Heparin-Induced Thrombocytopenia: The Dark Side of a Common Anticoagulant

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Learning Objectives

- Review the pathophysiology and clinical importance of heparin-induced thrombocytopenia (HIT)
- Discuss laboratory testing methodologies used for HIT diagnosis
- Discuss optimal laboratory approaches for accurate, timely, and cost-effective diagnosis of HIT
Heparin-induced thrombocytopenia (HIT) – historical aspects

- **1916**: Heparin discovered
- **1950's**: Heparin established as an anticoagulant
- **1958**: First publication associating heparin use with thrombosis
- **1964**: Immune basis for heparin-associated thrombosis is suggested
- **1970's**: Routine platelet counts are available
- **1977**: HIT with thrombosis
- **1979**: “White clot syndrome”
- **1984**: HIT “gold standard” assay developed

**Key Events**:
- **1916**: Weismann & Tobin, Arch Surgery
  - 10 cases over 3 years
  - 6 deaths, 2 amputations
- **1958**: Roberts et al, Surgery
- **1964**: Rhodes et al, Ann Surgery
  - Heparin-dependent antibodies were demonstrated
- **1977**: Towne et al, Arch Surgery
- **1984**: Sheridan et al, Blood
  - Serotonin release assay
Heparin

- Used as an anticoagulant for many decades to prevent and treat thrombotic events and for systemic anticoagulation
  - Venous thromboembolism, cardiopulmonary bypass, dialysis, percutaneous coronary intervention, etc.

- Heterogeneous mixture of sulfated saccharides that enhance antithrombin activity, resulting in potent inhibition of serine protease coagulation factors
Types of heparin

- Unfractionated heparin (UFH)
  - Heterogeneous
  - Many very large molecules
- Low molecular weight heparin (LMWW)
  - More homogeneous
  - Smaller, molecular weight <50% that of UFH
- Fondaparinux
  - Uniform, synthetic heparin with small size
Heparin-induced thrombocytopenia (HIT)

- Syndrome that occurs in ~1-5% of all heparin-exposed patients
- Immune-mediated
  - Formation of IgG antibodies to heparin-platelet factor 4 (PF4) complexes
    - PF4 is released from platelet granules and is positively charged
    - Heparin is negatively charged
    - Heparin-PF4 complexes represent a neoantigen, resulting in production of IgG by marginal zone B-cells
Heparin-induced thrombocytopenia (HIT)

- Immune complexes (heparin-PF4-IgG) cause platelet activation via the platelet Fcγ receptor for IgG (FcγRIIA)
  - Thrombocytopenia
  - High risk of thrombosis
    - Occurs in ~50% of untreated cases
    - May be venous (DVT, PE) or arterial (limb ischemia, stroke, MI)
      - Thrombotic complications can be fatal
  - Monocyte activation via FcγRIIA also appears to contribute to increased thrombotic potential
Heparin-Induced Thrombocytopenia

High risk of thrombosis

Heparin exposure

Seroconversion, 5-10 days

IgG antibodies form against heparin-PF4

Immune complexes bind to platelets

Platelet activation

Excessive thrombin generation

Heparin

PF4

IgG

High risk of thrombosis
Heparin-induced thrombocytopenia (HIT)

- Thrombosis
- Platelet activation with thrombocytopenia
- IgG antibodies to heparin-PF4 complexes
- All heparin-exposed patients
Risk factors for HIT

- Risk is related to amount of circulating PF4 and type and dose of heparin
  - Determines whether heparin-PF4 complexes (the HIT antigen) will form
- Higher risk in surgical patients than in medical patients
- Higher risk with UFH than with LMWH
- Estimated at 1-5% for surgical patients, and less than 1% for most medical patients
HIT diagnosis

- Clinical evaluation
  - Many heparin treatment protocols require platelet count monitoring in high-risk patients
  - HIT considered when platelet count drops 5-10 days after heparin initiation/exposure
  - Clinical scoring systems determine pre-test clinical probability
    - 4Ts system
      - Low scores have good negative predictive value (NPV): 97-99%
      - Intermediate and high scores have poor positive predictive value (PPV): 10-20% and 40-80%, respectively

HIT clinical scoring – 4T’s

Thrombocytopenia
- 0 points; <30% drop or nadir <10 x 10^9/L
- 1 point; 30-50% drop or nadir 10-19 X 10^9/L
- 2 points; >50 drop and nadir >=20 X 10^9/L

Timing of platelet drop
- 0 points; <4 days with no recent exposure
- 1 point; suspect 5-10 days but not documented, >10 days, <=1 day with last exposure 30-100 days prior
- 2 points; 5-10 days or <= 1 day if exposed in past 30 days

Thrombosis
- 0 points; none
- 1 point; progressive, recurrent, silent, suspected but not proven
- 2 points; proven new thrombosis or skin necrosis or acute systemic reaction to heparin

Other causes of thrombocytopenia
- 0 points; definite
- 1 point; possible
- 2 points; none

- Scoring
  Low = <4 points, good NPV
  Intermediate = 4-5 points, poor PPV
  High = 6-8 points, poor PPV

- Lab testing is needed for patients with intermediate or high 4T’s scores
HIT laboratory evaluation

- Immunoassays
  - ELISA is most common
    - Plate coated with heparin-PF4 or acceptable alternative used to capture heparin-PF4 antibodies from patient serum or plasma
      - Polyspecific detection – IgG/IgM/IgA
      - Monospecific detection – only IgG
    - Often used as an initial test
      - High sensitivity for HIT, inexpensive, simple to perform, fairly good turnaround time
  - Chemiluminescent immunoassay (CLIA) - recently FDA approved
- Rapid assays
Heparin-PF4 ELISA

- General performance characteristics

  **Negative results** *(you can’t have HIT with no antibodies)*
  - Excellent NPV (~99%)
    - No need to repeat negative testing if no change in clinical status

  **Positive results** *(you can have antibodies without HIT)*
  - Excellent sensitivity (95-100%)
  - Poor specificity (<90% or even <<<90%)
    - Specificity improved by considering optical density (OD) value and with use of IgG only ELISA
  - Poor PPV

Heparin-PF4 ELISA

- Results should be reported as an OD value with cutoff value rather than qualitatively (positive/negative)
- Higher OD values correlate with antibody titer and capacity for platelet activation
  - <0.4: antibody negative
  - 0.4 to 1.0: ~5% probability of HIT
  - 1.0 to 1.5: ~25% probability of HIT
  - >=2.0: ~90% probability of HIT

J Thromb Haemost 2008;6:1304-12
Heparin-PF4 ELISA

- Polyspecific (IgG/IgM/IgA) detection versus monospecific (IgG only) detection
  - HIT is caused IgG antibodies that activate platelets through the surface Fcγ receptor for IgG
    - Platelets are not activated by IgM or IgA antibodies
  - Some published studies suggest that the polyspecific assays have worse performance characteristics than IgG alone while others suggest that with use of optimal cutoffs the polyspecific ELISA has adequate sensitivity and specificity
  - Although IgG ELISAs may have better specificity, clinically irrelevant antibodies are still detected

J Lab Clin Med 2005;146:341-6
Blood 2016;127(5):546-57
Heparin-PF4 ELISA

- Potential for false-positive ELISA in specimens containing antiphospholipid antibodies
  - Thought to be due to autoantibodies against PF4
  - Negative in functional assays for HIT

J Thromb Haemost 2009;7:100-4
Rapid immunoassays

- Particle immunofiltration assay approved for use in the United States, although does not appear to be widely used
  - Qualitative (positive/negative) results based on color change, results in ~30 minutes

Advantages
  - Timely results, no special instrumentation, efficient processing of single patient samples

Utility similar to ELISA (useful for exclusion of HIT)
  - Recent publication suggests that diagnostic accuracy may be suboptimal for this assay (limited data)

Blood 2016;127(5):546-57
Case study 1

- A 75-year-old female received unfractionated heparin during admission for aortic valve replacement. Her baseline platelet count of 400 K/µL dropped to 150 K/µL 9 days after heparin was initiated and she has developed a DVT. Other potential causes of thrombocytopenia include an infection being treated with antibiotics.
  - 4T’s score: 7 (high probability, PPV 40-80%)
  - ELISA OD: 1.1 (cutoff 0.4, ~25% probability of HIT)
Case study 2

- A 60-year-old male being treated with LMWH for a DVT that occurred after knee replacement develops mild thrombocytopenia (130 K/µL, compared to his baseline of 200 K/µL) with unclear timing due to incomplete lab data. Imaging is inconclusive but suggests possible extension of his DVT. Other causes for his thrombocytopenia are considered possible but not probable (history of autoimmune disease, possible history of ITP).
  - 4T’s score: 4 (intermediate probability, PPV 10-20%)
  - ELISA OD: 3.2 (>90% probability of HIT)
HIT laboratory evaluation

- Functional platelet activation assays
  - Evaluate ability of patient serum or plasma antibodies to activate reagent platelets in the presence of heparin
  - Useful for
    - Evaluating unexpected ELISA results
    - Evaluating positive ELISA results
- Serotonin release assay (SRA) is the golden standard and most commonly used
  - >90% sensitivity and specificity for HIT due to demonstration of platelet activating properties of antibodies
- Other platelet activation assays (non-SRA)
Serotonin release assay

Heparin (reagent)

Patient sera (provides antibodies)

Washed platelets (reagent)

IgG
PF4
Heparin

Platelet activation

IgG
PF4
Heparin

Serotonin released by platelet dense granules

Detection and quantification of released serotonin (expressed as % of total serotonin)
Serotonin release assay (SRA)

- Serotonin detection methods
  - Radiolabeled serotonin (C$^{14}$ serotonin)
  - High-performance liquid chromatography (HPLC)
  - Mass spectrometry
  - Immunoassay
HPLC serotonin elution profile
Donor platelet total serotonin content

<table>
<thead>
<tr>
<th>Day of testing</th>
<th>Total platelet serotonin content (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>356</td>
</tr>
<tr>
<td>2</td>
<td>519</td>
</tr>
<tr>
<td>3</td>
<td>485</td>
</tr>
<tr>
<td>4</td>
<td>339</td>
</tr>
<tr>
<td>5</td>
<td>463</td>
</tr>
<tr>
<td>6</td>
<td>500</td>
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<tr>
<td>7</td>
<td>312</td>
</tr>
<tr>
<td>8</td>
<td>457</td>
</tr>
<tr>
<td>9</td>
<td>299</td>
</tr>
<tr>
<td>10</td>
<td>337</td>
</tr>
</tbody>
</table>
Serotonin release assay (SRA)

- Result interpretation; \( \geq 20\% \) serotonin release represents platelet activation

<table>
<thead>
<tr>
<th>Therapeutic concentration, supports HIT immune complex formation</th>
<th>Supra-therapeutic concentration, disrupts HIT immune complex formation</th>
<th>Overall interpretation is based on response to both heparin concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low heparin percent release</td>
<td>High heparin percent release</td>
<td>Interpretation</td>
</tr>
<tr>
<td>( \geq 20% )</td>
<td>( &lt;20% )</td>
<td>Positive</td>
</tr>
<tr>
<td>( &lt;20% )</td>
<td>( &lt;20% )</td>
<td>Negative</td>
</tr>
<tr>
<td>( \geq 20% )</td>
<td>( \geq 20% )</td>
<td>Indeterminate</td>
</tr>
</tbody>
</table>
Serotonin release assay (SRA)

- Indeterminate results
  - Obtained in a few percent of samples tested in the SRA
  - When reproducible usually indicate platelet activation by non-heparin-dependent (non-HIT) antibodies
    - HLA antibodies
    - Specific antibodies to platelet surface antigens
    - Immune complexes
  - Likelihood of HIT in these cases determined by combination of clinical impression and ELISA results
Other platelet activation assays (not serotonin release)

- Heparin-induced platelet activation (HIPA)
  - Visual evaluation of platelet activation/clumping
- Heparin-induced platelet aggregation
  - Light transmission or impedance aggregometry
- Heparin-induced platelet ATP release by luminography
- Heparin-induced platelet microparticle generation by flow cytometry
- Heparin-induced platelet P-selectin expression by flow cytometry
Thoughts on lab testing

- HIT lab testing is prompted by development of thrombocytopenia (on the correct timeline) in a heparin-exposed patient
  - In HIT, the platelet count drop is caused by heparin/PF4/IgG complex-mediated platelet activation
    - Detectability of the antibodies precedes thrombocytopenia
  - A negative ELISA indicates no antibodies and excludes HIT as the cause of thrombocytopenia
    - No need to repeat a negative ELISA in the absence of a new, additional drop in platelet count
## Correlation between ELISA and SRA

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>ELISA (OD) cutoff 0.4</th>
<th>SRA LH/HH (% release)</th>
<th>SRA Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.066</td>
<td>99/0</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>3.358</td>
<td>100/0</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>1.334</td>
<td>72/0</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>2.759</td>
<td>100/0</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>1.032</td>
<td>70/0</td>
<td>Positive</td>
</tr>
<tr>
<td>6</td>
<td>1.346</td>
<td>100/0</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>2.659</td>
<td>100/0</td>
<td>Positive</td>
</tr>
<tr>
<td>8</td>
<td>2.111</td>
<td>70/0</td>
<td>Positive</td>
</tr>
<tr>
<td>9</td>
<td>2.385</td>
<td>100/0</td>
<td>Positive</td>
</tr>
<tr>
<td>10</td>
<td>2.786</td>
<td>97/0</td>
<td>Positive</td>
</tr>
</tbody>
</table>
## Correlation between ELISA and SRA

<table>
<thead>
<tr>
<th>Sample ID</th>
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<th>SRA LH/HH (% release)</th>
<th>SRA Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.667</td>
<td>0/0</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>0.277</td>
<td>0/0</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>0.162</td>
<td>0/0</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>0.078</td>
<td>0/0</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>0.753</td>
<td>0/0</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>0.089</td>
<td>0/0</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>0.082</td>
<td>0/0</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>0.048</td>
<td>0/0</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>0.513</td>
<td>0/0</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>1.278</td>
<td>0/0</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Positive SRA results

- Although cutoff’s of 20% serotonin release are frequently used to define platelet activation in the SRA, samples with at least 50% release has been most closely correlated with risk of thrombosis in HIT in the literature.

- 95% of patients with clinical HIT in prospective studies have serotonin release >=50%.

- From local data; 1 month of positive SRAs (n = 71):
  - % release <50%: n = 9
  - % release >=50%: n = 62

Am J Hematol 2007;82:1037-43
Quality assurance for improbable results

- Local quality practices involve tracking ELISA/SRA correlations (when available) and researching any improbable pairs that are identified
  - Only 5 such pairs have been identified in the past year
    - Very strong positive ELISA with negative SRA
    - Negative ELISA with positive SRA
HIT diagnostic algorithm

Clinical impression and 4T’s scoring

- Low 4T’s score (<4 points)
  - HIT unlikely; lab testing not routinely performed

- Intermediate (4-5 points) or high (6-8 points) 4 T’s score: Lab testing needed for possible HIT
  - Order ELISA; initiate HIT treatment
    - Negative ELISA
      - HIT excluded; no further testing needed in most cases; modify clinical actions; look for alternative diagnosis
    - Positive ELISA
      - HIT possible/probable depending on OD value; order SRA
        - Negative SRA
          - HIT very unlikely; modify clinical actions
        - Positive SRA
          - HIT confirmed; continue HIT treatment
Case study conclusions

- **Case study 1**
  - 4T’s score: 7 (high probability, PPV 40-80%)
  - ELISA OD: 1.1 (cutoff 0.4, ~25% probability of HIT)
  - SRA: Negative

- **Case study 2**
  - 4T’s score: 4 (intermediate probability, PPV 10-20%)
  - ELISA OD: 3.2 (>90% probability of HIT)
  - SRA: Positive
Treatment

- For patients with intermediate or high 4T’s scores, treatment is initiated immediately, prior to availability of laboratory test results
  - Discontinue all heparin products, including line flushes
  - Discontinue warfarin
  - Initiate a non-heparin anticoagulant (unless contraindicated)
    - Note: treatments are more expensive than heparins and often with higher risk of bleeding complications
- Order laboratory testing
- If HIT is confirmed, heparin allergy should be added to patient’s chart
Natural history of HIT

- HIT is a transient immune response and antibodies become undetectable after ~3 months (median 40-100 days)
- There is no anamnestic response
  - If a prior HIT patient (now antibody negative) were re-exposed to heparin, seroconversion would again take 5-10 days
- Brief heparin re-exposure in an antibody negative patient with prior HIT may be safe, but should be avoided when possible
  - Consider if heparin is preferred anticoagulant for the indication
  - Use non-heparin anticoagulants whenever possible
- Rapid onset HIT occurs upon heparin re-exposure in a prior HIT patient who still has antibodies (heparin within past 100 days)

Blood 2016;128:348-59
Natural history of HIT

- Thrombosis
- Platelet activation with thrombocytopenia
- IgG antibodies to heparin-PF4 complexes
- All heparin-exposed patients
## Case example of treated HIT

<table>
<thead>
<tr>
<th>Date</th>
<th>ELISA (OD) cutoff 0.4</th>
<th>SRA LH/HH (% release)</th>
<th>SRA Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>07/01/16</td>
<td>Not done</td>
<td>100/0</td>
<td>Positive</td>
</tr>
<tr>
<td>07/11/16</td>
<td>2.773</td>
<td>99/0</td>
<td>Positive</td>
</tr>
<tr>
<td>08/24/16</td>
<td>2.280</td>
<td>67/0</td>
<td>Positive</td>
</tr>
<tr>
<td>09/21/16</td>
<td>Not done</td>
<td>22/0</td>
<td>Positive</td>
</tr>
<tr>
<td>11/04/16</td>
<td>Not done</td>
<td>16/0</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Rare spontaneous (autoimmune) HIT

- Develops in the absence of heparin exposure
- Risk factors are major surgery or infection/inflammation
  - Positively charged PF4 can bind to other polyanions such as nucleic acids, glycosaminoglycans, or bacterial lipopolysaccharide
  - Complexes of PF4 and non-heparin polyanions induce primary immunization
    - Resultant transient IgG antibodies allow opsonization and phagocytosis of PF4-coated bacteria that does not require prior antigen exposure
- HIT results when this mechanism is misdirected
  - Classic HIT – platelets are coated with heparin-PF4 complexes
  - Spontaneous HIT – platelets are coated with non-heparin polyanion-PF4 complexes

Avoid heparin programs

- An Avoid-Heparin Initiative has been studied over a 10-year period at a tertiary care hospital on Toronto, Ontario, Canada
  - Replaced UFH with LMWH whenever possible in prophylactic and therapeutic protocols
    - Rate of suspected HIT decreased by 42%
    - Rate of positive HIT ELISAs decreased by 63%
    - Rate of adjudicated HIT decreased by 79%
    - Rate of HIT with thrombosis decreased by 91%
    - HIT-related cost of care decreased by 83% ($266,938/year)

Blood 2016;127:1954-59
Conclusions

- Heparin-induced thrombocytopenia (HIT) is an immune-mediated syndrome of thrombocytopenia and thrombosis that occurs in a few percent of heparin-exposed patients.
- HIT is a clinicopathologic diagnosis, requiring both clinical and laboratory information.
- Algorithmic testing approaches utilizing immunoassays and functional assays maximize clinical performance and cost-effectiveness.