



Heparin Induced Thrombocytopenia (HIT)

- Clinical Features and Diagnosis

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Overview

- Pathogenesis
- Epidemiology + Clinical Features
- Diagnosis
 - Functional assays
 - Antigenic assays
 - Clinical Prediction rule for Pre-test probability
- Treatment
- Algorithm and future directions

HITS Background

Gollub et al *J Lab Clin Med* **59** (1962), pp. 430-435.

- Heparin-induced thrombocytopenia in man.

Rhodes et al *Surg Gynecol Obstet* 1973; 136:409-41

- Thrombocytopenia and thrombosis while receiving heparin
- Recurrence on re-exposure to heparin
- Patient Plasma + heparin → platelet aggregation

HITS Definition

Immune-mediated adverse drug reaction

Clinico-pathological diagnosis

- **Laboratory Features**

Strong platelet activating abs recognizing heparin/PF4

- **Clinical Features**

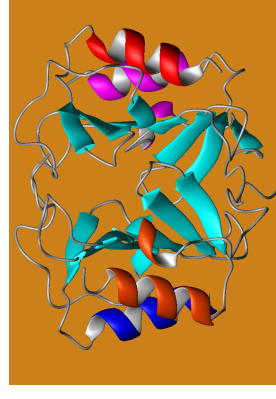
Thrombocytopenia (absolute or proportional)
Thrombosis (Arterial/Venous)

HIT Pathogenesis

Evident late 1970's that HIT was an immune disorder.

Amiral et al Thromb Haemost 1992;68:95-96

- Demonstrated platelet factor 4, tetrameric member C-X-C cytokine family to be target antigen.



- ? Small number other targets
- IL-8, NAP 2

HIT Pathogenesis

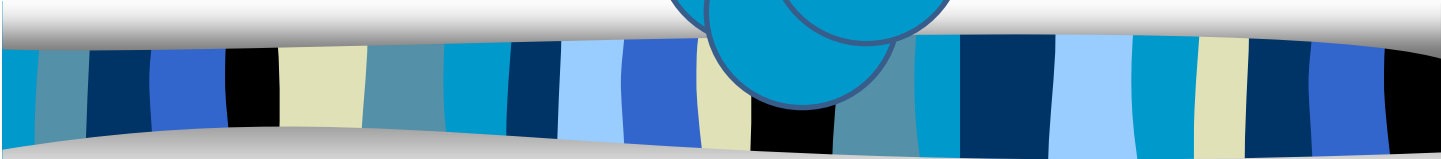
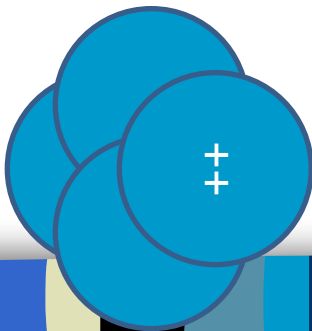
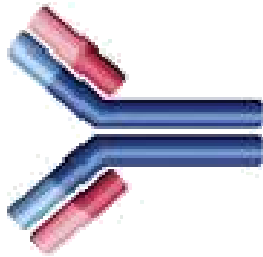
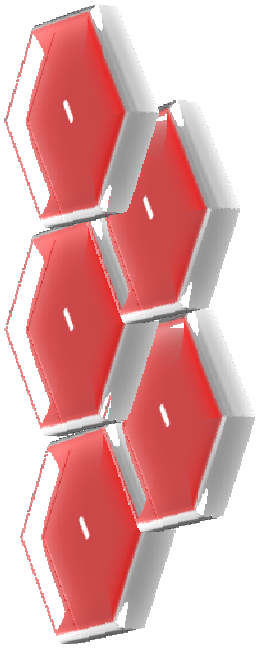
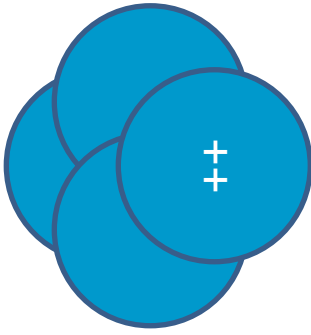
Generation of PF4/Heparin HIT target antigen

Greinacher et al Arterioscler Thromb Vasc Biol. 2006;26:2386-2393.

- HIT antigen requires approximation of 2 PF4 tetramers due to charge neutralization of PF4 by heparin
- HIT antibodies have highest affinity for largest complexes (more common with UFH)
- Minimum charge and length requirement of heparin

Rauova et al Blood. 2006;107: 2346-2353

- Formation large PF4/hep complexes optimal at 1:1 molar ratio.



HIT Pathogenesis

Characterization of HIT antibodies

- Majority of HIT antibody is polyclonal IgG₁ +/- IgG₂
- IgM and IgA can also be present (? Clinical significance)

Greinacher et al J Thromb Haem 2007 May 7

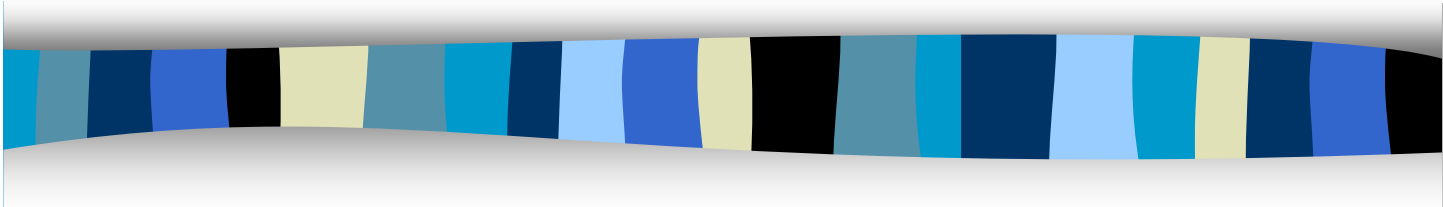
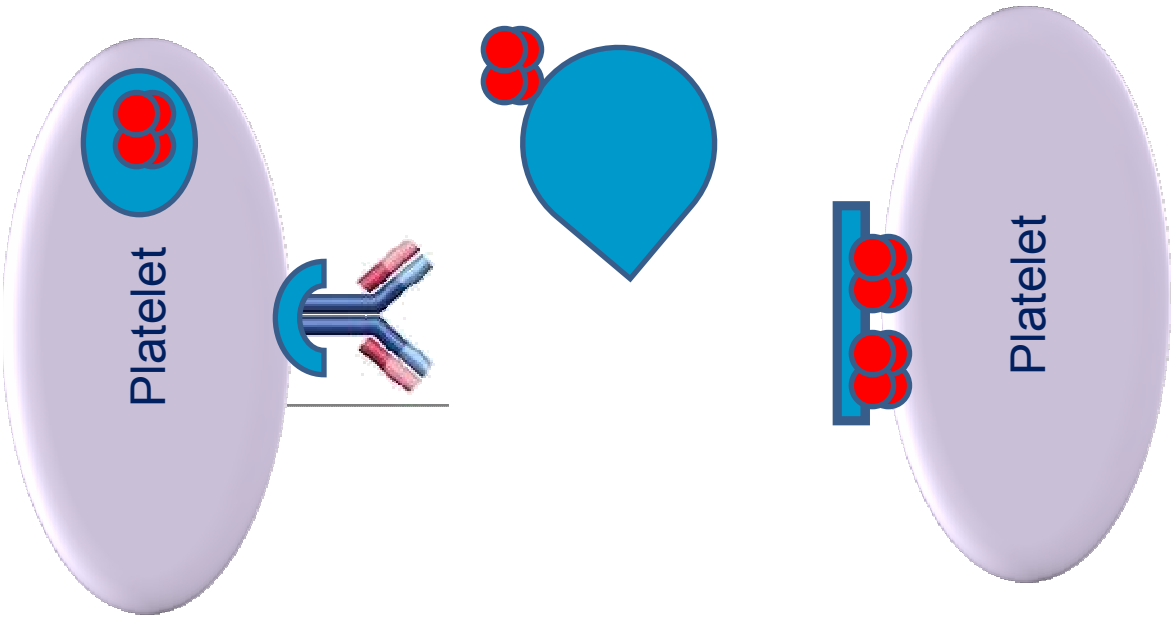
- 185 pts with HIT antibodies detected by EIA
- 168 (82.4%) IgG, 112 (54.9%) IgM, 57 (27.9%) IgA PF4/hep ab
- 17.6% had detectable IgM and/or IgA without detectable IgG.

HIT Pathogenesis

Platelet - HIT antibody / antigen interaction

Newman and Chong *Blood*. 2000;96:182-187

- Initial interaction of Fab region HIT ab with platelet associated PF4/heparin complex
- Fc region then binds to FcγRII receptor
- Platelet activation and degranulation results
- Released PF4 results in increased antigen formation





HIT Pathogenesis

Activation of coagulation cascade

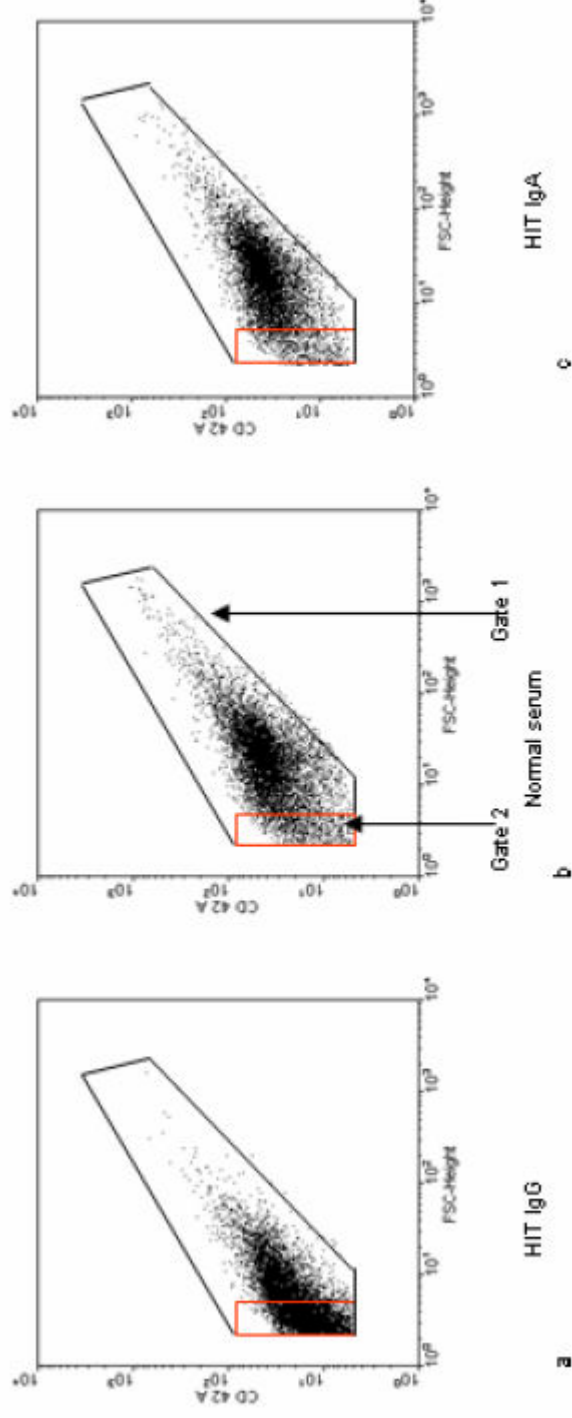
- Platelet activation results in release of procoagulant microparticles
- Procoagulant changes on endothelial cells

Marked increase in thrombin generation results

HIT Pathogenesis

Greinacher et al J Thromb Haem 2007 May 7

Microparticle formation



Iceberg Theory of HIT

Warkentin / Kelton Model

- Frequency of formation HIT antibodies significantly higher than incidence “clinical” HIT
- Not all PF4/heparin antibodies are platelet activators
- Patients with thrombocytopenia are most likely to develop thrombosis
- Ratio detectable HIT abs to clinical HIT varies by
 - Clinical setting
 - Type of heparin exposure
 - Assay used

Influence of clinical setting

Post operative > medical > obstetric

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Influence of type of heparin

Bartel et al Blood 2005 (106) 8: 2710

- Meta-analysis of trials comparing UFH LMWH
- Incidence UFH 2.6% vs UFH 0.2%
- Predominantly Surgical Studies

Influence of type of heparin

Morris et al Chest 2007 132 (4): 1131

- Meta-analysis trials comparing LMWH with UFH for treatment of venous thrombosis
- 13 trials involving 5200 pts
- UFH 1.5%, LMWH 1.2% $p=0.2$

Influence of Gender

Warkentin et al Blood 2006 108: 2937-2941

- Data from 6 prospective trials examined.
- OR for women 2.37 (1.37-4.1)

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Clinical Features

Thrombocytopenia

- >50% fall in platelet count occurs in >97% patients with HIT
- Moderate severity
- Onset
 - Typical Day 5-14
 - Rapid Day1
 - Delayed after cessation

QuickTime™ and a
TIFF (Uncompressed) decompressor
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Clinical Features

Thrombosis

- OR for thrombosis of 20-40
- Tends to effect the vascular bed for which treatment is being administered
- High rate of thrombosis in absence of alternative anticoagulation

Rarer manifestations

- Heparin injection site reactions
- Systemic reactions (hypotension/dyspnea/flushing)

To start or not to start

Problems with delaying alternative anticoagulation

- 25-50% patients with isolated HIT develop thrombosis
- Daily rate of thrombosis if treatment delay of up to 6%

Problems with starting alternative anticoagulation

- Falling incidence of confirmed HIT
- High bleeding rates (at therapeutic level)
- Difficulty with monitoring
- Limited availability, expense



Ideal diagnostic test for HIT

- Highly sensitive
- Identifies patients at risk of clinical events
- Rapid results
- Easy to perform
- Cheap



Current diagnostic tests for HIT

Platelet “activation” assays (functional)

- Washed platelet assays
- Platelet aggregation test

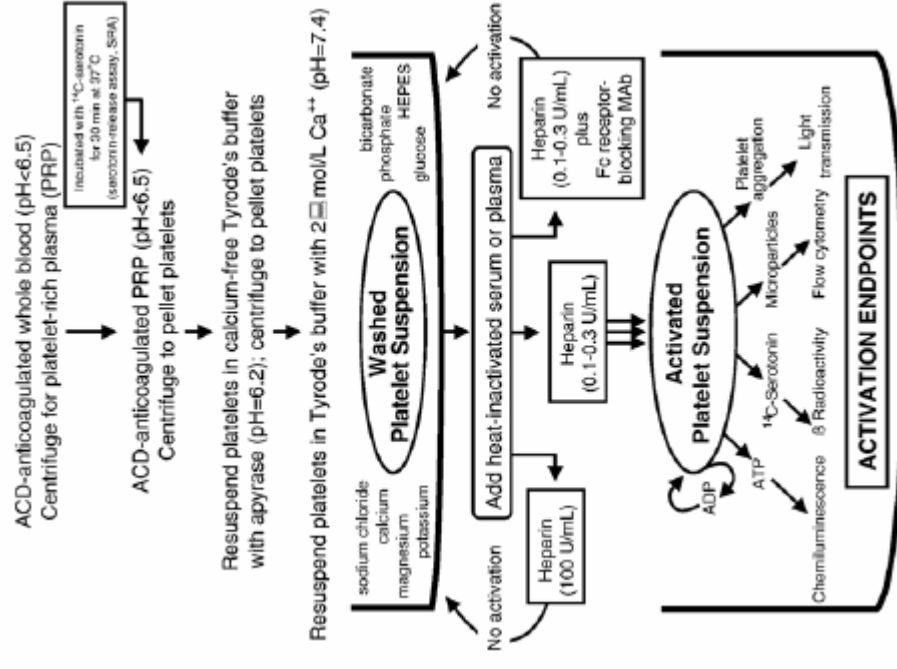
PF4-dependent “antigen” assays

- Solid phase enzyme immunoassays
- Rapid immunoassays

Platelet “activation” assays

Washed Platelet Assays

- Preparation washed platelet suspension
- Addition pt serum or plasma
- Measurement of platelet activation after addition heparin
- Use of controls to improve specificity



Platelet activation assays

Serotonin Release Assay (SRA)

Sheridan et al Blood 1986 Jan;67(1):27-30.

- Activation measured by release ^{14}C -serotonin

Heparin-induced platelet activation assay (HIPA)

Greinacher et al Thromb Haemost 1991; 66(6); 734-736

- Activation measured by visual endpoint

High sensitivity and specificity (95-99%)

- Limited availability
- Technically difficult assays

Platelet Aggregation Testing for HIT

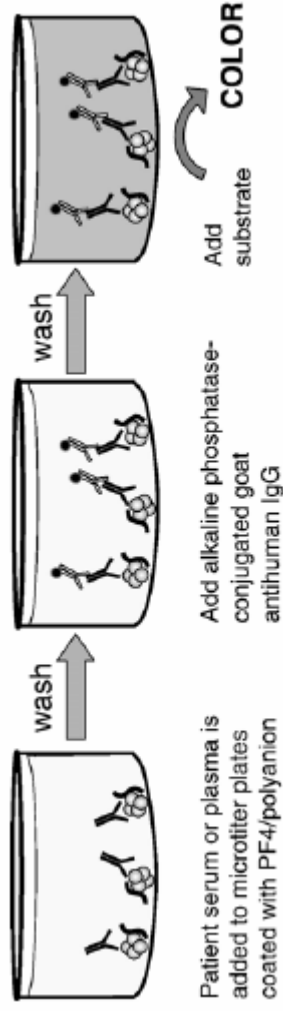
Chong et al *Thromb Haemost* 1993; 9(4): 344-350

- Measures ability patient serum/plasma to induce heparin dependent platelet aggregation.
- Reported sensitivity ranges from 30-80%.
- Use of “reactive” donor PRP increases sensitivity
- Specificity improved by use of “two-point” system
 - positive test at heparin concentration of 0.1-1.0 U/ml
 - negative test at high heparin concentration (100 U/ml)

PF4 dependent antigen assays

Solid phase immunoassays

- Microtitre wells coated with PF4
- Bound PF4 ab detected by conjugated anti-human Ig.
- Commercially available kits detect IgG, IgM, IgA
- High sensitivity, low specificity (40-50%)



Attempts to improve ELISA specificity

Alteration optical density cut-points

Zwicker et al *J Thromb Haemost* 2004; 2: 2133–7.

- Pts with isolated HIT OD ≥ 1.0 had a 6-fold risk of thrombosis c/w OD 0.4 to 0.99
- Absolute rate of thrombosis 9% in pts lower OD.

Janatpour et al *Am J Clin Pathol* 2007;127:429-433

- Improved specificity (85%), decreased sensitivity.

Supports move towards reporting actual figures

Attempts to improve ELISA specificity

Limiting to detection of IgG PF4 antibody alone

Warkentin et al J Lab Clin Med 146, Number 6

- Specificity improved from 79 to 84%.

Greinacher et al J Thromb Haem 2007 May 7




- Specificity improved from 46 to 54%

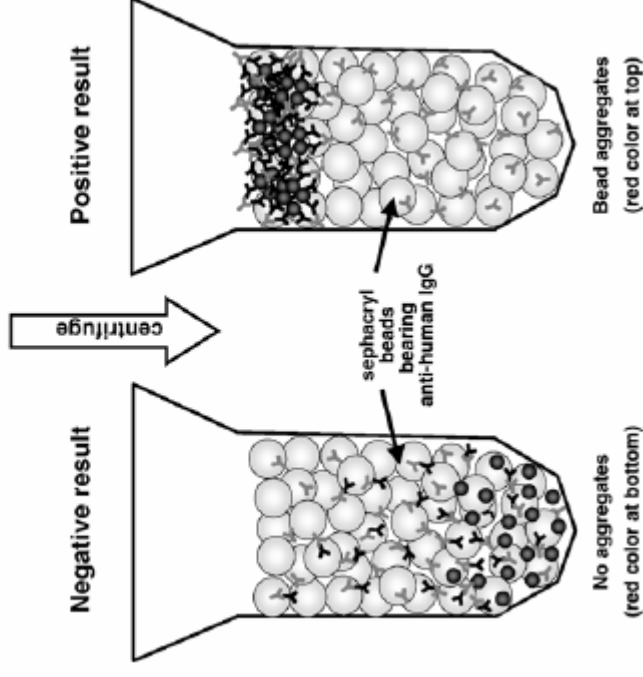
Modest increase in specificity with no loss of sensitivity

Rapid Immunoassay

Particle Gel Immuno-assay (ID-PaGIA HPF4 assay)

Particle Gel Immunoassay
(DiaMed ID-PaGIA Heparin/PF4 antibody test)

- test serum containing positive or negative HIT IgG 
- is mixed with red polystyrene beads coated with PF4-heparin 
- the test mixture is then added to the particle gel immunoassay tube containing anti-human IgG 



Particle gel Immunoassay

Eichler et al Br J Haematol 2002; 116 (4): 887-891.

- Positive in 94 of 100 samples from pts with confirmed HIT
- Negative in 39/40 samples in pts without HIT

Pouplard et al J Thromb Haemost 2007; Mar 14

- 213 pts with suspected HIT.
- Sensitivity 95.5% (20/21), Specificity 91.8% (175/191)

By itself inadequate to rule out or rule in HIT

Clinical Score for HIT

Prevalence of confirmed HIT is only 5-10%

- ? falling due to change pt population
- Low prevalence increases risk false positives

Stratification of pre-test probability by clinical features

- ? Ability to identify pts who do not need diagnostic testing
- Helps with estimation of post-test probability of HIT
- 4T's rule developed by McMaster group

“4T” Clinical prediction rule

“4 T’s”	2 points	1 point	0 points
Thrombocytopenia	Fall > 50% Nadir > 20	Fall 30-50% Nadir 10-19	Fall < 30% Nadir < 10
Timing of platelet count fall	Between 5-10d or < 1d (prior Rx 30 d)	Onset after day 10 < 1d (prior Rx 30 -100 d)	< 4 days No recent Rx
Thrombosis or other sequelae	New thrombosis Systemic reaction. Skin necrosis	Recurrent thrombosis Suspected thrombosis Skin reaction	None
Other cause for Thrombocytopenia	None apparent	Possible	Definite

High score 6-8, Intermediate 4-5, Low ≤3.

4T's Rule – Previous Results

	Low PTP	Intermediate PTP	High PTP
Lo (Centre 1)	2% (1/64)	29% (8/28)	100% (8/8)
Lo (Centre 2)	0% (0/55)	8% (11/139)	21% (9/42)
Pouplard	0% (0/74%)	11% (14/129)	80% (8/10)

Lo et al JTH 2006 4: 759–765

Pouplard et al J Thromb Haemost 2007; Mar 14

Local Study

- Retrospective review of pts referred for HIT testing at a single laboratory (Jan 2003 -Feb 2006).
- 4T's clinical score performed independently by 2 clinicians, unaware of lab results.
- Platelet aggregation testing for HIT and HPF4-PaGIA performed on all patients locally
- SRA, Commercial EIA and EIA-G, EIA-A, EIA-M performed McMaster, Ontario.

Overall Results

	n
Samples tested	124
Patients tested	115

Classification		% of patients tested
A	20	17.39
B	4	3.48
HIT classification (A+B)	24	20.87

Prevalence of HIT by 4T score

	Low PTP	Intermediate PTP	High PTP
RAH	0% (0/55)	15% (6/40)	90% (18/20)

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Pouplard	0% (0/74)	11% (14/129)	80% (8/10)
Total	0.5%	12%	80%

Performance of Platelet Aggregation Testing

	HIT +ve	HIT -ve
Plat Agg +ve	20	0
Plat Agg -ve	4	91

Sensitivity 83% (95% CI, 64 – 93)
Specificity 100% (95% CI, 96 – 100)

Performance of Diamed PaGIA

	HIT +ve	HIT -ve
PaGIA +ve	17	2
PaGIA-ve	7	89

Sensitivity 71% (95% CI, 51 – 85)

Specificity 98% (95% CI, 92 – 99)

Likelihood Ratio + = 32

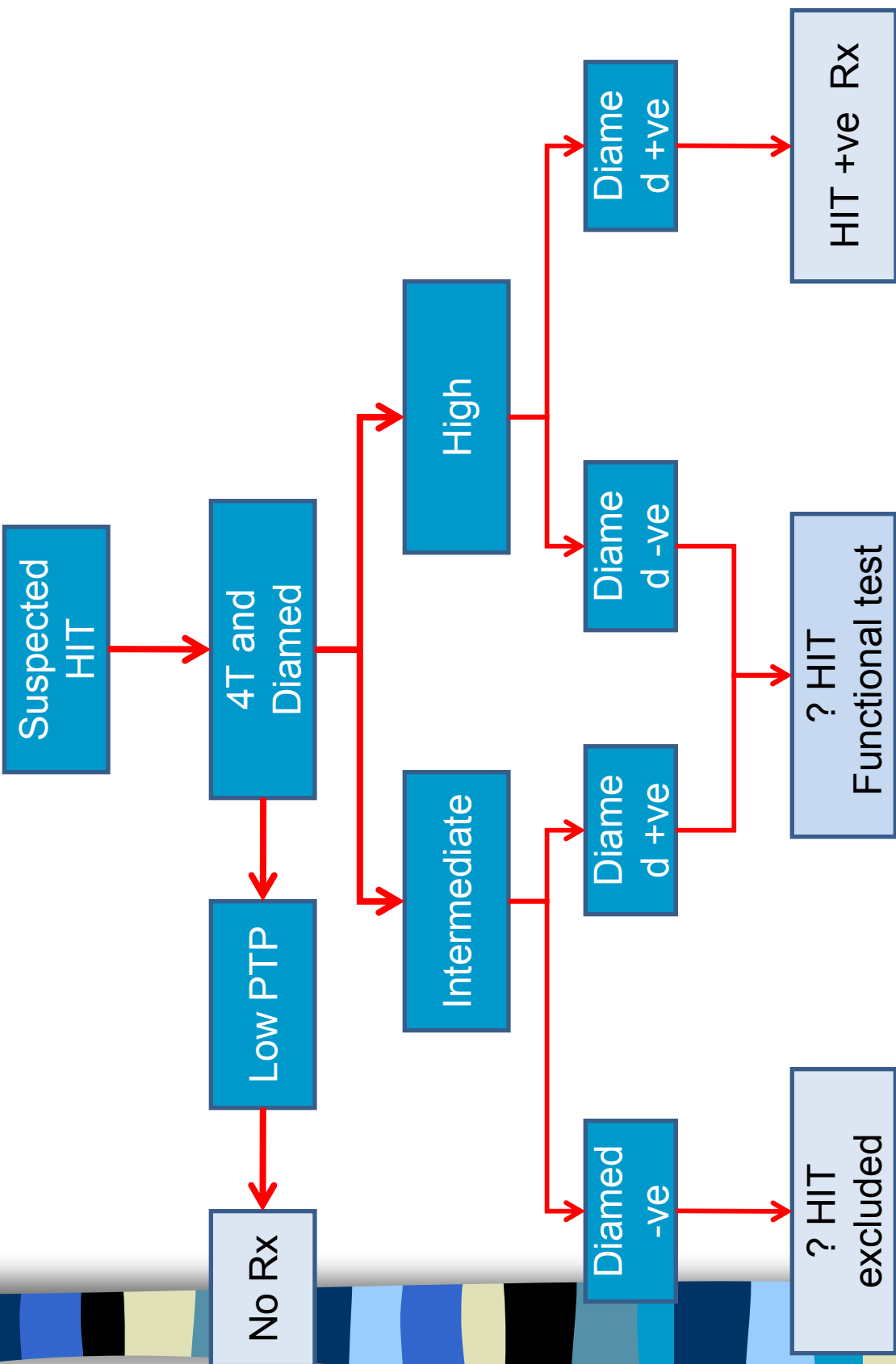
Likelihood ratio - = 0.3

Combination 4T's and DiaMed

4T Category	Pretest Probability	Post -test Probability	
		DiaMed +ve	DiaMed -ve
Low	0.00	0.00	0.00
Intermediate	0.15	0.85	0.05
High	0.90	0.99	0.73

Combination 4T's and Diamed

4T Category	Pretest Probability	Post -test Probability	
		Diamed +ve	Diamed -ve
Low	0.00	0.00 (0)	0.00 (0)
Intermediate	0.15	0.85 (0.58)	0.05 (0.01)
High	0.90	0.99 (0.98)	0.73 (0.16)



Diagnosis in pts post-bypass

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Pouplard et al BJH 2005

Treatment of HIT

Lepirudin

Previous dose recommendations

- Bolus 0.4mg/kg followed by infusion 0.15 mg/kg targetting aPTT 1.5 to 2.5

HAT trial *J Thromb Haemost.* 2005;3: 2428-2436

Mean lepirudin doses were

- 0.11 mg/kg/h in HIT patients with thrombosis
- 0.07 mg/kg/h in patients with asymptomatic HIT.

Tardy et al *Blood* ,2006, Vol. 108, No. 5, pp. 1492-1496.

Mean lepirudin dose 0.07 mg/kg

Treatment of HIT

Fondaparinux

