Heparin-Induced Thrombocytopenia: The Dark Side of a Common Anticoagulant

Kristi J. Smock, MD

Associate Professor of Pathology, Medical Director, ARUP Hemostasis/Thrombosis Laboratory, University of Utah
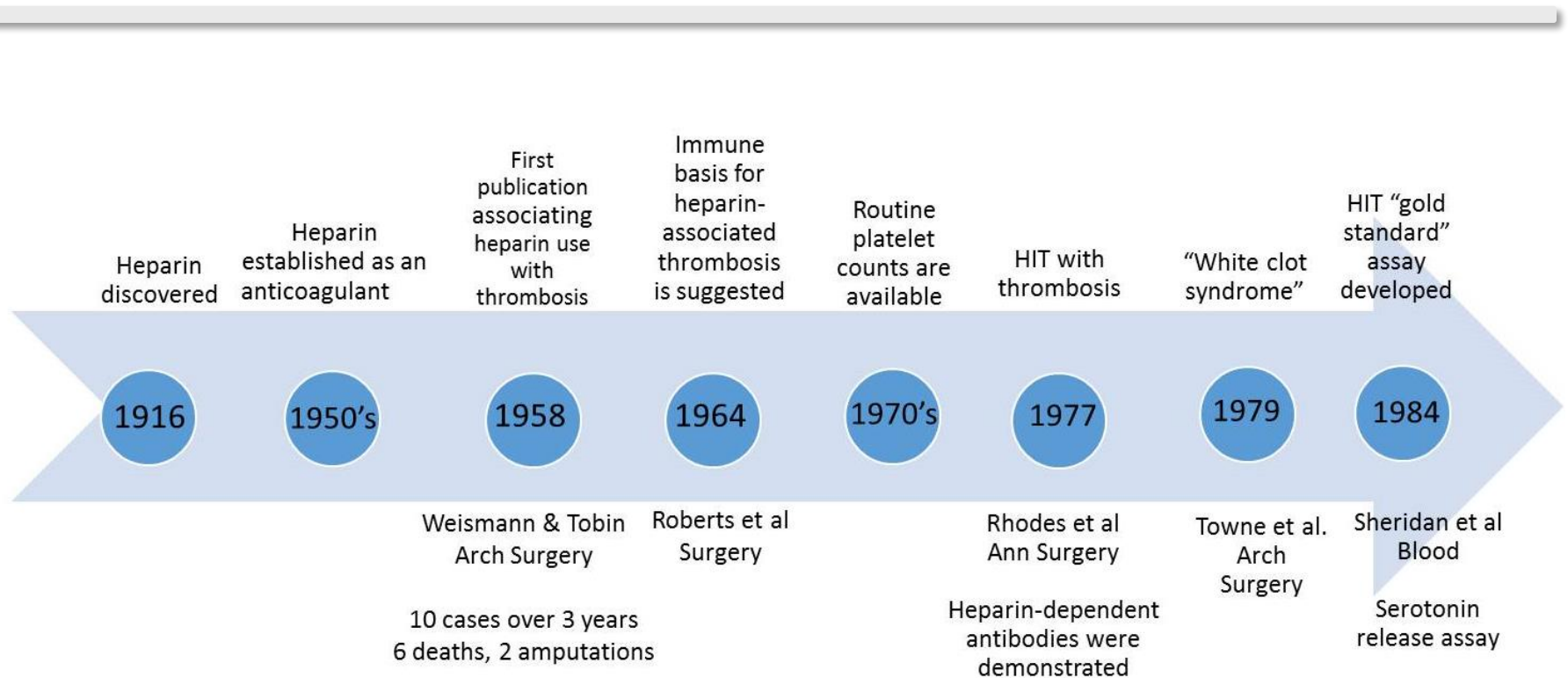
And

George M. Rodgers, MD, PhD

Professor of Medicine and Pathology, Medical Director, ARUP Hemostasis/Thrombosis Laboratory, University of Utah

Learning Objectives

❖ Review the pathophysiology and clinical importance of heparin-induced thrombocytopenia (HIT)

❖ Discuss laboratory testing methodologies used for HIT diagnosis

❖ Discuss optimal laboratory approaches for accurate, timely, and cost-effective diagnosis of HIT

Heparin-induced thrombocytopenia (HIT) – historical aspects

Heparin

❖ Used as an anticoagulant for many decades to prevent and treat thrombotic events and for systemic anticoagulation

  ❖ Venous thromboembolism, cardiopulmonary bypass, dialysis, percutaneous coronary intervention, etc.

❖ Heterogeneous mixture of sulfated saccharides that enhance antithrombin activity, resulting in potent inhibition of serine protease coagulation factors

Types of heparin

❖ Unfractionated heparin (UFH)

   ❖ Heterogeneous

   ❖ Many very large molecules

❖ Low molecular weight heparin (LMWW)

   ❖ More homogeneous

   ❖ Smaller, molecular weight <50% that of UFH

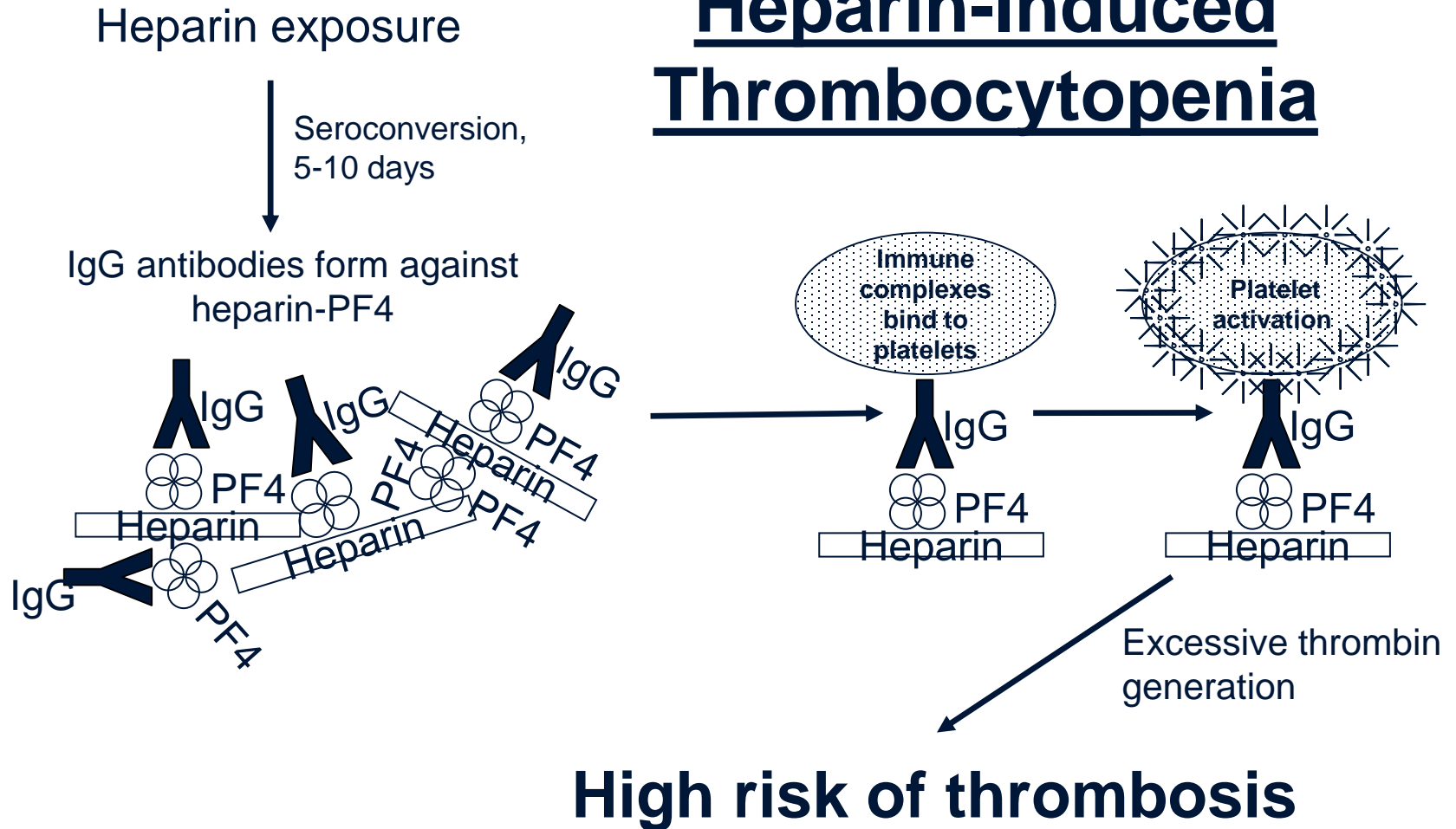❖ Fondaparinux

   ❖ Uniform, synthetic heparin with small size

Heparin-induced thrombocytopenia (HIT)

❖ Syndrome that occurs in ~1-5% of all heparin-exposed patients

❖ Immune-mediated

  ❖ Formation of IgG antibodies to heparin-platelet factor 4 (PF4) complexes

    ❖ PF4 is released from platelet granules and is positively charged

    ❖ Heparin is negatively charged

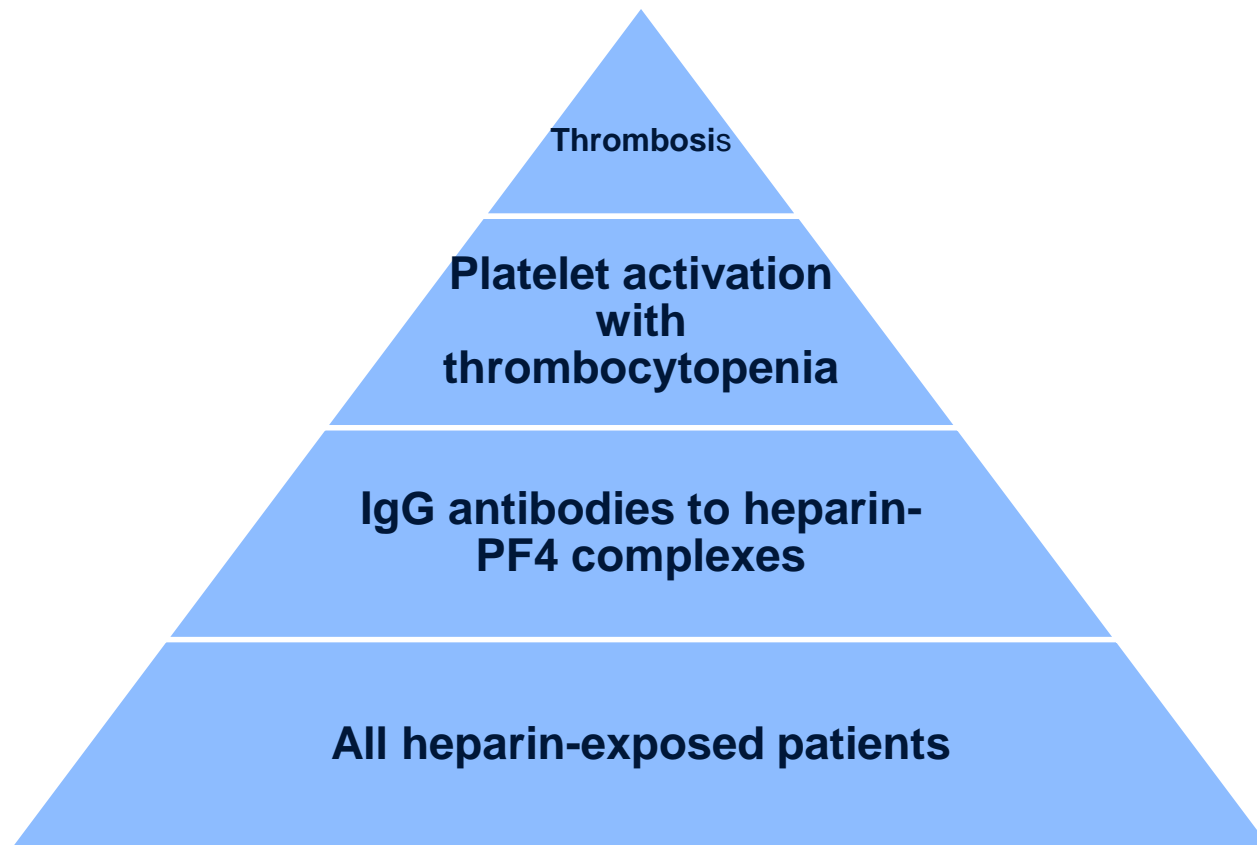   ❖ Heparin-PF4 complexes represent a neoantigen, resulting in production of IgG by marginal zone B-cells

Heparin-induced thrombocytopenia (HIT)

❖ Immune complexes (heparin-PF4-IgG) cause platelet activation via the platelet Fcγ receptor for IgG (FcγRIIA)

> ❖ Thrombocytopenia
>
> ❖ High risk of thrombosis
>
> > ❖ Occurs in ~50% of untreated cases
> >
> > ❖ May be venous (DVT, PE) or arterial (limb ischemia, stroke, MI)
> >
> > > ❖ Thrombotic complications can be fatal

❖ Monocyte activation via FcγRIIA also appears to contribute to increased thrombotic potential

Heparin-Induced Thrombocytopenia

Heparin exposure

Seroconversion, 5-10 days

IgG antibodies form against heparin-PF4

Immune complexes bind to platelets

Platelet activation

Excessive thrombin generation

High risk of thrombosis

Heparin-induced thrombocytopenia (HIT)

Risk factors for HIT

❖ Risk is related to amount of circulating PF4 and type and dose of heparin

   ❖ Determines whether heparin-PF4 complexes (the HIT antigen) will form

❖ Higher risk in surgical patients than in medical patients

❖ Higher risk with UFH than with LMWH

❖ Estimated at 1-5% for surgical patients, and less than 1% for most medical patients

HIT diagnosis

- ❖ Clinical evaluation

  - ❖ Many heparin treatment protocols require platelet count monitoring in high-risk patients

  - ❖ HIT considered when platelet count drops 5-10 days after heparin initiation/exposure

  - ❖ Clinical scoring systems determine pre-test clinical probability

    - ❖ 4Ts system

      - ❖ Low scores have good negative predictive value (NPV): 97-99%

      - ❖ Intermediate and high scores have poor positive predictive value (PPV): 10-20% and 40-80%, respectively

HIT clinical scoring – 4T's

Thrombocytopenia

- – 0 points; <30% drop or nadir <10 x 10^9/L

- – 1 point; 30-50% drop or nadir 10-19 X 10^9/L

- – **2 points; >50 drop and nadir >=20 X 10^9/L**

Timing of platelet drop

- – 0 points; <4 days with no recent exposure

- – 1 point; suspect 5-10 days but not documented, >10 days, <=1 day with last exposure 30-100 days prior

- – **2 points; 5-10 days or <= 1 day if exposed in past 30 days**

Thrombosis

- – 0 points; none

- – 1 point; progressive, recurrent, silent, suspected but not proven

- – **2 points; proven new thrombosis or skin necrosis or acute systemic reaction to heparin**

Other causes of thrombocytopenia

- – 0 points; definite

- – 1 point; possible

- – **2 points; none**

❖ <u>Scoring</u>
Low = <4 points, good NPV
Intermediate = 4-5 points, poor PPV
High = 6-8 points, poor PPV

❖ <u>Lab testing is needed for patients with intermediate or high 4T's scores</u>

HIT laboratory evaluation

- ❖ Immunoassays

  - ❖ ELISA is most common

    - ❖ Plate coated with heparin-PF4 or acceptable alternative used to capture heparin-PF4 antibodies from patient serum or plasma

      - ❖ Polyspecific detection – IgG/IgM/IgA

      - ❖ Monospecific detection – only IgG

    - ❖ Often used as an initial test

      - ❖ High sensitivity for HIT, inexpensive, simple to perform, fairly good turnaround time

  - ❖ Chemiluminescent immunoassay (CLIA) - recently FDA approved

  - ❖ Rapid assays

Heparin-PF4 ELISA

❖ General performance characteristics

<u>Negative </u>results **(you can't have HIT with no antibodies)**

❖ Excellent NPV (~99%)

❖ No need to repeat negative testing if no change in clinical status

<u>Positive results</u> **(you can have antibodies without HIT)**

❖ Excellent sensitivity (95-100%)

❖ Poor specificity (<90% or even <<<90%)

❖ Specificity improved by considering optical density (OD) value and with use of IgG only ELISA

❖ Poor PPV

N Engl J Med 2015 373;3:252-61

Heparin-PF4 ELISA

❖ Results should be reported as an OD value with cutoff value rather than qualitatively (positive/negative)

❖ Higher OD values correlate with antibody titer and capacity for platelet activation

    ❖ <0.4: antibody negative

    ❖ 0.4 to 1.0: ~5% probability of HIT

    ❖ 1.0 to 1.5: ~25% probability of HIT

    ❖ >=2.0: ~90% probability of HIT

Heparin-PF4 ELISA

❖ Polyspecific (IgG/IgM/IgA) detection versus monospecific (IgG only) detection

   ❖ HIT is caused IgG antibodies that activate platelets through the surface Fcγ receptor for IgG

      ❖ Platelets are not activated by IgM or IgA antibodies

   ❖ Some published studies suggest that the polyspecific assays have worse performance characteristics than IgG alone while others suggest that with use of optimal cutoffs the polyspecific ELISA has adequate sensitivity and specificity

   ❖ Although IgG ELISAs may have better specificity, clinically irrelevant antibodies are still detected

J Lab Clin Med 2005;146:341-6
Blood 2016;127(5):546-57

Heparin-PF4 ELISA

❖ Potential for false-positive ELISA in specimens containing antiphospholipid antibodies

    ❖ Thought to be due to autoantibodies against PF4

    ❖ Negative in functional assays for HIT

J Thromb Haemost 2009;7:100-4

Rapid immunoassays

❖ Particle immunofiltration assay approved for use in the United States, although does not appear to be widely used

    ❖ Qualitative (positive/negative) results based on color change, results in ~30 minutes

    ❖ Advantages

        ❖ Timely results, no special instrumentation, efficient processing of single patient samples

    ❖ Utility similar to ELISA (useful for exclusion of HIT)

        ❖ Recent publication suggests that diagnostic accuracy may be suboptimal for this assay (limited data)

Blood 2016;127(5):546-57

Case study 1

❖ A 75-year-old female received unfractionated heparin during admission for aortic valve replacement. Her baseline platelet count of 400 K/µL dropped to 150 K /µL 9 days after heparin was initiated and she has developed a DVT. Other potential causes of thrombocytopenia include an infection being treated with antibiotics.

    ❖ 4T's score: 7 (high probability, PPV 40-80%)

    ❖ ELISA OD: 1.1 (cutoff 0.4, ~25% probability of HIT)

Case study 2

❖ A 60-year-old male being treated with LMWH for a DVT that occurred after knee replacement develops mild thrombocytopenia (130 K/µL, compared to his baseline of 200 K/µL) with unclear timing due to incomplete lab data. Imaging is inconclusive but suggests possible extension of his DVT. Other causes for his thrombocytopenia are considered possible but not probable (history of autoimmune disease, possible history of ITP).

  ❖ 4T's score: 4 (intermediate probability, PPV 10-20%)

  ❖ ELISA OD: 3.2 (>90% probability of HIT)

HIT laboratory evaluation

❖ Functional platelet activation assays

  ❖ Evaluate ability of patient serum or plasma antibodies to activate reagent platelets in the presence of heparin

  ❖ Useful for

    ❖ Evaluating unexpected ELISA results

    ❖ Evaluating positive ELISA results

❖ Serotonin release assay (SRA) is the golden standard and most commonly used

  ❖ >90% sensitivity and specificity for HIT due to demonstration of platelet activating properties of antibodies

❖ Other platelet activation assays (non-SRA)

Serotonin release assay

Heparin (reagent)

Patient sera (provides antibodies)

Washed platelets (reagent)

Platelet activation

Serotonin released by platelet dense granules

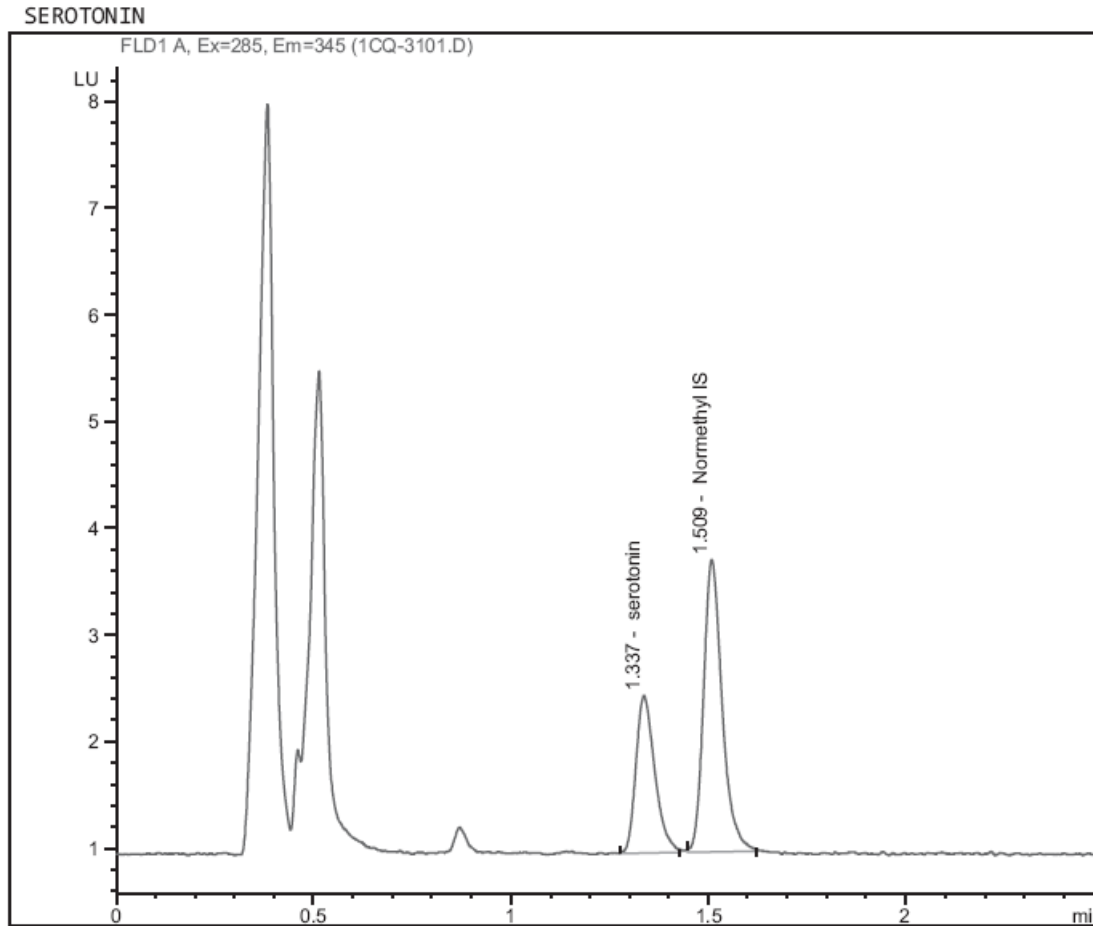**Detection and quantification of released serotonin (expressed as % of total serotonin)**

Serotonin release assay (SRA)

❖ Serotonin detection methods

    ❖ Radiolabeled serotonin (C^{14} serotonin)

    ❖ High-performance liquid chromatography (HPLC)

    ❖ Mass spectrometry

    ❖ Immunoassay

HPLC serotonin elution profile

SEROTONIN

FLD1 A, Ex=285, Em=345 (1CQ-3101.D)

1.337 – serotonin

1.509 – Normethyl IS

Donor platelet total serotonin content

Day of testing	Total platelet serotonin content (ng/mL)
1	356
2	519
3	485
4	339
5	463
6	500
7	312
8	457
9	299
10	337

Serotonin release assay (SRA)

❖ Result interpretation; >=20% serotonin release represents platelet activation

Therapeutic concentration, supports HIT immune complex formation	Supra-therapeutic concentration, disrupts HIT immune complex formation	Overall interpretation is based on response to both heparin concentrations
Low heparin percent release	**High heparin percent release**	**Interpretation**
>=20%	<20%	Positive
<20%	<20%	Negative
>=20%	>=20%	Indeterminate

Serotonin release assay (SRA)

❖ Indeterminate results

    ❖ Obtained in a few percent of samples tested in the SRA

    ❖ When reproducible usually indicate platelet activation by non-heparin-dependent (non-HIT) antibodies

        ❖ HLA antibodies

        ❖ Specific antibodies to platelet surface antigens

        ❖ Immune complexes

    ❖ Likelihood of HIT in these cases determined by combination of clinical impression and ELISA results

Other platelet activation assays (not serotonin release)

❖ Heparin-induced platelet activation (HIPA)

  ❖ Visual evaluation of platelet activation/clumping

❖ Heparin-induced platelet aggregation

  ❖ Light transmission or impedance aggregometry

❖ Heparin-induced platelet ATP release by luminography

❖ Heparin-induced platelet microparticle generation by flow cytometry

❖ Heparin-induced platelet P-selectin expression by flow cytometry

Thoughts on lab testing

❖ HIT lab testing is prompted by development of thrombocytopenia (on the correct timeline) in a heparin-exposed patient

   ❖ In HIT, the platelet count drop is caused by heparin/PF4/IgG complex-mediated platelet activation

      ❖ Detectability of the antibodies precedes thrombocytopenia

   ❖ A negative ELISA indicates no antibodies and excludes HIT as the cause of thrombocytopenia

      ❖ No need to repeat a negative ELISA in the absence of a new, additional drop in platelet count

Correlation between ELISA and SRA

Sample ID	ELISA (OD) cutoff 0.4	SRA LH/HH (% release)	SRA Interpretation
1	3.066	99/0	Positive
2	3.358	100/0	Positive
3	1.334	72/0	Positive
4	2.759	100/0	Positive
5	1.032	70/0	Positive
6	1.346	100/0	Positive
7	2.659	100/0	Positive
8	2.111	70/0	Positive
9	2.385	100/0	Positive
10	2.786	97/0	Positive

Correlation between ELISA and SRA

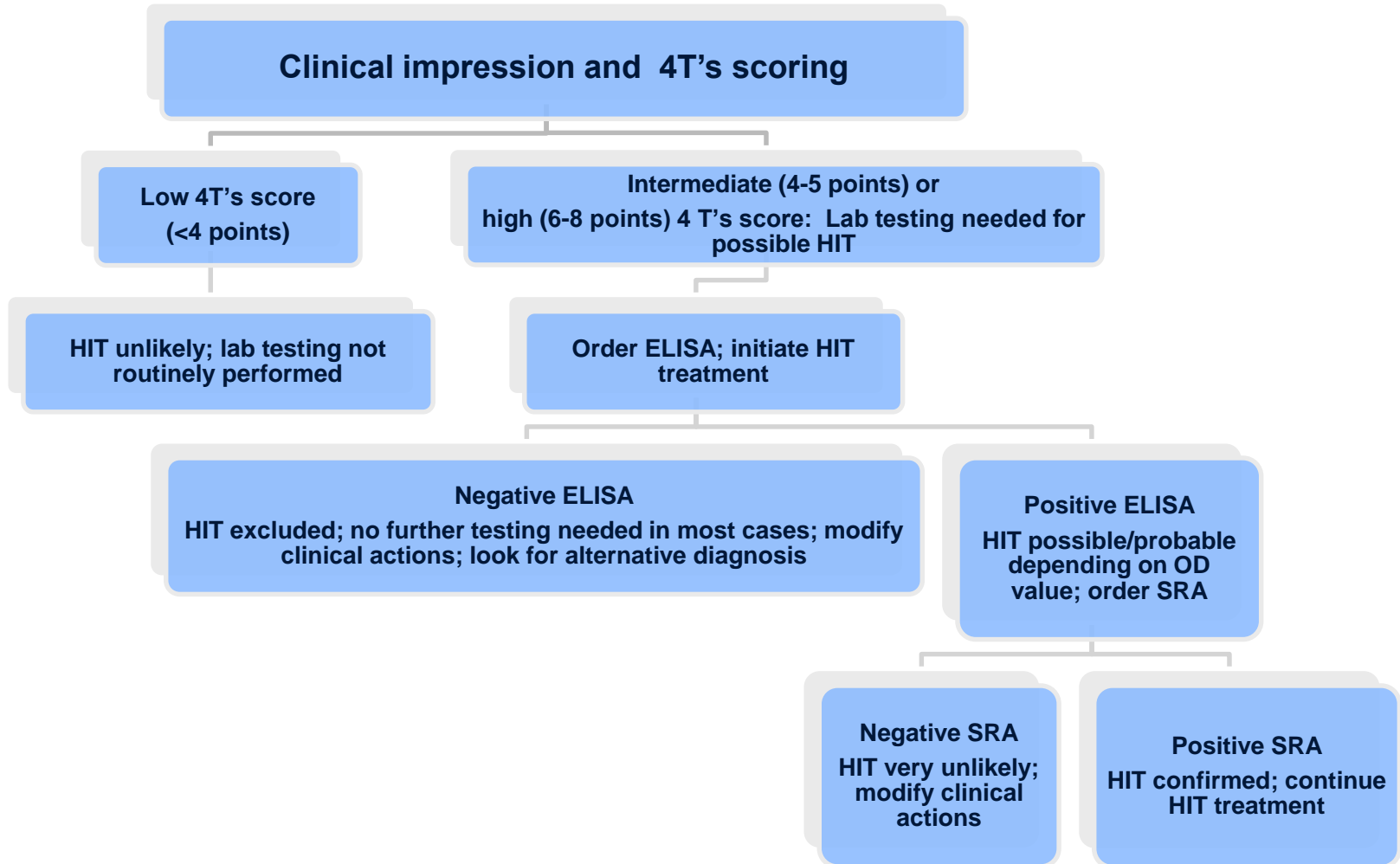| Sample ID	ELISA (OD) cutoff 0.4	SRA LH/HH (% release)	SRA Interpretation
1	0.667	0/0	Negative
2	0.277	0/0	Negative
3	0.162	0/0	Negative
4	0.078	0/0	Negative
5	0.753	0/0	Negative
6	0.089	0/0	Negative
7	0.082	0/0	Negative
8	0.048	0/0	Negative
9	0.513	0/0	Negative
10	1.278	0/0	Negative

Positive SRA results

❖ Although cutoff's of 20% serotonin release are frequently used to define platelet activation in the SRA, samples with at least 50% release has been most closely correlated with risk of thrombosis in HIT in the literature

    ❖ 95% of patients with clinical HIT in prospective studies have serotonin release >=50%

❖ From local data; 1 month of positive SRAs (n = 71)

    ❖ % release <50%: n = 9

    ❖ % release >=50%: n = 62

Quality assurance for improbable results

❖ Local quality practices involve tracking ELISA/SRA correlations (when available) and researching any improbable pairs that are identified

    ❖ Only 5 such pairs have been identified in the past year

        ❖ Very strong positive ELISA with negative SRA

        ❖ Negative ELISA with positive SRA

HIT diagnostic algorithm

Clinical impression and 4T's scoring

Low 4T's score (<4 points)

Intermediate (4-5 points) or high (6-8 points) 4 T's score: Lab testing needed for possible HIT

HIT unlikely; lab testing not routinely performed

Order ELISA; initiate HIT treatment

Negative ELISA

HIT excluded; no further testing needed in most cases; modify clinical actions; look for alternative diagnosis

Positive ELISA

HIT possible/probable depending on OD value; order SRA

Negative SRA

HIT very unlikely; modify clinical actions

Positive SRA

HIT confirmed; continue HIT treatment

Case study conclusions

- ❖ Case study 1
  - ❖ 4T's score: 7 (high probability, PPV 40-80%)
  - ❖ ELISA OD: 1.1 (cutoff 0.4, ~25% probability of HIT)
  - ❖ SRA: Negative
- ❖ Case study 2
  - ❖ 4T's score: 4 (intermediate probability, PPV 10-20%)
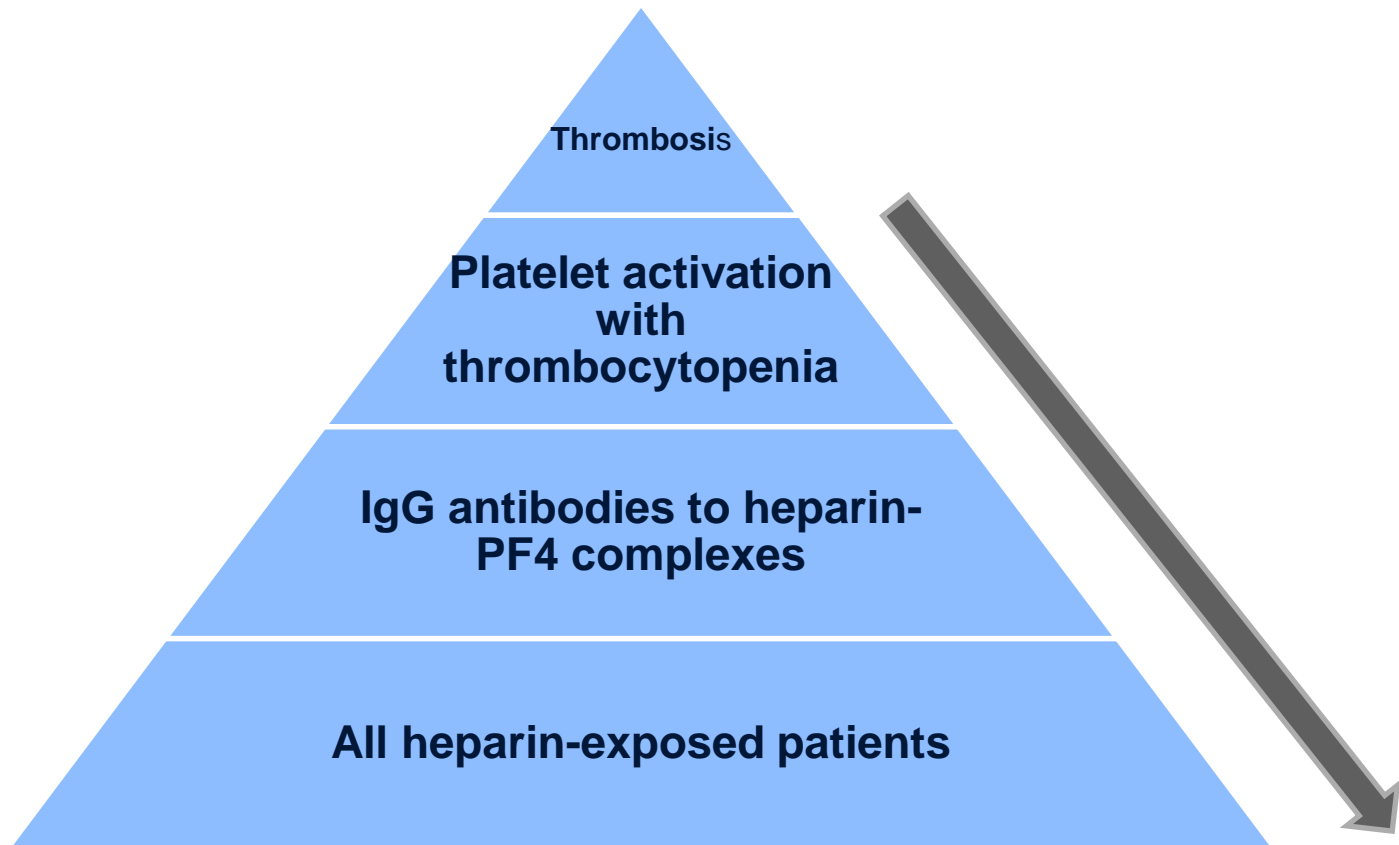 - ❖ ELISA OD: 3.2 (>90% probability of HIT)
  - ❖ SRA: Positive

Treatment

❖ For patients with intermediate or high 4T's scores, treatment is initiated immediately, prior to availability of laboratory test results

  ❖ Discontinue all heparin products, including line flushes

  ❖ Discontinue warfarin

  ❖ Initiate a non-heparin anticoagulant (unless contraindicated)

    ❖ Note: treatments are more expensive than heparins and often with higher risk of bleeding complications

  ❖ Order laboratory testing

  ❖ If HIT is confirmed, heparin allergy should be added to patient's chart

Natural history of HIT

❖ HIT is a transient immune response and antibodies become undetectable after ~3 months (median 40-100 days)

❖ There is no anamnestic response

    ❖ If a prior HIT patient (now antibody negative) were re-exposed to heparin, seroconversion would again take 5-10 days

    ❖ Brief heparin re-exposure in an antibody negative patient with prior HIT may be safe, but should be avoided when possible

        ❖ Consider if heparin is preferred anticoagulant for the indication

        ❖ Use non-heparin anticoagulants whenever possible

❖ Rapid onset HIT occurs upon heparin re-exposure in a prior HIT patient who still has antibodies (heparin within past 100 days)

Natural history of HIT

Pyramid levels from top to bottom:
- Thrombosis
- Platelet activation with thrombocytopenia
- IgG antibodies to heparin-PF4 complexes
- All heparin-exposed patients

Case example of treated HIT

Date	ELISA (OD) cutoff 0.4	SRA LH/HH (% release)	SRA Interpretation
07/01/16	Not done	100/0	Positive
07/11/16	2.773	99/0	Positive
08/24/16	2.280	67/0	Positive
09/21/16	Not done	22/0	Positive
11/04/16	Not done	16/0	Negative

Rare spontaneous (autoimmune) HIT

❖ Develops in the absence of heparin exposure

❖ Risk factors are major surgery or infection/inflammation

    ❖ Positively charged PF4 can bind to other polyanions such as nucleic acids, glycosaminoglycans, or bacterial lipopolysaccharide

    ❖ Complexes of PF4 and non-heparin polyanions induce primary immunization

        ❖ Resultant transient IgG antibodies allow opsonization and phagocytosis of PF4-coated bacteria that does not require prior antigen exposure

        ❖ HIT results when this mechanism is misdirected

            ❖ Classic HIT – platelets are coated with heparin-PF4 complexes

            ❖ Spontaneous HIT – platelets are coated with non-heparin polyanion-PF4 complexes

Avoid heparin programs

❖ An Avoid-Heparin Initiative has been studied over a 10-year period at a tertiary care hospital on Toronto, Ontario, Canada

   ❖ Replaced UFH with LMWH whenever possible in prophylactic and therapeutic protocols

      ❖ Rate of suspected HIT decreased by 42%

      ❖ Rate of positive HIT ELISAs decreased by 63%

      ❖ Rate of adjudicated HIT decreased by 79%

      ❖ Rate of HIT with thrombosis decreased by 91%

      ❖ HIT-related cost of care decreased by 83% ($266,938/year)

Conclusions

❖ Heparin-induced thrombocytopenia (HIT) is an immune-mediated syndrome of thrombocytopenia and thrombosis that occurs in a few percent of heparin-exposed patients

❖ HIT is a clinicopathologic diagnosis, requiring both clinical and laboratory information

❖ Algorithmic testing approaches utilizing immunoassays and functional assays maximize clinical performance and cost-effectiveness

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