Diagnosis of Drug-Induced Immune Thrombocytopenia (DITP)

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Disclosure for Mervyn Sahud, MD

<table>
<thead>
<tr>
<th>Role</th>
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<tr>
<td>Research Support/P.I.</td>
<td>No relevant conflicts of interest to declare</td>
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<td>Consultant</td>
<td>No relevant conflicts of interest to declare</td>
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<td>Major Stockholder</td>
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<td>Speaker Bureau</td>
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<td>Honoraria</td>
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Presentation does not include any discussion of off-label use of a drug or medical device
Laboratory Diagnosis of DITP4

Historical Notes

- Rosenthal 1928
- Grandjean 1948
- Ackroyd 1948
- Bolton 1956

Re-challenged Platelet Quinidine
PRP Plus Quinine Causes Lysis of Platelets
Sedormid Lally(isopropyl-acetylurea
1. Inhibition of Clot Retraction
2. PRP Plus Drug Causes Agglutination
3. Agglutination of Normal Platelets in Presence of Drug and Serum
Quinine and Quinidine Induced Antibodies to Platelets Causing Platelet Destruction in the Presence of Complement via Platelet Drug Antibody Complex

Methods Formerly Used For Ddab Detection

- Platelet Agglutination
- Clot Retraction Inhibition
- Complement Fixation
- “Platelet Factor 3” Release
- $^{51}$Cr Release

Platelet Agglutination

A. Normal platelet-rich plasma plus quinine 0.1 mg per ml
B. Patient platelet plasma (obtained post-recovery) plus quinine 0.1 mg/ml

Detection of quinine-dependent platelet antibodies in a patient with quinine-induced thrombocytopenia using platelet agglutination as the end point
Clot Retraction Inhibition

Complement Fixation

“Platelet Factor 3” Release

- Karpatkin M, Siskind GW, Karpatkin S. The platelet factor 3 immunoinjury technique re-evaluated. Development of a rapid test for antiplatelet antibody. Detection in various clinical disorders, including immunologic drug-induced and neonatal thrombocytopenias.

<p>| Drug-associated Thrombocytopenia with Detectable Drug-dependent Antiplatelet Antibody |
|---------------------------------|-------------------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Age / Sex</th>
<th>Drug</th>
<th>Antibody Without The Drug</th>
<th>Antibody With The Drug</th>
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<tbody>
<tr>
<td>65 F</td>
<td>Quinidine</td>
<td>0</td>
<td>1:24</td>
</tr>
<tr>
<td>46 F</td>
<td>Quinidine</td>
<td>0</td>
<td>1:24</td>
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<tr>
<td>55 F</td>
<td>Quinidine</td>
<td>0</td>
<td>1:32</td>
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<tr>
<td>47 M</td>
<td>Quinidine</td>
<td>0</td>
<td>1:16</td>
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<tr>
<td>12 F</td>
<td>Quinidine</td>
<td>0</td>
<td>1:12</td>
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- Washed platelets are incubated with patient serum in the presence and absence of drug.
- Resulting platelet activation is detected by the ability of platelets to shorten the clotting time of plasma (using Russell’s viper venom).
Platelets are labeled with $^{51}$Cr sodium chromate and washed.

Patient serum is added with and without test drug.

Fresh normal serum is added as a source of complement.

After one hour at 37°C, released chromium is measured and expressed as a percent of total $^{51}$Cr.

Sensitivity for antibody detection is increased by pre-treatment of test platelets with papain.

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**51Cr Release Assay**

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**Detection of “Quinine-type” Drug-Dependent Antibodies: Caveats**

- Drug should be used at a concentration of 1.0 mg per ml, if solubility permits.

- Solubilization of drug can sometimes be facilitated by suspending in 1% albumin.

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**Detection of “Quinine-type” Drug-Dependent Antibodies: Caveats**

- Soluble drug should be present at all steps of the assay (some antibodies dissociate in the absence of drug).

- Drug metabolites formed in vivo can occasionally be the sensitizing agents. In such cases, urine obtained from a person taking the suspect drug can sometimes be used as a source of metabolites for antibody detection.

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Peripheral blood smear in a patient with May-Hegglin anomaly, a form of dominantly-inherited thrombocytopenia characterized by giant platelets and neutrophil inclusions.

**Flow Cytometry Assay**

1. Washed platelets are combined with patient serum with and without quinine.

2. After 1 hour incubation at room temperature, platelets are washed with buffer or buffer containing quinine.

3. FITC labeled anti-human secondary antibody is added (+/- quinine) and allowed to incubate 30 minutes at room temperature.

4. Sample is diluted and analyzed on a FACScan.

**Glycoprotein-1b – IX Complex**

Representative Titer:
Source: Serum
Mean fluorescence intensity (MFI) ratio [with drug/no drug] at various serum dilutions. A positive reaction is defined as a MFI ratio of 2.0 or greater.

Representative Histogram:
Source: Serum (1:50)
Antibody binding to platelets in presence of buffer, quinine, or quinidine.

Detection of Platelet Reactive Drug-dependent Antibodies By Flow Cytometry

Naproxen-Induced Thrombocytopenia - Detection Of Antibody Using Urinary Metabolites And Naproxen Glucuronide

“Naturally Occurring” Antibodies

*Naturally occurring* antibodies that recognize ligand-occupied GPIIb/IIIa appear to be the cause of thrombocytopenia occurring in patients treated with ligand-mimetic inhibitors.
1. Solid phase assay (PF4 ELISA)

2. Platelet activation assays
   - Serotonin release
   - Platelet aggregation

**HIT Antibody Detection**

- Unfractionated heparin (or polyvinyl sulfonate, a heparin-mimetic for the purpose of this assay) is incubated with platelet PF4 at an optimum ratio to produce macromolecular complexes.
- These complexes are plated in microtiter trays and used as targets for antibody detection using an ELISA endpoint.
- Specificity of a positive result can be confirmed by showing that the reaction is inhibited by high dose heparin.
- Several commercial kits are available.

**Characteristics of the PF4 ELISA Assay**

- Can be done in a few hours
- Highly specific and sensitive
- Not all patients with antibodies have HIT
- Laboratory results plus clinical findings make a diagnosis
Stronger HIT antibodies can be detected by their ability to induce platelet aggregation in the presence of heparin. Some hospital laboratories offer this test because it can be done locally.

However, the platelet aggregation test is lacking in sensitivity (30-85% relative to the SRA) and specificity (HLA antibodies can cause positive reactions).

**IT IS DANGEROUS TO RELY ON THIS TEST FOR DETECTION OF HIT ANTIBODIES!!**

**The Serotonin Release Assay (SRA)**

- Two highly sensitive platelet donors
- $^{14}C$-serotonin incorporated into platelet dense granules
- Test serum heated inactivated
- Incubation at room temperature for 60 minutes with gentle agitation
- After centrifugation, released radioactivity is measured and expressed as percent of total radioactivity.

*Sheridan and Kelton. Blood 1986, 67, 2719*

**Take Home Messages...**

- The serotonin release test (SRA) is technically demanding and requires referral of the patient sample to a reference laboratory.
- It is less sensitive than the PF4 ELISA for antibody detection, but patients with a positive SRA are more likely to have HIT than those who are SRA negative.
- Why SRA-positive antibodies are more likely to cause clinical HIT is not fully understood but is probably related to their ability to activate platelet and induce procoagulant activity.
From Bench To Bedside Diagnosis

HIT  Non-HIT (DITP)

Thank You