs. LMWH vs. Fondaparinux

**Unfractionated Heparin**
- Antithrombin
  - Factor Xa
  - Inhibition of Factor Xa

**Low Molecular Weight Heparin**
- Pentasaccharide sequence
  - Factor Xa
  - Inhibition of Factor Xa

**Fondaparinux**
- Synthetic pentasaccharide sequence
  - Factor Xa
  - Minimal 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
Mechanism of HIT

1. Platelet factor 4 (PF4) from platelet α-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 α-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>Postoperative patients</td>
<td></td>
</tr>
<tr>
<td>Heparin, prophylactic dose</td>
<td>1-5</td>
</tr>
<tr>
<td>Heparin, therapeutic dose</td>
<td>1-5</td>
</tr>
<tr>
<td>Heparin, flush 1</td>
<td>0.1-1</td>
</tr>
<tr>
<td>LMWH, prophylactic or therapeutic dose</td>
<td>0.1-1</td>
</tr>
<tr>
<td>Cardiac surgery patients</td>
<td>1-3</td>
</tr>
<tr>
<td>Medical</td>
<td></td>
</tr>
<tr>
<td>Patients with cancer</td>
<td>1</td>
</tr>
<tr>
<td>Heparin, prophylactic or therapeutic dose</td>
<td>0.1-1</td>
</tr>
<tr>
<td>Intensive care patients</td>
<td>0.4</td>
</tr>
<tr>
<td>Heparin, flush 1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Obstetrics patients</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

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><strong>Score = 2</strong></th>
<th><strong>Score = 1</strong></th>
<th><strong>Score = 0</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>Thrombocytopenia</td>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>Thrombosis (or other thrombotic phenomena) (deletions only)</td>
<td>Thrombosis (or other thrombotic phenomena) (deletions only)</td>
<td>Thrombosis (or other thrombotic phenomena) (deletions only)</td>
</tr>
<tr>
<td>Other causes for thrombocytopenia (gamma only)</td>
<td>Other causes for thrombocytopenia (gamma only)</td>
<td>Other causes for thrombocytopenia (gamma only)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>T</strong></th>
<th><strong>4</strong></th>
<th><strong>Score</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>4</td>
<td>Score</td>
</tr>
<tr>
<td>Thrombosis (or other thrombotic phenomena) (deletions only)</td>
<td>4</td>
<td>Score</td>
</tr>
<tr>
<td>Other causes for thrombocytopenia (gamma only)</td>
<td>4</td>
<td>Score</td>
</tr>
</tbody>
</table>
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


**4T or HEP score?**

**Table 3: HEP Expert Probability (HEP) Score**

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Magnitude of fall in platelet count</td>
<td>-1</td>
</tr>
<tr>
<td>2. Platelet count at diagnosis</td>
<td>-2</td>
</tr>
<tr>
<td>3. Time of clinical presentation of symptoms</td>
<td>-1</td>
</tr>
<tr>
<td>4. Duration of symptoms</td>
<td>-2</td>
</tr>
<tr>
<td>5. Presence of pulmonary embolism</td>
<td>-1</td>
</tr>
<tr>
<td>6. Presence of cerebrovascular event</td>
<td>-1</td>
</tr>
<tr>
<td>7. History of autoimmune disease</td>
<td>-1</td>
</tr>
<tr>
<td>8. History of sun sensitivity</td>
<td>-1</td>
</tr>
</tbody>
</table>

**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.
HIT Laboratory Testing

Antigen Assays
- Detect HIT antibodies
  - ELISA
  - SRA

Functional Assays
- Detect presence of platelet activation by HIT antibodies
  - 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  “Immuonassay” PF4 ELISA

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

Patient Plasma or Serum Sample

PF4 coated ELISA plate

Tagged goat anti-human Ig

Add substrate

Detect absorbance

2015 CAP Proficiency Test

<table>
<thead>
<tr>
<th>Heparin-induced Thromboplastin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>PF4 coated PF4 IgG</td>
<td>98</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1</td>
</tr>
<tr>
<td>Negative Control</td>
<td>1</td>
</tr>
<tr>
<td>Cut-off 2</td>
<td>2</td>
</tr>
<tr>
<td>Cut-off 3</td>
<td>12</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>3</td>
</tr>
<tr>
<td>Enzyme</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Heparin-induced Thromboplastin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>PF4 coated PF4 IgG</td>
<td>15</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1</td>
</tr>
<tr>
<td>Negative Control</td>
<td>1</td>
</tr>
<tr>
<td>Cut-off 2</td>
<td>5</td>
</tr>
<tr>
<td>Cut-off 3</td>
<td>6</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>2</td>
</tr>
</tbody>
</table>

Platelet Activation Assay

**14C - Serotonin Release Assay**
Platelet Activation Assay

Load Dense Granules With 14C-Serotonin

**No Release**

Heparin - High

Heparin - Low

Serum

Measure 14C-Serotonin Released
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**
  - 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 use in 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)
- HIT 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 feature: 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 enzymes-immunoassay and platelet activation test reactivities

Theodore E. Warshak,1,5. J.A. An, I. Shepro,1,6 F. Victor,1 and Kopcow1,6 Nain A. Cowles,1,7 and Adam Gang1,2

1Department of Pathology and Molecular Medicine, 2Department of Medicine, and 3Department of Surgery, McMaster University, Hamilton, ON, Canada

Key Points

• Repeated plasma exchange removes sufficient HIT IgG to achieve negative SPA despite ongoing strong positive EIA.
• Sensitivity-diluted HIT sera tested in both SPA and EIA show that SPA 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 cardiothoracic surgery in patients who have serologically confirmed HIT or aortic HIT. In this situation, a negative platelet activation test (PAT) assay is helpful in guiding the decision whether to proceed with surgery. We report a case of a patient with acquired HIT anticoagulated with unfractionated heparin who was referred for TPE and cardiothoracic surgery. We describe a novel approach to TPE, using serial serial serum samples in a patient with recent (presumed) HIT who underwent central TPE prior to surgery, as well as for 15 other serologically HIT cases. We obtained the post-TPE serial sera tests (SPA and SPA-DAF) in 7 cases—sensitive SPA-negative continue to be strongly positive by EIA-IgG. This dissociation between SPA and SPA-DAF continues even after a TPE. Sensitivity of TPE for HIT antibodies is not dependent on euglobulin fraction assay for HIT antibodies. Indications that patients will respond to HIT undergoing repeat central TPE before heparin reexposure should be further evaluated by serial serial antibody studies (ASIA, ASIA-D) rather than their SPA and TPE antibody positivity.

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

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
2012 CS HIT Project

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)
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

W4T score – 3 points
PF4 IgG – 0.64
SRA – 69%

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

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

<table>
<thead>
<tr>
<th>Date</th>
<th>BCW</th>
<th>SRA</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/14</td>
<td>2.36</td>
<td>0%</td>
<td>Strong IgG</td>
<td>Strong IgM</td>
</tr>
<tr>
<td>8/22</td>
<td>1.34</td>
<td>0%</td>
<td>Strong IgG</td>
<td>Absent IgM</td>
</tr>
</tbody>
</table>

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!