HEPARIN INDUCED THROMBOCYTOPENIA (HIT) AND THE ROLE OF SEROTONIN RELEASE ASSAY (SRA)

PRESENTER:

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DISCLOSURES

• No relevant disclosures

OBJECTIVES

• Recall clinical presentation and pathology of heparin induced thrombocytopenia (HIT)
• Review the HIT Diagnostic algorithm including the clinical scoring system
• Recall the roles and limitations of the HIT
• Apply results of serotonin release assay (SRA) to patient management
HIT BACKGROUND

• Most common immune mediated drug-induced thrombocytopenia (DITP)
• Incidence 1-3% (therapeutic heparin doses)
• Complex
• Difficult to confirm diagnosis
• Difficult to manage
• Most important DITP
• Thrombosis risk – up to 50% with HIT

CASE EXAMPLE

• 73-year-old male admitted to hospital for community-acquired pneumonia
  • Received antibiotic therapy
• Receiving subcutaneous unfractionated heparin (UHF) deep vein thrombosis prophylaxis
CASE EXAMPLE

DAYS 50 100 150 200 250 300
PLATELET COUNT (X10^9/L)

HEPARIN

Anti-heparin-PF4 antibody strongly positive
Dx isolated HIT

CASE EXAMPLE

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Design</td>
<td>Retrospective</td>
<td>Retrospective</td>
<td>Prospective</td>
</tr>
<tr>
<td>Therapy</td>
<td>D/C heparin, 21/113 received other Rx</td>
<td>D/C heparin or substitute warfarin</td>
<td>D/C heparin and/or substitute warfarin</td>
</tr>
<tr>
<td>NEW THROMBOSIS</td>
<td>21/113 (18%)</td>
<td>32/62 (52%)</td>
<td>32/139 (23%)</td>
</tr>
</tbody>
</table>

- Patients with isolated HIT have high risk of thrombosis
- Prophylactic therapy with a DTI should be considered
HIT DIAGNOSTIC ALGORITHM
CLINICAL PRESENTATION WHEN TO SUSPECT HIT

• Thrombocytopenia during heparin exposure and in the right clinical circumstance

• Type of heparin:
  • Low molecular weight heparin (LMWH)
  • Unfractionated heparin (UFH)

• Heparin dose:
  • Prophylactic dose
  • Therapeutic dose
  • Heparin bolus during cardiac bypass procedures (CPB)

• Clinical circumstance
  • Cardiac bypass surgery (coronary bypass, cardiac valve repair and replacement)
  • Orthopedic surgery (arthroplasty)
  • General Surgery
  • Thromboprophylaxis during pregnancy (rare)

HIT RECOGNITION

TIMING
THROMBOCYTOPENIA
THROMBOSIS
EXCLUDE OTHER CAUSES OF THROMBOCYTOPENIA
HIT PRE-TEST PROBABILITY SCORING

**SUSPICION OF HIT BASED UPON THE “4 T’s”**

<table>
<thead>
<tr>
<th>SCORE</th>
<th>PRE-TEST PROBABILITY SCORE CRITERIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>Nadir 20-100, or &gt; 50% platelet fall</td>
</tr>
<tr>
<td>Timing of onset of platelet fall</td>
<td>Day 5-10, or ≤ day 1 with recent heparin</td>
</tr>
<tr>
<td>Thrombosis or other sequelae</td>
<td>Proven thrombosis, skin necrosis or ASR</td>
</tr>
<tr>
<td>Other cause of platelet fall</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)

**HIT PRE-TEST PROBABILITY SCORING**

Other cause for Thrombocytopenia

No alternative explanation for platelet fall is evident

Possible other cause is evident:

- Sepsis without proven microbial source
- Thrombocytopenia associated with initiation of ventilator
- Other

Drug implicated in drug-induced immune thrombocytopenia (D-ITP)

**Relatively Common:** Glycoprotein IIb/IIIa antagonists (abciximab, eptifibatide, tirofiban); quinine, quinidine, sufa antibiotics, carbamazepine, vancomycin

**Less Common:** Azithromycin, amitriptyline, amoxicillin/ampicillin/cephalexin, cephalosporins (cefazolin, cefaclor, ceftriaxone), clonidine, ciprofloxacin, droperidol, furosemide, heparin, heparinoid, indomethacin, lidocaine, metronidazole, propafenone, phenytoin, propranolol, procainamide, tetracyclines, vancomycin, tilmethoprim. Note: This is a partial list.
Suspect Heparin Induced Thrombocytopenia (HIT)

Calculate 4Ts Score
PREDICTIVE VALUE OF 4TS

• NEGATIVE PREDICTIVE VALUE (NPV) FOR LOW PROBABILITY (SCORE <3)
  99.8% [95% (CI) 97–100%]

• POSITIVE PREDICTIVE VALUE (PPV) FOR NON LOW PROBABILITY | OVERALL 9 – 17%
  Intermediate (score 4-5) 14% (95% CI 9–22%)
  High (score 6-8) 64% (95% CI 40–82%)

### Suspect HIT

**Calculate 4Ts Score**

#### LOW PROBABILITY (<3)
- Hit lab testing: **NOT NEEDED**
- Non-heparin a/c: **DISCONTINUE**
- Heparin: **CONTINUE/RESUME HEPARIN**

- **NPV 99.8% 95% (CI) 97-100%**

#### INTERMEDIATE/HIGH PROBABILITY
- **PPV 4-5: 14% 95% CI 9–22%**
- **PPV 6-8: 64% 95% CI 40–82%**
Suspect HIT

Calculate 4Ts Score

INTERMEDIATE/ HIGH PROBABILITY

Hit lab testing:
ORDER IMMUNOASSAY
Heparin:
DISCONTINUE
Non-heparin a/c:
INITIATE

PPV 4-5: 14% 95% CI 9–22%
PPV 6-8: 64% 95% CI 40–82%

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Suspect HIT
Calculate 4Ts Score
INTERMEDIATE/ HIGH PROBABILITY
Hit lab testing: ORDER IMMUNOASSAY
Heparin: DISCONTINUE
Non-heparin a/c: INITIATE

Immunoassay Result
NEGATIVE
NPV >98%

Non-heparin a/c: DISCONTINUE
Heparin: CONTINUE/RESUME
<table>
<thead>
<tr>
<th>Suspect HIT</th>
<th>Calculate 4Ts Score</th>
<th>Immunoassay Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTERMEDIATE/ HIGH PROBABILITY</td>
<td>Hit lab testing: ORDER IMMUNOASSAY</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heparin: DISCONTINUE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-heparin a/c: INITIATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>POSITIVE</td>
<td></td>
</tr>
</tbody>
</table>

Are these pathogenic antibodies?
Suspect HIT
Calculate 4Ts Score

INTERMEDIATE/HIGH PROBABILITY
Hit lab testing: ORDER IMMUNOASSAY
Heparin: DISCONTINUE
Non-heparin a/c: INITIATE

Immunoassay Result
POSITIVE
Are these pathogenic antibodies
Order functional assay

HIT LABORATORY ASSAYS

<table>
<thead>
<tr>
<th>Category</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polytypic</td>
<td>&gt;95%</td>
<td>50-89%</td>
</tr>
<tr>
<td>IgG-specific</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGIA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functional</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIPA</td>
<td>&gt;90%</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>SRA</td>
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</tbody>
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• HIPA: heparin induced platelet aggregation
• SRA: serotonin release assay
MAYO CLINIC SRA METHOD

• Fresh platelet donors known to be reactive in the assay
• Standard washed platelet preparation (labor intensive)
• Incubate donor platelets with patient serum (which contains the heparin/platelet factor 4, H/PF4 antibodies) and unfractionated heparin
• If the H/PF4 antibodies are pathogenic, platelet activation occurs and platelet contents are released
  • Measure serotonin released in activated platelets
  • Light chain mass spectrometry (non-radioactive)

INTERPRETATION

<table>
<thead>
<tr>
<th>Serotonin release</th>
<th>Low heparin</th>
<th>High heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>&lt;20%</td>
<td>&lt;20%</td>
</tr>
<tr>
<td>Positive</td>
<td>&gt;20%</td>
<td>&lt;20%</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>&gt;20%</td>
<td>&gt;20%</td>
</tr>
</tbody>
</table>
Correlation of H/PF4 (IgG) ELISA with SRA Positive Test Result

<table>
<thead>
<tr>
<th>Optical Density</th>
<th>Heparin Inhibition</th>
<th>SRA Negative</th>
<th>SRA Indeterminate</th>
<th>SRA Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.4</td>
<td>N/A</td>
<td>230 (98.5%)</td>
<td>1 (0.5%)</td>
<td></td>
</tr>
<tr>
<td>0.4 – &lt; 1.0</td>
<td>13/15</td>
<td>210 (85%)</td>
<td>2 (8%)</td>
<td></td>
</tr>
<tr>
<td>1.0 – 1.5</td>
<td>14/15</td>
<td>8 (22%)</td>
<td>1 (3%)</td>
<td></td>
</tr>
<tr>
<td>1.5 – 2.0</td>
<td>10/11</td>
<td>5 (33%)</td>
<td>1 (7%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 2.0</td>
<td>18/18</td>
<td>1 (6%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Positive ELISA and Negative SRA: N=1

- 4Ts score moderate (4):
  - Post chemotherapy thrombocytopenia on UFH prophylaxis
- H/PF4 IgG ELISA OD: 2.001 with 100% heparin inhibition
- Mayo SRA and SRA (send out test) by radioactive: negative
- Example of a non-platelet activating H/PF4 antibody
HIT ELISA NEGATIVE AND SRA INDETERMINATE

- Indeterminate: n=1
  - Acute myeloid leukemia, UFH prophylaxis
  - 4Ts: 2
  - HIT IgG: <0.4
  - Mayo and mail out (radioactive) SRA: indeterminate
  - Heparin was discontinued due to post-chemotherapy thrombocytopenia
  - No evidence of thrombosis.

WHAT ABOUT SRA NEGATIVE HIT?

- Reports of patients with high clinical suspicion of HIT and positive HIT immunoassay but negative SRA
  - Case reports and series published
  - Careful clinical evaluation is critical in diagnosis and management of such patients
CONCLUSIONS

• Heparin induced thrombocytopenia is a complex clinicopathologic syndrome
• The initial evaluation of patients with thrombocytopenia who are on heparin should consist of a thorough clinical evaluation: 4Ts scoring system and follow guidelines
• Likelihood of HIT should influence decision making on
  • heparin interruption
  • initiation of a non-heparin anticoagulation
  • Laboratory testing
• A positive immunoassay result should prompt confirmation of pathogenicity of the antibody in HIT
• Functional assay serotonin release assay is currently the gold standard for detection of platelet activating antibodies
  • Rare cases of SRA negative HIT and cases of negative ELISA but positive SRA