PIASTRINOPENIA DA EPARINA (HIT): DIAGNOSI E TERAPIA

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MANAGEMENT OF HIT

CLINICAL AND LAB DIAGNOSIS

- **HIT definition & Clinical Suspicion**
- **Epidemiology & Risk Factors**
- **Pathophysiology**
- **Clinical Features & Diagnosis**
- **Laboratory Testing of HIT**
- **Therapy: Alternative anticoagulants**

Treatment & Prevention of HIT:

American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition)

T. E. Warkentin, A. Greinacher, A. Koster, A. M. Lincoff

Chest 2008; 133: 340S-380S

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HEPARIN-INDUCED THROMBOCYTOPENIA

DEFINITIONS OF HIT

HIT can be defined as any clinical event best explained by platelet-activating, platelet Factor 4 (PF4)-heparin reactive antibodies ("HIT antibodies") in a patient who is receiving or who has recently received, UFH or LMWH

CLINICAL SUSPICION OF HIT
Is Based upon the 4 *T's*

- ***T*hrombocytopenia:** a platelet count-fall of > 50% (usual nadir, 20-150.000/uL)
- ***T*iming of thrombocytopenia**: consistent with heparin induced immunization.
- ***T*hrombosis** or other sequelae (DIC ?)
- o***T*her explanations** for thrombocytopenia lacking

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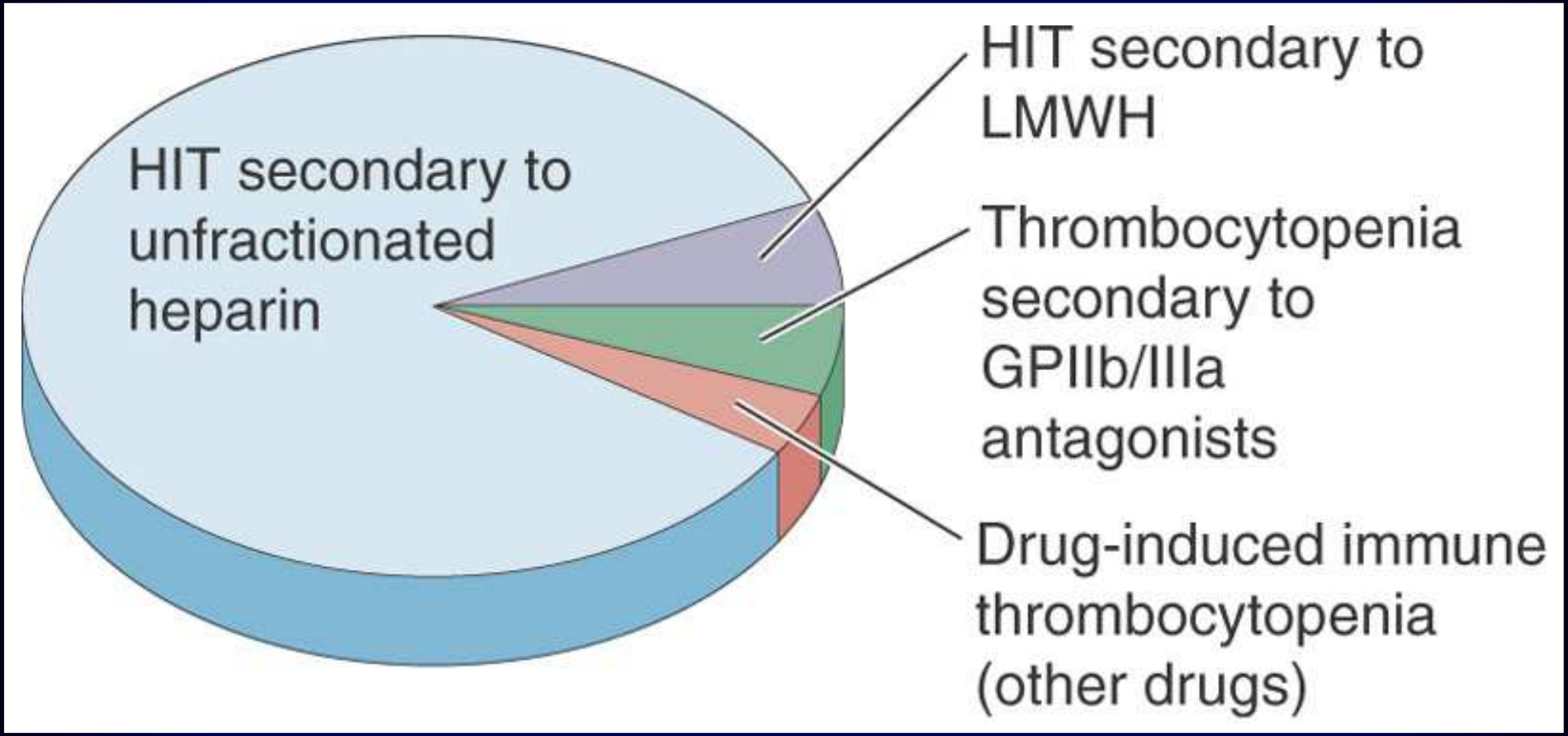
FREQUENCIES OF DRUG-INDUCED THROMBOCYTOPENIAS



Factors Influencing Incidence of HIT

Heparin preparation: bovine lung unfractionated heparin > porcine intestinal unfractionated heparin > (porcine-derived) LMW heparin

Patient population: postoperative > medical > obstetric patients/children/neonates

Duration of heparin: progressive increase in frequency as heparin continues from 5 to 14 days

Gender: female > male (1.5–2.0:1)

Intensity of platelet count monitoring

Laboratory testing for HIT antibodies

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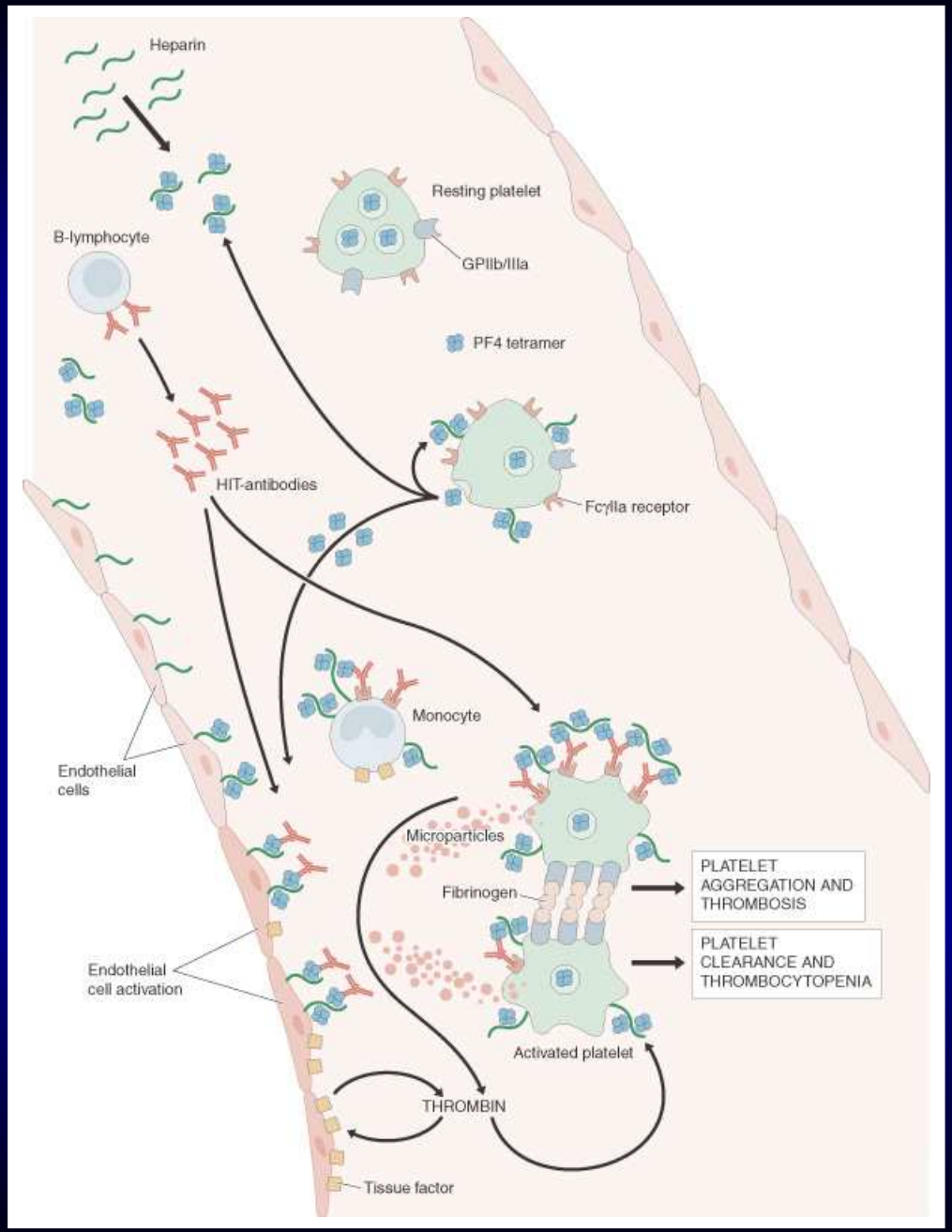
PATHOPHYSIOLOGY OF HIT

HYPERCOAGULABILITY & THROMBOSIS

- **PF4-Heparin complexes**
- **Platelet activation**
- **Platelet-derived microparticles**
- **Endothelial & Monocyte activation**
- **Thrombin generation**


Heparin
Resting platelet
GPIIb/IIIa
B-lymphocyte
PF4 tetramer
HIT-antibodies
FcγIIa receptor
Monocyte
Endothelial cells
Microparticles
Fibrinogen
PLATELET AGGREGATION AND THROMBOSIS
PLATELET CLEARANCE AND THROMBOCYTOPENIA
Endothelial cell activation
Activated platelet
THROMBIN
Tissue factor

THROMBIN GENERATION IN HIT

Thrombin generation and heparin-induced thrombocytopenia

B. TARDY-PONCET,*† M. PIOT,† C. CHAPELLE,† G. FRANCE,* L. CAMPOS,* O. GARRAUD,‡ H. DECOUSUS,†§ P. MISMETTI† and B. TARDY†§

J Thromb Haemost 2009; **7**: 1474–81.

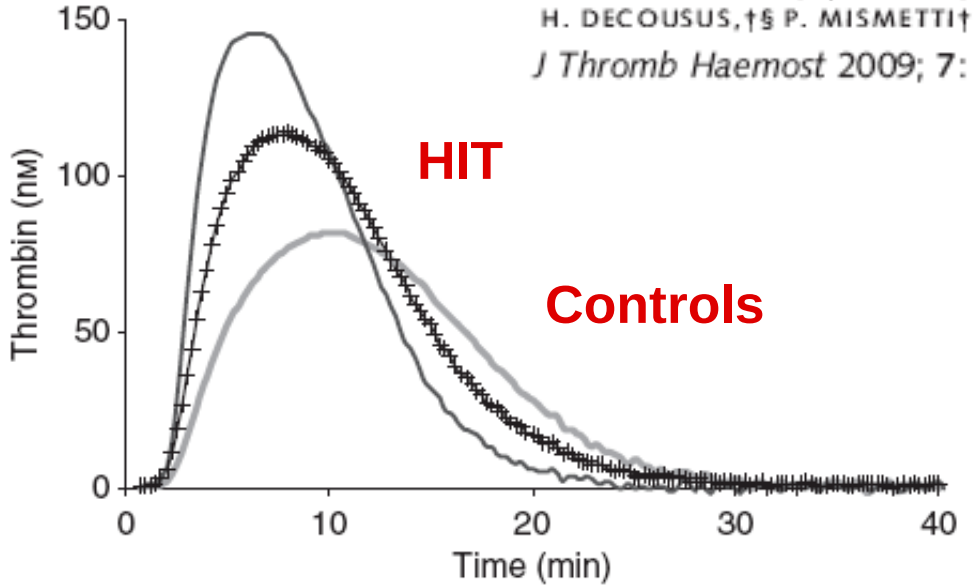
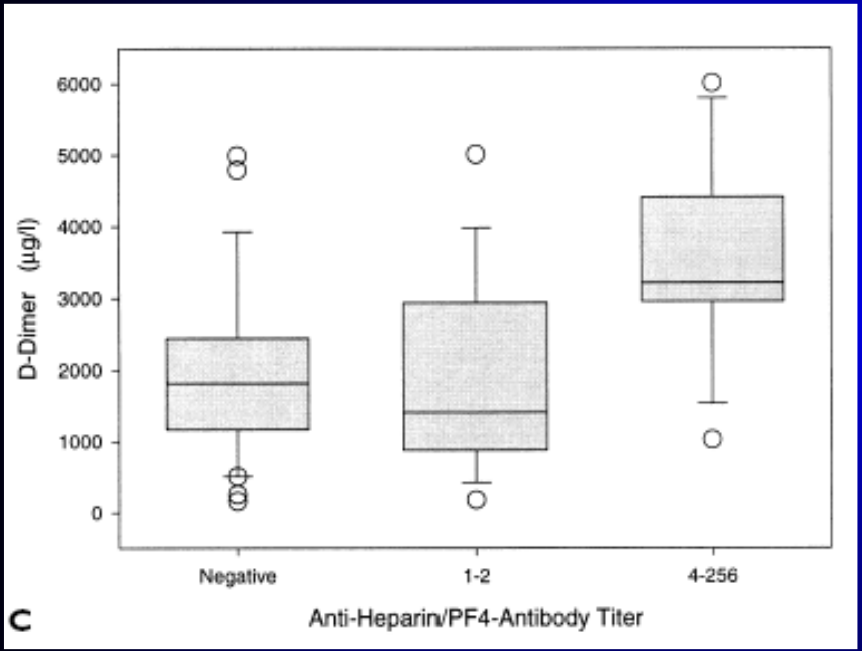


Fig. 1. Thrombogram profiles obtained on mixtures of patient platelet-poor plasma (PPP) and donor platelet-rich plasma (PRP) without the addition of UFH, for all patients with suspected heparin-induced thrombocytopenia (HIT) (n = 48) (cross line), and with the addition of unfractionated heparin (UFH) (0.2 U mL^{-1} final dilution) for HIT-negative patients (n = 25) (gray line) and HIT-positive patients (n = 23) (black line). The curves represent computer-generated averages of all individual curves registered.

D-DIMER & HIT

D-Dimer levels and HIT Antibodies



Factors predicting high levels of coagulation markers in suspected HIT.

Factor	F1+2	TAT	D-dimers
Anti-heparin/PF4-antibody titre	0.0036	0.0176	0.0003
Infection/sepsis	0.0159	0.0228	<0.0001
Peripheral artery disease	0.0002	n.s.	n.s.
Thromboembolism while on heparin	n.s.	0.0058	n.s.
Cancer	n.s.	n.s.	n.s.
Coronary artery disease	n.s.	n.s.	n.s.
Renal insufficiency	n.s.	n.s.	n.s.
Surgery in the preceding 48 hrs	n.s.	n.s.	n.s.

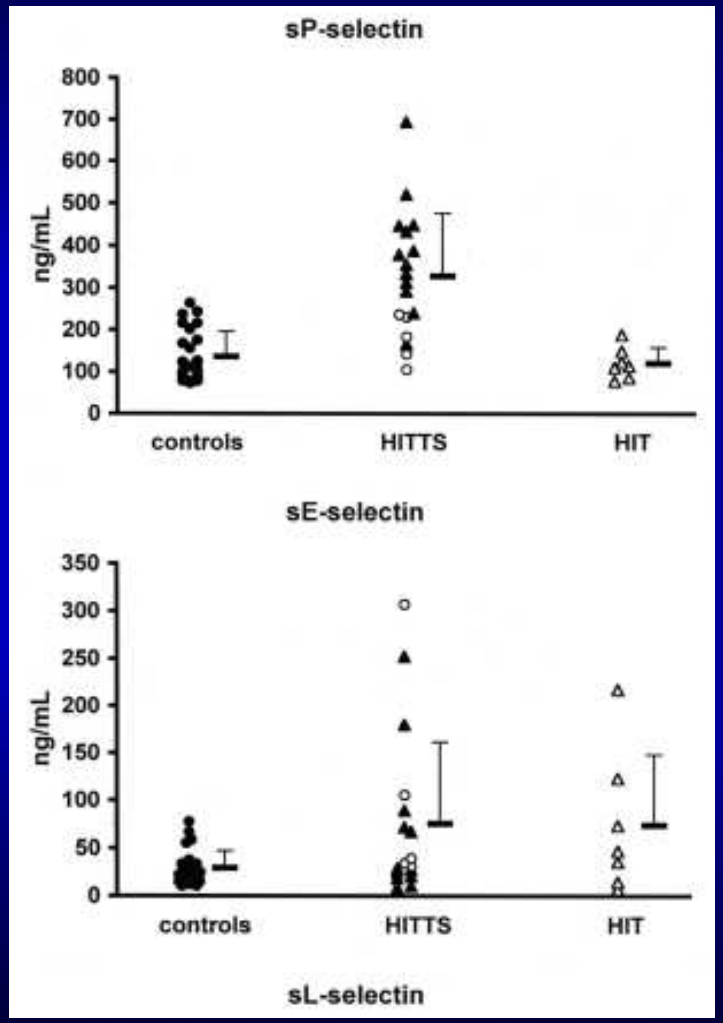
P values are reported. n.s. indicates "not significant" ($\alpha = 0.05$).

Chilver-Stainer L et al Thromb Haemost 2004

E-SELECTIN & P-SELECTIN IN HIT

P-SELECTIN

E-SELECTIN



CELLA et al Haematologica 2001

PROTHROMBOTIC STATE IN HIT

Odds Ratio for thrombosis

DISEASE	O.R.
HIT (platelets < 150×10^9)	36
V Leiden	6.6
PC PS deficiency	12
ATIII deficiency	24
APA syndrome	5

Warkentin: N Engl J Med 1995; Am J Med 1996

MANAGEMENT OF HIT

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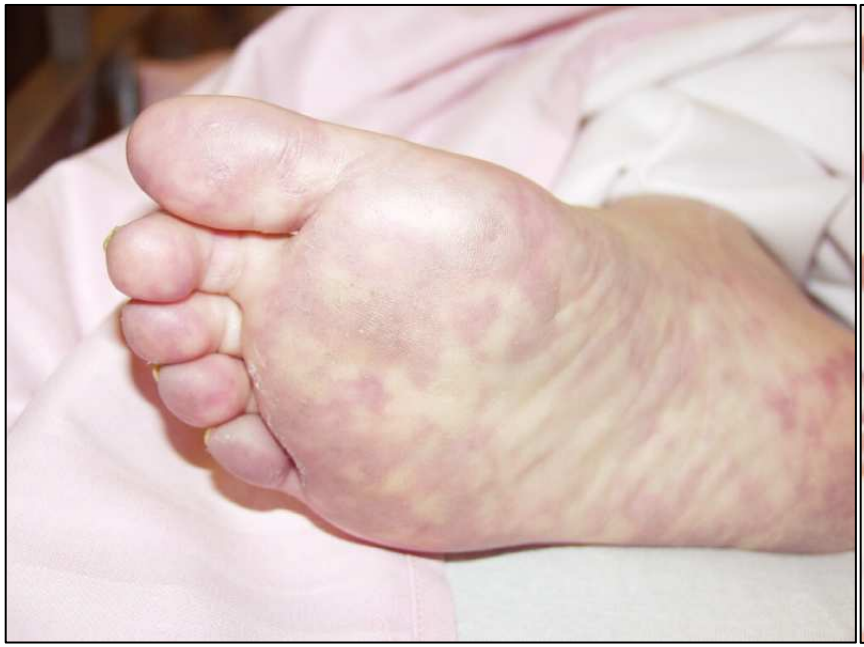
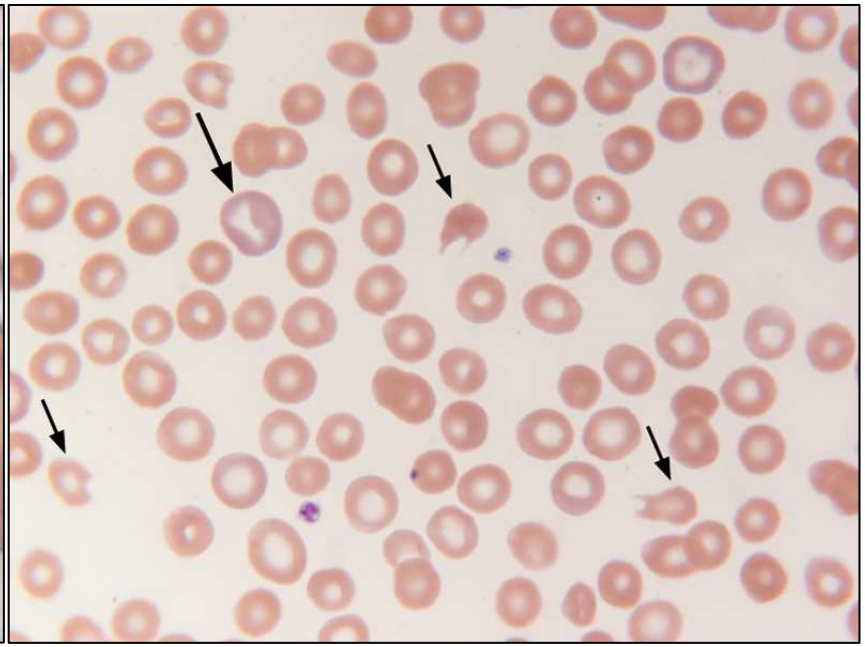
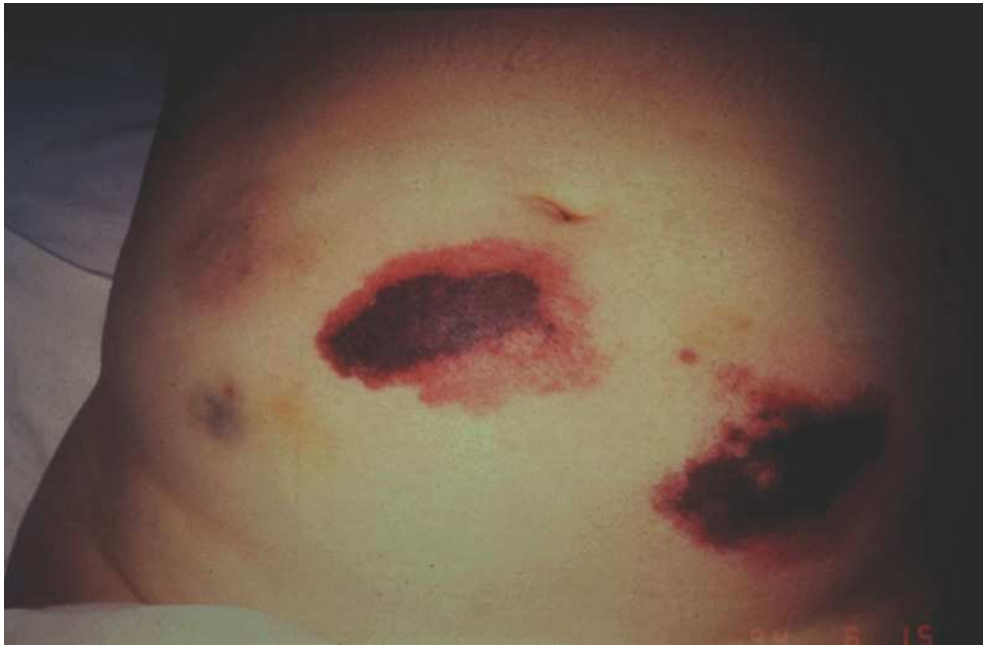
CLINICAL PRESENTATIONS
OF HIT PATIENTS

In the management of the patients with HIT we should always consider the two main conditions:

- **Isolated HIT (thrombocytopenia only)**
- **HIT-associated thrombosis**







CLINICAL PRESENTATIONS OF HIT PATIENTS (I)

Clinical Features of HIT

Thrombocytopenia

Definition: Platelet count fall (usually >50%) in an appropriate temporal relationship to heparin therapy that is not more readily explained by another disorder.

Timing:

Typical-onset HIT: platelets begin to fall usually 5–10 days after an immunizing exposure to heparin (most often, intraoperative or perioperative heparin).

Rapid-onset HIT: an abrupt platelet count fall can occur when heparin is given to a patient who already has HIT antibodies resulting from a recent exposure.

Recovery after stopping heparin: variable, median 4 days to platelet count rise to >150 × 10^9/L; but 10% of patients take more than a week to recover.

Thrombosis

Venous thrombosis: deep venous thrombosis > pulmonary embolism > adrenal vein thrombosis (causing adrenal hemorrhagic infarction) > cerebral venous thrombosis > other visceral venous thrombosis

Arterial thrombosis: limb artery thrombosis > thrombotic stroke > myocardial infarction > mesenteric artery thrombosis > thrombosis in other arteries

Microvascular thrombosis: either secondary to coumarin (e.g., warfarin-induced venous limb gangrene or "classic" warfarin-induced skin necrosis) or disseminated intravascular coagulation (DIC) alone

Intracardiac thrombosis: intra-atrial or intraventricular thrombi

CLINICAL PRESENTATIONS OF HIT PATIENTS (II)

Clinical Features of HIT

Heparin-Induced Skin Lesions (at Heparin Injection Sites)

Erythematous plaques
Skin necrosis

Acute Systemic Reactions

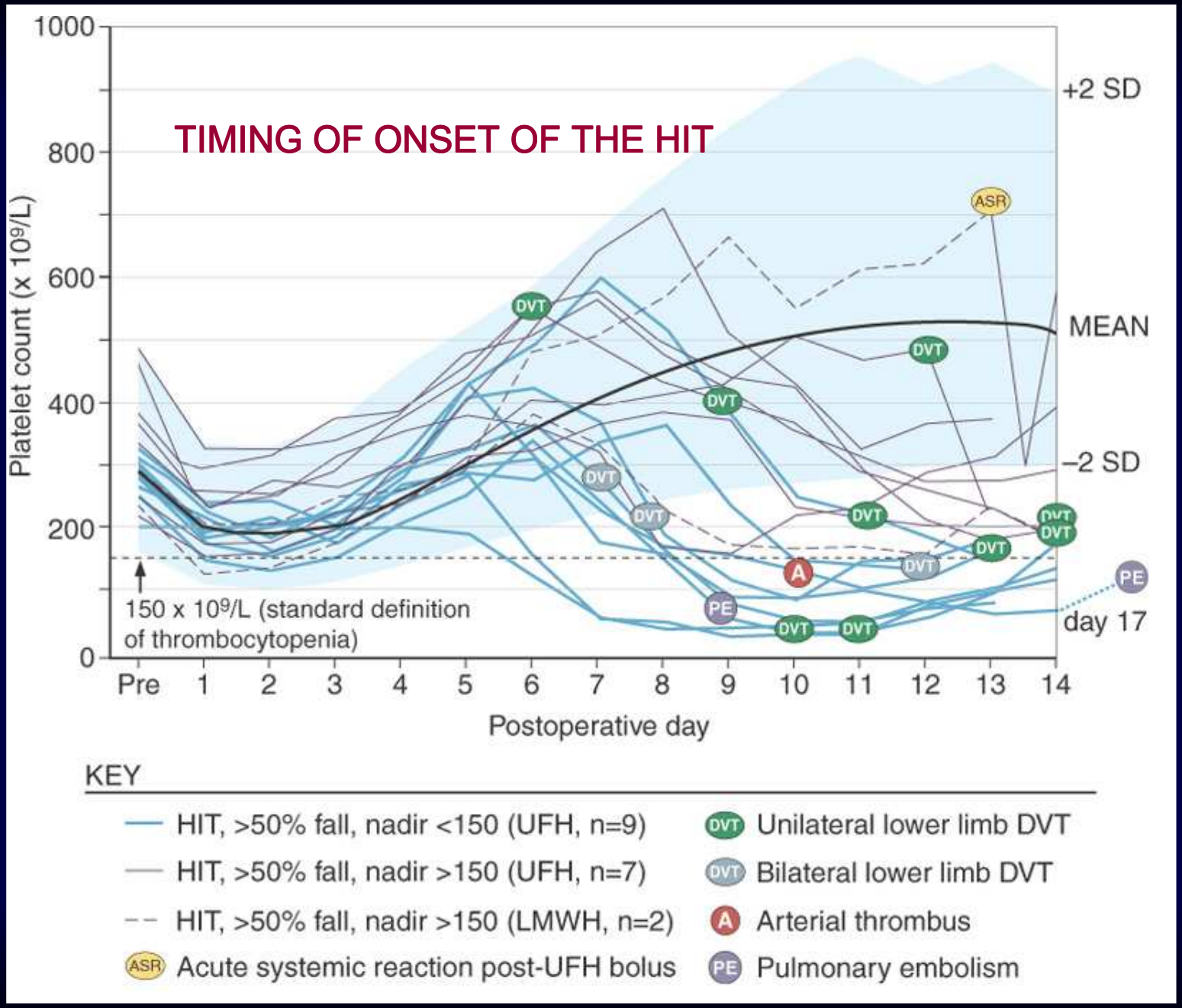
One or more of the following beginning 5–30 min after an intravenous unfractionated heparin bolus:
Inflammatory: chills, rigors, fever, flushing
Cardiorespiratory: tachycardia, hypertension, tachypnea, dyspnea, chest pain or tightness, cardiopulmonary arrest
Gastrointestinal: nausea, vomiting, large-volume diarrhea
Neurologic: headache, transient global amnesia

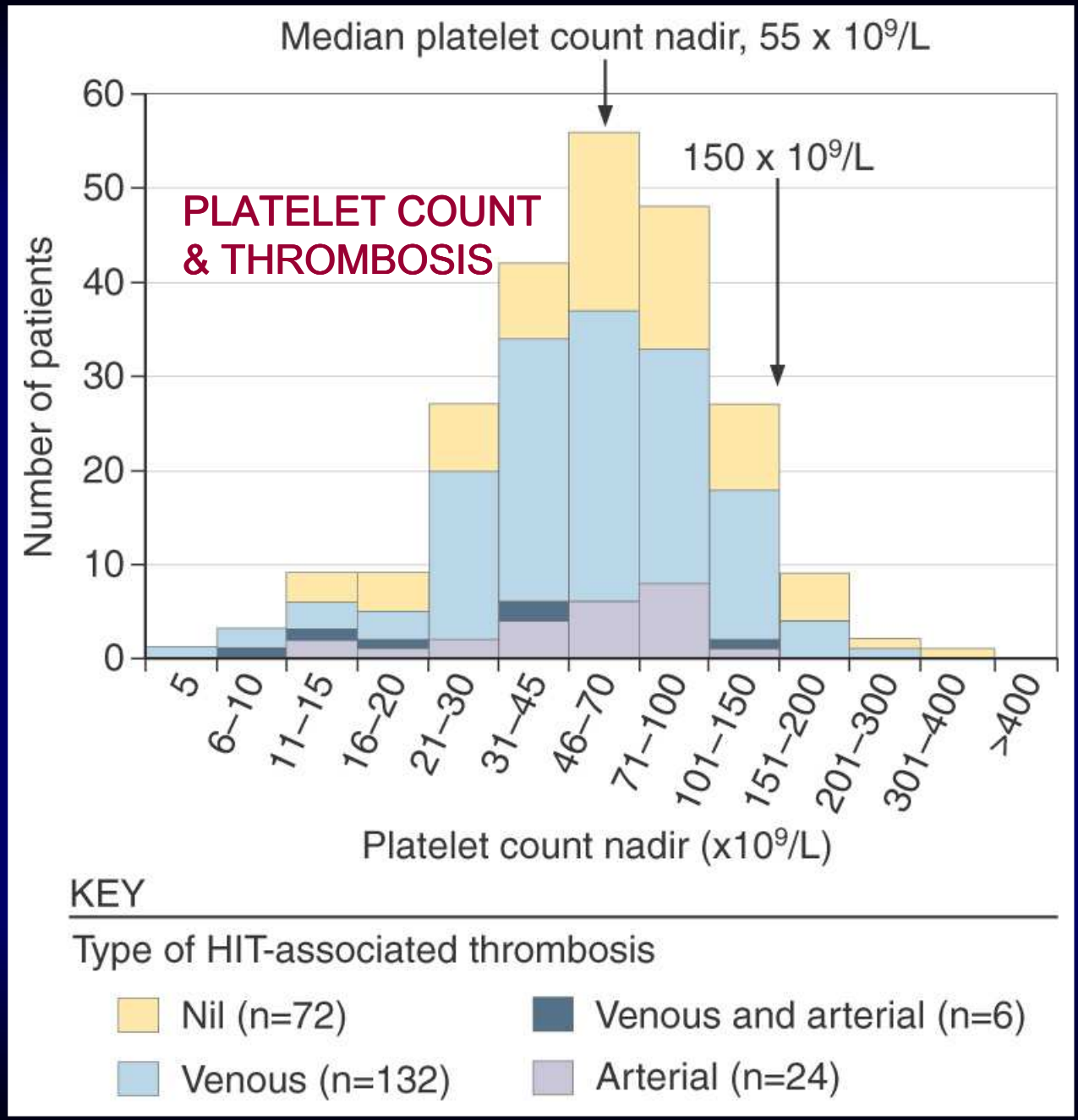
Decompensated DIC

One or more of the following (in the absence of another explanation):
Prothrombin time (International Normalized Ratio) increase
Fibrinogen decrease
Microangiopathic blood film (see also Fig. 69–5)
Circulating normoblasts (rare)

Preserved Hemostasis

Petechiae and other clinical evidence of thrombocytopenic bleeding are generally not present in HIT, even when severe thrombocytopenia is present.


TIMING OF ONSET OF THE HIT
Platelet count (x 10⁹/L)
1000
800
600
400
200
0
+2 SD
MEAN
–2 SD
day 17
150 x 10⁹/L (standard definition of thrombocytopenia)
Pre
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Postoperative day
ASR
DVT
A
PE
KEY
HIT, >50% fall, nadir <150 (UFH, n=9)
HIT, >50% fall, nadir >150 (UFH, n=7)
HIT, >50% fall, nadir >150 (LMWH, n=2)
Acute systemic reaction post-UFH bolus
Unilateral lower limb DVT
Bilateral lower limb DVT
Arterial thrombus
Pulmonary embolism


Median platelet count nadir, 55 x 10⁹/L
150 x 10⁹/L
PLATELET COUNT
& THROMBOSIS
Number of patients
60
50
40
30
20
10
0
5
6–10
11–15
16–20
21–30
31–45
46–70
71–100
101–150
151–200
201–300
301–400
>400
Platelet count nadir (x10⁹/L)
KEY
Type of HIT-associated thrombosis
Nil (n=72)
Venous (n=132)
Venous and arterial (n=6)
Arterial (n=24)

THROMBOSIS DURING HIT

EVENT	O.R.
Pulmonary embolism	93
Arterial thrombosis	41
venous or arterial thrombosis	37
Any venous thrombosis	16.5

Warkentin 1995 modified

PROBABILITY OF HIT DIAGNOSIS

ACCORDING TO THE 4T's SCORE

Suspicion of HIT based upon the 4T's	Score	2	1	0
Thrombocytopenia	☐	>50% platelet fall to nadir ≥20	nadir 10-19, or 30-50% platelet fall	nadir <10, or <30% platelet fall
Timing of onset of platelet fall or thrombosis	☐	day 5-10, or ≤day 1 with recent heparin (within past 30 days)	>day 10 or timing unclear; or ≤day 1 with recent heparin (past 30-100 days)	day ≤4 (no recent heparin)
Thrombosis or other sequelae	☐	proven thrombosis, skin necrosis, or acute systemic reaction	progressive or recurrent thrombosis, or erythematous skin lesions	none
o**T**her cause(s) of platelet fall	☐	none evident	possible	definite
Total pre-test probability score	☐	periodic reassessment as new information can change pre-test probability (e.g., positive blood cultures)		

High = 6-8 --- Intermediate = 4-5 --- Low = 0-3

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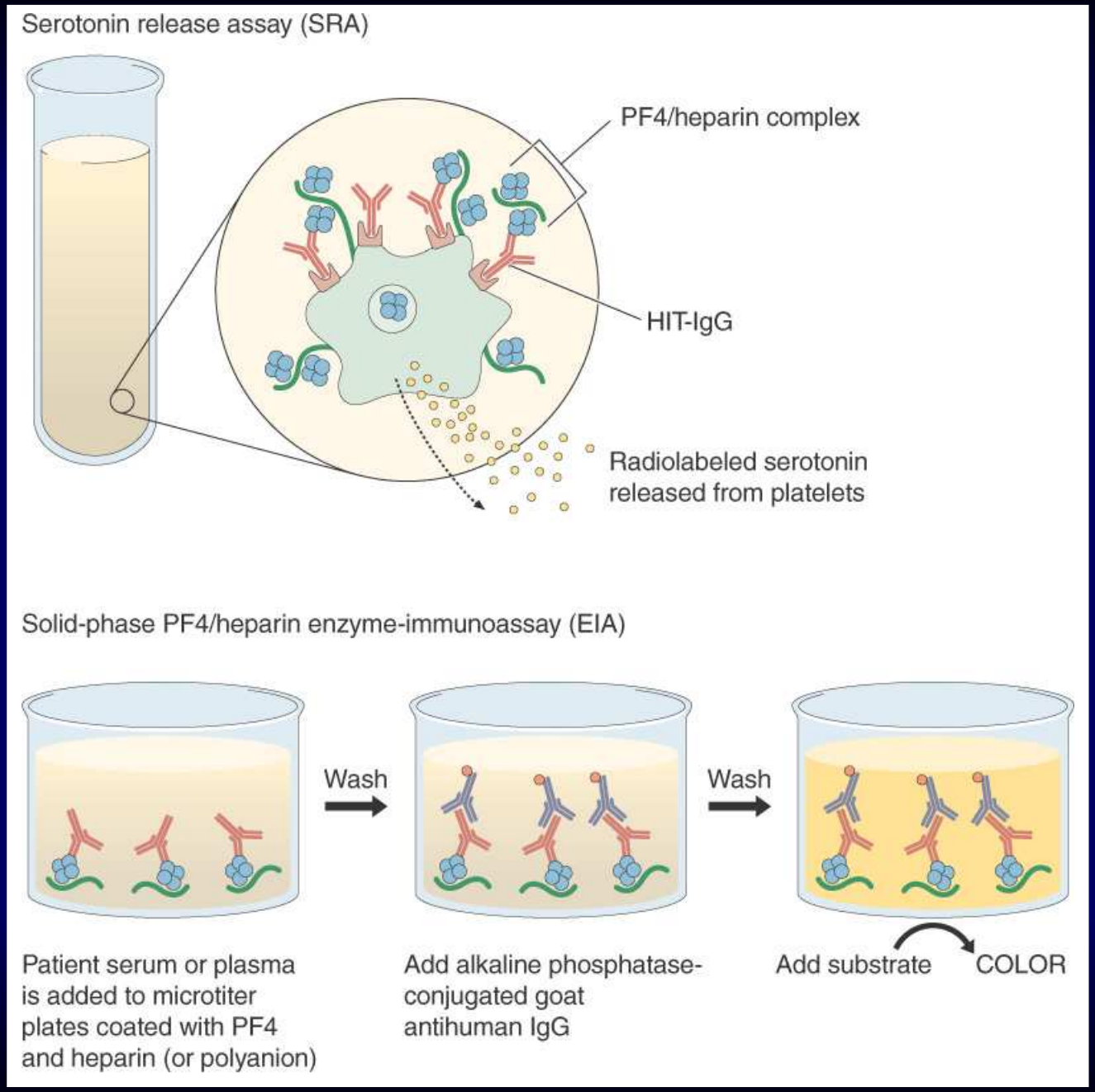
Commonly used tests for HIT

A) Functional Assays

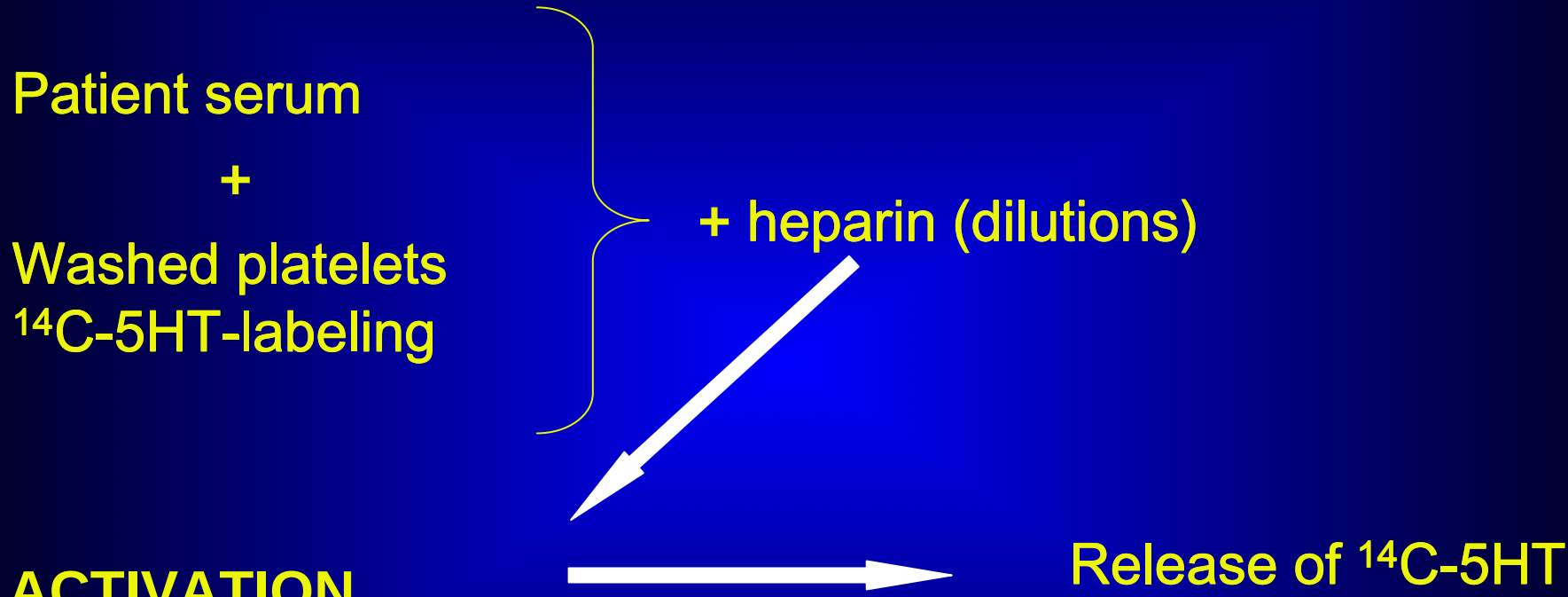
Platelet activating antibodies

B) Immunological Assays

Antigen specific antibodies

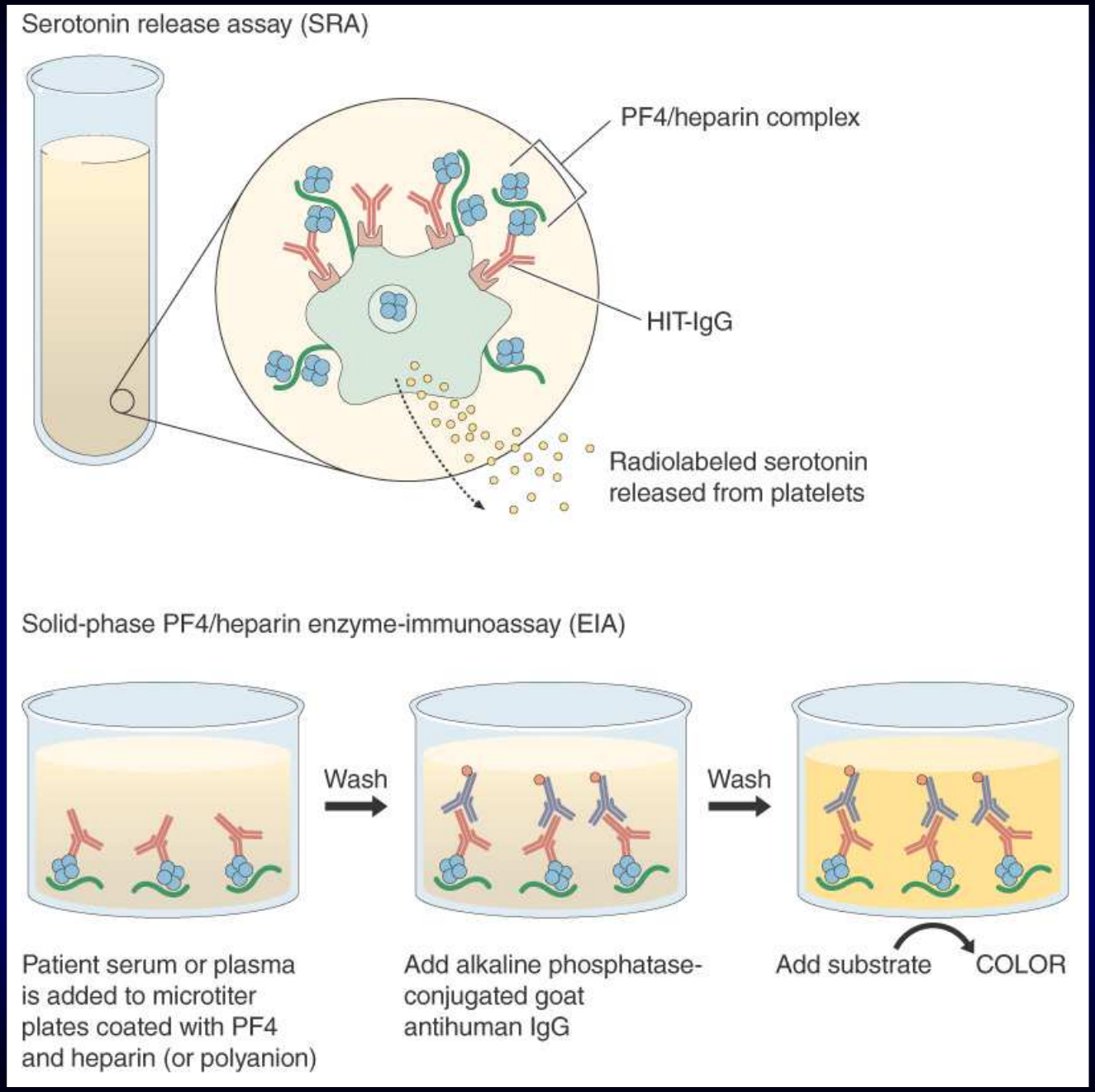

Serotonin release assay (SRA)
PF4/heparin complex
HIT-IgG
Radiolabeled serotonin
released from platelets
Solid-phase PF4/heparin enzyme-immunoassay (EIA)
Wash
Wash
Patient serum or plasma
is added to microtiter
plates coated with PF4
and heparin (or polyanion)
Add alkaline phosphatase-
conjugated goat
antihuman IgG
Add substrate
COLOR

SEROTONIN RELEASE ASSAY SRA
(gold standard)



+ Sensitive
- Donor: critical
- Radio-labeled material

↑ ^{14}C = ↑ Anti-heparin Abs


Serotonin release assay (SRA)
PF4/heparin complex
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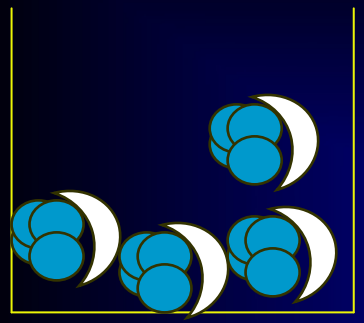
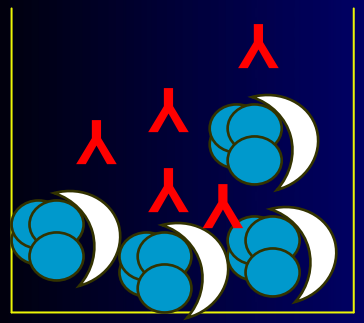
Radiolabeled serotonin
released from platelets
Solid-phase PF4/heparin enzyme-immunoassay (EIA)
Wash
Wash
Patient serum or plasma
is added to microtiter
plates coated with PF4
and heparin (or polyanion)
Add alkaline phosphatase-
conjugated goat
antihuman IgG
Add substrate
COLOR

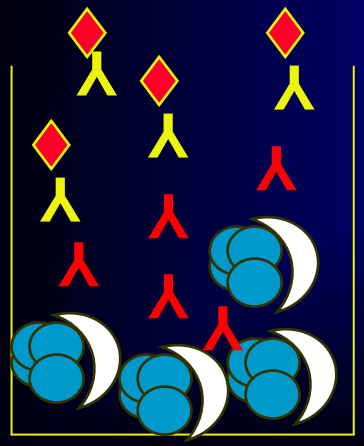
ELISA TEST: *H/PF4/Ab*



Heparin/PF4

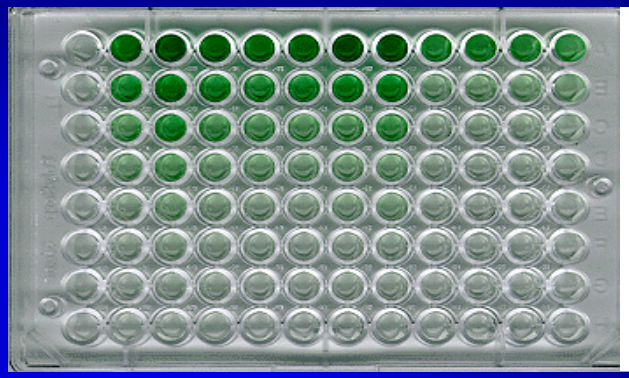


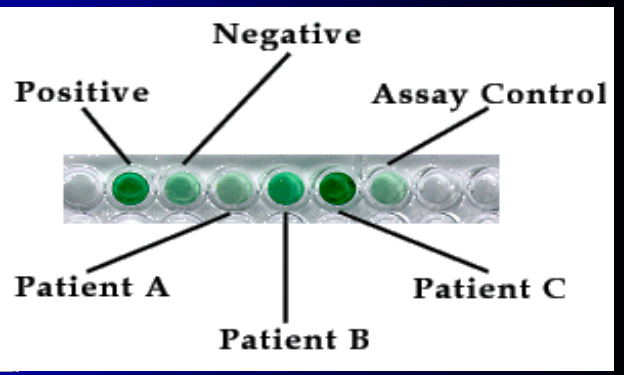
HIT-IgG



Peroxidase
Conjugated IgG

PF4/HEPARIN ELISA





+) > sensitive than functional tests

-) specific for clinical HIT

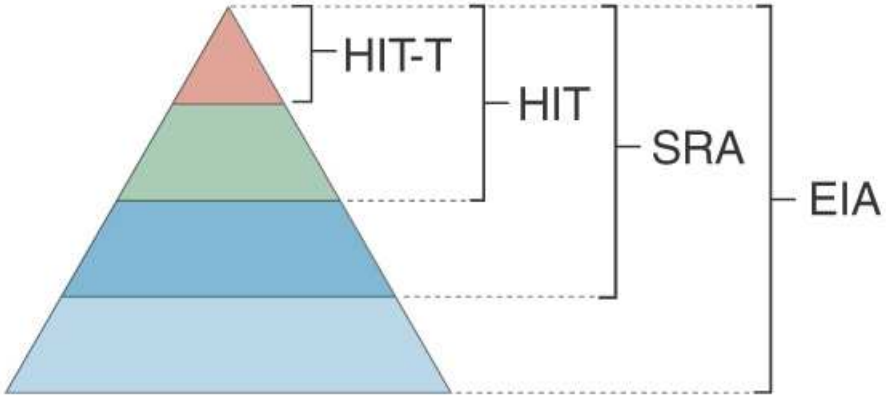
+) rapid & easy

+) no problem with donors

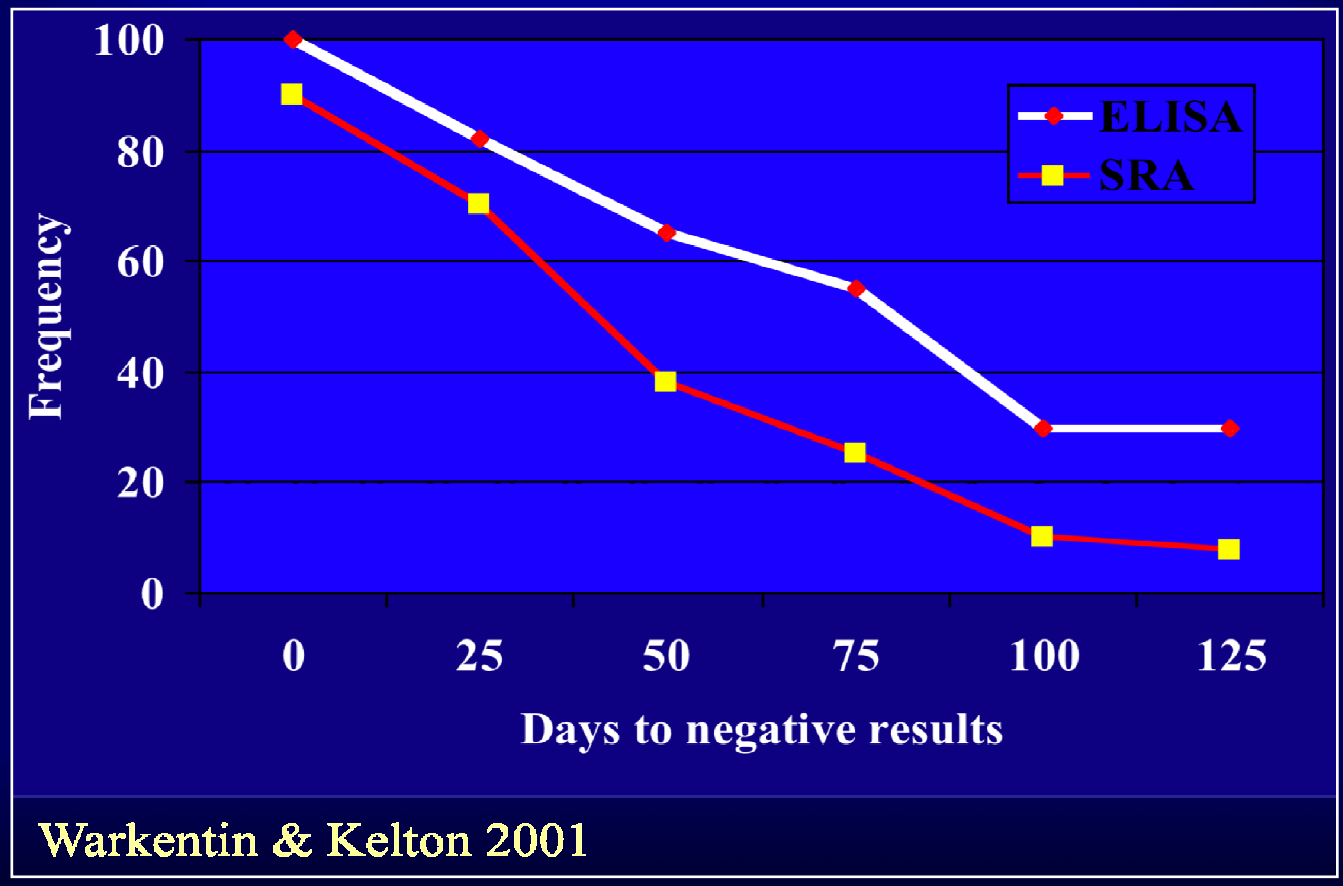
-) only anti PF4/heparin Abs

Frequency of HIT and HIT antibodies after orthopedic surgery

Event	UFH (n=332)	LMWH (n=333)	P value
HIT-thrombosis (HIT-T)	3.6%	0.3%	<0.001
HIT (>50% platelet fall)	4.8%	0.6%	<0.001
Platelet-activating IgG antibodies (positive SRA)	9.9%	2.9%	0.010
Anti-PF4/heparin IgG antibodies (positive EIA)	15.6%	6.5%	0.011



Antibodies positivity over the time



Warkentin & Kelton 2001

Commonly used tests for HIT

A) Immunoassays

a) Solid-Phase or Fluid-Phase EIA

- PF4/heparin ELISA

  Heparin: STAGO, Polyanion: GTI AESKU

b) Rapid Immunoassays

- ID-H/PF4 test: Particle gel immunoassay

c) Emergency Immunoassays

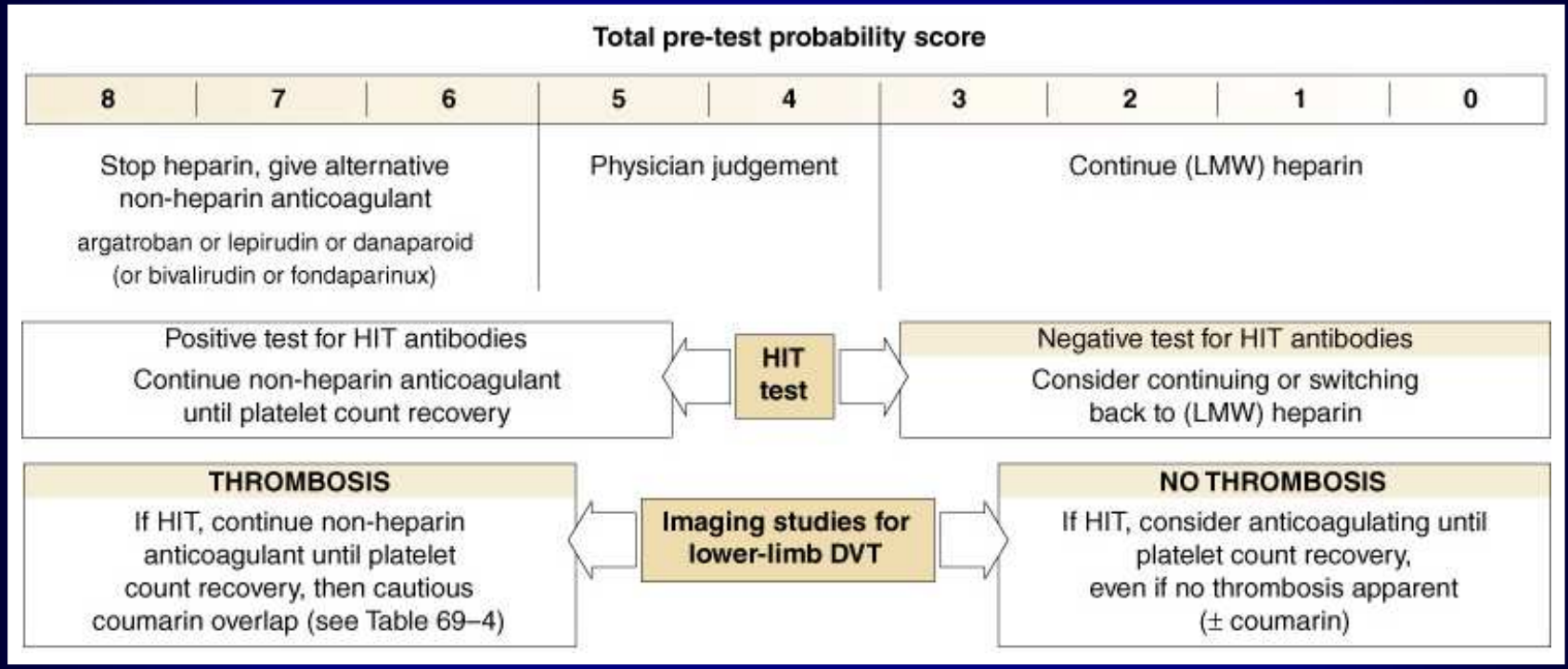
- HemosIL AcuStar IgG

ASSAYS' SENSITIVITY AND SPECIFICITY

ASSAYS	Feasibility	Specificity	Sensitivity	Donor	Instrument
FUNCTIONAL					
PAT	+++	90%	40-80%	critical	Aggregometer
^{14}C-SRA	++	95%	100%	critical	b-camera
HIPA	+++	90%	80%	critical	Microwell plate
FCA (Annexine, etc)	++	90%	50-80%	critical	Flow cytometer
IMMUNOASSAY					
PF4/heparin	++++	90%	90%	no	Microwell plate reader
ID-H/PF4	++++	90%	90%	no	Microcentrifuge

Fabris F: SISET 2010

DIFFERENTIAL DIAGNOSIS ALGORITHM WITH APPROACH TO TREATMENT



DIFFERENTIAL DIAGNOSIS OF HIT

List of other Conditions

- **DIC (Cancer and Infections)**
- **APS - TTP**
- **Mechanical consumption: in ECC, Prosthetic valves, Dialysis**
- **Post-transfusion Thrombocytopenia**
- Acute Thrombosis associated Thrombocytopenia

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RECOMMENDATIONS FOR TREATMENT OF HIT PATIENTS (A)

- **Discontinuation of Heparin and use a non-heparin anticoagulant**
- **Dangers of WARFARIN (caveat): it is the best to avoid/postpone the use of OAC or to administer VK if Warfarin was already started**
- **Avoid Platelet Transfusions**

Recommendations for Avoiding Coumarin-Induced Venous Limb Gangrene and Skin Necrosis Syndromes in Patients with HIT

1. In a patient who has already begun receiving coumarin when acute HIT is recognized, reverse coumarin anticoagulation with intravenous or oral vitamin K.
2. Delay coumarin anticoagulation until the platelet count has recovered to *at least* 100×10^9/L (preferably, 150×10^9/L).
3. Begin coumarin only in low, maintenance doses (e.g., initial dose ≤5 mg warfarin).
4. Administer coumarin only during overlapping alternative anticoagulation (minimum, 5-day overlap).
5. Do not stop the alternative anticoagulant until the platelet count has normalized and reached a stable plateau, with at least the last 2 days in the target therapeutic range.

RECOMMENDATIONS FOR TREATMENT OF HIT PATIENTS (B)

- **Alternative anticoagulants:**
  - **a) Direct Thrombin Inhibitors (DTI)**
    - **LEPIRUDIN - ARGATROBAN**
    - **BIVALIRUDIN**
  - **b) Anti-Xa: DANAPAROID**
  - **c) FONDAPARINUX**

THERAPEUTIC REGIMENS ACCORDING TO DRUGS

Alternative Anticoagulants for Treating HIT: Main Characteristics

Drug	Structure and function	Usual Starting Dose*	Usual Half-Life	Elimination	Adverse Events (Selected List) and Other Comments
Lepirudin	Bivalent DTI (hirudin)	(±0.4 mg/kg); 0.15 mg/kg/hr IV infusion (0.10 mg/kg/hr for isolated HIT)†	80 min	Predominant renal	Bleeding; post–IV bolus anaphylaxis
Bivalirudin	Bivalent DTI (hirudin analogue)	0.15 mg/kg/hr IV infusion	25–35 min	Enzymic (80%); renal (20%)	Bleeding
Argatroban	Univalent DTI (arginine derivative)	2 µg/kg/min IV infusion	40–50 min	Predominant hepatobiliary	Bleeding; prolongs INR more than the bivalent DTIs (complicates coumarin overlap)
Danaparoid	Mixture of GAGs with predominant anti-FXa activity	2250 U bolus‡; 400 U/hr × 4 hr; then 300 U/hr × 4 hr; then continue at 200 U/hr	25 hr (anti-FXa activity)	Partial renal	Bleeding; weak in vitro cross-reactivity against HIT antibodies seen in 15–40% of patient sera, usually without clinical significance; withdrawn from U.S. market (2002)
Fondaparinux	AT-binding pentasaccharide (indirect thrombin inhibitor)	? 7.5 mg SC qd (dosing not established)	17 hr (anti-FXa activity)	Partial renal	Cross-reactivity against HIT antibodies is absent; minimal experience, and thus effective dose in HIT is unknown

MANAGEMENT OF HIT
CONCLUSIONS

- **HIT is a rare clinical disorder but several patients can be exposed to acute and recurrent life-threatening thrombosis**

- **Early diagnoses of HIT with specific tests can help to start immediately appropriate therapy and reduce mortality**

Questions

Comments

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Thanks

OTHER SLIDES

Comments

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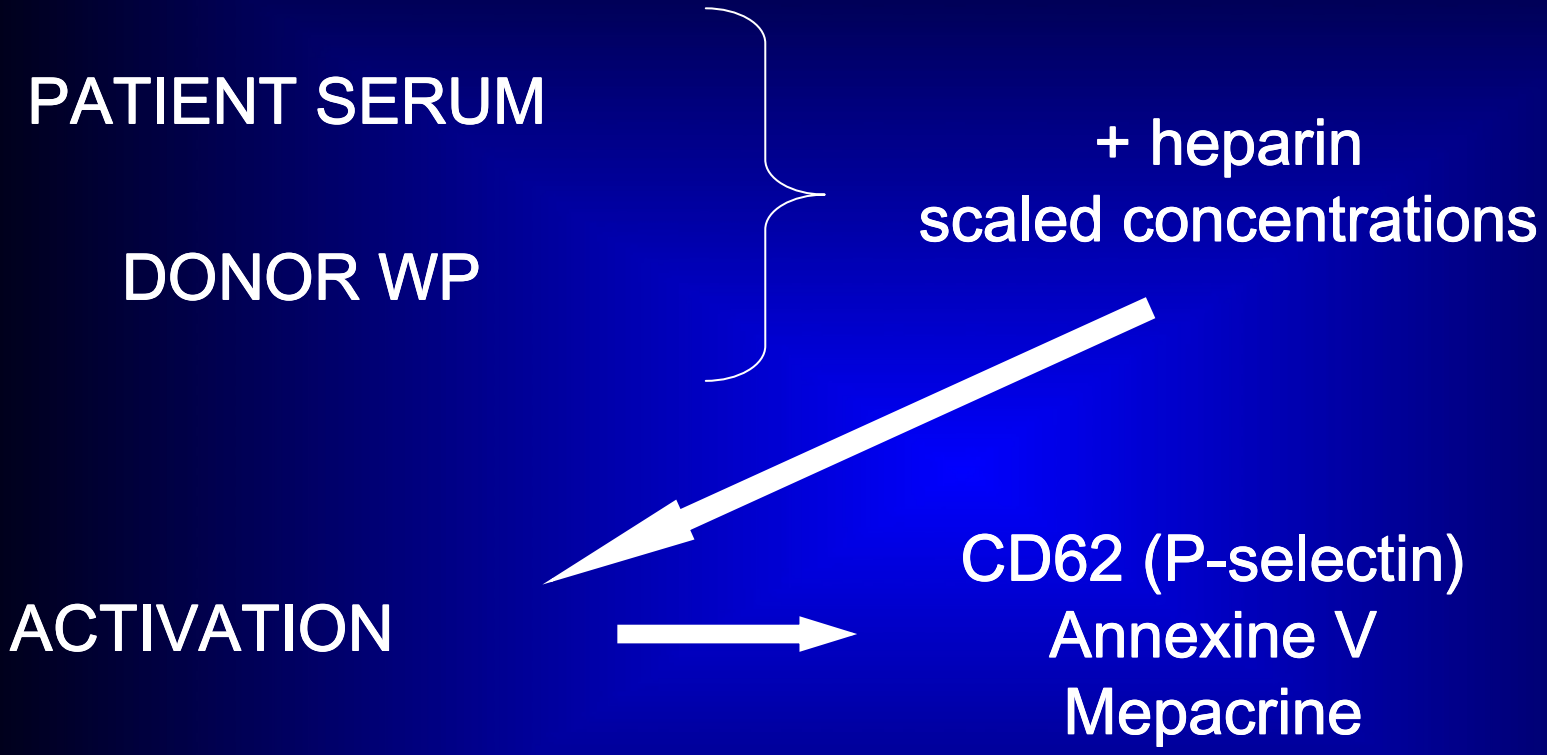
Thanks

FLOW CYTOMETRY ASSAY

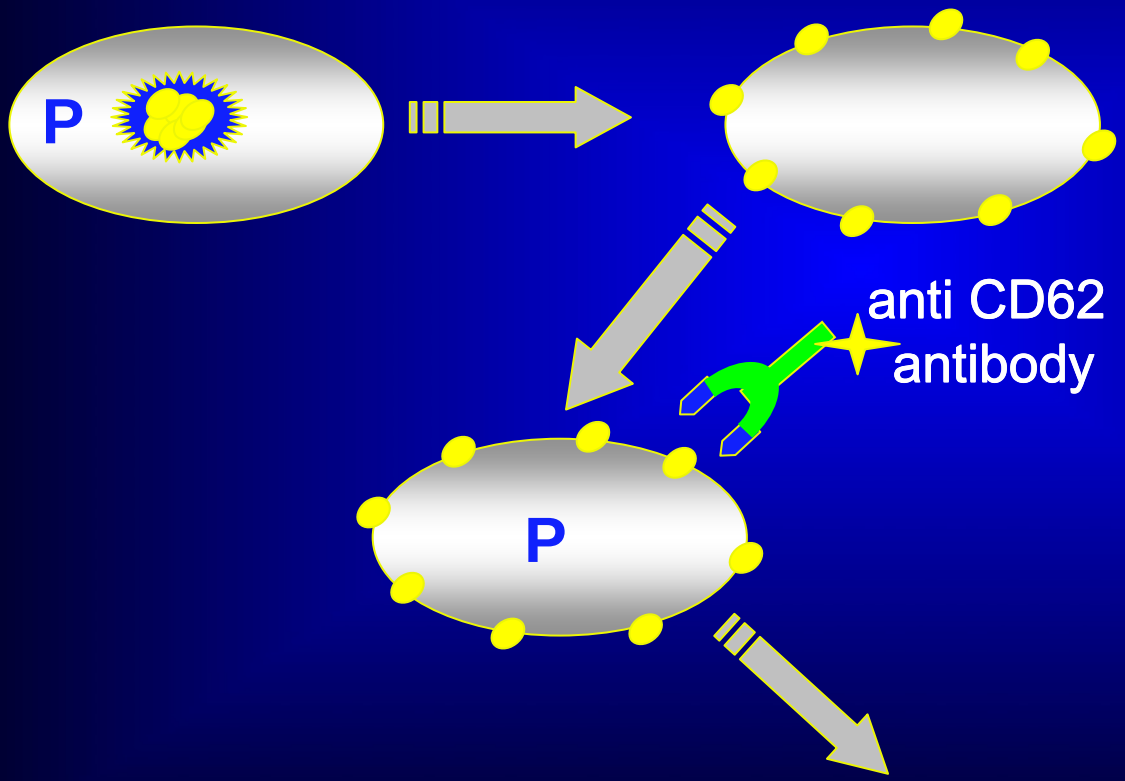


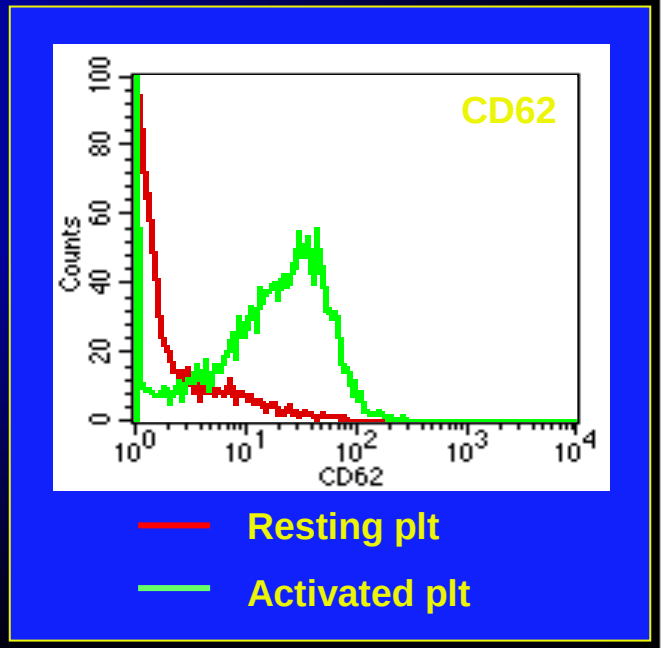
- Critical variability in platelet donor
- MoAb costs
- Flow cytometer

CD62 (P-selectin)

Resting platelets

Activated platelets





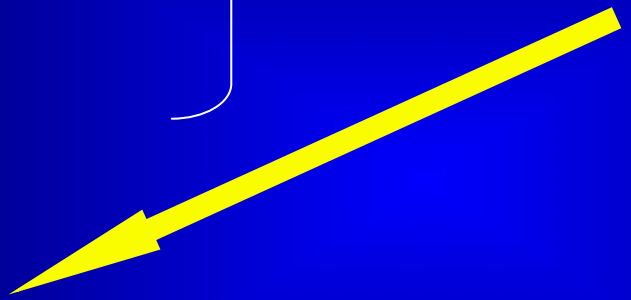
HIPA

PATIENT SERUM

+

DONOR WP

+ heparin
scaled concentrations



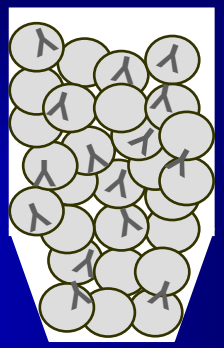
ACTIVATION → AGGLUTINATION

Critical variability in platelet donor
Low costs; Feasibility
High sensitivity and specificity

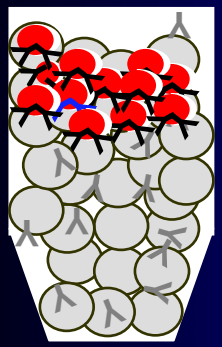
PARTICLE GEL IMMUNOASSAY
(ID-Hep/PF4 Antibody test)

Red polystyrene beads coated with PF4-heparin
+
Patient serum

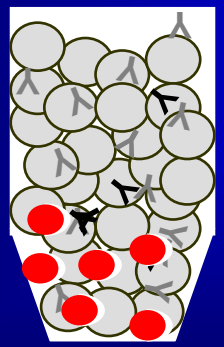
Particle gel tube containing anti-IgG



centrifuge

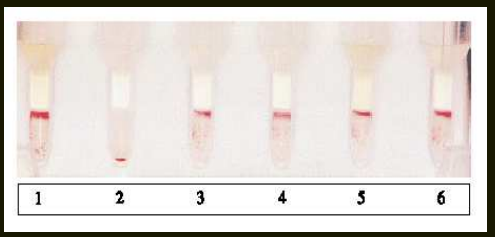


POSITIVE RESULT



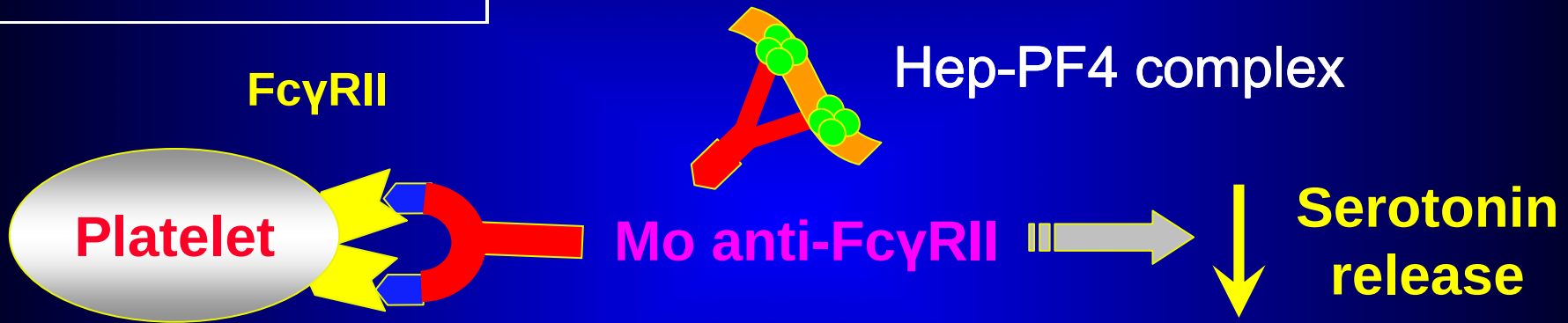
NEGATIVE RESULT

Rapid and easy
Good sensitivity
Not pathogenetic

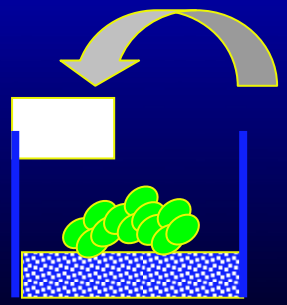


Confirmatory tests for heparin associated antibodies in HIT

FUNCTIONAL



ELISA



HIT Treatment Outcomes of Alternate Anticoagulation

Study, Year	Regimen	*N*	Mean Days of Alternative Anticoagulant	% HIT-Antibody Positive	New Thrombosis Rate (RRR*)	Amputation Rate (RRR*)	Composite End Point (RRR*)	Major Bleed (% per Day Alternative Anticoagulant Given)†
HIT-Associated Thrombosis								
HAT-1/2, 2000 [104]	Lep: 0.4 mg/kg bolus + 0.15 mg/kg/hr	113	13.3	100%	10.1 (63%)	6.5% (38%)	21.3% (55%)	1.4%
HAT-3, 2004 [117]	Lep: bolus + 0.15 mg/kg/hr	98	14.0	100%	6.1% (78%)	5.1% (51%)	21.5% (55%)	1.5%
DMP, 2003 [118]	Lep: bolus + 0.15 mg/kg/hr	496	12.1	77%	5.2% (NA)	5.8% (NA)	21.9%	0.45%
Arg-911, 2001 [102]	Arg: 2 μg/kg/min	144	5.9	65%	19.4% (35%)	11.8% (−8%)	43.8% (22%)	1.9%
Arg-915, 2003 [103]	Arg: 2 μg/kg/min	229	7.1	NA	13.1% (62%)	14.8% (−36%)	41.5% (27%)	0.9%
RCT vs. dextran, 2001 [106]	Danap: bolus + infusion 200 U/hr	25	6‡	83%	12.0% (77%)	NA	20.0% (62%)	0%

*RRR (relative risk reduction, expressed as percent) compared with historical controls (not shown).
†Calculated by dividing major bleed rate by number of mean days of alternative anticoagulant given.
‡Median (data provided by Dr. Harry Magnani, Organon NV).
§Data limited to on-treatment observation period.
‖Data limited to on-treatment observation period + 1 day.
Abbreviations: Arg, argatroban; DMP, drug monitoring program (postmarketing study); Danap, danaparoid; HAT, heparin-associated thrombocytopenia (prospective lepirudin study); Lep, lepirudin; NA, not available; RCT, randomized controlled trial.

HIT Treatment Outcomes of Alternate Anticoagulation

Study, Year	Regimen	*N*	Mean Days of Alternative Anticoagulant	% HIT-Antibody Positive	New Thrombosis Rate (RRR*)	Amputation Rate (RRR*)	Composite End Point (RRR*)	Major Bleed (% per Day Alternative Anticoagulant Given)†
Isolated HIT								
HAT1–3, 2002 [119]	Lep: 0.10 mg/kg/hr	111	13.5	100%	2.7% (NA)‡	2.7% (NA)‡	9.0% (NA)‡	1.1%‡
DMP, 2002 [118]	Lep: 0.10 mg/kg/hr	612	11.0	66%	2.1% (NA)‖	1.3% (NA)‖	≥15.7% (NA)‖	0.5%‖
Arg-911, 2001 [102]	Arg: 2 μg/kg/min	160	5.9	65%	8.1% (64%)	1.9% (5%)	25.6% (34%)	0.6%
Arg-915, 2003 [103]	Arg: 2 μg/kg/min	189	5.1	NA	5.8% (75%)	4.2% (−45%)	28.0 (28%)	1.0%

*RRR (relative risk reduction, expressed as percent) compared with historical controls (not shown).
†Calculated by dividing major bleed rate by number of mean days of alternative anticoagulant given.
‡Median (data provided by Dr. Harry Magnani, Organon NV).
§Data limited to on-treatment observation period.
‖Data limited to on-treatment observation period + 1 day.
Abbreviations: Arg, argatroban; DMP, drug monitoring program (postmarketing study); Danap, danaparoid; HAT, heparin-associated thrombocytopenia (prospective lepirudin study); Lep, lepirudin; NA, not available; RCT, randomized controlled trial.