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The Laboratory Diagnosis of HIT

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Clinical probability HIT score

Try to prevent HIT laboratory investigation without clinical score!

4 T's pretest probability HIT scoring

Warkentin 2003

Thrombocytopenia	> 50% fall or platelet nadir 20-100 x 10^9/L	2
	30-50% fall or platelet nadir 10-19 x 10^9/L	1
	fall <30% or platelet nadir <10 x 10^9/L	0
Timing	Clear onset between days 5 and 10 or less than 1 day (if heparin exposure within past 100 d)	2
	Consistent with immunisation but not clear (e.g. missing platelet counts) or onset after day 10	1
	Platelet count fall too early (without recent heparin exposure	0
Thrombosis or other sequelae (e.g. skin lesions)	New thromb. Skin necr. Post hep. acute syst. reaction	2
	Progressive or recurrent thromb. Suspected thromb.	1
	None	0
Other causes	No other causes	2
	Possible other causes	1
	Definite other cause is present	0

Score 6-8=high; 4-5=intermediate; 0-3=low

Table 2 HIT Expert Probability (HEP) Score

Cuker et al 2010

Clinical feature	Score
1. Magnitude of fall in platelet count (measured from peak platelet count to nadir platelet count since heparin exposure)	
a. < 30%	−1
b. 30%–50%	1
c. > 50%	3
2. Timing of fall in platelet count	
For patients in whom typical onset HIT is suspected	
a. Fall begins < 4 days after heparin exposure	−2
b. Fall begins 4 days after heparin exposure	2
c. Fall begins 5–10 days after heparin exposure	3
d. Fall begins 11–14 days after heparin exposure	2
e. Fall begins > 14 days after heparin exposure	−1
For patients with previous heparin exposure in last 100 days in whom rapid onset HIT is suspected	
f. Fall begins < 48 h after heparin re-exposure	2
g. Fall begins > 48 h after heparin re-exposure	−1
3. Nadir platelet count	
a. $\leq 20 \times 10^9$ L^{-1}	−2
b. $> 20 \times 10^9$ L^{-1}	2
4. Thrombosis (Select no more than one)	
For patients in whom typical onset HIT is suspected	
a. New VTE or ATE ≥ 4 days after heparin exposure	3
b. Progression of pre-existing VTE or ATE while receiving heparin	2
For patients in whom rapid onset HIT is suspected	
c. New VTE or ATE after heparin exposure	3
d. Progression of pre-existing VTE or ATE while receiving heparin	2
5. Skin necrosis	
a. Skin necrosis at subcutaneous heparin injection sites	3
6. Acute systemic reaction	
a. Acute systemic reaction after intravenous heparin bolus	2
7. Bleeding	
a. Presence of bleeding, petechiae or extensive bruising	−1
8. Other causes of thrombocytopenia (Select all that apply)	
a. Presence of a chronic thrombocytopenic disorder	−1
b. Newly initiated non-heparin medication known to cause thrombocytopenia	−2
c. Severe infection	−2
d. Severe DIC (defined as fibrinogen < 100 mg dL^{-1} and D-dimer > 5.0 μg mL^{-1})	−2
e. Indwelling intra-arterial device (e.g. IABP, VAD, ECMO)	−2
f. Cardiopulmonary bypass within previous 96 h	−1
g. No other apparent cause	3

VTE, venous thromboembolism; ATE, arterial thromboembolism; DIC, disseminated intravascular coagulation; IABP, intra-aortic balloon pump; VAD, ventricular assist device; ECMO, extracorporeal membrane oxygenation.

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A clinical score is not enough to diagnose HIT

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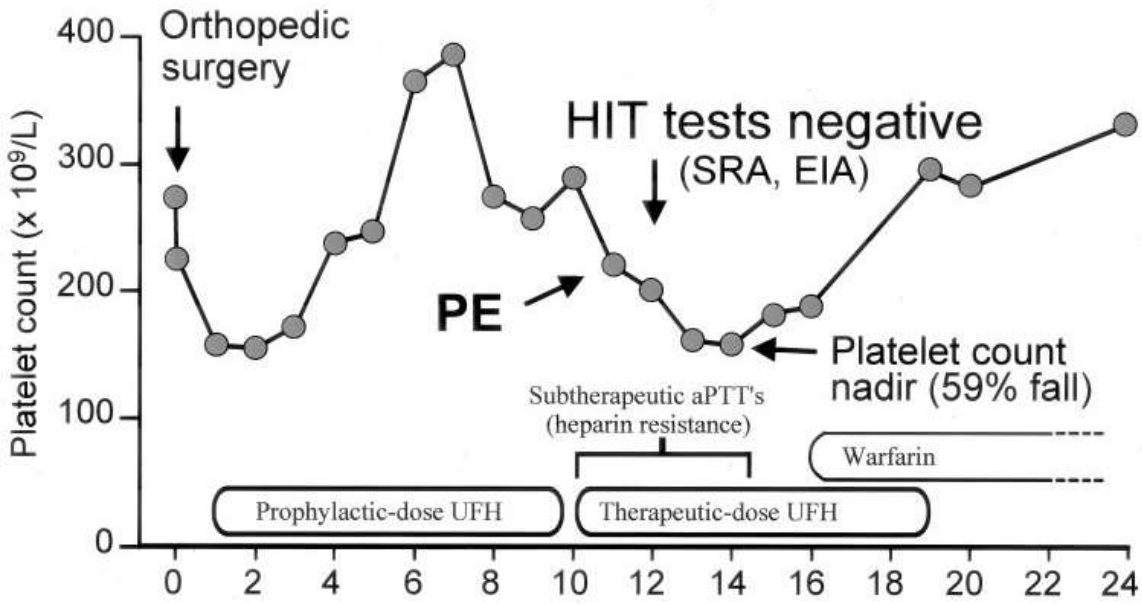
4T score high
HIT test negative



Warkentin Chest 2005

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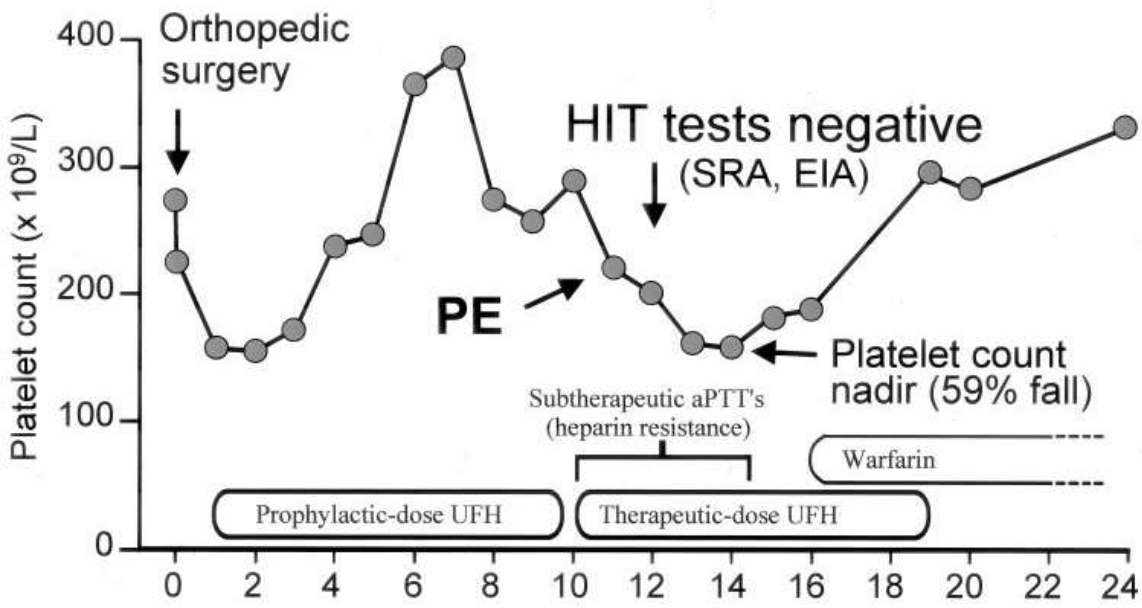
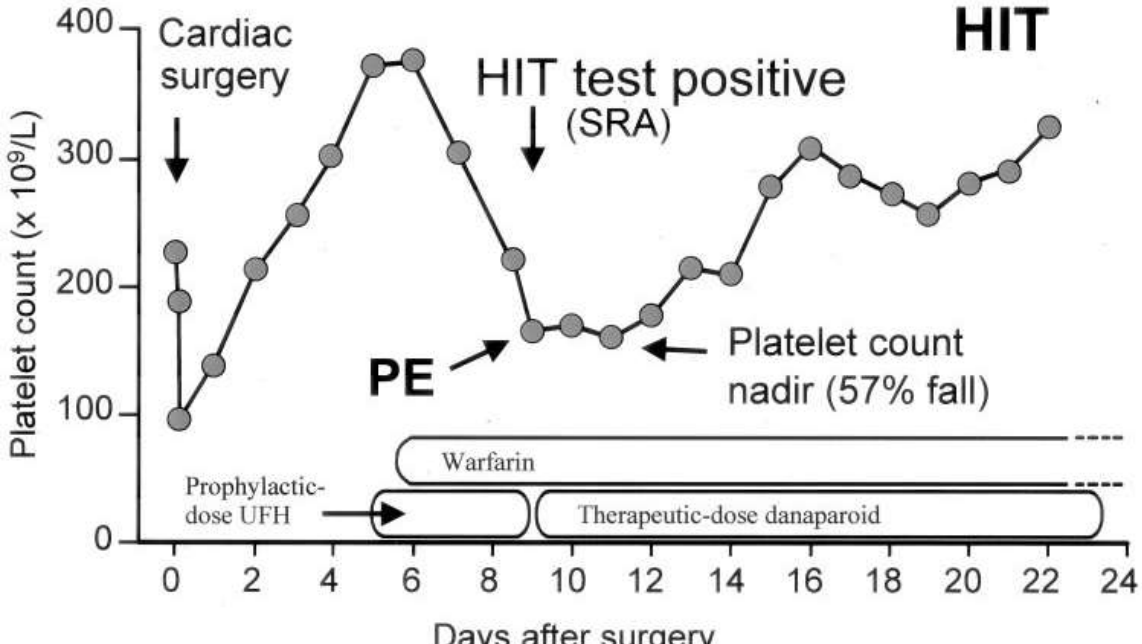
4T score high
HIT test negative

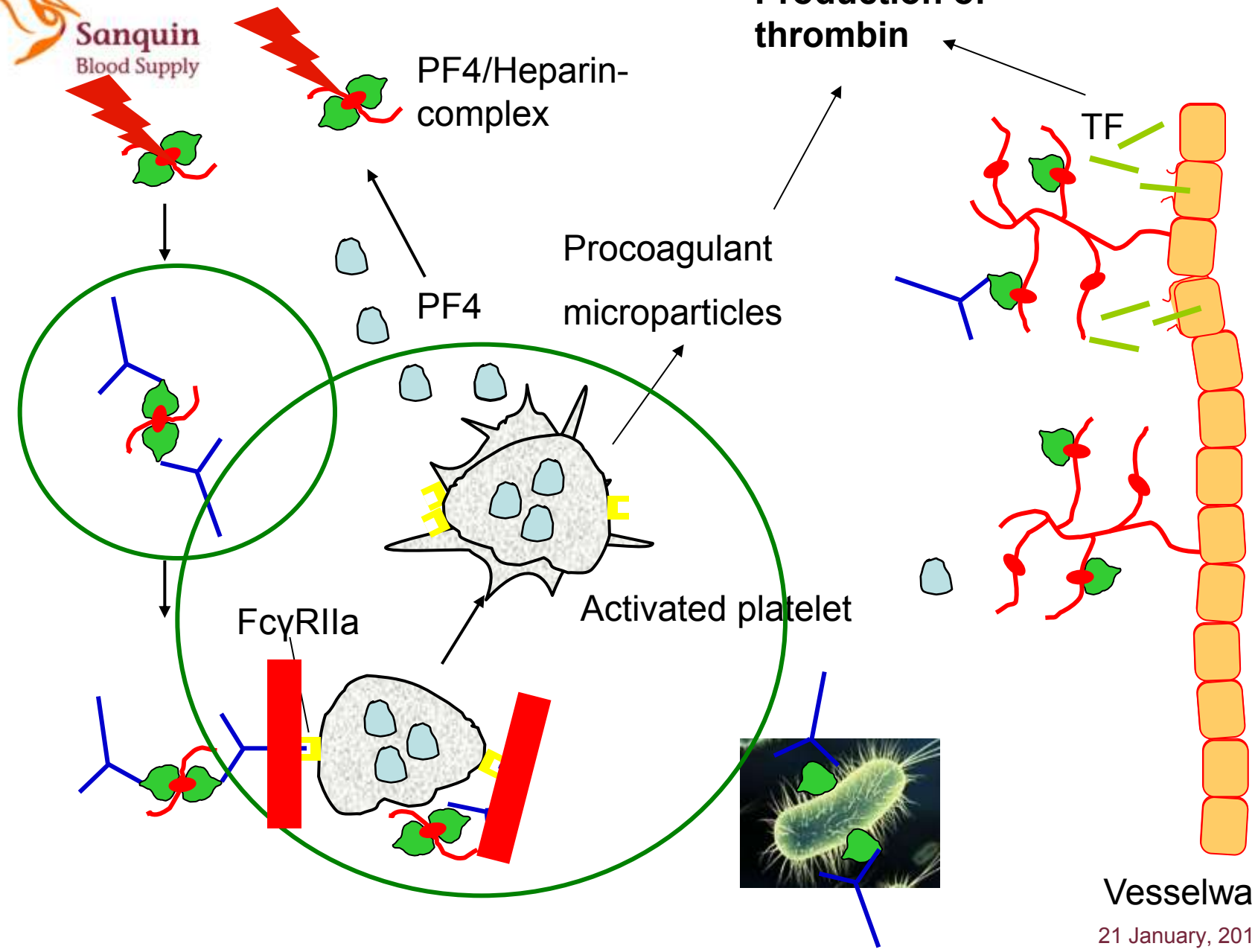


4T score high
HIT test positive

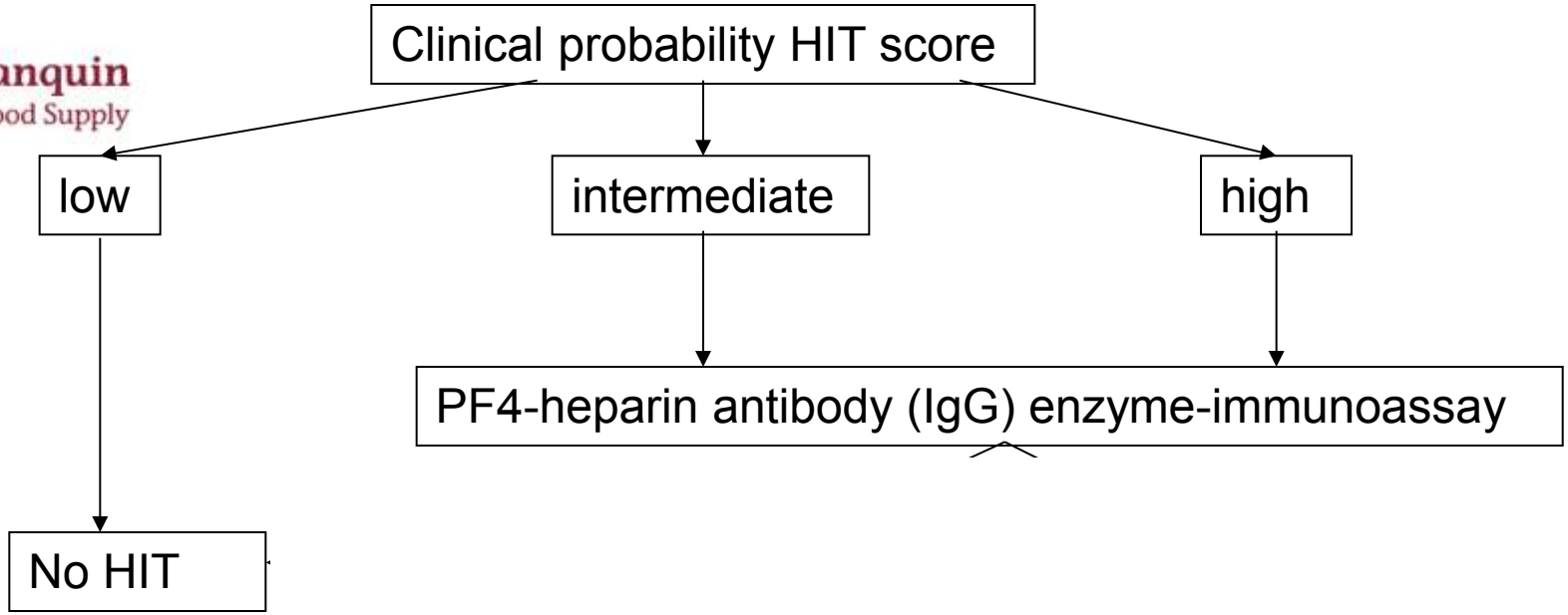


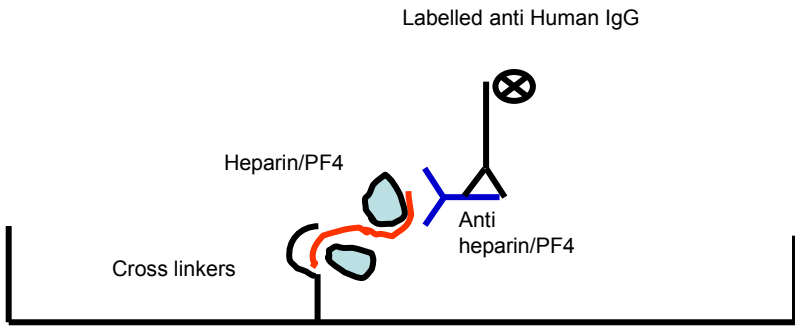
Warkentin Chest 2005

HIT



Amiral 1995, Greinacher 2006/2008, Ziporen 1998


Clinical probability HIT score
low
intermediate
high
PF4-heparin antibody (IgG) enzyme-immunoassay
No HIT



Heparin/PF4 ELISA

Enzyme Immunoassays

PF4/heparin-complex specific antibody detection methods

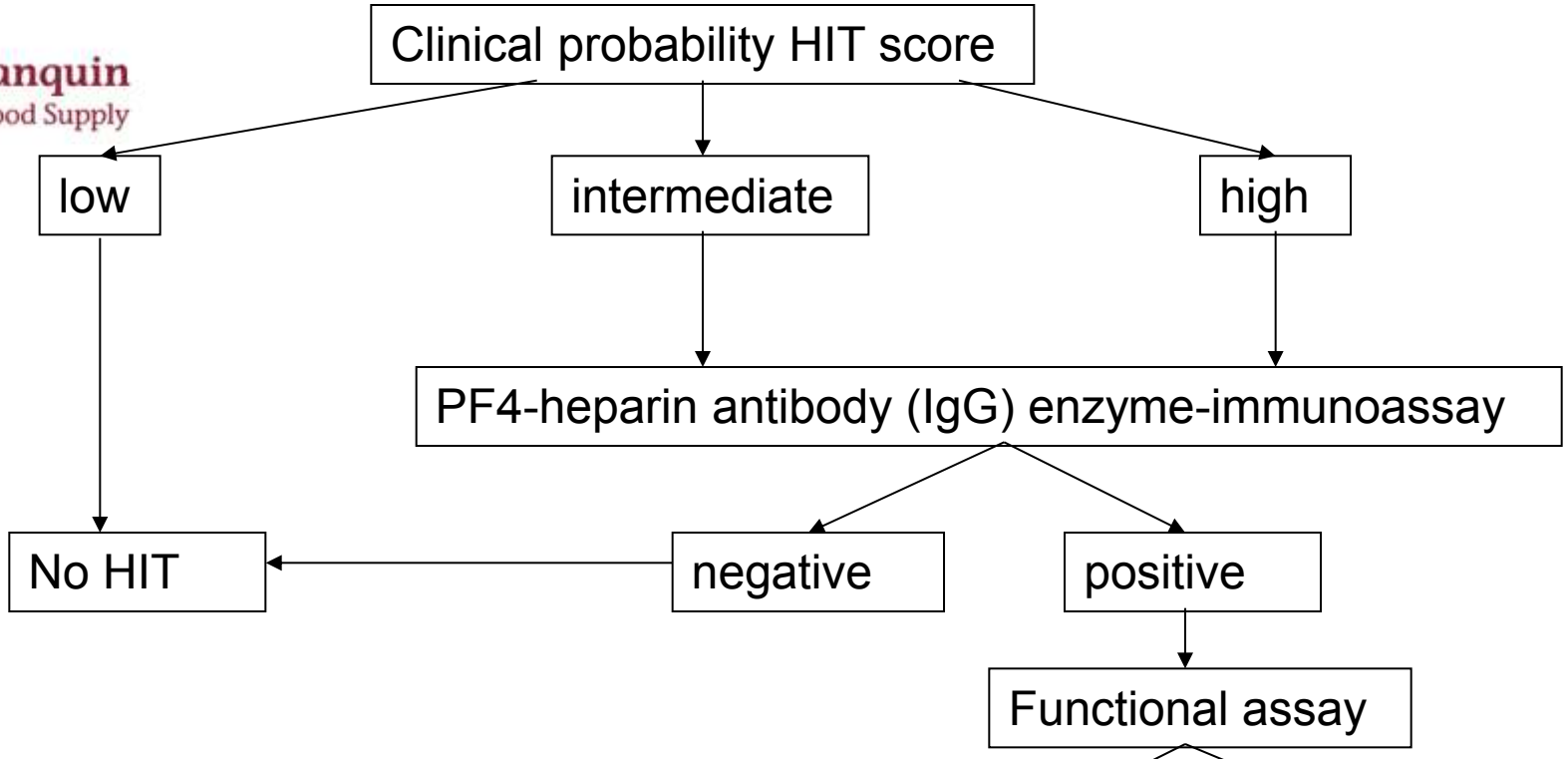
Pro's
- commercially available
- Easy to perform
- Sensitivity 99%

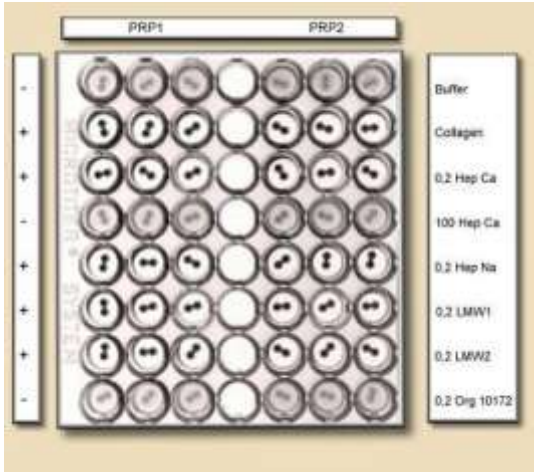
Con's
- Do not differentiate between pathogenic antibodies and clinically irrelevant antibodies
- Specificity 10 to 90% depending on the O.D values

Enzyme immunoassays

- IgG specific methods are more specific than Igtotal methods.

- PF4/heparin complexes can be destructed by adding overdoses heparin.
    - Inhibition of reaction with high concentration heparine
    - Increases PPV (ELISA pos/clinic neg: 34% can be ruled out with inhibition)
    - However, is not always clear


Clinical probability HIT score
low
intermediate
high
PF4-heparin antibody (IgG) enzyme-immunoassay
No HIT
negative
positive
Functional assay



Heparin Induced Platelet Activation Assay (HIPAA)

Greinacher et al 1991

Platelet activation methods

Functional methods: gold standard

- Heparin induced platelet activation assay (HIPAA)
- Serotonin release assay (SRA)

Platelet activation methods

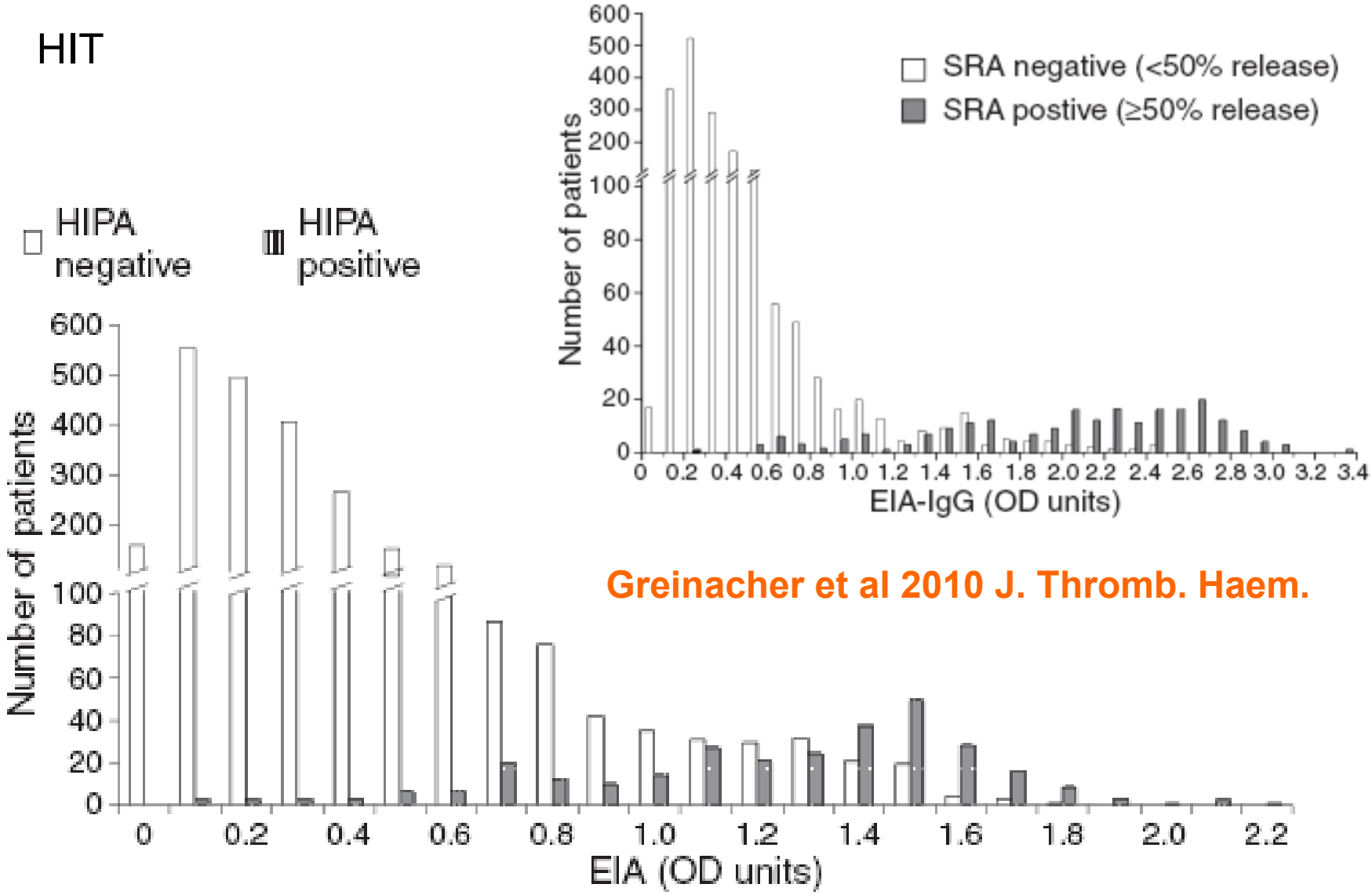
Pro's	specificity 99%	
	sensitivity 94%	
Con's	HIPAA	Not an easy test to perform
		For each test, fresh platelet suspensions of at least four donors are needed
	SRA	Needs radioactive reagents

PPV increases by:

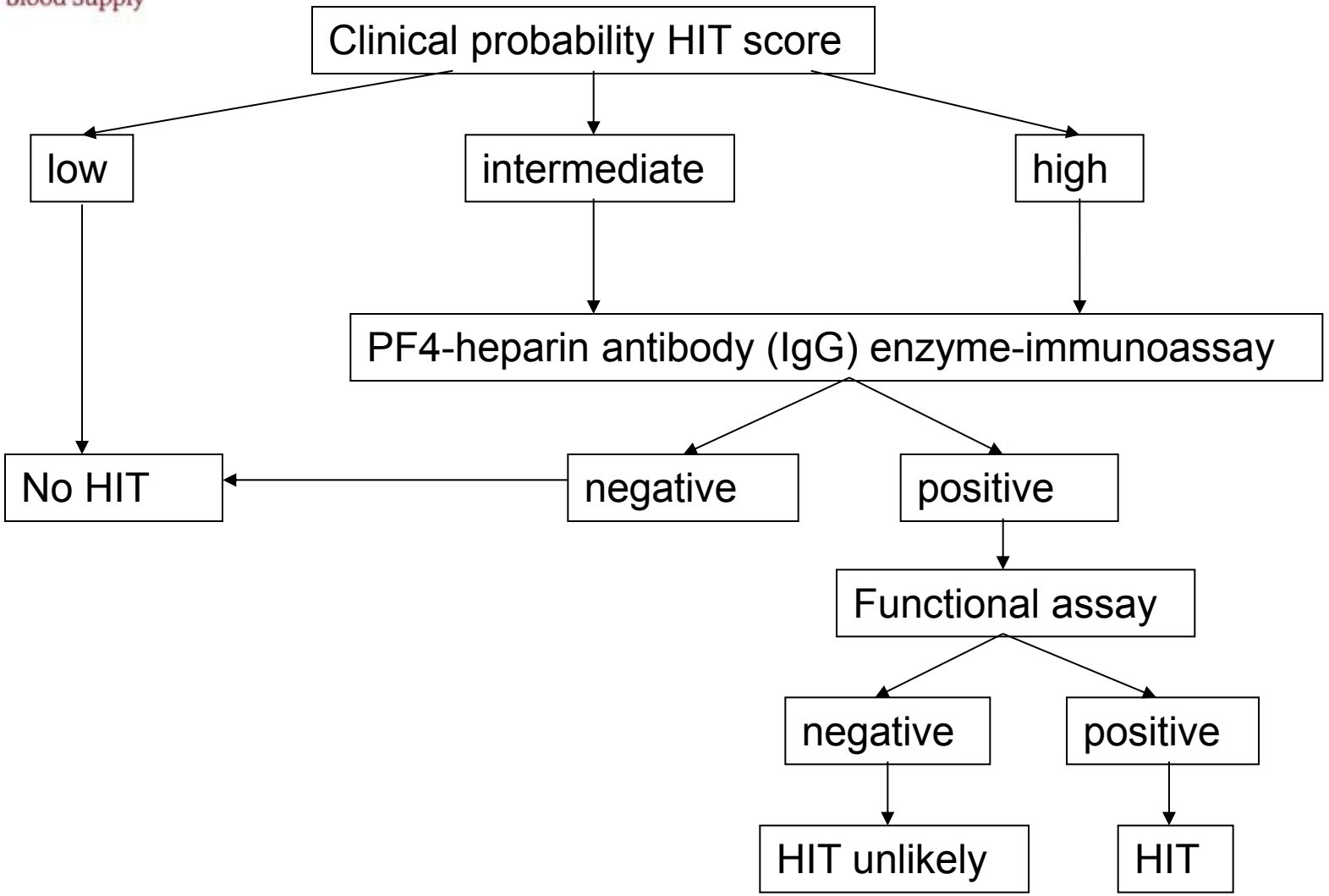
Inhibition with high concentration heparin

and with MoAb Blocking FcyRIIa

HIT



Greinacher et al 2010 J. Thromb. Haem.


Clinical probability HIT score
low
intermediate
high
PF4-heparin antibody (IgG) enzyme-immunoassay
No HIT
negative
positive
Functional assay
negative
positive
HIT unlikely
HIT

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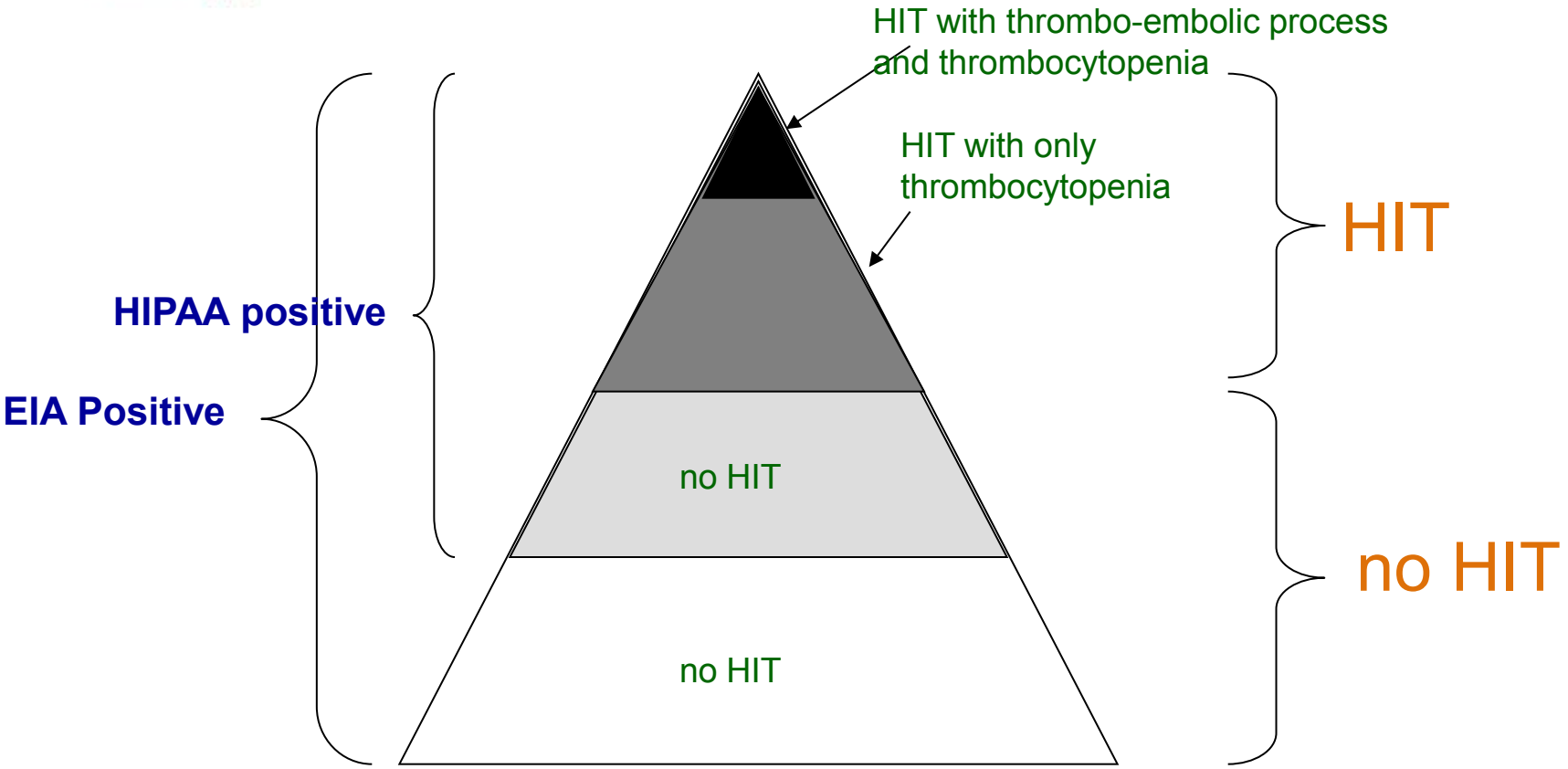
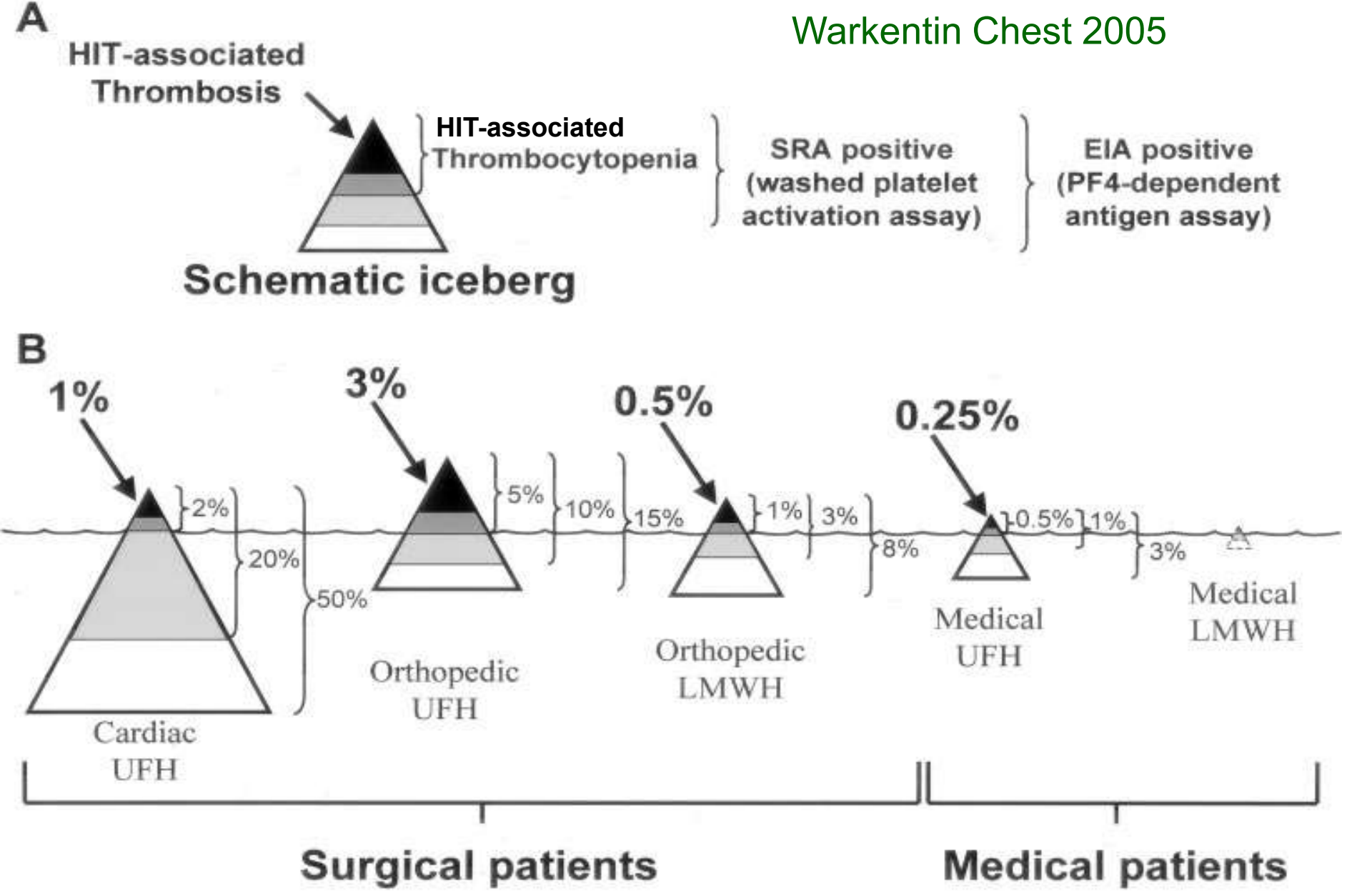
Some considerations…

Pyramid for patients with antibodies



Explanation pyramid figure Warkentin et al.

Warkentin Chest 2005



Clinical consequences

clinical probability score	EIA	HIPAA	Heparin treatment	conclusion
low				no HIT

Follow up also depends on how frequent a test is performed.

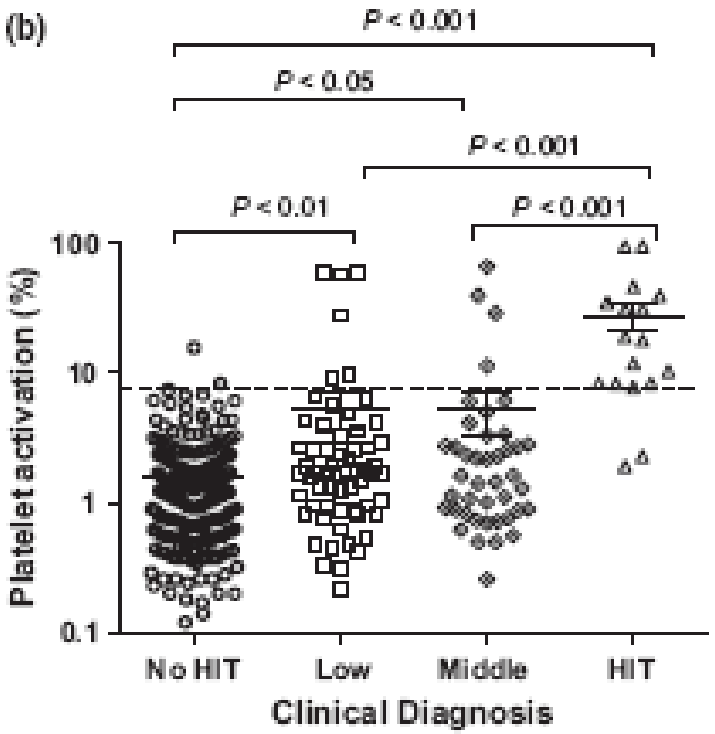
For instance EIA each day

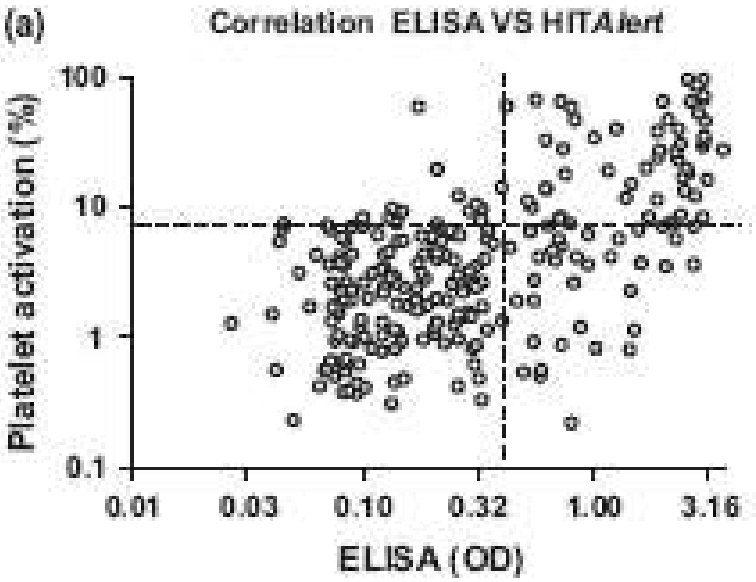
Functional assay once a week

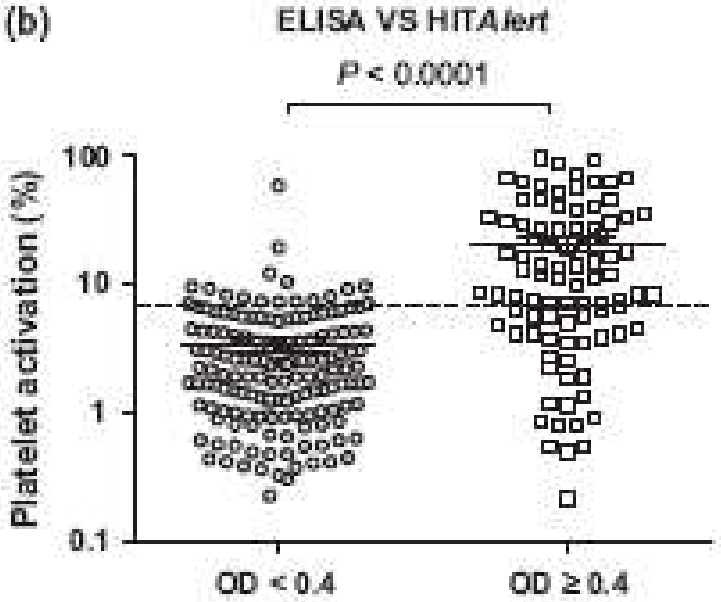

Thank you

	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)
HemosILHIT-Ab (cut-off: 1.0 U/mL)	100% (83.2% to 100%)	75.6% (66.1% to 86.4%)	100% (93.9% to 100.0%)	45.5% (29.6% to 60.0%)
HemosIL AcuStar-Ab (cut-off: 1.0 U/mL)	98.1% (89.7% to 99.9%)	82.1% (77.9% to 85.7%)	99.7% (98.3% to 100.0%)	41.8% (32.9% to 51.1%)
HemosIL AcuStar-IgG (cut-off: 1.0 U/mL)	96.2% (86.7% to 99.5%)	96.5% (93.8% to 97.8%)	99.5% (98.1% to 99.9%)	78.1% (66.0% to 87.5%)
In house IgG-ELISA (cut-off OD 0.5)	98.1% (89.7% to 99.9%)	86.4% (82.6% to 89.6%)	99.7% (98.3% to 100%)	47.2% (37.5% to 57.0%)
HemosILHIT-Ab (cut-off: 3.85 U/mL)	95% (75.1% to 99.9%)	98.9% (94.5% to 99.9%)	98.9% (97.02% to 100%)	95% (91.1% to 98.9%)
HemosIL AcuStar-Ab (cut-off: 2.45 U/mL)	98.1% (89.7% to 99.9%)	95.7% (93.2% to 97.5%)	99.7% (99.2% to 100.2%)	75.1% (71.1% to 79.1%)
HemosIL AcuStar-IgG (cut-off: 0.57 U/mL)	98.1% (89.7% to 99.9%)	93.4% (90.5% to 95.7%)	99.7% (99.2% to 100.2%)	66.2% (61.8% to 70.6%)
In house IgG-ELISA (cut-off OD 0.77)	94.3% (84.1% to 98.8%)	93% (90.2% to 95.5%)	99.2% (98.4% to 100%)	64.5% (60.1% to 68.9%)

Althaus et al. Thrombosis Research 2013







Garritsen et al.

Flow cytometric evaluation of HIT

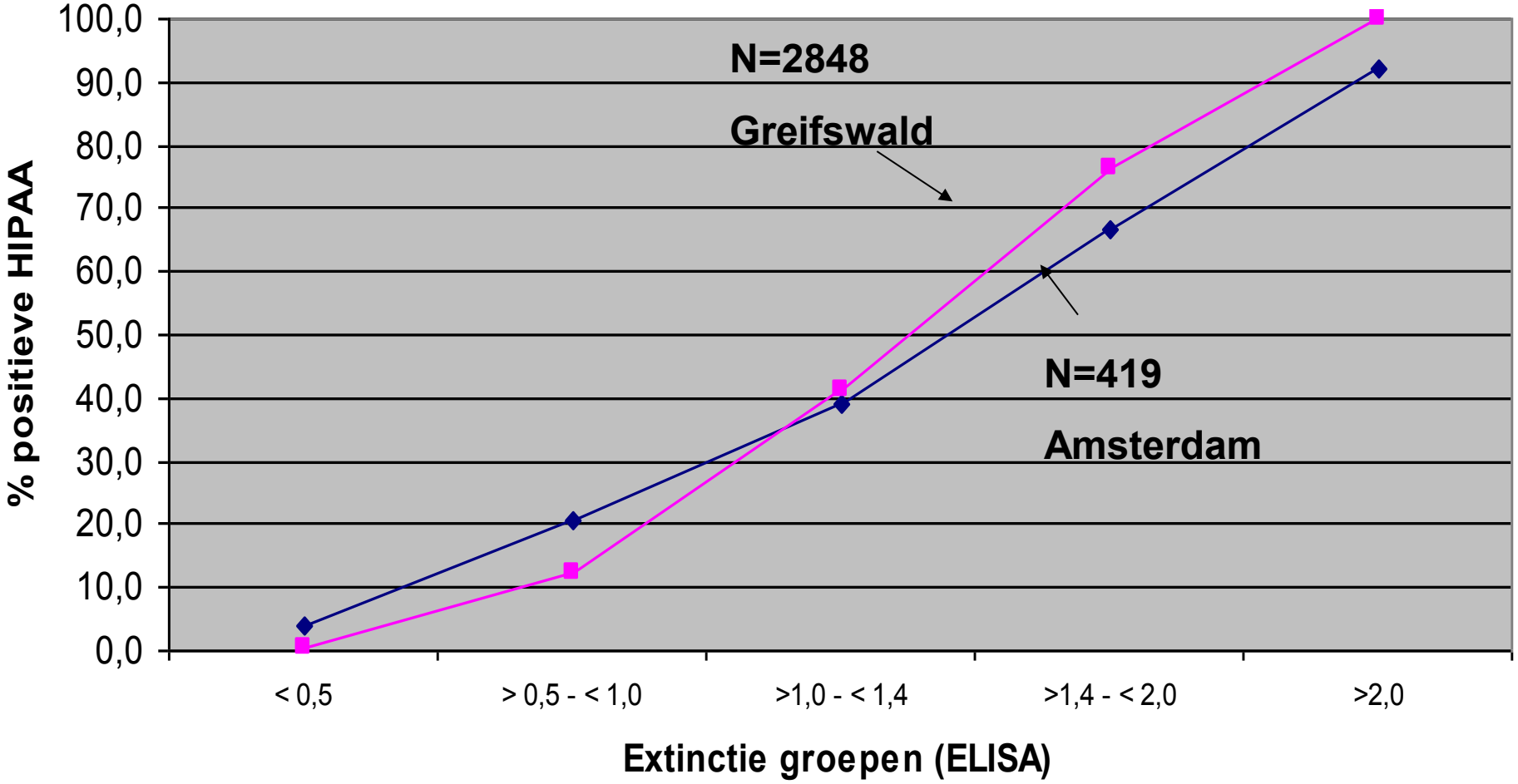
Int Jnl Lab Hem 2014

HemosIL AcuStar-IgG

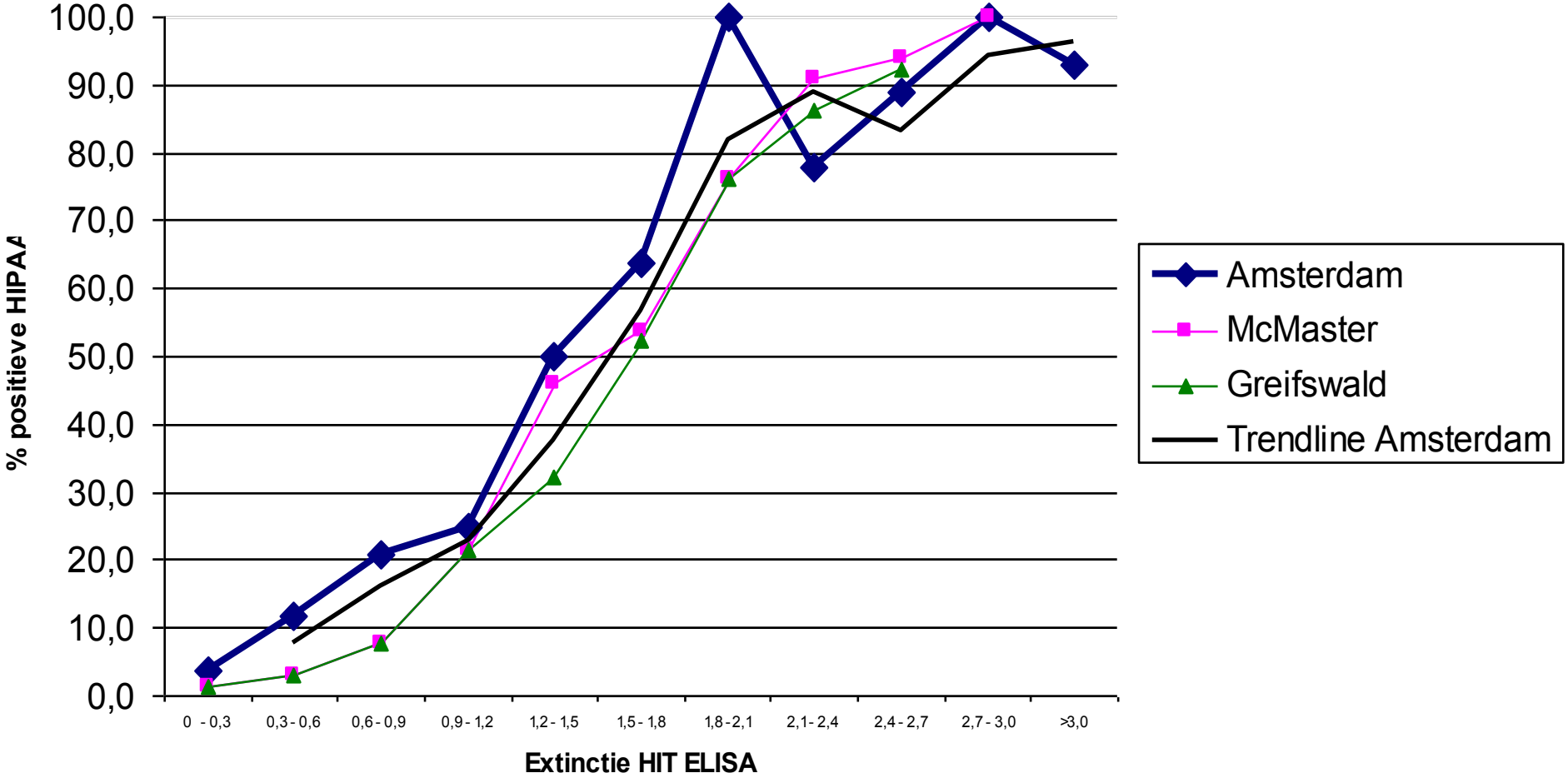
		HIPA negative	HIPA positive	HIPA negative	HIPA positive
n = 116 plasma samples	negative (0–0.99 U/mL)	76	0	93	0
	weak positive (1.00-1.99 U/mL)	13	0	0	0
	positive (2.00-3.99 U/mL)	3	0	0	2
	Strong positive (> 4.0 U/mL)	4	20	3	18
n = 332 serum samples	negative (0–0.99 U/mL)	249	1	289	2
	weak positive (1.00-1.99 U/mL)	32	0	8	6
	positive (2.00-3.99 U/mL)	16	2	3	3
	Strong positive (> 4.0 U/mL)	3	29	0	21
n = 448 serum and plasma samples	negative (0–0.99 U/mL)	325	1	382	2
	weak positive (1.00-1.99 U/mL)	45	0	8	6
	positive (2.00-3.99 U/mL)	19	2	3	5
	Strong positive (≥ 4.0 U/mL)	7	49	3	39

	serum van patienten			
trombocyten	hep 0,2	hep 0,2	hep 100	FcRII MoAb
donor 1	10 min	10 min	neg	35
donor 2	15 min	15 min	neg	40
donor 3	10 min	10 min	neg	neg
donor 4	15 min	10 min	neg	neg

Extinctie ELISA versus % positieve HIPAA diagnostiek en validatie sera t/m 15-1-2012 Vergelijking onze resultaten met de resultaten van Greinacher et al.



Extinctie HIT ELISA Asserechrome versus positieve HIPAA
prospectief validatie/ diagnostiek sera
Vergelijking onze HIPAA resultaten met HIPAAresultaten van Greinacher (Greifswald) en SRA resultaten van McMaster



Bakchoul and Greinacher 2012

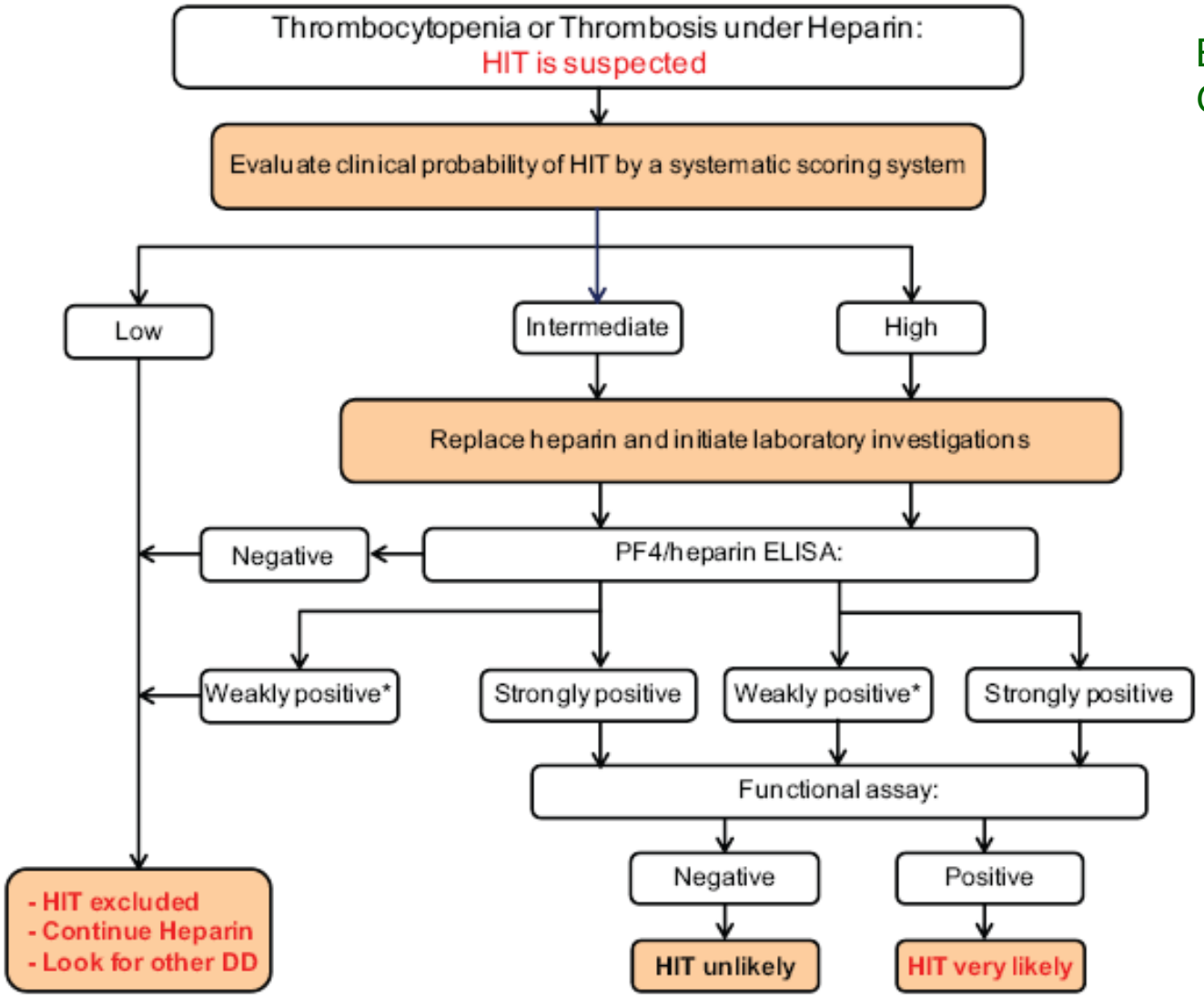


Figure 1. A suggested approach to diagnosis and initial management of patients with suspected HIT. This approach to the diagnosis and initial management of patients with suspected HIT is based on clinical assessment supported by complementary laboratory investigations. Results of Immunoassays can be devided into neagtive, weakly positive (OD < 1.0) and strong positive (OD>1.0). The decision whether weakly positive results need to be further verfied using functional assays or not depends on the clinical probabilty. As indeterminate results may be occasionally obtained using laboratory tests, re-evaluating the clinical probability of HIT in an individual patient may be helpful to overcome some diagnostic uncertainty.