

Platelet Function, Coagulation, and Effects of Cardiopulmonary Bypass.

Presented By:

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Overview

- Platelet physiology and review of general concepts in hemostasis
- Review of cell-based theory vs cascade theory on coagulation and its relation to platelet function.
- How does cardiac bypass affect platelet function?
- Possible therapies targeting platelet protection.



Platelet Physiology:

- Discoid shaped cells with 7-10 day lifespan.
- Contain both α and dense granules
- Activation can be maximal, or partial depending on degree of stimulus which is a key point in our discussion about CPB.



Platelet Physiology:

- α -granules contain adhesive ligands, PF4 and coagulation factors including Factor V and VIII
- Dense granules contain calcium, ADP and serotonin, and require stronger signal for release



Hemostasis:

- Two types – Arterial and Venous
- Arterial bleeding is typically more concerning in perioperative setting and is our focus today.
- Arterial flow → high mechanical shear forces that oppose clot formation
- Platelets, thrombin, fibrinogen, collagen, and the coagulation factors all must come together in a complex and interrelated manner.



Hemostasis cont.....

- The initiation of arterial hemostasis depends primarily on platelets and requires:
- **Activation** and conformational change
- **Adhesion** to subendothelial matrix (GPIb, VWF, GPIIb/IIIa) and generation of stable platelet – platelet bonds
- **Aggregation** and **recruitment** of coagulation factors to direct development of a stable fibrin clot

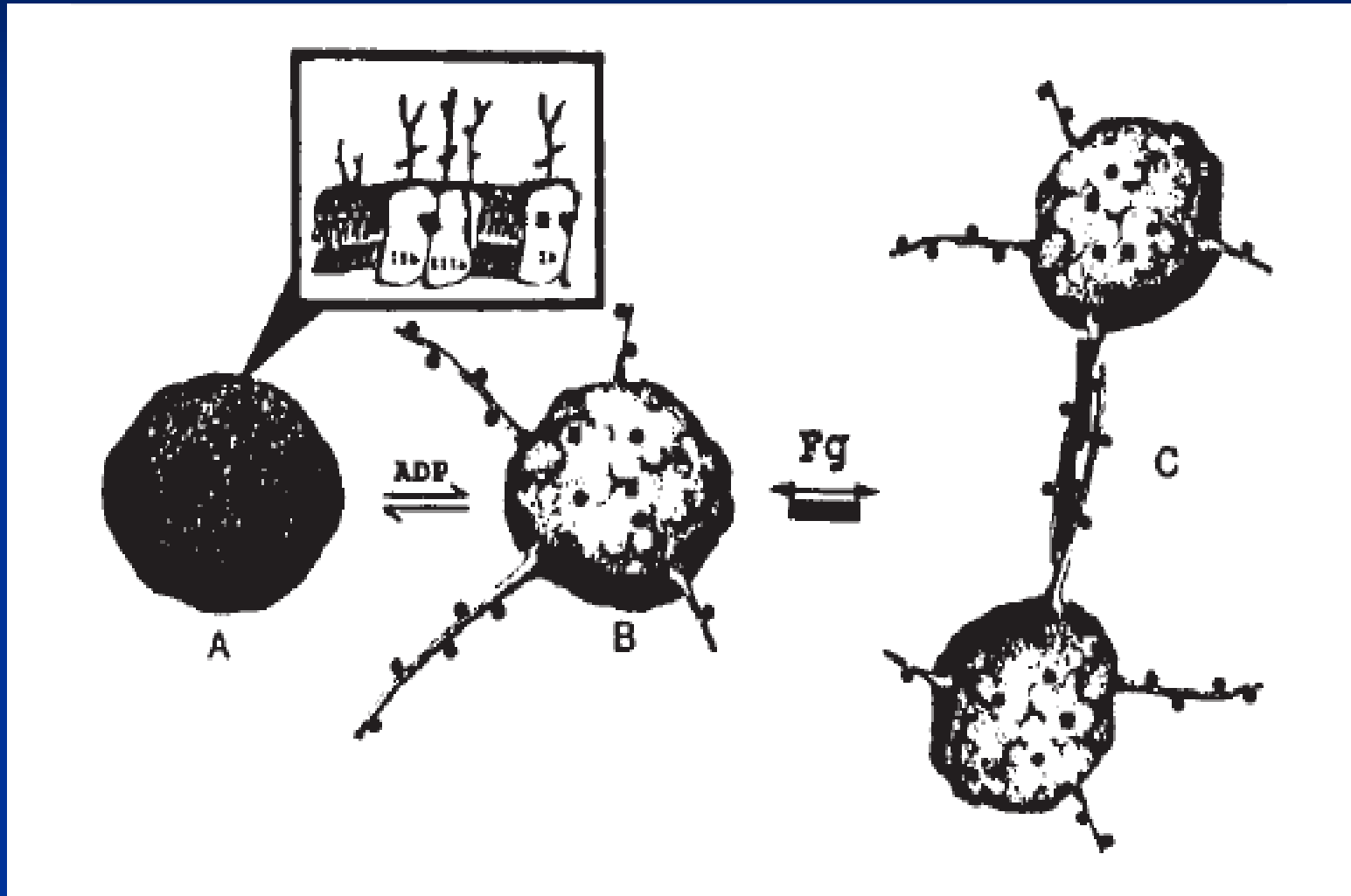


Platelet Activation:

- Activation induces the platelet to change shape and involves release of α -granules and / or dense granules
- Activators include: Fibrinogen, Collagen, **Thrombin**, ADP and “Cardiopulmonary bypass”
- Results of Activation:
 - Recruitment
 - Vasoconstriction
 - Release of messaging factors
 - Acceleration of fibrin formation
 - Clot protection



Platelet Activation



Frojmovic et al, American Heart Journal 1998.

Activation:

- Basic structure of platelet plug is the platelet-ligand-platelet matrix
- Fibrinogen and VWF serve as links
- Both act to bind at GIIb/IIIa receptors, of which the resting platelet has 50,000

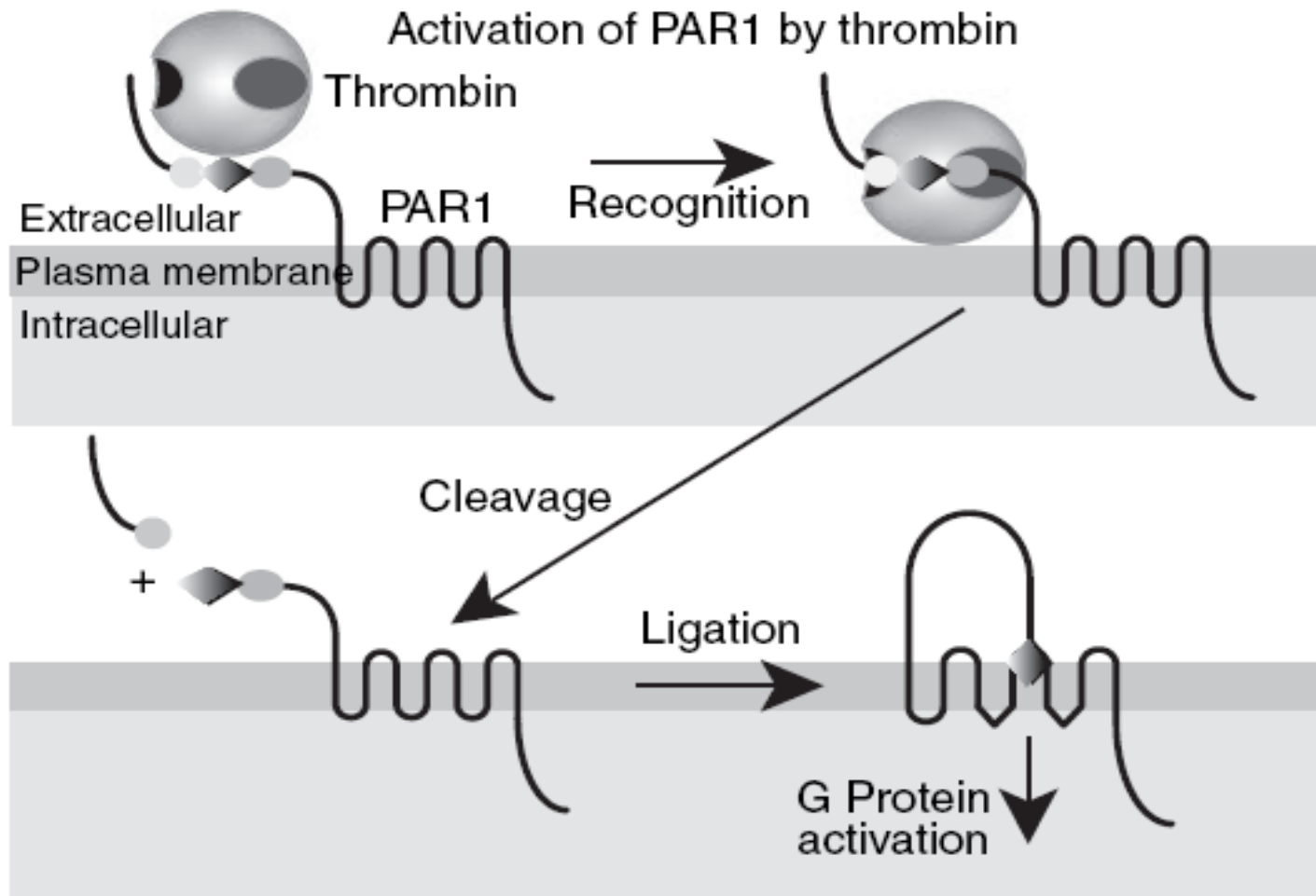


Activation:

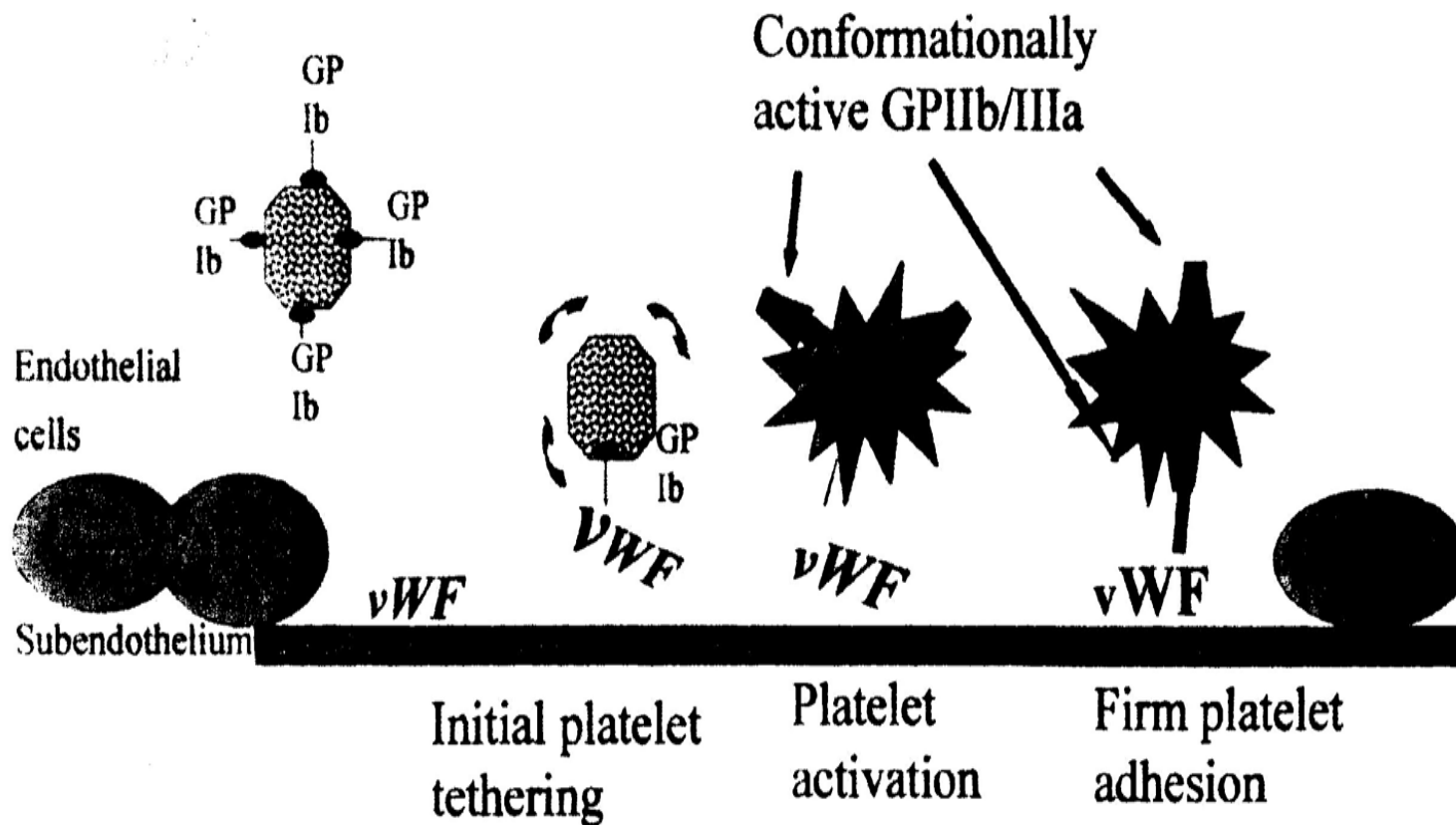
- Thrombin is one of the most potent activators of platelets.
- Activation is thought to occur via PARs
 - G-protein receptor which is cleaved upon thrombin binding
 - Cleavage of ligand exposes intracellular binding sites, allowing signal cascade to begin
 - Potential target for drug therapy.



Thrombin and PARs:



Platelet Activation & Adhesion:



C.S. Rinder, Hematologic effects of Cardiopulmonary bypass. (Cardiopulmonary bypass: practice and principles by Gravlee et al. 2nd edition 2000.)

Aggregation:

- Aggregation begins once the initial platelet layer has formed. The substances mentioned (ADP, Thromboxane etc) all act to recruit more platelets and incorporate more fibrinogen.
- This is where aspirin and clopidigrel have their activity.



Recap:

- Discussed platelet physiology and covered basics of hemostasis
- Platelets form first line defense against arterial hemorrhage.
- Requires activation, adhesion, and aggregation.
- This leads to interaction with coagulation factors and the coagulation cascade.



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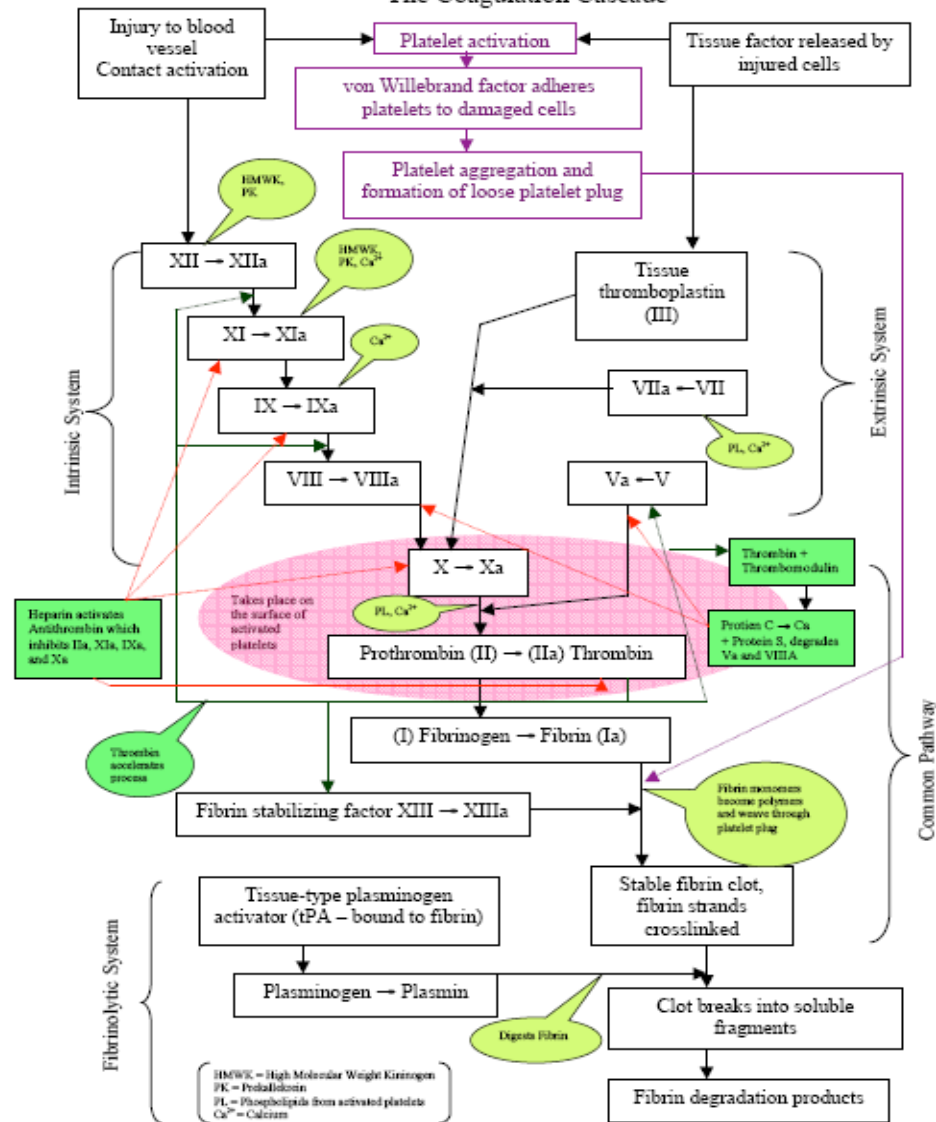


Cell based model:

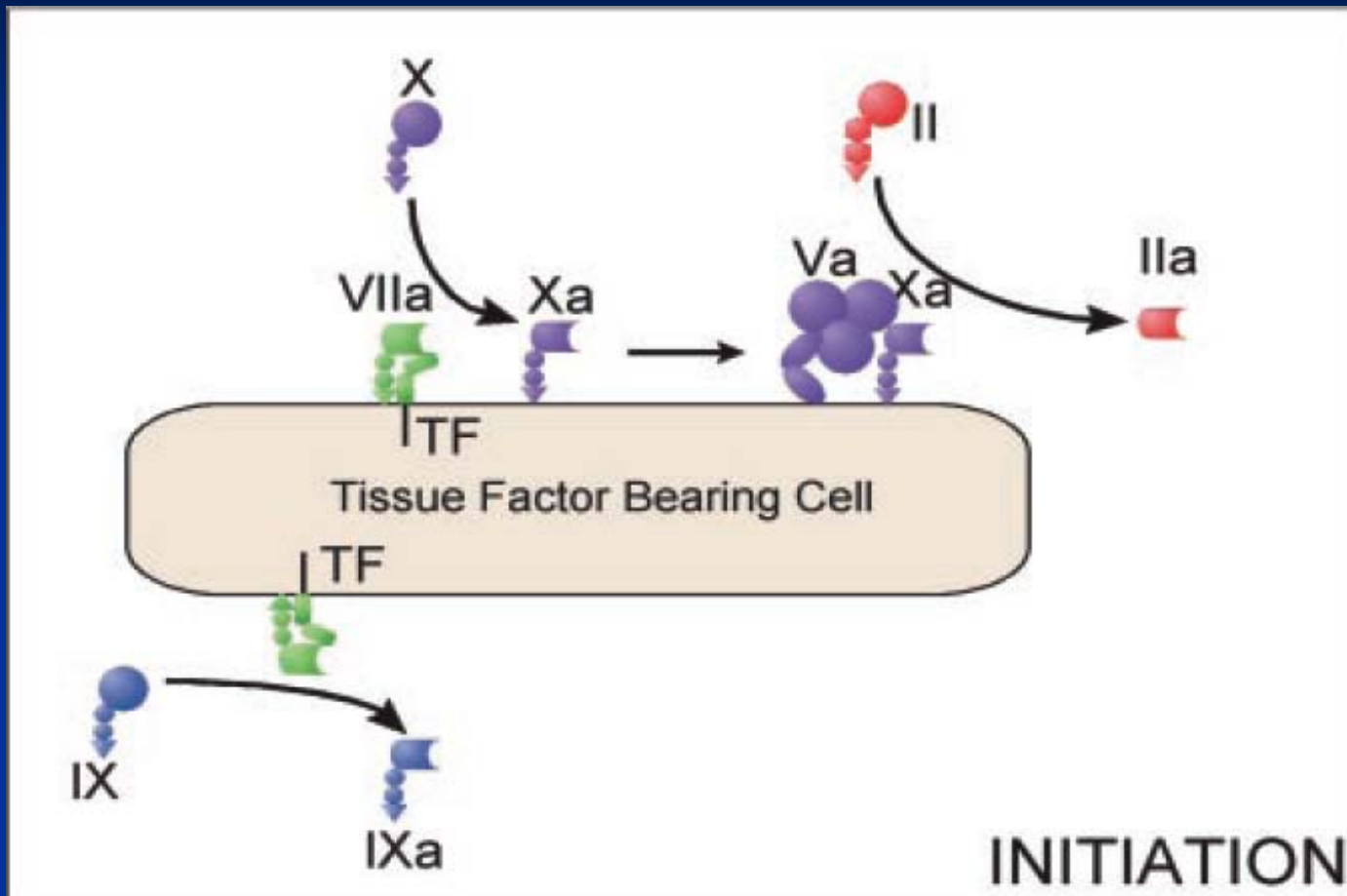
- Coagulation cascade now viewed more accurately as a series of proteolytic events localized to the activated platelet surface.
- Exposure to vessel endothelium initiates a cascade of proenzyme reactions eventually leading to thrombin and fibrin formation in close association with activated platelets.
- Key point: Consider these reactions as occurring in the shelter of the platelet membranes.



The Coagulation Cascade

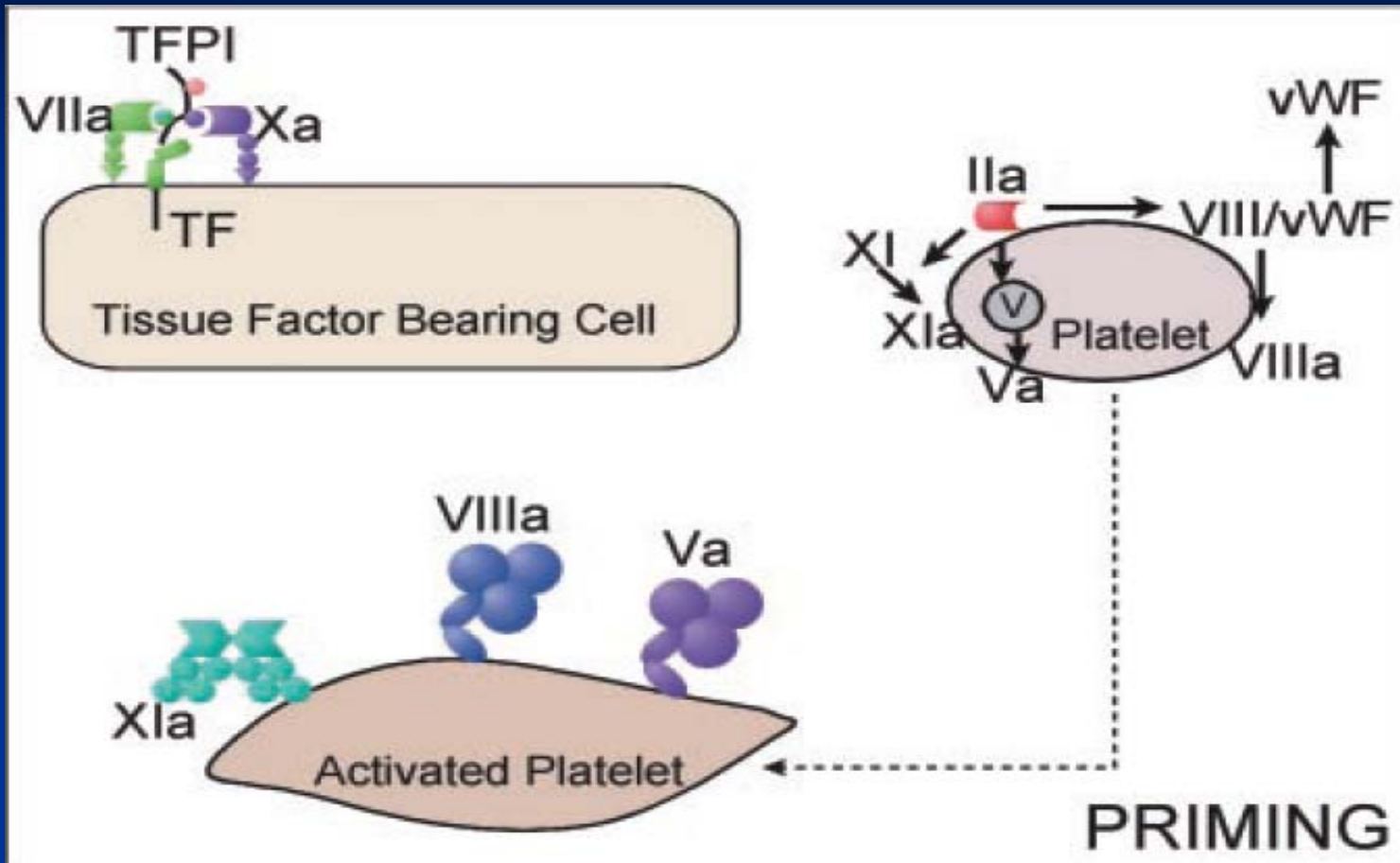


Initiation Phase:



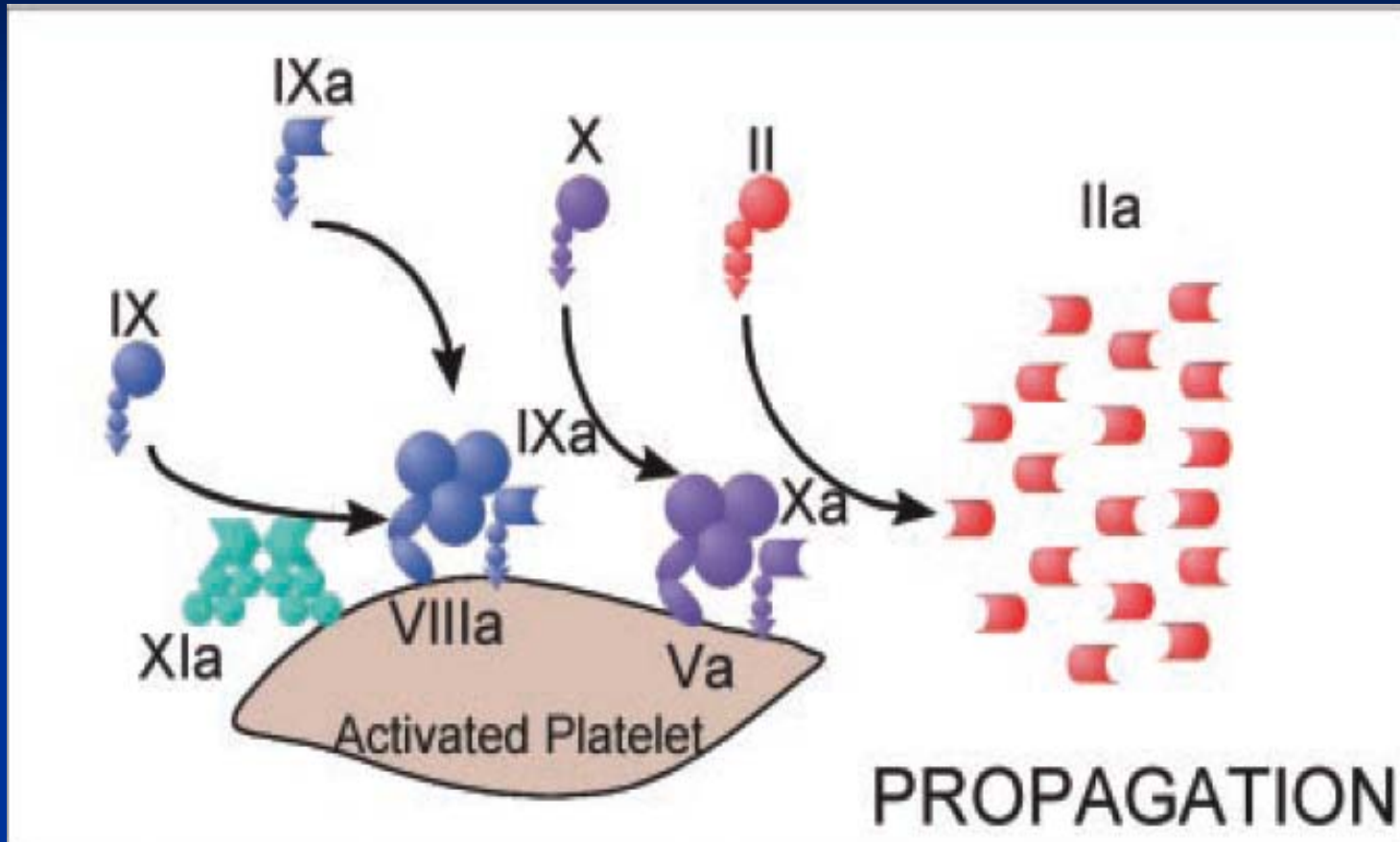
Factor VIIa bound to TF activates factor IX and also factor X. Factor Xa then activates factor V on the TF-bearing cell, complexes with factor Va, and converts a small amount of II to IIa

Priming Phase:



Small amount of initial IIa activates platelets, causing release of α granule contents including factor V, activates factor V, activates factor XI, and activates factor VIII by cleaving it from vWF.

Propagation Phase:



Factor IXa generated by factor VIIa/TF binds to the activated platelets and subsequently activates factor X to Xa. The formation of the "tenase" complex, comprising factors VIIIa, IXa, and calcium ions on the platelet surface, leads to the large-scale generation of factor Xa.

Cell based model cont...

- Formation of the “Xase” complex is a key point in the cascade
- Provides kinetic advantage for Factor X activation-13 million fold more efficient
- Allows interaction of Factor Xa and Va in a **protected platelet environment**
- Tight geometry of matrix prevents “invasion” by TFPI, and Antithrombin (even with heparin)



Cell based model cont...

- End result → prothrombinase complex followed by the Thrombin Burst
- Thrombin cleaves fibrinogen to fibrin
- Platelet provides a foundation for protease / factor interactions.



Recap:

- Cell based coagulation theory:
- Important changes incorporate the dependence of the entire cascade reaction on platelet activation and membrane signaling pathways.
- Generation of tenase complex in contact with activated platelets allows for kinetically superior reaction times and improved clot formation.



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Platelet Dysfunction and Bypass

- Who cares if the platelets are not working?
- If there is some bleeding we can just transfuse blood and platelets right?
- That is what the blood bank is for anyway.
- Well.....maybe this is not the best approach.



Problems with transfusion

- Speiss et al retrospectively analyzed data collected during 6 RCT's that were evaluating aprotinin and bypass, looking for effects of platelet transfusion.
- Results from logistic regression analysis demonstrated increased risk of stroke and death associated with platelet transfusion.

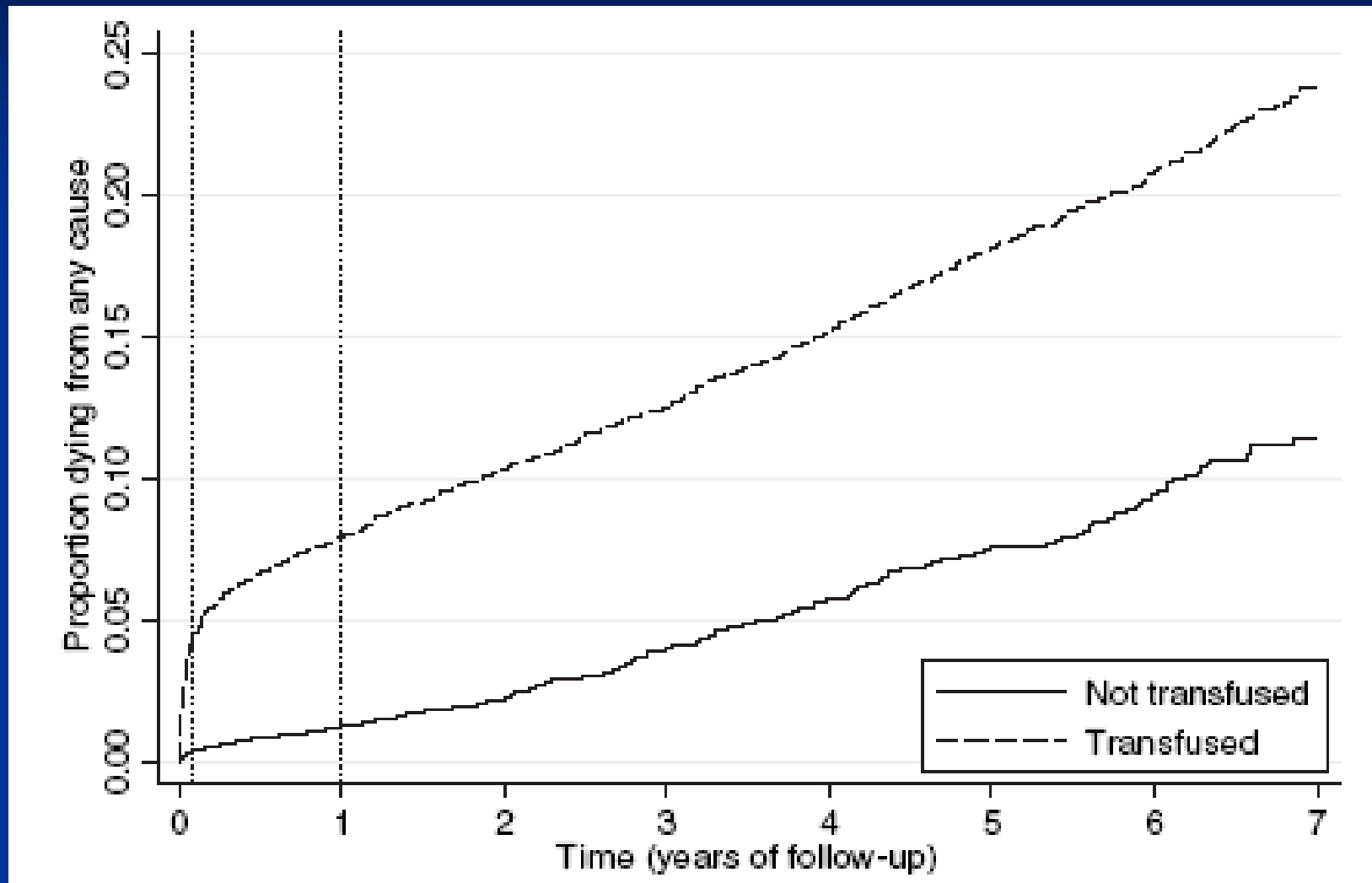


Effects of RBC transfusion

- Murphy et al recently published results from a large retrospective cohort study looking at both infectious outcomes and ischemic outcomes associated with transfusion.
- Results: Significantly increased risk of infection, ischemic outcomes, and death for those patients transfused.



Effects of RBC transfusion



Circulation 2007;116:2544-52

What causes platelet dysfunction?

- Platelet activation via interaction with cardiopulmonary bypass circuit.
- Mechanical shear from circuit
- Hemodilution (if extreme)
- Hypothermia
- Heparin induced platelet dysfunction

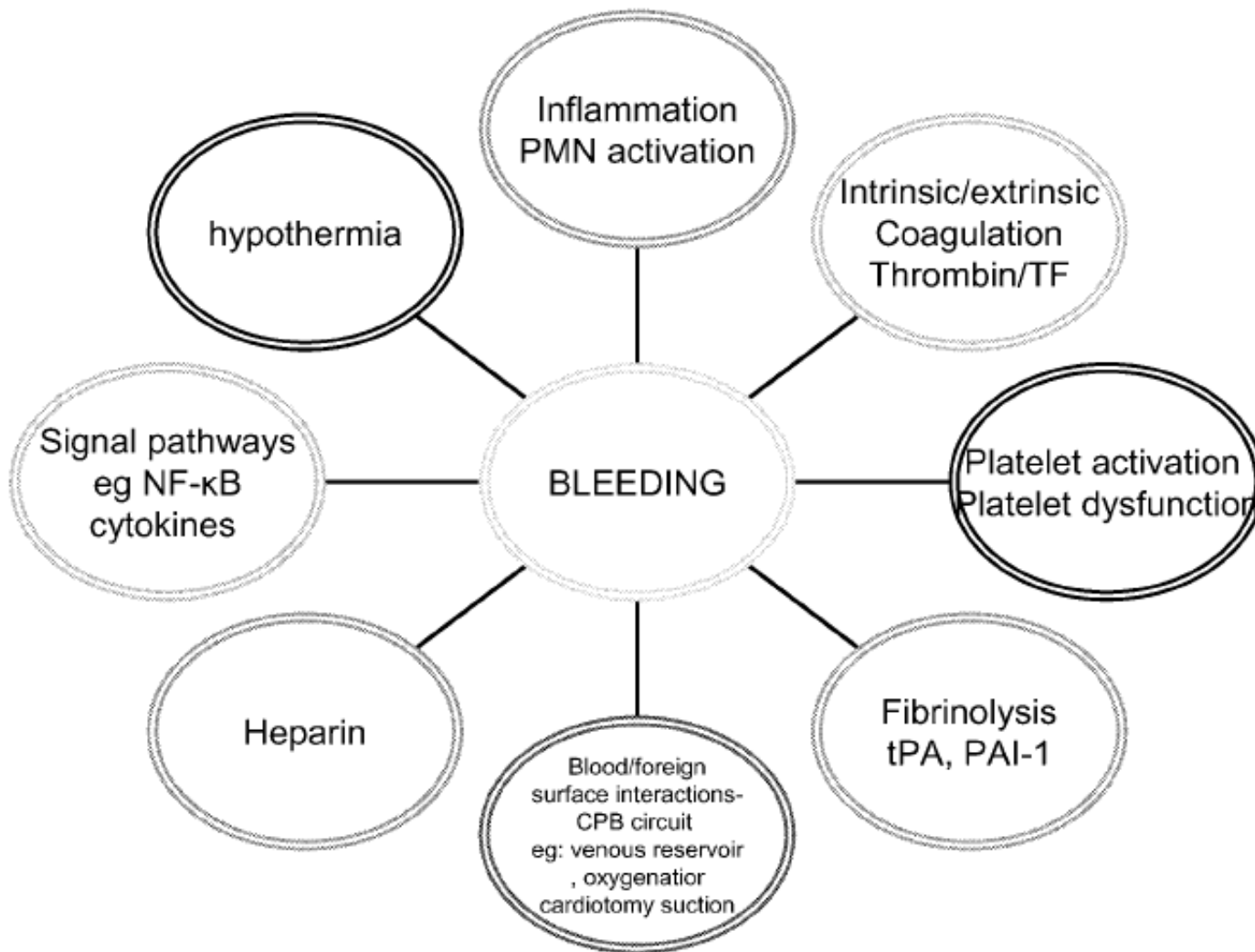


Cardiopulmonary Bypass:

- Artificial heart lung machine, requiring blood to be pumped through an extracorporeal circuit.
- Exposure to circuit causes coagulation defects, with platelet dysfunction considered a key player.
- Ironically, CPB causes both thrombosis and coagulation deficits
- Re-operation rates for bleeding are 2-6%.



CPB induced bleeding:



Bypass induced platelet activation:

- CPB activates platelets
- Studies demonstrate α -granule release
- Activation allows platelet binding to subendothelium, bypass circuit, and circulating cells
- Potential loss of GPIIb/IIIa and GPIb receptors

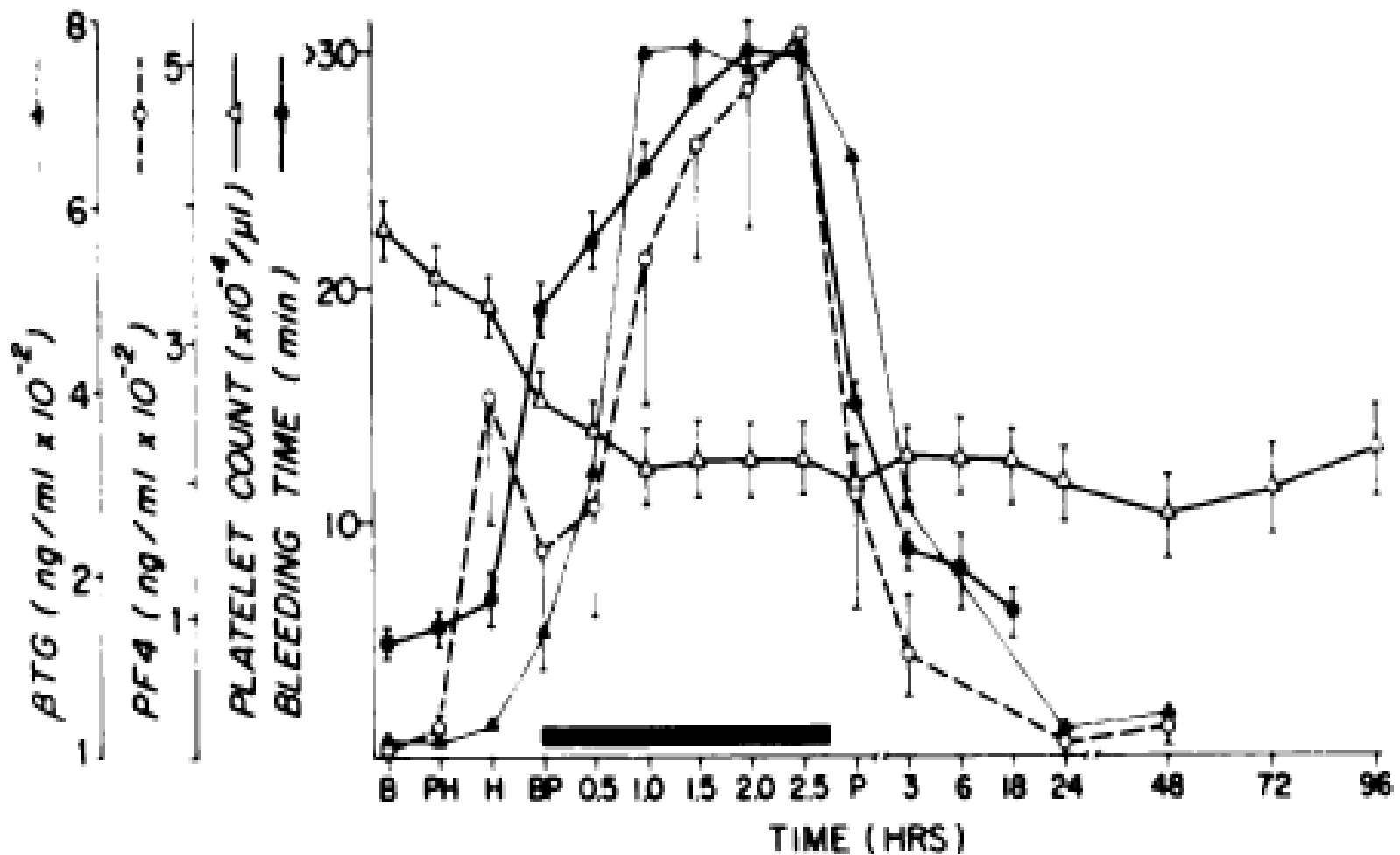


Bypass induced platelet activation:

- Partial to complete activation causes shape changes and degranulation → leading to weakened platelet pool and poor clot formation
- Activation allows generation of small amounts of fibrin along circuit laying the groundwork for further platelet binding and activation.
- Receptor dysfunction causes the need for increased agonist concentrations to achieve activation post-bypass.



Platelets during bypass:



Platelet membrane effects from CPB:

Table 1. Changes in Platelet Surface Molecules Associated With Cardiopulmonary Bypass

Molecule	Description	Effect of CPB [Reference No.]
GPIb(CD42b)	Binds von Willebrand factor. Important in interactions with endothelium under high shear rates.	Decreased [6-9]
GPIIb/IIIa(CD41a/CD61a)	Binds fibrinogen.	Decreased [6-8]
GPIV	Binds thrombospondin and collagen.	Increased [8]
HLA-ABC	A major histocompatibility complex class-1 molecule.	Increased [8]
CD31	Also present on endothelium, subpopulation of T-cells, monocytes, and neutrophils	Decreased [10]
CD62P(P-selectin)	Also present in Weibel Pallade bodies of endothelial cells. Both platelets and endothelium can also secrete a soluble form of P-selectin	Increased [10, 11]
CD63(GP53)	An integral protein of platelet lysosomes detectable on their surface on activation.	No change [7]

CPB = cardiopulmonary bypass.

Hemodilution:

- Circuit prime is responsible for a significant dilution effect-but most often insufficient to explain clotting defects.
- Platelet count reduction not entirely explained by hemodilution alone
- Current theory involves platelet interactions with circuit and heparin induced dysfunction / activation.



Hemodilution

- Fibrinogen binds to both circuit and platelets.
- Result → additional platelet binding and sequestration along bypass circuit.
- Continuous low grade activation may preferentially act on younger / stronger platelets
- This adds to the hemodilution effect, and reduces functional platelet pool.



Hypothermia and Platelets:

- Wolberg et al examined effects hypothermia and coagulation.
 - Coagulation enzyme activity only mildly reduced at 33-36° C
 - Significant reductions below 33°C
- However, both platelet adhesion and aggregation markedly reduced even at 33° C – 36°C.



**Boldt et al. Normothermic versus hypothermic
cardiopulmonary bypass: do changes in coagulation differ?
Ann Thorac Surg 1996;62:130–5.**

- Study demonstrated that aggregatory function of platelets returned to normal in normothermic group ($>35^{\circ}\text{C}$) group by the next day, but in hypothermic group (28°C) function remained impaired beyond 24 hours.
- Also demonstrated that thrombocytopenia was increased in hypothermic group.



Heparin and Platelet Function:

- Heparin is the most common anti-coagulant for CPB
- Profound anticoagulation required to prevent widespread clotting.
- Heparin binds anti-thrombin III speeding activity against thrombin, Factor Xa and IXa
- Effective against systemic thrombin, but not necessarily **surface bound** thrombin.



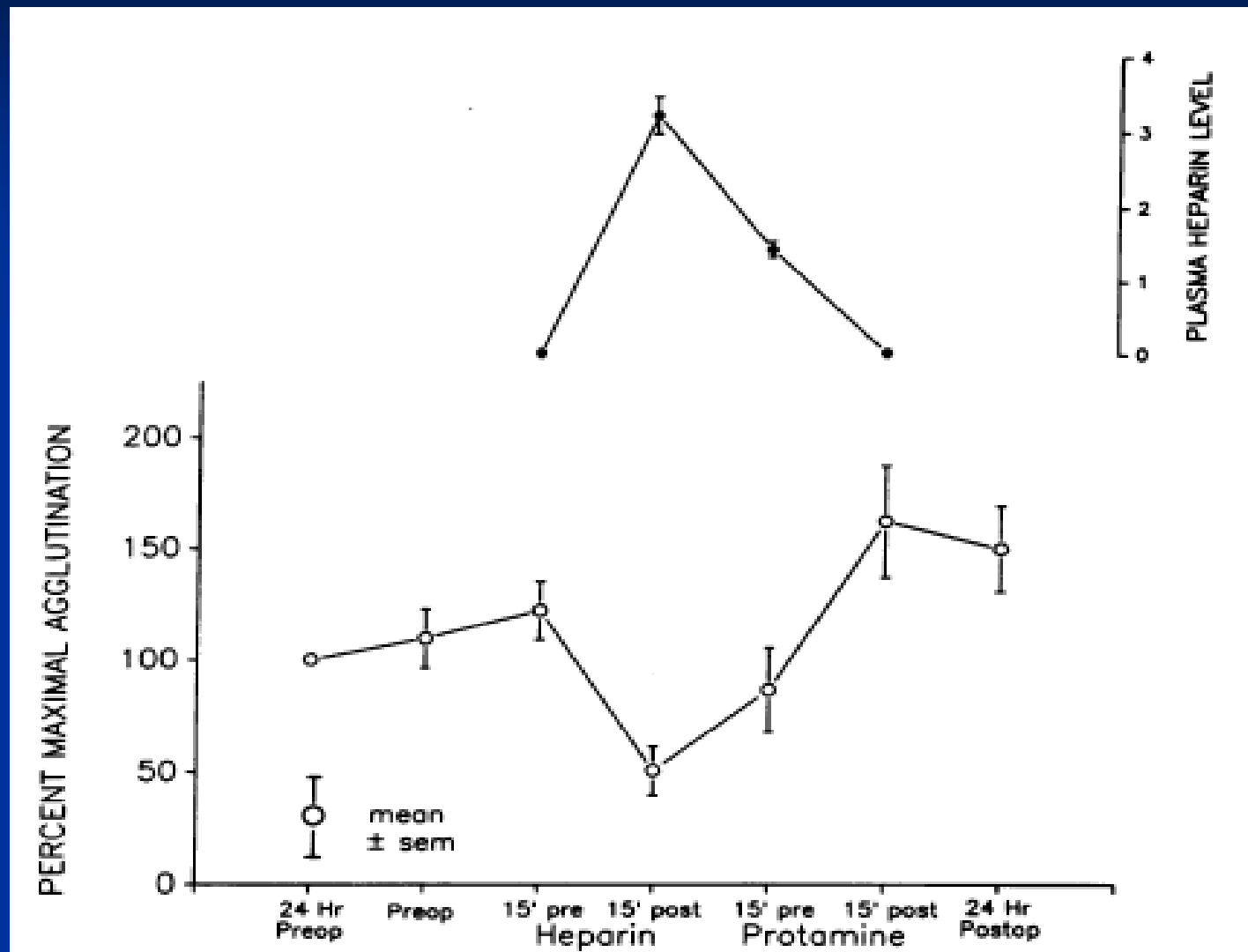
Heparin and Platelet Function:

- Sobel et al demonstrated direct heparin binding to platelets resulting in dysfunction in 1991:
- Studied blood samples from a small group of patients undergoing CPB, as well as in vitro studies.
- Demonstrated direct heparin binding to platelets at GPIIb site, thus disrupting ability to adhere via VWF – platelet interactions.

J Clin Invest 1991;87:1878-93.



Heparin and Platelet Function:



J Clin Invest 1991;87:1878-93.

Heparin and Platelet Function:

- Khuri et al also demonstrated similar effects of heparin on platelets in 1995
 - Found prolonged bleeding time and reduced ability to release thromboxane which has been shown to be a reliable in vivo marker for activation
 - These results along with those of Sobel et al point to the disruption of the GPIb – vWF binding ability of platelets secondary to heparin

KHURI ET AL
HEPARIN EFFECTS ON PLATELETS AND FIBRINOLYSIS

Ann Thorac Surg
1995;60:1008-14

Heparin and Platelet Function:

- Despite these results the clinical effect of heparin on platelets is not clear, as well as duration of effect after protamine reversal.
- Future considerations will likely examine the use of different anticoagulants during bypass → possibly direct thrombin inhibitors or hirudins to avoid some of the negative affects of heparin.



Protamine and Platelets:

- Griffin and Rinder (2001), using in vitro techniques demonstrated that protamine itself has direct binding capabilities
 - Excess protamine can cause platelet dysfunction by inhibition of thrombin activation sites, as well as disruption of GPIb-VWF interaction
- Heparin / Protamine complex also inhibits platelet function
- Suggest that there is a small window available to reverse heparin without causing further platelet dysfunction.
- Anesth Analg 2001;93:20-7.

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What else can we improve?

- Improved platelet protection.
 - Heparin Coated Circuits
 - Off Pump CABG
- Specific reversible platelet inhibitors
 - Iloprost
 - NO
 - Aprotinin and PAR inhibitors



Clinical use of Heparin Coated CPB in CABG

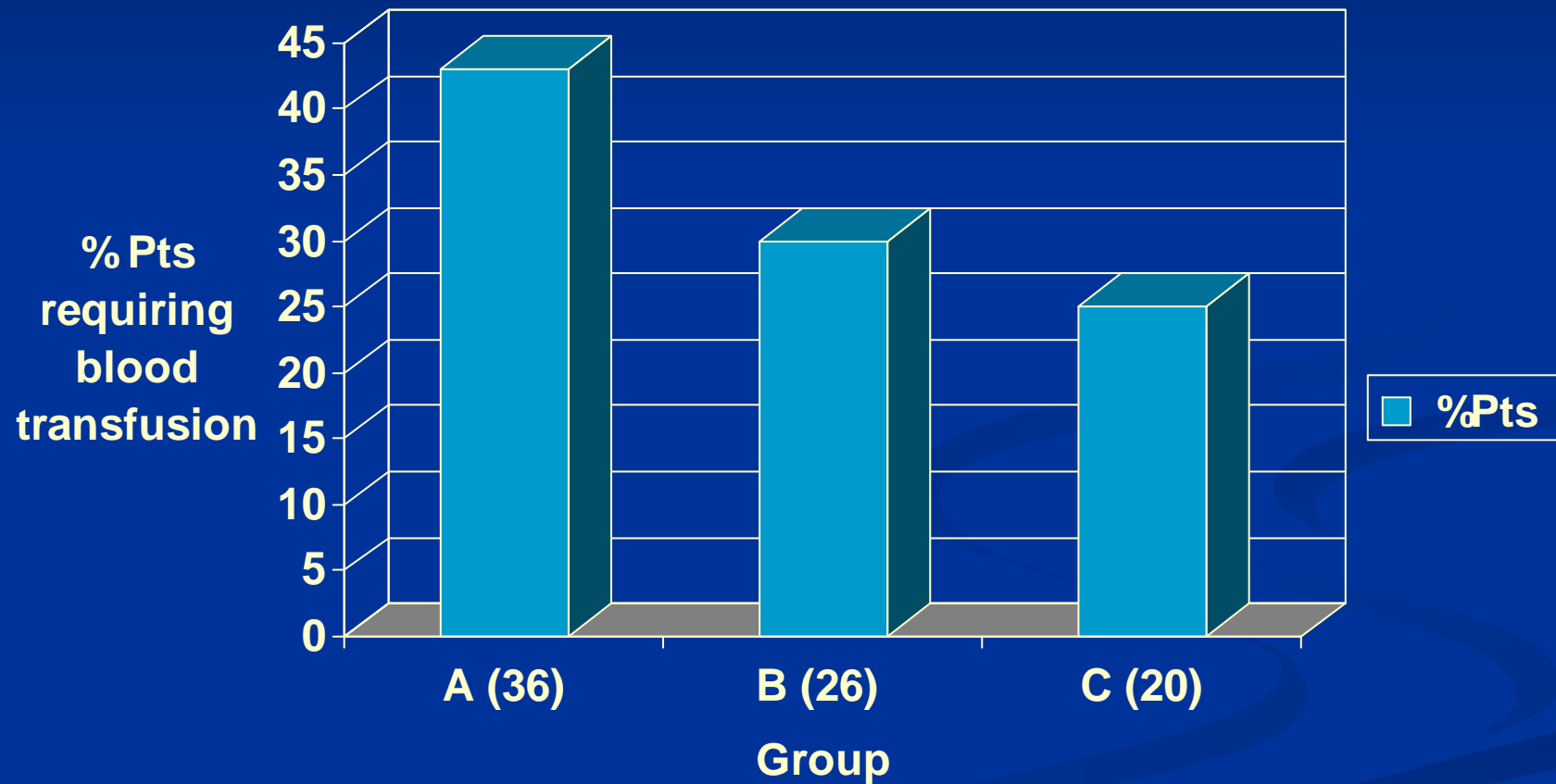
Mirow et al. Thoracic and Cardiovascular Surgery 2001; 49:131-136

- Prospective, RCT compared:
 - Standard heparin (ACT target 480)
 - Standard heparin + LMW heparin coated circuit (ACT target 480)
 - Low dose heparin + LMW heparin coated circuit. (ACT target 240)
- 243 total patients enrolled



Clinical use of Heparin Coated CPB in CABG

Mirow et al. Thoracic and Cardiovascular Surgery 2001; 49:131-136



A = Standard heparin; B= Standard heparin + circuit coating;
C=Low dose heparin + circuit coating

Clinical use of Heparin Coated CPB in CABG

Mirow et al. Thoracic and Cardiovascular Surgery 2001; 49:131-136

- Additional endpoints noted:
 - Reduction in measured levels of platelet activation (BTG)
 - Significant reduction in amount of blood transfused in study group.

2003 retrospective study of 1300 patients found similar results.

- Ovrum et al. Journal of Cardiothoracic Surgery 2003;18:140-146



Off Pump CABG

- By avoiding CBP, theoretically you can avoid the shear stress and activation previously discussed
- Lower Heparin doses required
- Less Hypothermia
- Elimination of Cross Clamping
- However- technically more difficult



Off Pump CABG

- Number of studies, retrospective and prospective have compared OPCAB to CPB.
- Impact of Off-pump Coronary Artery Bypass Surgery on Postoperative bleeding: Current best available evidence
 - Raja and Dreyfus. Journal of Cardiothoracic Surgery 2006; 21:35-41



Impact of Off-pump Coronary Artery Bypass Surgery on Postoperative bleeding: Current best available evidence

Raja and Dreyfus. *Journal of Cardiothoracic Surgery* 2006; 21:35-41

- 65 RCT's evaluated, 19 specifically looked at blood loss and transfusion requirements.
- 9 of 19 trials showed significant reduction in transfusion rates.
- Remainder showed decreased or similar rates between groups, but not statistically significant.
- Did not specifically evaluate platelet function.



Hemostasis in Off-pump compared to On-pump CABG: Prospective Randomized Study.

Vedin et al. *Ann Thorac Surg* 2005; 80:586-593

- Examined 31 patients in prospective, RCT for OPCAB vs CBP.
- Measured hemostatic parameters as well as markers of endothelial activation (D-Dimer, Fibrinogen, VW factor, etc).
- No specific platelet activation markers measured.
- Results mixed.



Hemostasis in Off-pump compared to On-pump CABG: Prospective Randomized Study.

Vedin et al. *Ann Thorac Surg* 2005; 80:586-593

- No significant difference in intra-operative or post-operative bleeding.
- D-Dimer, platelet counts, VWF, and fibronectin all measured.
- Authors noted a trend toward reduction in markers of endothelial activation, but not significant.

Ann Thorac Surg 2005; 80:586-593



Nitric Oxide and Iloprost

- NO, prostacyclin (PGI₂) and Iloprost (PGI₂ analog) are potent platelet inhibitors both in vitro and in vivo.
- Can these be used to anesthetize the platelets during CPB?



Combined administration of nitric oxide gas and iloprost during cardiopulmonary bypass reduces platelet dysfunction: a pilot clinical study.

Chung et al. J Thorac Cardiovasc Surg. 2005 Apr;129(4):782-90.

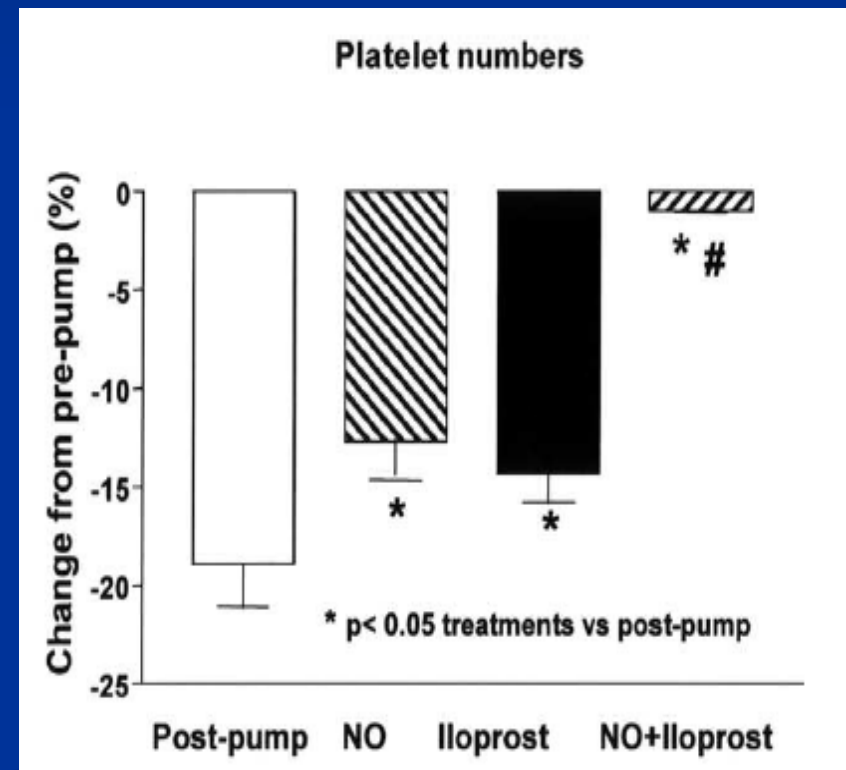
- Pilot study to examine effects of NO and Iloprost on platelet function and hemostasis in CPB.
- Prospective RCT of 25 patients
- Control, NO, PGI₂, and NO + PGI₂
- Standard CPB. ACT of >400
- NO @ 20ppm and Iloprost (2ng/kg/min) given through pump.



Combined administration of nitric oxide gas and iloprost during cardiopulmonary bypass reduces platelet dysfunction: a pilot clinical study.

Chung et al. J Thorac Cardiovasc Surg. 2005 Apr;129(4):782-90.

- Decreased chest tube output with NO, and NO + Iloprost*
- Platelet aggregation well preserved with NO + Iloprost, along with reduction in markers for platelet activation.



PARs

- Signaling pathway for Thrombin
- Specific PAR antagonists exist, but are in preclinical trials
- Day et al conducted trial to examine effects of Aprotinin on PAR activity.
- Clinical Inhibition of the Seven-Transmembrane Thrombin Receptor (PAR1) by Intravenous Aprotinin During Cardiothoracic Surgery. *Circulation* 2004;110;2597-2600



Clinical Inhibition of the Seven-Transmembrane Thrombin Receptor (PAR1) by Intravenous Aprotinin During Cardiothoracic Surgery. *Circulation* 2004;110;2597-2600

- 30 patients for elective CABG enrolled
- Either received Aprotinin (high dose) or saline infusion
- Examined thrombin formation, platelet aggregation, PAR-1 expression and function.



Clinical Inhibition of the Seven-Transmembrane Thrombin Receptor (PAR1) by Intravenous Aprotinin During Cardiothoracic Surgery. *Circulation* 2004;110;2597-2600

Overall results:

- First trial to demonstrate thrombin activation via PAR-1 can be inhibited clinically.
- Result in net anti-thrombotic effect, but platelet activity for other activators is maintained (ADP, Collagen, Fibrin)
- Potential ability to block platelet activation during bypass, and preserve function post bypass.
- Aprotinin no longer available, but this study may pave the way for development of PAR inhibitors



Desmopressin:

- Synthetic vasopressin analogue
- Increases plasma levels of Factor VIII, vWF and tissue plasminogen activator.
- Has been looked at in multiple studies with mixed results, but studies **do not** justify the use of DDAVP as **prophylaxis** against bleeding associated with cardiac bypass.



Desmopressin:

- Studies do support use in patients with vW disease, mild Haemophilia A, or platelet dysfunction with response to test dose.
- Dosing is 0.3 mcg / kg and should be re-dosed at 6 hours.



Concluding Remarks:

- Coagulation is extremely complex.
- Platelets play a key role.
- CBP markedly disrupts this process.
- Ideally one could give a quickly reversible platelet inhibitor during CBP, and reverse it along with heparin thus protecting the platelet function.
- We will have to wait and see...





THE END.