Advances in the Diagnosis of Heparin Induced Thrombocytopenia

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A Case-based Workshop: Clinical and Laboratory Aspects of Hemophilia and Thrombosis
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DISCLOSURES

Relevant Financial Relationship(s)

Patents/Pending Patents: Versiti, Retham Technologies

Invention Disclosure: Mayo Clinic

Equity Ownership: Retham Technologies

Advisory Board: Veralox Therapeutics

Off Label Usage:

• Nothing to Disclose
Case

- Patient had protracted thrombocytopenia and developed thrombosis
- **SRA-negative** in the early time point. Could this ab have been detected early?

Outline: Heparin-induced Thrombocytopenia

- Background

- HIT Diagnosis: Current assays

- Advances in functional testing

- “Next Gen” HIT diagnostic testing

- Implications for Understanding HIT pathophysiology

- Conclusions
Heparin-induced Thrombocytopenia

- Some patients (<1%) treated with heparin develop heparin induced thrombocytopenia/thrombosis

- HIT is characterized by low platelet counts and a relatively high (~30%-50%) thrombosis rate

- A number of patients undergo digit/limb amputations and have strokes

- >50 HIT patients are diagnosed each day in the US. ~5 patients die (10% mortality)

Dhakal et al, Lancet Haem, May 2018

Model to explain platelet activation induced by HIT antibodies

Model on Left: Does not explain (1) Prothrombotic state days/weeks after Heparin cessation
(2) Spontaneous HIT/Delayed Onset HIT/Persistent HIT

Padmanabhan et al, Blood 2015
HIT Diagnostic Assays: Antigen Assays

- Highly Sensitive (>99%)
- Non-specific (PPV only 30-50%)
- More recently released automated ELISAs* appear to have lower sensitivity than solid phase ELISAs


Functional HIT assays: Serotonin Release Assay

- Superior for identifying disease-positive patients
- Radioactive. Technically demanding. Few reference labs perform it
- Empiric treatment with alternative anticoagulants until SRA results are obtained (typically 2-5 day TAT)
- While SRA results are pending, unwarranted bleeding risk due to alternative anticoagulant use in patients without HIT
- **Goal:** Develop an accurate functional HIT assay that is also technically-simple
**Novel HIT assay: PF4-Dependent P-selectin Expression Assay (PEA)**

Basis: PF4 bound to platelets is the target recognized by activating ("pathogenic") HIT antibodies.

- **Platelets** + PF4 ➔ **Patient Serum** ➔ P Selectin Expression (Flow cytometry)

Uses 20-fold fewer platelets than the SRA (1M vs ~21M)

**SRA**: Low dose heparin is added
**No PF4 is used**

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**Utility of the PEA: Preliminary Studies**

- The PEA has equal or greater accuracy than the SRA*

- PEA detects pathogenic antibodies earlier than the SRA, and in some cases even the PF4 ELISA**

- "SRA-negative HIT" is being recognized as a real entity***

- Independent validation of the utility of PF4-enhanced functional assays for earlier and more accurate diagnosis has been obtained by other groups****

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**Jones et al, Chest, 2017; Samuelson Bannow, Warad et al, Blood 2020
***Pandya et al, Th Res, 2018; Warkentin et al, Int J Lab Hem, 2019
****Vayne et al, Th & Haem, 2019: Vayne et al, BJH, 2017
Predefined HIT classification

<table>
<thead>
<tr>
<th>HIT ELISA OD</th>
<th>4Ts Score</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0-3 (Low)</td>
<td>4-5 (Intermidiate)</td>
<td>6-8 (High)</td>
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<tr>
<td>≤ 0.300 or &lt;0.4</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>0.301(or 0.4)-0.999</td>
<td>Negative</td>
<td>Negative</td>
<td>Indeterminate</td>
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<tr>
<td>1.000-1.999</td>
<td>Negative</td>
<td>Indeterminate</td>
<td>Positive</td>
</tr>
<tr>
<td>≥2.000</td>
<td>Indeterminate</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Indeterminates were considered **disease-negative** in the primary analysis.

Indeterminates are considered **disease-positive** in a sensitivity analysis.
**Results**

**HIT-pos**

![Graph showing serotonin release or P-selectin expression (%)](image)

**HIT-neg**

![Graph showing serotonin release or P-selectin expression (%)](image)

**HIT-Indeterminate**

![Graph showing serotonin release or P-selectin expression (%)](image)

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Samuelson Bannow, Warad et al, Blood, Sep 2020

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**PEA facilitates earlier detection of HIT abs**

![Graph showing platelet count over time](image)

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BV- Bivalirudin

Samuelson Bannow, Warad et al, Blood, Sep 2020
Back to the case:
PF4-enhanced assays are positive early in the course

Where Next?
“Next Gen” HIT testing: Decentralize Functional Testing

Functionally testing: Send out

Results from Ref Lab

In-hospital testing: Functional HIT IVD

(1) Long-term Stable Platelets
(2) PF4
(3) Assess Platelet Activation
Stabilized platelets support PF4-dependent HIT antibody-mediated platelet activation

PA- Proprietary Analyte (Platelet granule component)

Test System has high accuracy

N=31
Sensitivity: 100%
Specificity: 100%
Test System is Highly Specific

Implications: Understanding HIT pathophysiology

“Garden variety” HIT with isolated thrombocytopenia
HIT with Thrombosis
Severe phenotypes (Delayed, Spontaneous etc.)

Why do some but not other HIT abs activate platelets in the no-heparin SRA?

One possibility: Addition of heparin potentiates platelet responses to subthreshold doses of agonists?

Heparin-independent, PF4-dependent platelet activation in the PEA

HIT is an Autoimmune Disease

Healthy humans possess preexisting inactive/tolerant PF4/heparin-specific B cells. Breakdown of tolerance leads to antibody production

*Zheng et al, Blood 2014
**Gao et al, Blood, 2011 May 5;117(18):4946-52
Conclusions

• The data support the view that PF4 in complex with platelets is recognized by pathogenic HIT antibodies

• While heparin is important for disease initiation, HIT can be perpetuated in the absence of heparin

• PEA, a technically-simple assay is at least as accurate as the SRA for identification of patients with HIT and detects pathogenic antibodies earlier in the course of disease

Conclusions

• Test systems using PF4-treated platelets will likely become increasingly important for early and accurate diagnosis (e.g. PEA, PF4-SRA). Still, they will likely not be as widely available as frontline assays such as ELISAs.

• We need a new generation of functional “near-patient” diagnostic assays that are technically simple and highly accurate to enable timely and appropriate management

• HIT may need to be viewed as an autoimmune disease