Case 1

- 65 year-old woman
  - CABG x3
- Post-op day 4
  - Platelets 215K
- Post-op day 6
  - post-op infection
  - antibiotics via PICC line
  - heparin flushes to maintain line patency
Post-op day 8
- pain, swelling left arm
- arm dusky, pulses present
- venogram showed extensive thrombosis
- IV heparin initiated
- arm worsened – cold, blue
- platelet count 43K
Case 1 (continued)

◆ Hematology consulted
  ● dyspnea, positive VQ scan
  ● Platelet count 17K
  ● HIT ELISA positive
  ● Direct Thrombin Inhibitor (DTI) initiated, but later held to allow t-PA treatment for impending gangrene
  ● arm improving - t-PA discontinued
  ● DTI resumed 1-2 days later
Case 1 (continued)

- **Post-op day 13**
  - platelet count 103K
  - warfarin 5 mg begun

- **Post-op day 14**
  - DTI discontinued

- **Post-op day 16**
  - breast pain with new necrotic lesion
  - warfarin discontinued; DTI restarted
  - vitamin K started
Case 1 (continued)

◆ Post-op day 21
  • breast lesion healed, arm saved
  • platelets 201K, warfarin resumed

◆ Post-op day 26
  • discharged

◆ Four months later
  • repeat CABG on heparin
  • no recurrence of HIT
  • HIT ELISA negative
Problems with heparin as an anticoagulant

- bleeding
- protamine toxicity (reversal)
- variable anticoagulant response
- failure to suppress platelet activation – may actually contribute
- antigenicity - risk of heparin-induced thrombocytopenia
Heparin-Induced Thrombocytopenia

- autoimmune, drug-induced condition
- 0.1–3.0% of heparin-treated patients
- antibodies to heparin-PF4 complex
- thrombocytopenia ± thrombosis
- high morbidity and mortality
- substantially increased health care costs
- high risk of litigation
Pathophysiology of HIT

PF-4 binds to surface of platelet following activation

Complexes of heparin (GAG) and PF-4 molecules form

IgG binds to the PF-4/heparin complex

Fc stimulation leads to the generation of procoagulant-rich microparticles

IgG/PF-4/heparin complex activates via the Fc receptor

Courtesy of Dr. John Kelton
HIT Factoids

- UFH (1-3%) > LMWH (0.1-0.5%)
- bovine > porcine
- high dose > low dose
- intravenous > subcutaneous
- surgical > medical
- females > males
- antibodies are transient
- usually not re-stimulated after re-exposure
- if re-stimulated, antibodies not formed more quickly
HIT is not a rare disease
(if you look for it)

~14% of all patients screened test positive

J.L. Francis (unpublished data)
Clinical diagnosis of HIT
When to suspect it

- relevant clinical symptoms
  - fall in platelets, thrombosis, allergic reactions
- in the setting of current or recent heparin exposure
  - any preparation, dose or route
- diagnosis is primarily clinical
- depends on awareness and vigilance
Clinical diagnosis of HIT
Timing of thrombocytopenia

Case 1

Typical-onset HIT (within 4 to 14 days)

Heparin exposure
Case 2

- 70 year-old male with aortic valve disease
  - underwent bypass surgery for aortic valve repair
  - received LWMH post-op days 1-6

- Day 9
  - discharged on coumadin
Case 2 (continued)

Day 30

- re-presented with a painful leg
- Doppler -> Femoral DVT
- platelets 420K
- heparin bolus administered
- acute systemic reaction – dyspnea, tachycardia, hypotension

Day 31

- platelets 47K
Case 2 (continued)

Day 31
- IVC filter placed
- VQ scan negative for PE
- HIT ELISA positive
- Direct Thrombin Inhibitor started

Days 32-35
- Prompt improvement in platelet count

Day 37
- Discharged; platelets 340K
Clinical diagnosis of HIT

Timing of thrombocytopenia

Heparin exposure

Rapid-onset HIT (previous heparin exposure)

Typical-onset HIT (within 4 to 14 days)

Case 1

Case 2

Timing of thrombocytopenia

Clinical diagnosis of HIT

Heparin exposure
Case 3

- AB underwent gastric bypass surgery for morbid obesity. He received s.c. heparin (5000 u bid) for prophylaxis. Platelets 290K on discharge.
- Day 5 – presented to ER with severe left arm pain - acute radial artery occlusion. Received IV heparin, coumadin and thrombo-embolectomy (platelets 150K).
- Day 6 – developed ulnar artery thrombosis. Platelet count 120K.
- Heparin discontinued – HIT ELISA +ve; SRA +ve.
- Treated with danaparoid. Platelet count recovered.
Case 4

- GF underwent CABGx3 and had uneventful post-op course. Platelet count normal on discharge at day 6.
- Day 13 – presented to ER with right foot pain and ischemic changes to a big toe. Platelet count 70K but heparin therapy started anyway.
- Day 14 – Platelets 45K -> Hem Consult -> Platelet count 45K. HIT ELISA +ve; heparin d/c.
- Started argatroban therapy – platelet count recovered without further issues
- Day 22 - discharged
Clinical diagnosis of HIT
Timing of thrombocytopenia

Heparin exposure

Days

Rapid-onset HIT (previous heparin exposure)

Typical-onset HIT (within 4 to 14 days)

Delayed-onset HIT (average of 9 days after heparin is stopped)

Case 1

Case 2

Cases 3 & 4
Clinical diagnosis of HIT
Degree of thrombocytopenia

◆ >50% fall in platelet count - not absolute thrombocytopenia
◆ smaller falls may occur
◆ diagnosis more difficult after bypass surgery
  ● transient thrombocytopenia is normal
  ● pre- and post-op heparin use (difficult to determine appropriate “pre-heparin” platelet count)
Timing of thrombocytopenia post-CPB

Post-op day

Normal Pattern
Timing of thrombocytopenia post-CPB

Typical post CPB HIT

Platelet count secondary fall

Post-op day
Clinical diagnosis of HIT

Thrombosis

- may be arterial or venous (1:4)
- upper extremity thrombosis common
- venous limb gangrene
- frequently result in amputations
- will supervene in 50% of thrombocytopenic patients if untreated
Platelet counts and Thrombosis

Standard definition of thrombocytopenia

Median platelet count nadir = 59 x 10⁹/L

Warkentin TE. Semin Hematol 1998, 35: 9-16
Clinical diagnosis of HIT

Key components

- timing of thrombocytopenia
- degree of thrombocytopenia
- thrombosis
- associated clinical syndromes
- supportive laboratory data (HIT is a clinicopathologic syndrome)
Laboratory diagnosis of HIT
Available assays

**Activation assays**
(Functional antibodies)
- Serotonin Release Assay
- Platelet Aggregation Assay
- Flow Cytometry

**Antigen assays**
(all antibodies)
- Enzyme-Linked Immunoassay (ELISA)
- Particle Gel IA
- Chemiluminescence
- Latex Enhanced IA
Laboratory diagnosis of HIT
Most widely available assay

Activation assays (Functional antibodies)
- Serotonin Release Assay
- Platelet Aggregation Assay
- Flow Cytometry

Antigen assays (all antibodies)
- Enzyme-Linked Immunoassay (ELISA)
- Particle Gel IA
- Chemiluminescence
- Latex Enhanced IA
Laboratory diagnosis of HIT

Immunoassays

◆ **Advantages**
  - very sensitive (exclude HIT)
  - large numbers of tests easily performed
  - may be (relatively) rapidly performed

◆ **Disadvantages**
  - not cost-effective if volumes low
  - frequently positive without symptoms of HIT
  - carry a danger of over-diagnosis
Laboratory diagnosis of HIT
Improving immunoassay specificity

- estimating the pre-test probability of HIT
  – the ‘4-T’ score

1. Thrombocytopenia
2. Timing of thrombocytopenia
3. Thrombosis (or other sequelae of HIT)
4. Other causes for platelet fall unlikely
## Pre-test probability of HIT

The “4 T’s”

<table>
<thead>
<tr>
<th>Thrombocytopenia</th>
<th>2 points</th>
<th>1 point</th>
<th>0 point</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;50% fall or nadir &lt;100 (&gt;30% fall)</td>
<td>30-50% fall or nadir 10-19</td>
<td>&lt;30% fall or nadir &lt;10</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Timing compatible with HIT</th>
<th>Yes (d 5-10) or &lt;d4 (recent heparin)</th>
<th>Possible (&lt;d10)</th>
<th>No (&lt;d4 with no recent heparin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombosis</td>
<td>Yes</td>
<td>Possible / Silent</td>
<td>No</td>
</tr>
</tbody>
</table>

| Other Dx                  | No                                     | Possible        | Likely |

| Probability of HIT | High: 6 - 8 | Moderate: 4 - 5 | Low: 0 - 3 |
Laboratory diagnosis of HIT
Improving immunoassay specificity

- estimating the pre-test probability of HIT
- detecting only IgG antibodies
  - only IgG antibodies can activate platelets
  - reduces “false positives”
  - better correlation with functional assays and clinical HIT
Laboratory diagnosis of HIT
Improving immunoassay specificity

- estimating the pre-test probability of HIT
- detecting only IgG antibodies
- reporting the OD value
  - many patients have low or borderline (0.4-0.6) values
  - values >1.0 are considered ‘strong’
  - high OD values are more likely to be associated with an IgG – and therefore pathogenic – antibody
  - increase in OD of 1.0 unit doubles risk of thrombosis
GTI OD >1.0 more likely to correlate with positive SRA
Laboratory diagnosis of HIT
Antibody titer and thrombin generation

Chilver-Stainer et al., Thromb Haemost, 91: 276-282, 2004
Laboratory diagnosis of HIT
Improving immunoassay specificity

- estimating the pre-test probability of HIT
- detecting only IgG antibodies
- reporting the OD value
- heparin neutralization step
  - repeat ELISA after mixing with 100 u/ml heparin
  - may improve diagnostic specificity with weak antibodies
  - may falsely classify strong antibodies as negative
  - should be used with caution – especially in patients with clinical signs consistent with HIT
Laboratory diagnosis of HIT
Problems with laboratory testing

- local testing often not available STAT
- may be unavailable locally
- variable sensitivity and specificity
- physicians unaware of type of test used
- tests may be positive without clinical HIT
- may result in OVER diagnosis of HIT
Spectrum of HIT following cardiovascular surgery

- Thrombosis 0.2%–1.0%
- Thrombocytopenia 1%–3%
- Antibody formation 50%+
Factors affecting H-PF4 antibody formation after CABG

- heparin type?
- heparin dose?
- platelet activation on oxygenator?
- inflammation?
HIT antibodies and cardiac surgery

Type of heparin?

Percent of patients

### HIT antibodies and cardiac surgery

**Heparin dose and/or CPB?**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Antibody Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td><strong>All patients</strong></td>
<td>207</td>
<td>87</td>
</tr>
<tr>
<td><strong>CPB</strong></td>
<td>166</td>
<td>70</td>
</tr>
<tr>
<td><strong>OPCAB</strong></td>
<td>41</td>
<td>17</td>
</tr>
</tbody>
</table>

HIT antibodies and cardiac surgery
The result of heparin during CABG?

![The Evolution-OFF Trial](image)

JL Francis et al (unpublished data)
Post-CABG H-PF4 antibodies

Unanswered questions

- result of pre and/or post-op heparin?
- result of intra-op heparin?
- partially non-specific response to CABG?
- is there a downside to HIT antibody formation?
Are ‘asymptomatic’ H-PF4 antibodies clinically significant?

- **Mattioli 2000**: 50% increase in MACE in H-PF4 +ve patients following heparin therapy for unstable angina
- **Francis & Palmer 2002**: post CABG +ve H-PF4 associated with ↑ LOS
- **Williams 2003**: +ve H-PF4 independent predictor of MI in ACS
- **Rice & Francis 2003**: observation of Delayed Onset HIT
- **Levine & Francis 2004**: rapid onset HIT in ER
- **Bennet-Guerrero et al 2005**: ve H-PF4 pre-CABG doubles risk of death and ↑ LOS
- **Kress et al 2007**: +ve H-PF4 pre-CABG predicts for adverse outcomes
- **Selleng et al 2010**: IgM antibodies associated with non-thrombotic adverse events
Post-CABG H-PF4 antibodies increase risk of later HIT

Day HIT test requested

HIT antibody formation

Patients re-presenting to the ER

Discharge

J.L. Francis (unpublished data)
Frequency of H-PF4 antibodies in the ER

- Recent history: 9.9%
- Remote (no) history: 3.3%

Immunobiology of HIT Antibodies

- Unusual kinetics, typical of neither 1° or 2° immune responses
- Relatively early IgG response suggesting prior immunization
- General activation of a host defense system triggered by inflammation
- H-PF4 antibody titers usually decrease rapidly
- Risk of HIT dependent on patient-related factors
Case 5

- 60 year-old man underwent knee replacement; Uneventful post-op course
- Day 8 - discharged on coumadin with therapeutic INR and platelet count of 300K
- Day 10 - readmitted with low grade fever, platelets = 40K
- Day 11 – platelets = 20K; Doppler = left DVT; HIT-ELISA +ve (OD=3.0); SRA +ve
- Took 20+ days of argatroban to recover platelets
- NO history of heparin exposure
**The emerging significance of H-PF4 antibodies**

- Cases of “spontaneous” HIT with positive serology despite lack of heparin exposure
- False positive ELISA in patients with APS and SLE
- Frequency of +ve ELISA in normal blood donors
- Association with periodontal disease in non-heparin treated blood donors
- Bind to PF4-coated bacteria promoting phagocytosis
- H-PF4 antibodies may represent an evolutionary step between innate and specific immunity
The central role of thrombin in HIT

Thrombin is intensely procoagulant and must be inhibited specifically.

- Endothelial activation
- Fibrinogen conversion
- Platelet activation
- Acceleration of coagulation

Thrombin
How is thrombin formed in HIT?

Platelet

alpha granule

PF-4 binds to surface of platelet following activation

PF-4/heparin complex

Complexes of heparin (GAG) and PF-4 molecules form

IgG binds to the PF-4/heparin complex

IgG

FC receptor

microparticles

Fc stimulation leads to the generation of procoagulant-rich microparticles

IgG/PF-4/heparin complex activates via the Fc receptor
How is thrombin formed in HIT?

- Anti-H-PF4 IgG
- Heparin/GAG
- Fc\λR1/Fc\λ Receptor
- PF4 Tetramer
- MEK
- erk1/2
- TF
- TF+MP

Platelet Factor 4 (PF4) bound to heparin.

Heparin anchors to the platelet surface via non-specific, low affinity interactions.

Platelet Aggregation Induced by Immune Complexes: Dependence on Fc Receptor

Bevacizumab + VEGF + Heparin

Collagen added to confirm aggregation competence
Immune complexes cause thrombotic thrombocytopenia in mice expressing CD32A.

Platelet Count ($\times 10^3/\mu L$)

- Baseline
- WT
- hFc

$P < 0.01$
Treatment of HIT

Principles

- interrupt the immune response
  - discontinue heparin
- inhibit thrombin activity
  - treat active thrombosis
  - prevent new thrombosis
- do no harm
Platelet counts in HIT
May not recover without treatment

Platelet count over time with Heparin and Direct Thrombin Inhibitor.
Just stopping heparin may not prevent thrombosis

All patients initially presented with thrombocytopenia without thrombosis

Cumulative thrombosis rate (%)

Days after HIT recognized

52.8%
Case 6

- 57 year old man – surgery for multiple bone fractures
- Day 3:
  - DVT – treated with heparin
- Day 7:
  - leg swollen, dyspnea
  - V/Q scan – multiple PE
  - Platelet count 40K
Case 6 (continued)

- Heparin discontinued
  - IVC filter placed
  - Warfarin 10 mg initiated
- After 4 days of warfarin
  - PT = 25 secs
  - Black, necrotic lesion on thigh
- Several days later
  - Lesion progressed
  - Sepsis, death
Coumadin should not be used as sole treatment for HIT.

Procoagulant

Factor II
Factor VII
Factor IX
Factor X

Anticoagulant

Protein C
Protein S
Direct Thrombin Inhibitors

Lepirudin

- Half-life: 50-120 min
- Metabolism: Kidney
- FDA approval: Yes

Argatroban

- Half-life: 40-50 min
- Metabolism: Liver
- FDA approval: Yes

Bivalirudin

- Half-life: 25 min
- Metabolism: Plasma
- FDA approval: No
HIT is caused by antibodies to heparin-PF4 complex
associated with falling platelet count, with or without venous or arterial thrombosis
laboratory tests may be positive without clinical HIT
antibodies common post-CABG – danger of over-diagnosis
may be partly an autoimmune phenomenon
HIT antibodies may predict for poorer outcome
direct thrombin inhibitors are treatment of choice
source of thrombin remains unclear