All Things Heparin

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Agenda:

- The Basics
- “Politics” of Testing
- Why Correlate?
- Testing Strategies
- Warning: A Reminder
- “New” Heparins
- The Future?

The Basics

Discovery of Heparin

Discovered in 1916 –

- Jay McLean working under William Howell (Johns Hopkins) extracted phosphatides from canine liver that demonstrated anticoagulant activity.
- Howell continues the work and names the compound “heparin”
- Presents a water extraction method at national meeting in 1922.

Discovery of Heparin

Initial clinical trials
In 1924; caused
headaches,
fevers,
and nausea due to
impurities.

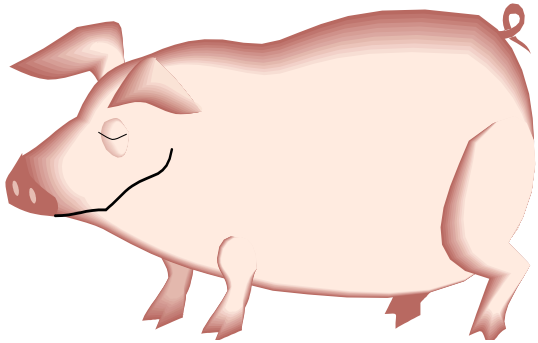
Discovery of Heparin

- By the 1930s, several researchers were investigating heparin.
- Erik Jorpes at Karolinska Institutet published research on the structure of heparin in 1935, which made it possible for the Swedish company Vitrum AB to launch the first heparin product for use in 1936.
- Between 1933 and 1936, Connaught Medical Research Laboratories, (University of Toronto), perfected a technique for producing safe, non-toxic heparin that could be administered to patients in a salt solution.
- The first human trials of heparin - May 1935,
- Prior to 1933, heparin was available, but in small amounts, and was extremely expensive, toxic, and of no medical value.

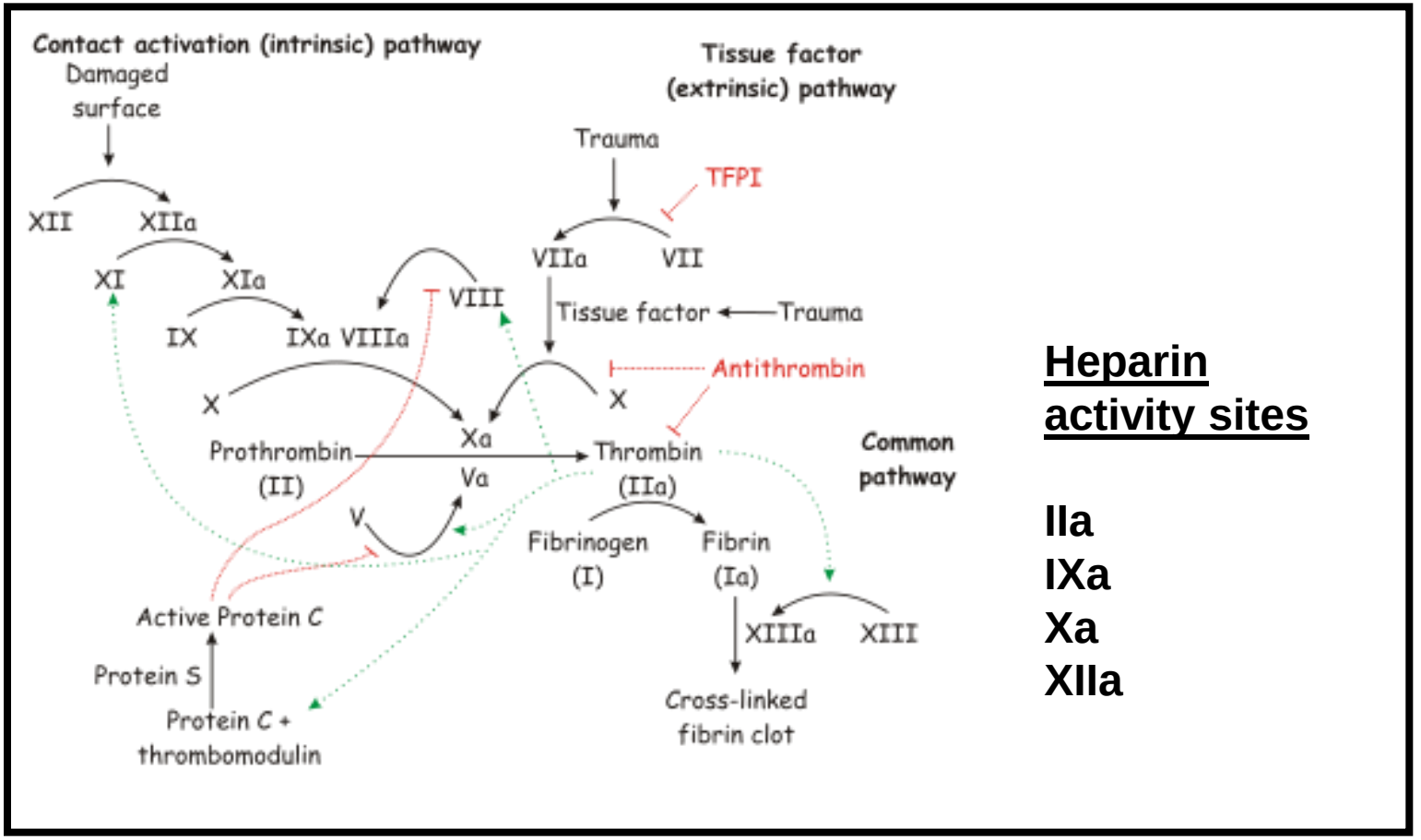
Discovery of Heparin

- Occurs naturally in human basophils and mast cells (only released at sites of tissue injury).
- Also derived from mucosal tissue of slaughtered animals: porcine intestine and bovine lung.
- Leading manufacturer in USA: SPL

"MOO!" ——



Heparin Activity “Sites”



Heparin activity sites

IIa
IXa
Xa
XIIa

Heparin Activity

- Binds to AT III - Ability of ATIII to inactivate coagulation enzymes is enhanced by 1000 times
- Catalyzes inactivation of Factors IIa, IXa, Xa, & XIIa
- Also binds endothelial cells, PF 4, and platelets.
  - **Platelets reduce the anticoagulant effect of heparin by protecting surface factor Xa from inhibition by heparin/AT complex**
  - **The binding of heparin to von Willebrand factor also inhibits von Willebrand factor-dependent platelet function.**

Heparin Science

- Half-life: 60 - 360 minutes
- First order kinetics: The (rapid) saturable phase of heparin clearance: binding to endothelial cell receptors and macrophages (also depolymerization).
- Renal elimination: The (slower) nonsaturable clearance is also largely renal.
- At therapeutic doses, a considerable proportion of heparin is cleared through the rapid, saturable, dose-dependent mechanism.
- “Law of Diminishing Returns”
  - Give more heparin
  - Reduced response

Heparin Response Is NOT Linear

- These kinetics make the anticoagulant response to heparin nonlinear at therapeutic doses, with both the intensity and duration of effect rising disproportionately with increasing dose.

- The apparent biological half-life of heparin increases from approximately 30 min following an IV bolus of 25 U/kg, to 60 min with an IV bolus of 100 U/kg, to 150 min with a bolus of 400 U/kg.

Other Effects of Heparin

"The anti-inflammatory action of heparin: Heparin as an antagonist to histamine, bradykinin and prostaglandin E1"

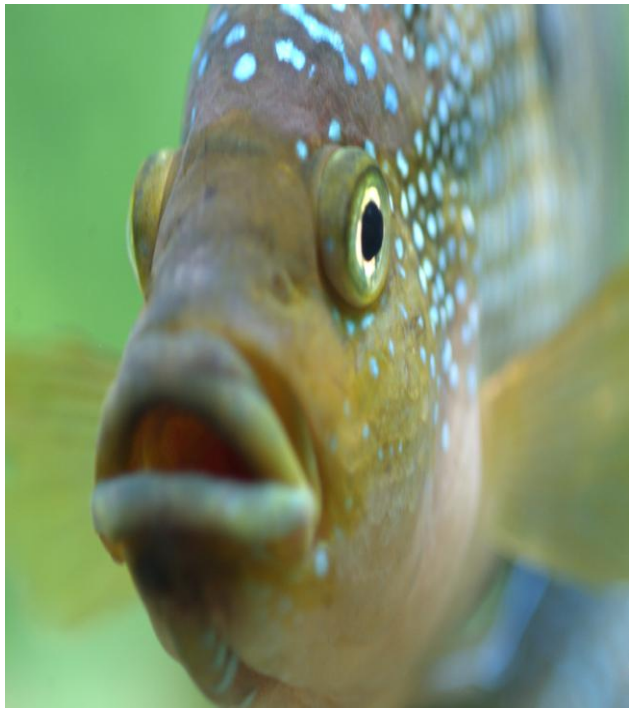
- Heparin may act on the vascular endothelium, and may prove to be useful in the control of edema in inflammation.

Dr. Jean Carr B.Sc., M.B.Ch.B.a, Assistant Professor
Department of Physiology College of Medicine, University of Saskatchewan 1979

Heparin Neutralization

Protamine:

- Discovered in 1878 by Meischer while investigating proteins in mammalian cells (salmon sperm).
- Originally found to enhance effect of insulin.
- Connaught connection through E. Jorpes.



Heparin Neutralization

"Protamine has a very short (approximately 5 minutes) half-life after a single 250-mg dose in adult patients. This short half-life could underlie recurrent anticoagulation after initial apparent reversal of heparin."

John Butterworth, MD, et al…

"Rapid disappearance of protamine in adults undergoing cardiac operation with cardiopulmonary bypass"

Ann Thorac Surg **2002;74:1589-1595© 2002 The Society of Thoracic Surgeons**

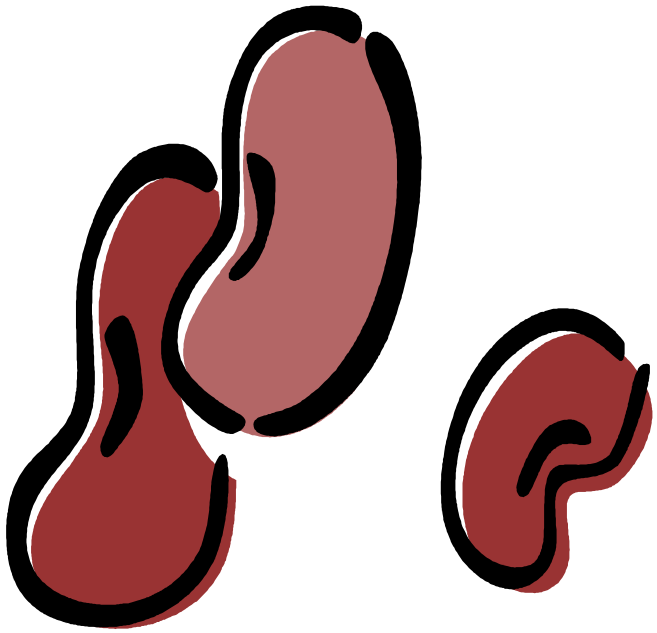
Protamine Problems

- Severe adverse reactions, such as hypotension and bradycardia, can be minimized by administering protamine slowly (*ie*, over > 1 to 3 min).

- Patients who have previously received protamine-containing insulin, have undergone a vasectomy, or have a known sensitivity to fish are at an increased risk to develop anti-protamine antibodies and to experience allergic reactions, including anaphylaxis.

Heparin Neutralization

- Dosing: 1 mg/ 100 IU Heparin
- After 30 minutes last heparin bolus: 0.5 -0.75 mg/ 100 IU heparin
- Heparin-Protamine complex excreted via kidneys in animal studies



Heparin Limitations/ Side Effects

All of the non-hemorrhagic limitations are caused by the AT-independent, charge-dependent binding properties of heparin to proteins and surfaces.

- Can induce immune-mediated platelet activation (leading to HIT)
- The effect on bone metabolism (leading to heparin-induced osteoporosis).

Heparin Resistance (HR)

- If the patient does not reach the targeted ACT range, patient is said to be “heparin resistant”.
- Actually, an alteration of the heparin response by the patient.
- Treatment:
  - FFP
  - Recombinant AT

What is ATIII?

- AT III is a glycoprotein that functions normally as a natural anticoagulant, by providing a slow inhibition of coagulation enzymes.
- In the presence of heparin, AT undergoes a conformational change that results in a 1000-fold increase in inhibitory activity.
- AT deficiency is a rare (0.16%) but serious medical condition. These patients can see an relative risk for VTE of 7 to 8 compared to the normal population underscoring the importance of adequate AT levels.

Predictors of possible HR

- AT III ≤ 60% preoperatively
- Sub-Q Heparin therapy
- IV Heparin Therapy
- Platelet Count ≥ 300,000 cell/ mm^3
- Age ≥ 65 years
- Aprotinin and nitroglycerin reported to cause drug-induced HR

Perfusion, December 1999, Vol 14, number 6, pp 437-442

Alternative Forms of HR

- Another scenario is due to the combination of increased levels of heparin-binding proteins and increased heparin clearance.
- Rarely, this form of heparin resistance is caused by low levels of antithrombin.
- No-ATIII - Unlike the other heparin-resistant patients, these subjects do not respond to AT III supplementation aimed at reaching supranormal AT III activity values.

Alternative Forms of HR

- Another limitation occurs when large amounts of acute phase reactants are circulating in acutely ill patients as they bind heparin making it unavailable to bind to AT.

- Neonates: Functional antithrombin levels are low at birth and may be further reduced in the sick neonate. Levels normally reach adult values at ~3 months of age. **(Br J Haematol, 119, 295-309)**

- This is also a problem in patients with malignancy and post partum. **(Case Reports in Anesthesia Mar 2012)**

Frequency of HR

- Research 2002 Milan Italy: 20.8% of patients identified as HR.
- Lab Hematol. 2003;9:125-131: 22%
- Percentage is rising as time goes on.

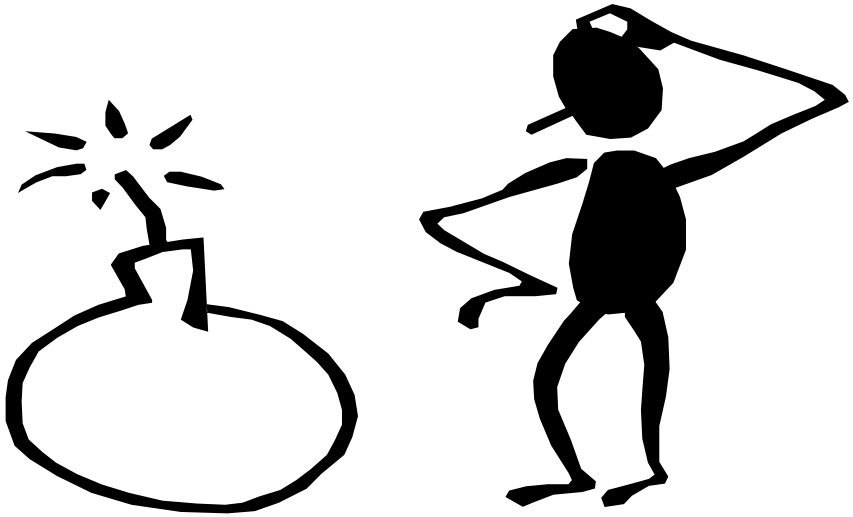
Heparin Rebound

- Defined as the reappearance of anticoagulant activity after adequate neutralization with protamine.
- May contribute to excessive postoperative bleeding after cardiac surgery.
- Protein- and tissue-bound UFH is released slowly and produces a heparin rebound phenomenon.
- Different interpretations of heparin rebound has lasted for more than 45 years,
  - **Kolff (1956) - "…a treacherous hemorrhagic phenomenon…"**
  - **Sise and colleagues – (1961) as "…rebound hypercoagulable state…"**
- Up to 18 hours post-op

(Guidelines on Perioperative Blood Transfusion Sep 2008)

“Politics” of Testing

Monitor heparin activity?????



Monitor heparin levels?????

Monitoring Heparin

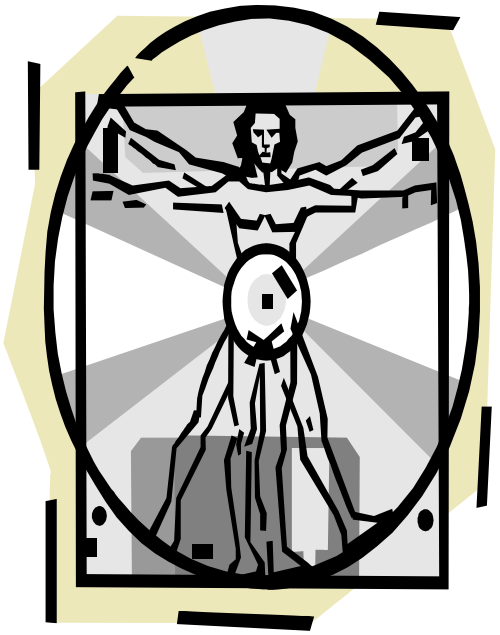
Free AT + Heparin → AT-Heparin

AT-Heparin + FXa (in Excess) →
AT-Heparin-FXa + FXa (Residual)

FXa (Residual) + Substrate →
Signal (Chromogen or Fluorescence)

Monitoring Heparin

The signal generated is proportional to the amount of residual Factor Xa (or thrombin) remaining after neutralization by the AT-heparin complex and is thus inversely proportional to the concentration of heparin.



Anti-FXa (Therapeutic Ranges)

Source	Reported Equiv (U/mL)
Levine et al,1994	0.35 - 0.67
Kitchen and Preston,1996	0.29 - 0.47
Baker et al,1997	0.30 - 0.60
Taylor et al,1999	0.25 - 0.45
Kitchen et al, 2000	0.25 - 0.38
	0.28 - 0.49
	0.24 - 0.39
	0.29 - 0.44
	0.29 - 0.49

Corresponds to a heparin level of 0.2 to 0.4 U/mL by protamine titration or 0.3 to 0.6 or 0.7 U/mL by heparin anti-factor Xa analysis.

10.1378/chest.121.1.303 *CHEST January 2002 vol. 121 no. 1 303-304*

Anti-FXa Assays

- Done in the Clinical Laboratory
- Chromogenic assay shows most promise as a POC test (glucose testing)
- Possible inaccuracy from a step:

  "Dextran sulfate in Factor Xa reagent overestimates heparin activity after Protamine reversal due to liberation of PF4-bound heparin."

  Journal of Thrombosis and Haemostasis Abstracts from XXth ISTH Congress 6-12 Aug 2005

Factor Assays

Factor assays:

II,

V,

VII,

X,

XI,

XII, XII

Fibrinogen

Drawbacks:

- Expensive
- Time-consuming

"STAT"

Monitoring Heparin Activity

- The anticoagulant response to heparin is unpredictable because of variable nonspecific binding to endothelial cells, monocytes, and plasma proteins.
- The anticoagulant response is unique to each individual.
- Tests available: ACT and aPTT

ACT and aPTT

- The ACT test will only detect a factor abnormality when there is a 95% or more decrease in single factor activity (less than 5% normal factor activity).
- The aPTT test can detect a factor abnormality with a 70% or more decrease in single factor activity (less than 30% normal factor activity).

First Test

Lee – White Clotting time

Drs. Paul White and Roger Lee developed the test in 1913 at Mass General.

Addition of 0.4 mL of blood to a glass tube and invert every 30 seconds.

(Lee demonstrates that it is safe to give group O blood to patients of any blood group, and that blood from all groups can be given to group AB patients. The terms "universal donor" and "universal recipient" are coined.)

Activated Clotting Time

Hattersley – 1966

- Add blood to glass tube with “dirt”, shake:
  - Diatomaceous earth activator
  - Intrinsic pathway activated
  - Still a manual method
  - Place in heat block
  - Visual and subjective clot detection

Gold Standard of 480 Seconds

- Bull and colleagues analyzed several heparinization protocols, and a target ACT of 480 seconds evolved as the standard of care for patients undergoing CPB.
- Among these included a calculation of heparin requirements based on the patient's heparin response and metabolism.
- At the same time that Bull, et al, were defining ACT target ranges, automation of the original Hattersley ACT had occurred

Activated Partial Thromboplastin Time (aPTT)

- Developed in 1953 by K. Brinkhous, et al, at UNC as test for hemophilia in plasma.

  J. Lab. Clin. Med. **41** (4): 637–47)
- The test is termed "partial" due to the absence of tissue factor from the reaction mixture.
- PTT measures the integrity of the intrinsic system (contact activation - Factors XII, XI, VIII, IX) and common clotting pathways.
- “Activated” refers to Kaolin that serves to activate the contact-dependent Factor XII.

Another View of aPTT

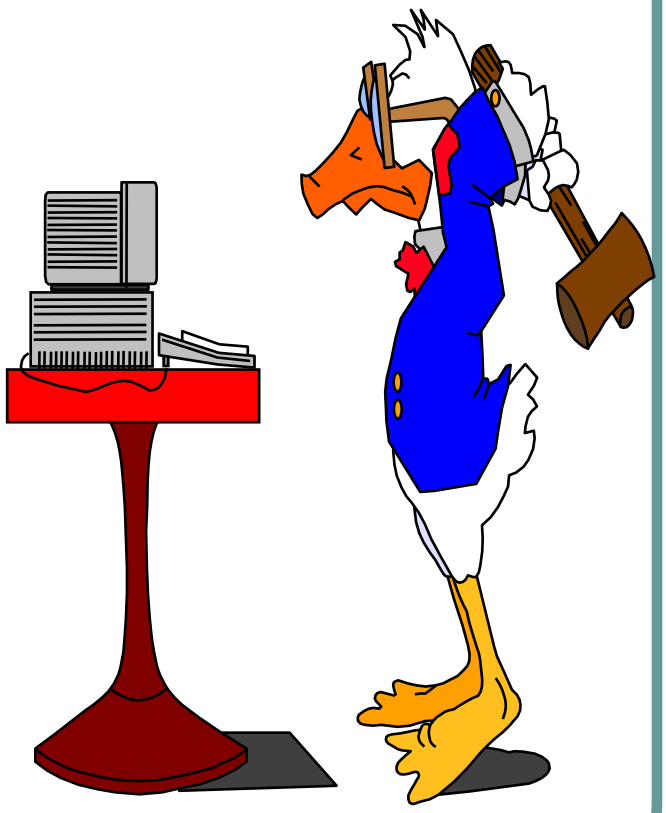
- “A fixed therapeutic range for the aPTT of 1.5 to 2.5 times the control value has become widely accepted, but the evidence supporting this range is weak and the clinical validity of using the aPTT for predicting thrombotic or bleeding events is questionable.”

- “Unfractionated heparin dose appears to be more important than the aPTT in predicting clinical efficacy.”

Thromb Haemost. 2006 Nov; 96(5):547-52

Variables in aPTT Testing

- Preanalytical Problems:
  - Time of sampling – diurnal variations
  - Site of Sampling – contamination by proximity to infusion site
  - Citrate concentration – higher concentrations are problematic
  - Sample transport – ideal is 2-4 C
  - Centrifugation – delay by 1 hour can cause release of PF4 from platelets.



Variables in aPTT Testing

- Biological:
  - Altered intravascular volume
  - Increased inflammatory proteins
  - Altered heparin half-life due to hepatic or renal disease
  - Increased VIII and decreased ATIII
  - Lupus anticoagulant
  - Reduced concentrations of other coagulant proteins

"Monitoring unfractionated heparin with the aPTT: Time for a Fresh Look" John W. Eikelboom, Jack Hirsh; 2006 Schattauer GmbH, Stuttgart

Is the aPTT doomed?

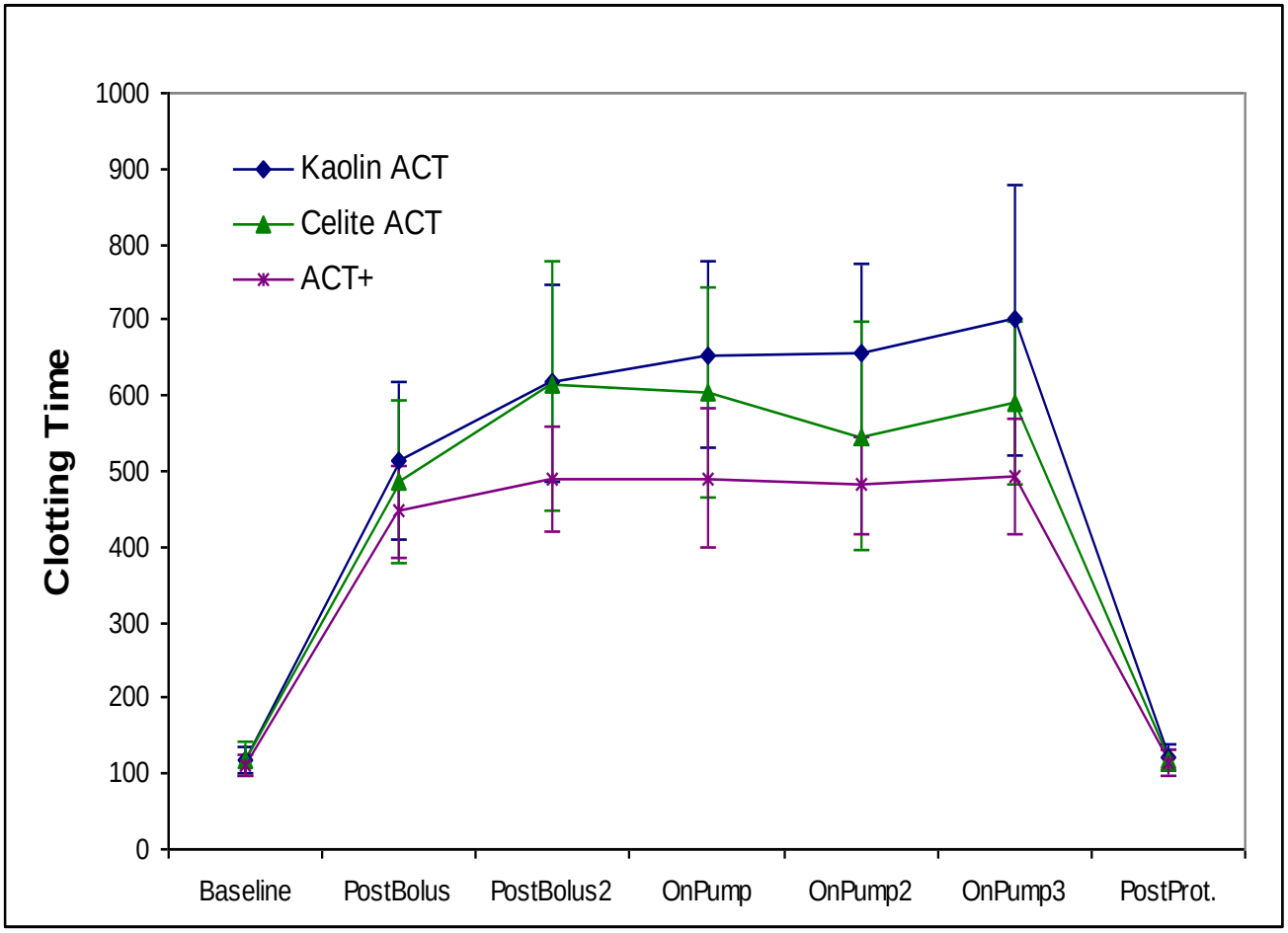
"Correlation of aPTT with the anti-Xa is the preferred method for establishing unfractionated heparin therapeutic ranges because it takes into account patient physiological factors that influence the response to heparin.

"These include the patient's factor VIII, fibrinogen and anti-thrombin III levels, lupus-like anticoagulants and other drugs the patient might be taking. Spiking normal pooled plasma with various heparin concentrations does not account for patient physiological variables."

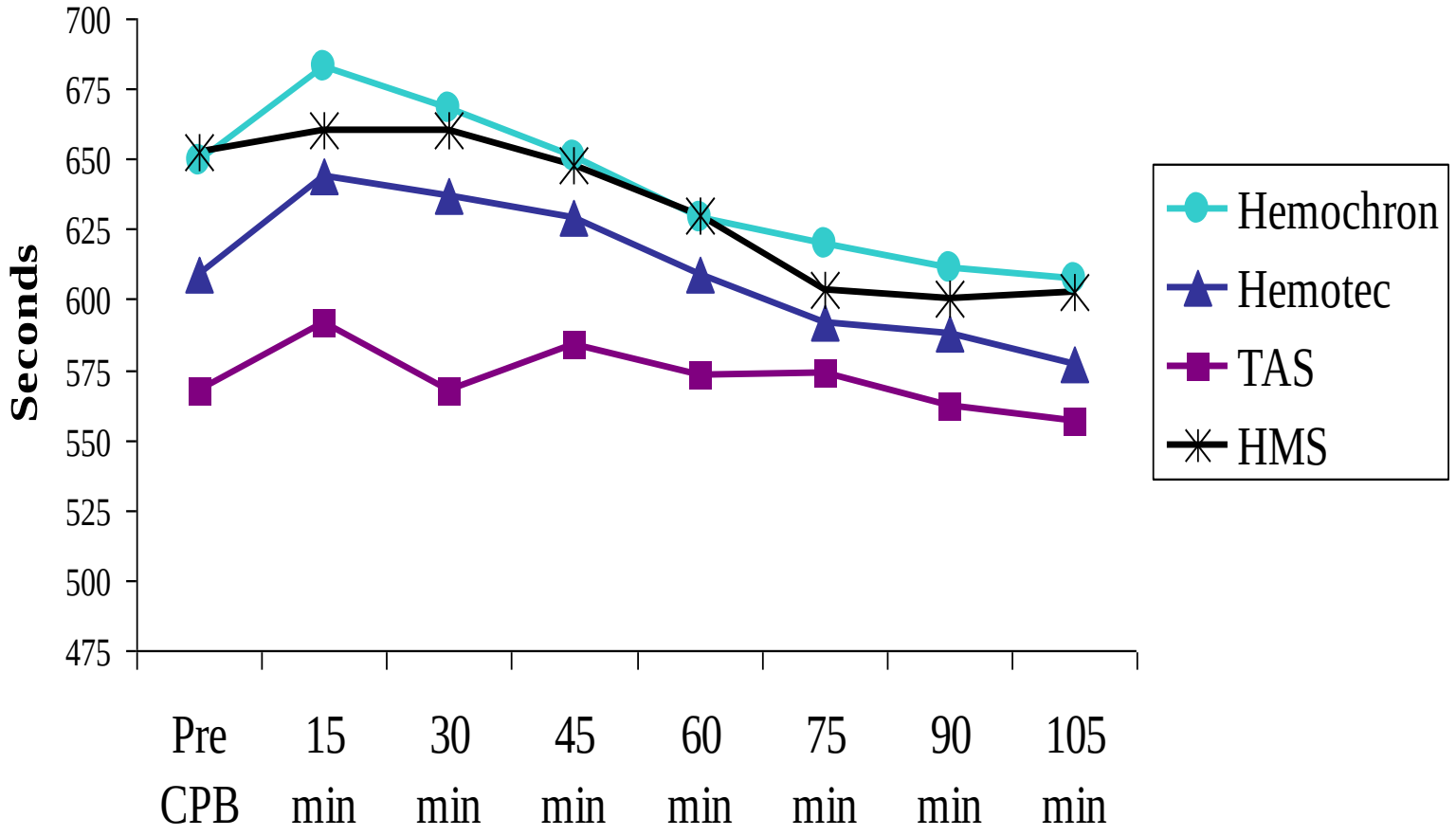
Northwest Hospital Clinical Laboratory statement – Nov 2008

Why Do Method Correlations ?

Same Manufacturer…



Different Manufacturers …



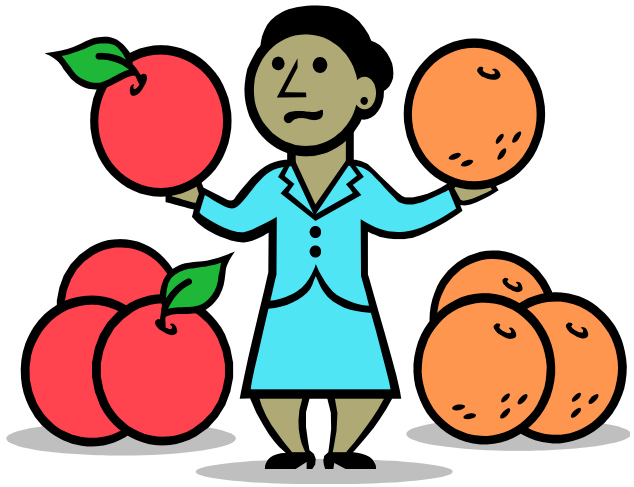
The Initial Correlation

- When comparing two systems, the correlation generally resulted in a close comparison.
- Whole blood coagulations assays do not follow the same rules.

The Initial Correlation

Methods differ:

- Endpoint detection system
- Clotting activators
- Sample size
- No standard method
- Hemodilution and hypothermia
- Platelet count & function



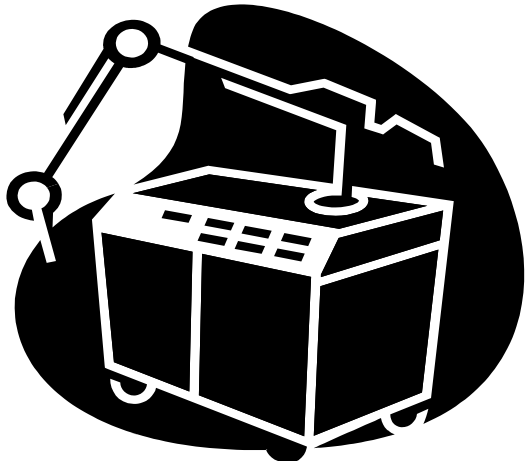
Why Correlate????

Anticoagulation

- Nursing assessment
  - Monitor ACT q 1-2 hours
    - via Hemochron®
  - Maintain ACT range 150-200”
  - Monitor for active bleeding
  - Monitor circuit for cracks and clotting

ACT Testing – Endpoint Detection

- Clot formation
  - Impedance
  - Optical
  - Timing



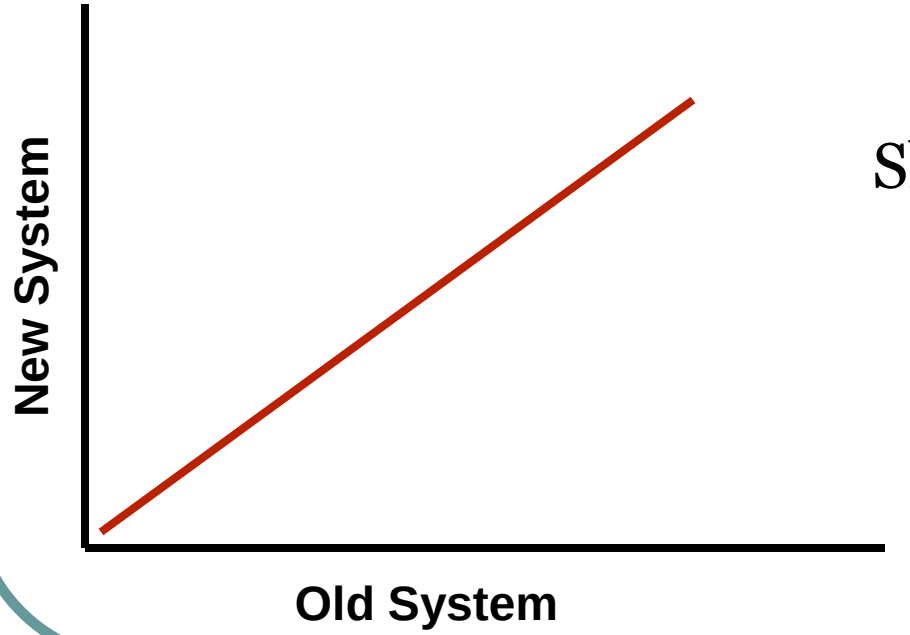
- Electrochemical
  - Conversion of a thrombin substrate other than fibrinogen
  - Electrochemical sensor detection of conversion

No “Bad” Correlations…

- Methods do correlate to a degree but the average difference and bias will be of greatest importance.
- Unlike standard single analytes that correlate to a very high degree and have traceable standards.
- Coagulation is the culmination of a “Cascade”

The “r” Value (usually)

If the “Slope” and the “r” (correlation coefficient) both equal “1”, then the tests are ***identical***.

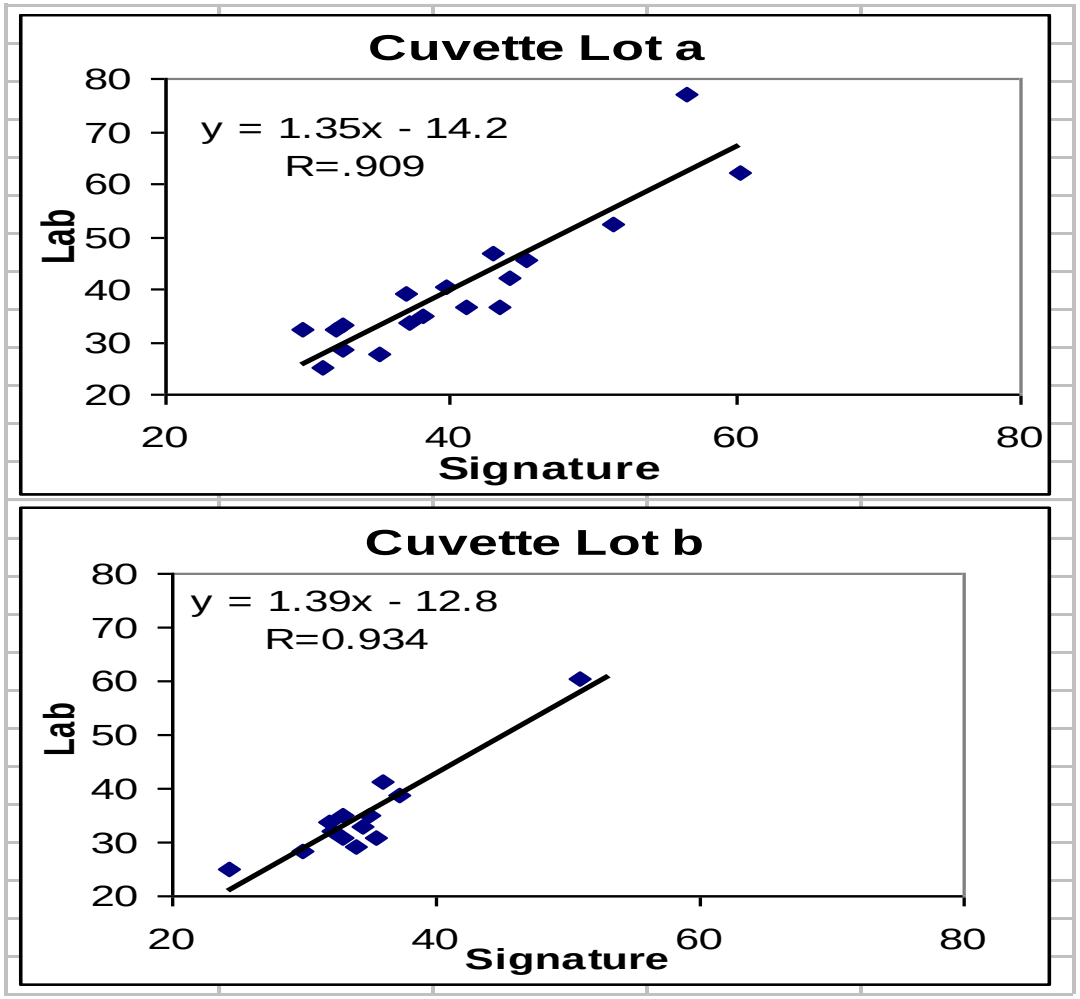


Slope = 1.0

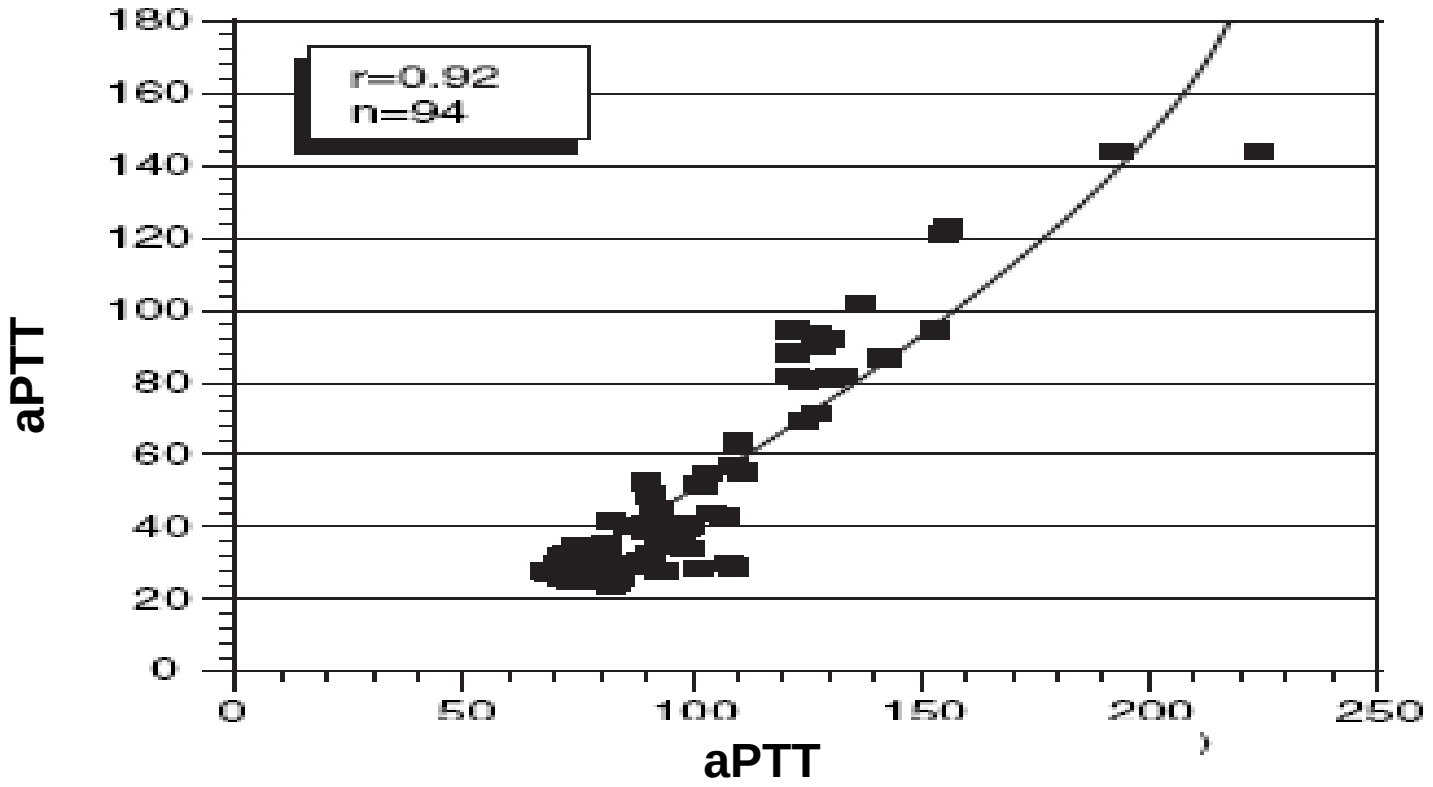
r = 1.0

} Perfect match

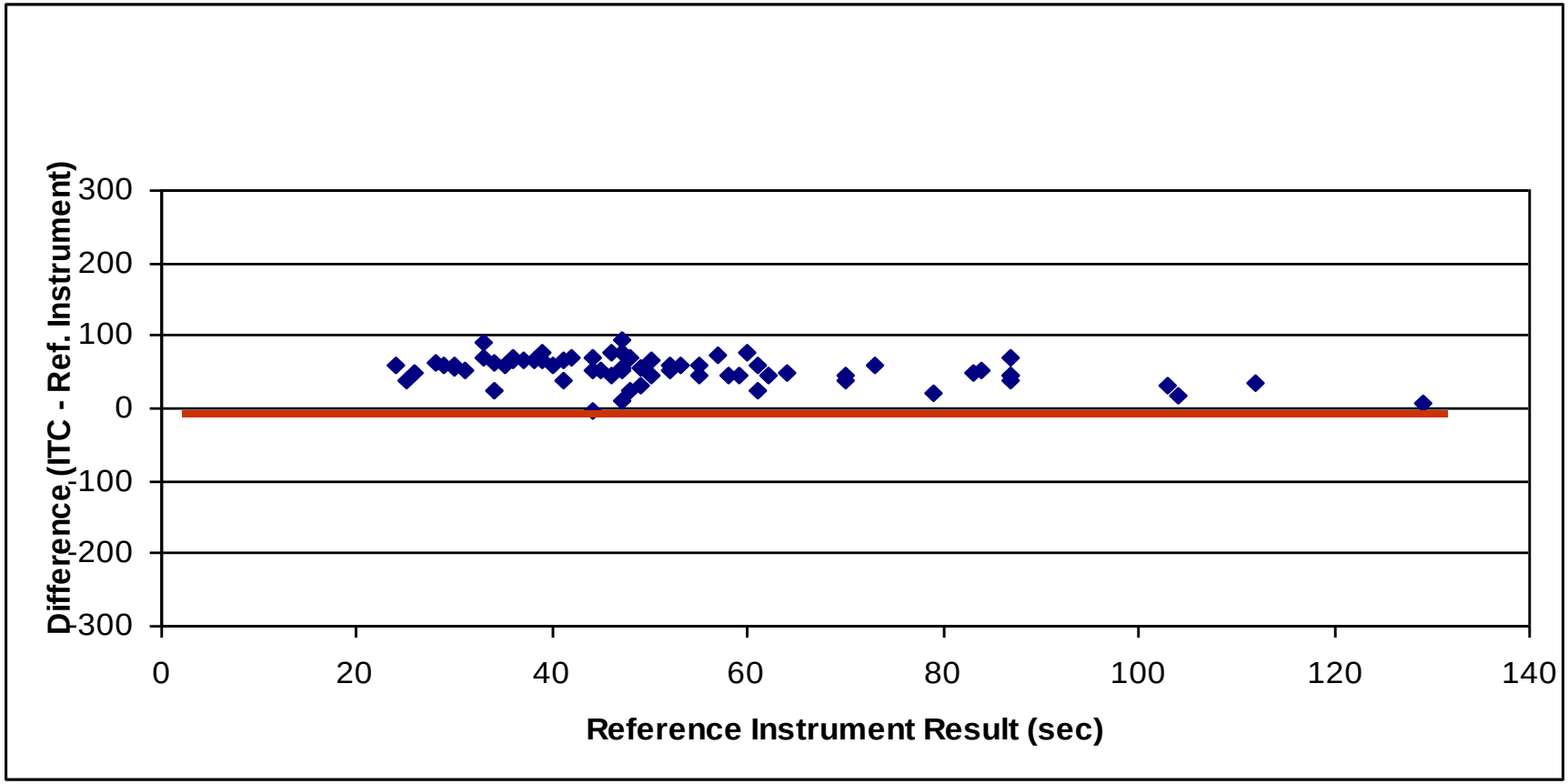
“r” Value in WB Testing



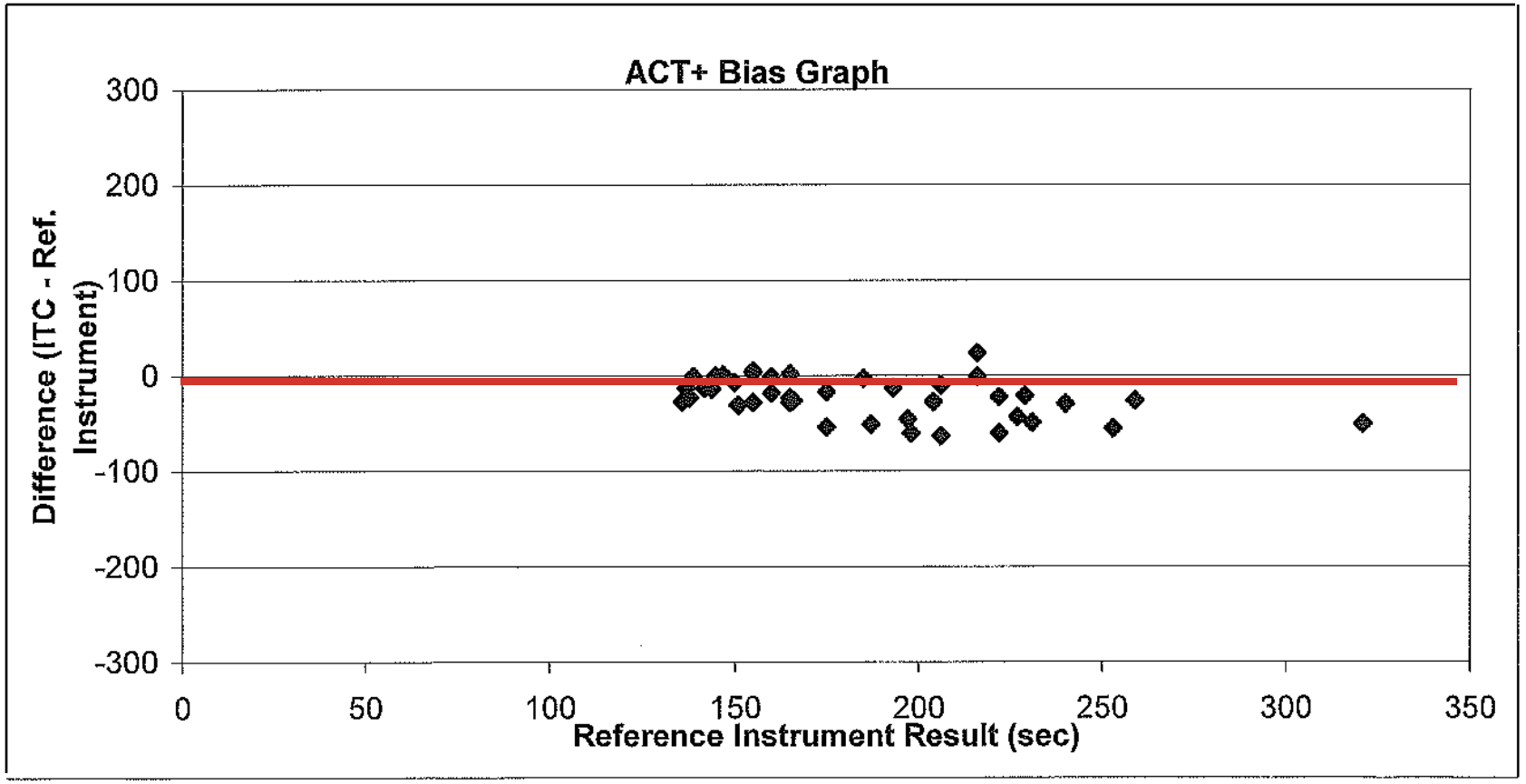
A Correlation Study



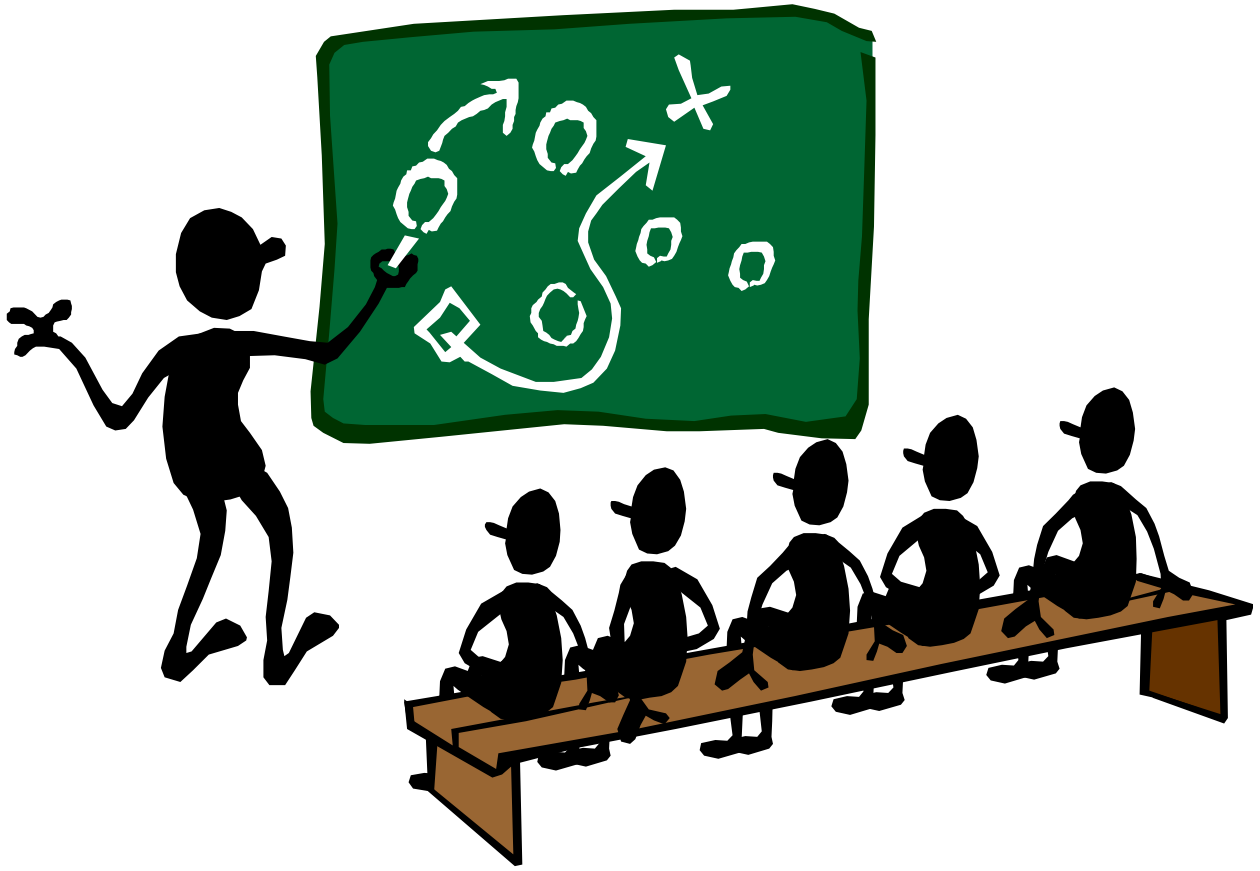
Bias Graph #1


Difference (ITC - Ref. Instrument)
300
200
100
0
-100
-200
-300
0
20
40
60
80
100
120
140
Reference Instrument Result (sec)

Bias Graph #2


ACT+ Bias Graph
Difference (ITC - Ref. Instrument)
300
200
100
0
-100
-200
-300
0
50
100
150
200
250
300
350
Reference Instrument Result (sec)

Testing Strategies



When To Sample…ACT

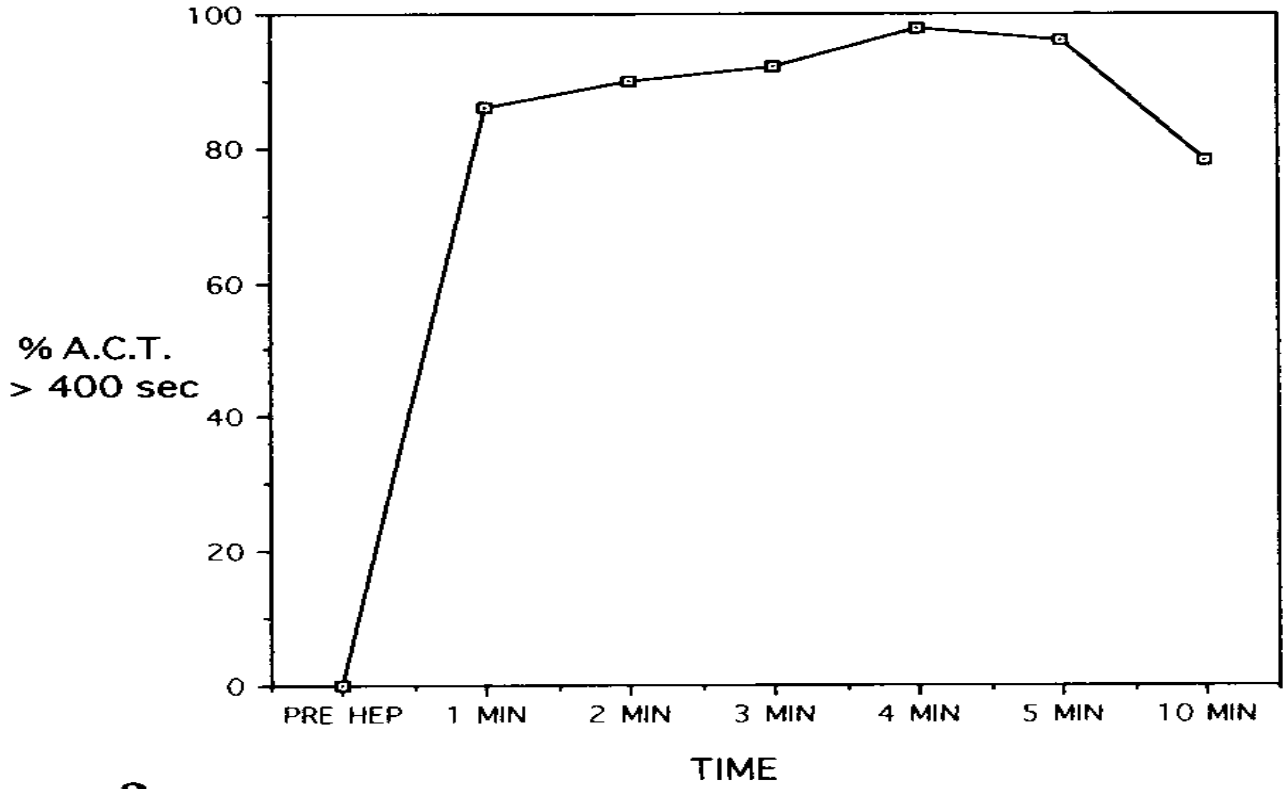


Figure 2
% ACT > 400 sec / Sampling Time.

When to Sample…ACT

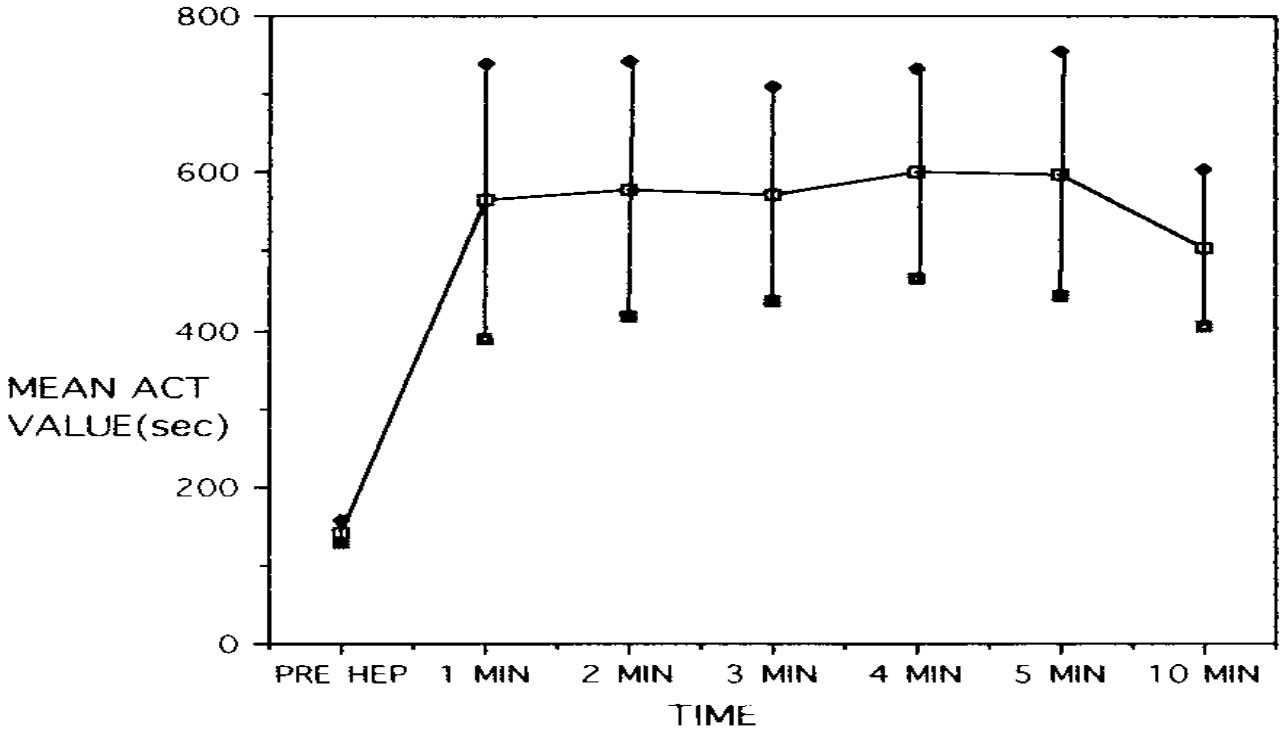
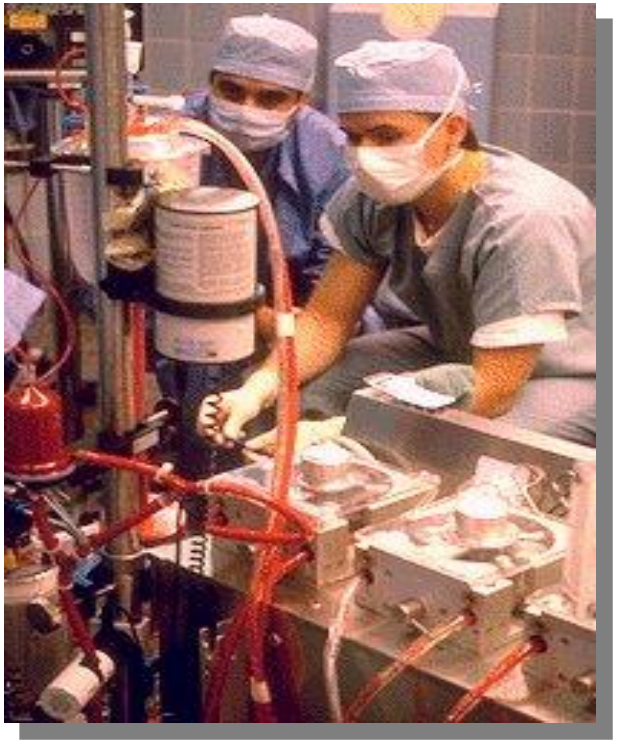


Figure 1
Time/Mean ACT Value (sec) ± STDEV.

ACT : CVOR

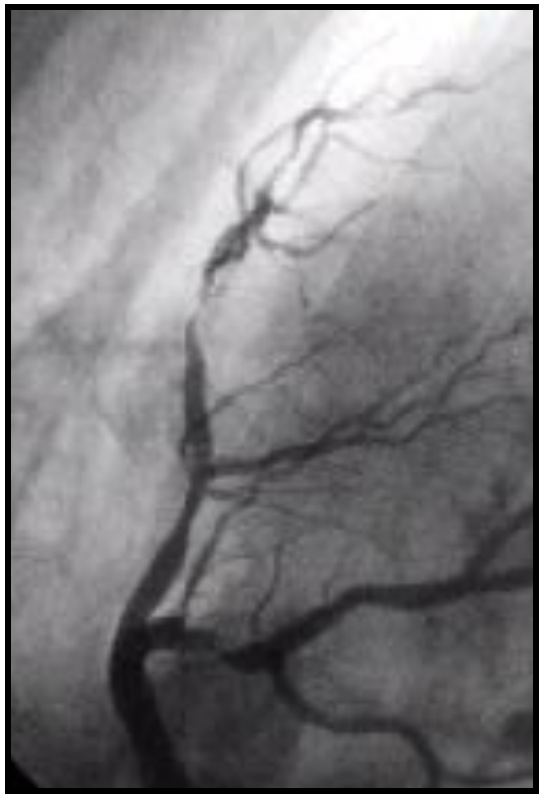
- Baseline
- 1-5 minutes post-bolus
- Prior to bypass initiation
- At 30 minute intervals
- After protamine



ACT - CCL

Cardiac Catheterization Lab

- Balloon angioplasty
- Vascular stenting
- Electrophysiology Laboratory
- Arrhythmia Ablation

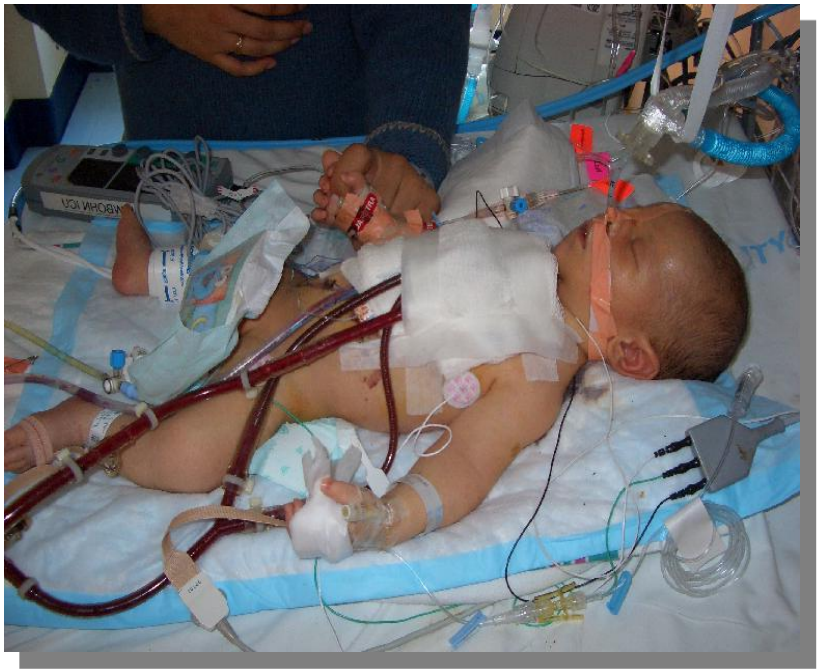


Monitoring

- Baseline
- 5 min post-bolus
- Pre-designated target range
- Prior to sheath pull

ACT : ECMO

- 15 minutes intervals when administering blood products
- 60 minutes + after heparin adjustment



When To Sample…aPTT

- Initial bolus of 80 units/kg
- Immediately followed by an initial infusion at a rate of 18 units/kg/h. All doses and rates were calculated based on total body weight (TBW).
- Monitoring at 6-hour intervals after initial bolus and any rate adjustment
- Therapeutic range achieved after 2 consecutive results in therapeutic range
- Testing reduced to once daily

A Reminder … Warning

Disseminated Intravascular Coagulation - DIC

- Seen in L&D, CVOR, NICU and in sepsis;
- Common cause – massive overload of “foreign” proteins;
- Inappropriate activation and consumption of coagulation factors;
- ~ 33% survival rate;
- Prevention: none;
- Hypercoagulable phase followed by hypocoagulable phase.

DIC in the Neonate

- No universal criteria for diagnosis;
- First clinical signs: uncontrolled bleeding and uncontrolled clotting;
- Causes: Cardiovascular compromise and infection;
- Reduced rate: proactive management and optimal intensive care.

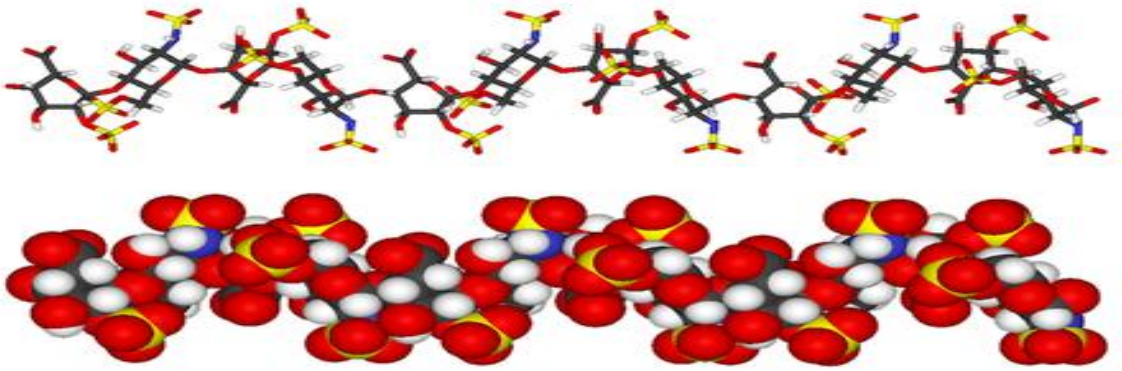
“New” Heparins

Where Did LMWHs Come From?

- LMWHs are derived from UFH by chemical or enzymatic depolymerization
- LMWH fractions prepared from standard commercial-grade heparin have been shown to have a progressively lower effect on aPTT as they are reduced in molecular size, while still inhibiting activated factor X (*ie*, factor Xa).
- Theory developed in 1976:"… any heparin molecule, irrespective of chain length, would catalyze the inactivation of serine protease coagulation enzymes equally, provided that it contained the high-affinity binding site for AT."

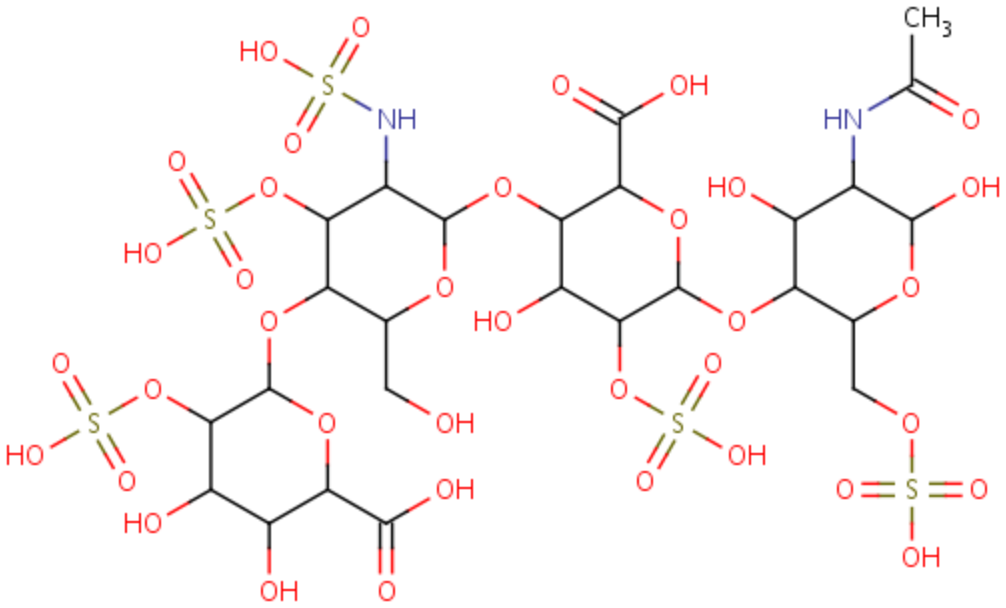
Johnson, EA, Mulloy, B The molecular weight range of commercial heparin preparations. ;,-127

Heparin Structure



Heparin molecule is 5-40 kDa in size

LWMH Structure

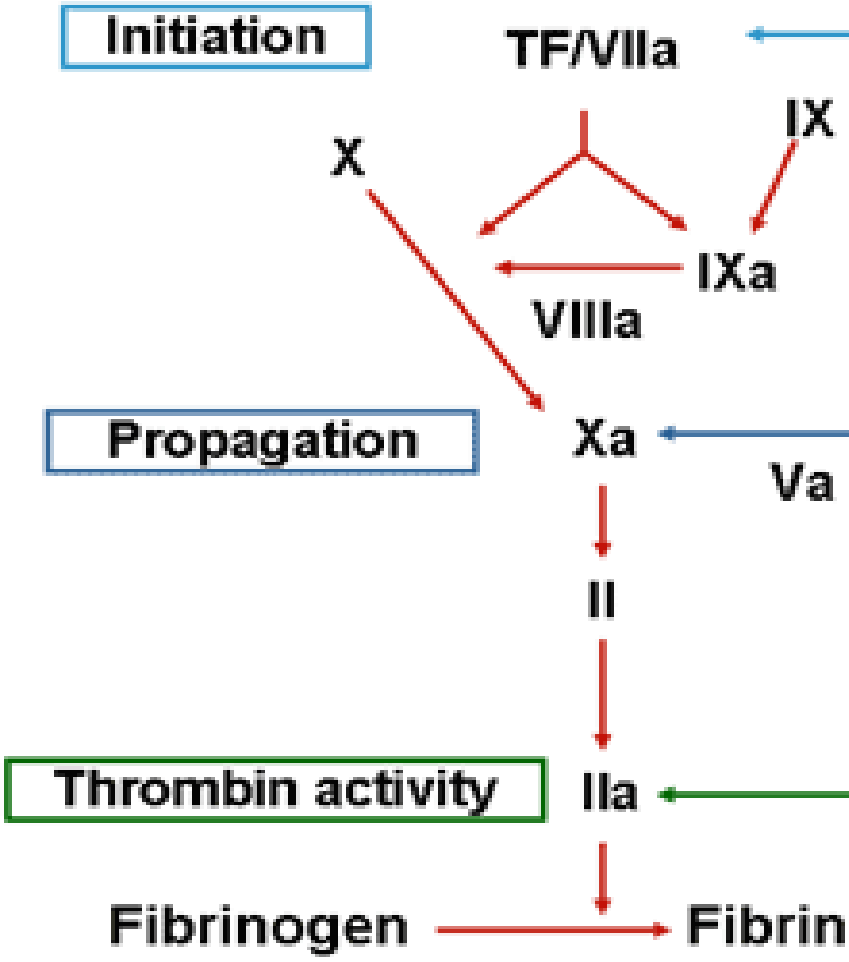


Enoxaparin – av. wt 4.5 kDa

LMWHs

- Average ~ 4.5 kDa
- Smaller risk of osteoporosis in long-term use
- Smaller risk of heparin-induced thrombocytopenia
- Less effect on thrombin
- Sub-Q administration
- Manufacturers' claim – no monitoring
- Some forms do not affect aPTT or INR
- Only form of monitoring: Factor Xa activity
- Effects on time not testing

New Anticoagulants



Drug (most in development)

- Tissue factor pathway inhibitor (TFPI) (recombinant)
- Nematode anticoagulant peptide (NAPc2)
- Active-site-blocked factor VIIa (FVIIa)

- Factor Xa inhibitors
- Direct factor Xa inhibitors:
- Rivaroxaban
- Apixaban
- YM150
- DU-176b
- LY517717
- Indirect factor Xa
- Inhibitors: fondaparinux
- Biotinylated idraparinux
- Oral heparins
- Inhibitors of factor VIIIa and Va
- Protein C
- Activated protein C
- Soluble thrombomodulin

- Hirudin
- Bivalirudin
- Argatroban
- Dabigatran
- Orally available heparins

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Adapted with permission from Weitz I, et al. *Chest*. 2001;119:95S–107S.

LMWH – Pediatric Application Canadian Recommendations

- Enoxaparin (< 2 months)
  - Prophylactic: 0.75 mg/kg/dose; q 12 hr;
  - Treatment: 1.5 mg/kg/dose; q 12 hr;
  - Pre-term: 2mg/kg; q 12 hr;
  - Term; 1.7 mg/kg; q 12 hr;
- Dalteparin
  - Prophylactic: 92 U/kg/dose; q 24 hr;
  - Treatment: 129 U/kg/dose; q 24 hr.

Another Anti-Xa (March 15, 2010)

(Atlanta, Georgia) **—** An investigational factor Xa inhibitor, **betrixaban** code named PRT-054,21 (Portola Pharmaceuticals/Merck), was safe and well tolerated when three doses of the drug--40 mg, 60 mg, and 80 mg--were compared with **warfarin** in a dose-finding phase 2 trial, **EXPLORE-Xa**, in patients with atrial fibrillation (AF), attendees of the **American College of Cardiology** (ACC) **2010 Scientific Sessions** heard today.

Dabigatran (DTI) In Canada

KINGSTON, ON — Prescriptions of new oral anticoagulants in Ontario jumped 20-fold over two years starting with the Canadian approval of **dabigatran etexilate** (Pradaxa, Boehringer Ingelheim) for atrial fibrillation, according to an analysis published today in *CMAJ Open* [1].

The far greater use of **warfarin** tapered off proportionately, while prescriptions of **rivaroxaban** (Xarelto, Bayer Pharma/Janssen Pharmaceuticals), available for less than half the study time, rose only a little.

(Oct 16,2013)

Reversal of LMWHs

- Enoxaparin – (Lovenox®, Xaparin®, Clexane®) protamine neutralizes by 66%.
- Fondaparinux – not reversed
- Tinzaparin – (Innohep®) protamine does neutralize
- Dalteparin - 1 mg/100 anti-Xa IU of dalteparin

Latest News: Oct 17, 2013

"A possible antidote (**PRT4445**, Portola Pharmaceuticals) to the anticoagulant effects of the new oral factor Xa inhibitors reversed the effects of one such agent, **apixaban** (Eliquis, Bristol-Myers Squibb/Pfizer), by >90% within two minutes of an initial IV bolus and maintained that level throughout a two-hour infusion[1]."

Neutralization of DTIs

- Lepirudin, Bivalrudin, and Argatroban
- No neutralization
- Treatment: Moderate Bleeding:
  - Infuse blood products
  - hemodialysis
- Treatment: Severe bleeding
  - Factor VIIa
  - Activated prothrombin complex concentrate

Future????

- Will LMWHs overtake heparin in:
  - CVOR
  - CCL
  - Treatment for PE, DVT
  - ECMO
  - Vascular OR

Questions?

THANK YOU !!!!!!


Gracias


Ευχαριστώ


Obrigado!


धन्यवाद


Köszönettel


Merci


תודה


شكراً


Teşekkürler