

Influence of Massive Transfusion on Coagulopathy & Improved Safety

Jerard Seghatchian

- Exsanguination and uncontrolled haemorrhage within 6 hours are the leading cause of early death in traumatic injury. Early aggressive correction of coagulopathy and optimal resuscitation are aimed at restoring adequate blood volume within safe limit to allow: adequate haemostasis; optimal oxygen carrying capacity; oncotic pressure ; blood chemistry and reversing the lethal triad of hypothermia, acidosis and acute traumatic coagulopathy (ATC).
- Massive transfusion itself carries a significant mortality (40%), which increases with the number of units transfused. The challenge of ensuring safe & effective therapy to improve patient survival rates in massive transfusion, is ongoing. Controversy exists over the optimal ratio of blood components with respect to clinical outcomes and haemostatic effectiveness. Inadequate transfusion is associated with poor outcomes but empirical over transfusion result in unnecessary donor exposure with increase rate of sepsis, infusion of some BRMs and multi organ failure ,leading to rapid death.
- Avoidance strategies such as the restrictive use of blood components or use of early alternative therapies, such as tranexamic acid effects help(CRASH-2 Trial, 2010). However in massive transfusion the use of warm fresh whole blood [WFWB] instead of stored components, containing hosts of “Biological Response Modifiers”, might be the most effective and safer option to improve survival rate & minimise collateral damage.

Acknowledgments: Arthur Bode; John Freedman; Tor Hervig

Massive Transfusion : Back to Basics

- Massive Transfusion is the replacement of blood volume with:
- Litres of crystalloid to maintain MAP and perfusion of tissues in primary resuscitation. (Dilutive)
- Ten or more units of pRBC in first 24 hours to restore oxygenation of tissues. (dilutive and pro-inflammatory)
- Add-on of Platelet and/or plasma as bleeding continues, usually after first 5-6 units of pRBC. (functionality issues & BRMs)

Massive Transfusion:

Scale of Problems & Remedial Actions

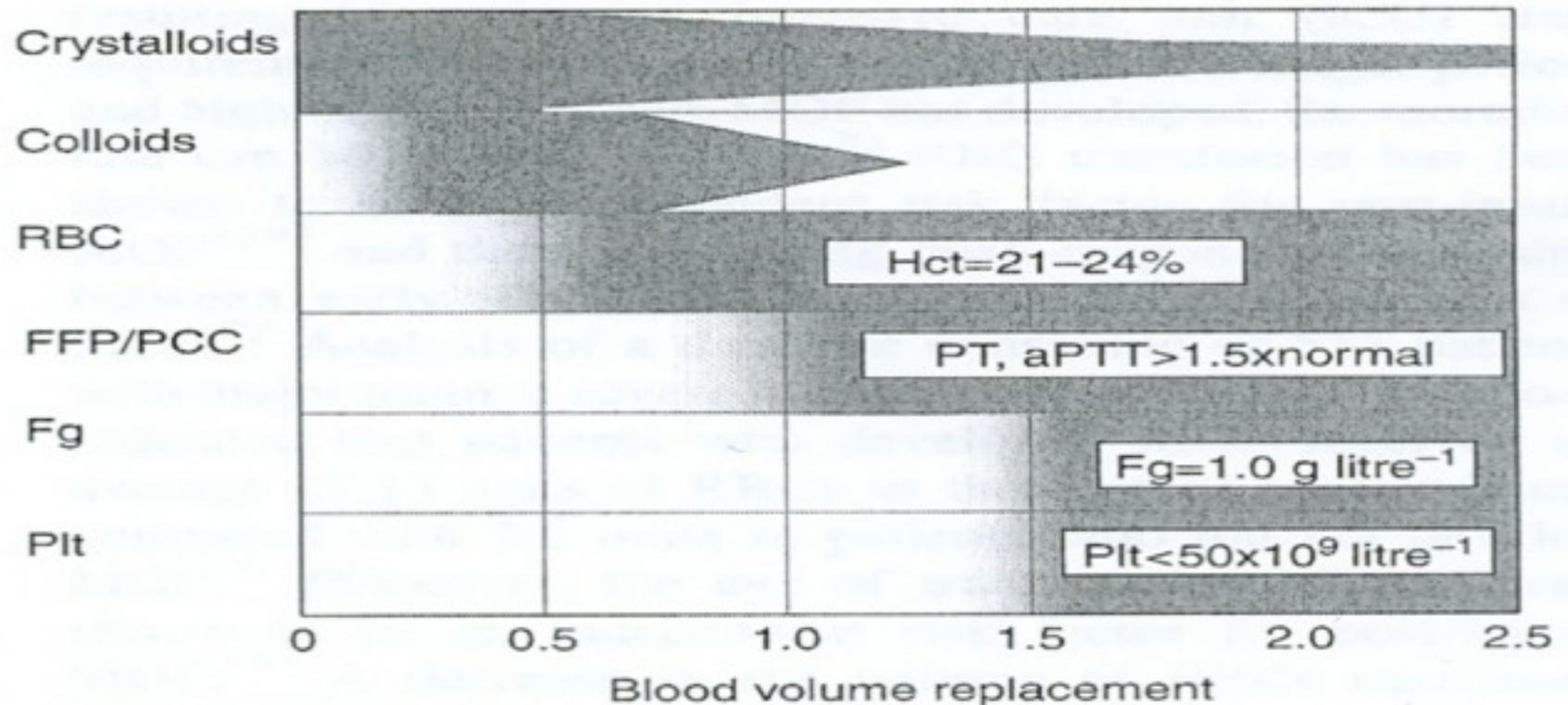
- Massive haemorrhage, in multiple traumatic injuries, is the most immediate threat to civilian / military injured persons (death within 3-6 hours). (timely actions matters!)
- Pre-hospital care is focused on the prompt control of bleeding and timely consultant led “Damage Control Trauma Resuscitation”.
- The early use of “ Shock Packs”, followed by “3Ps” of Best Clinical Practice: i) **P**atient Clinical Response; ii) **P**rovision of good Laboratory diagnostic Support; iii) **P**oint of care Testing.

- MT may be defined by volume or rate. A useful definition is >4U of RBC/hour, or 10U RBC in 24 hour period.
- Rapid use of blood components, apart from the usual risk of TTI/TR, may result both in infusion of various BRMs, present in large amounts in even "purest" stored components & serious volume overload.
- Less recognised risk is false serological laboratory results, due to previous use of ABO non-identical blood before patient transfer, leading to the anomalous ABO assignment.

The 3 Important pointers in Massive TX

- **State of the patient:** stages of trauma-induced coagulopathy, DIC, haemorrhagic shock, base deficit, hypothermia, hypoperfusion, poor tissue oxygenation, electrolyte imbalance, endothelial activation, Protein C activation, complement activation, platelet activation/refractoriness,, clotting factor consumption, hyperfibrinolysis pre-existing bleeding diathesis, on-board meds.
- **State of the bag content:** apart from provision of competent rbc & plt & plasma proteins, it also conveys leucocytes/over-aged apoptotic/fragmented cells or microparticles, imbalance of clotting factors vs regulators, degraded proteins, citrate, potassium, BRMs (i.e. C3a/ C5a, cytokines, etc), antibodies, bacteria, viruses, prions.
- **State of the union:** improved survival/safety.

The Conventional Medical Intervention: (ensure care delivered meets quality standard)



Protocol: Identify/manage surgical bleeding; prevent/reverse hyperthermia-acidosis and correct coagulopathy using “shock pack”. Consider: Calcium Chloride (1gm IV slowly); antifibrotic agents (Tranexamic acid 10mg/kg IV), DDAVP in uremia/vWD (0.3 ug/kg IV); Cell salvage as appropriate & rVIIa 4.8mg (one vial) if patient meets criteria acceptability and lack of contraindications.

Main Substances Used in MT:

1. Volume Expanders

- Liters of Crystalloid or Colloidal Solutions: (no cells or plasma)– normal saline or hypertonic saline, are **dilutive** but no citrate or active interference with haemostasis.
- Lactated Ringers (NaCl, Ca₂Cl, KCl, Na–Lactate) **dilutive**, no citrate but may aggravate neutrophil activation and cell injury unless L–isomer Lactate used.
- Dextran or Hespan (hypertonic NaCl with poly–dextrose or hydroxyethyl starch) as volume expander and “sealer of endothelium”, causes less **dilution** but significant neutrophil activation.

Alam HB and Rhee P; Surg Clin N Am 87: 55–72, 2007

Colloids Impacts on coagulation!

- HES infusion lead to significant falls in clotting factors, vWF:Ag & function and PLT mainly due to volume expansion & haemodilution, while fibrinolysis remains normal.
- Hetastarch produces significant abnormalities in some haemostasis laboratory results when infused at clinically relevant doses, without any clinical bleeding.

Strauss RG et al: Transfusion 42(1):27-36, 2002 (40-50)

2. Characteristics of RBC in Massive Transfusion:-dilutive& contain BRMs.

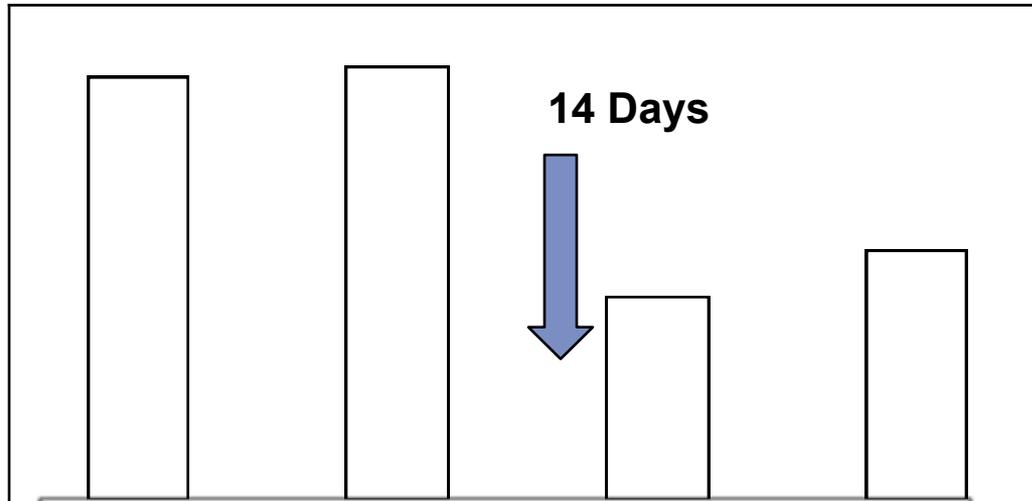
- **Packed RBC:** contains erythrocytes(1 – 120 days old) from 450mL blood packed to HCT > 80% in residual plasma volume of ~50mL.
- One unit of PRBCs typically will raise the haematocrit by 3–4% and the blood hemoglobin concentration by 1 g/d.
- RBC also contains some residual WBC/ PLT & microparticles, soluble antigens & pro-inflammatory cytokines, ~3grams of citrate, variable amount of free potassium & additive and substances leaching out from the packs. Citrate is metabolised in 5 minutes if liver is functional(hypocalcemia), but potassium may cause cardiac arrhythmia .

3. Impacts of Storage Lesion in RBC on MT

- Aged Packed RBCs have a limited ability to augment oxygen delivery to tissues. Patients NOT receiving such transfusions have significantly better outcomes. *Gould S et al; Am J Crit Care;16 (1):39-48, 2007.*
- Levels of Annexin V binding and procoagulant phospholipid activity (PS), toward the end of the storage period of PRBC units, are similar to levels seen in disease states associated with significant vasoocclusive pathophysiology. *Cardo LJ. et al; Transfus Apher Sci;38(2):141-7, 2008.*
- Fresher pRBC (<14 days) is better than aged products. *Spinella PC et al; Crit Care; 13:R151, 2009.*
- Ageing RBCs gradually expose pro-coagulant PS on their surface, as well as losing some adhesive membrane proteins (i.e. CD 47, measured by FACS) & parallel increase of Soluble CD 47 & soluble cytosolic Annexin V, in supernatant, measured by ELISA. *(Bessos,H. Seghatchian,J. Trasci , 2003).*

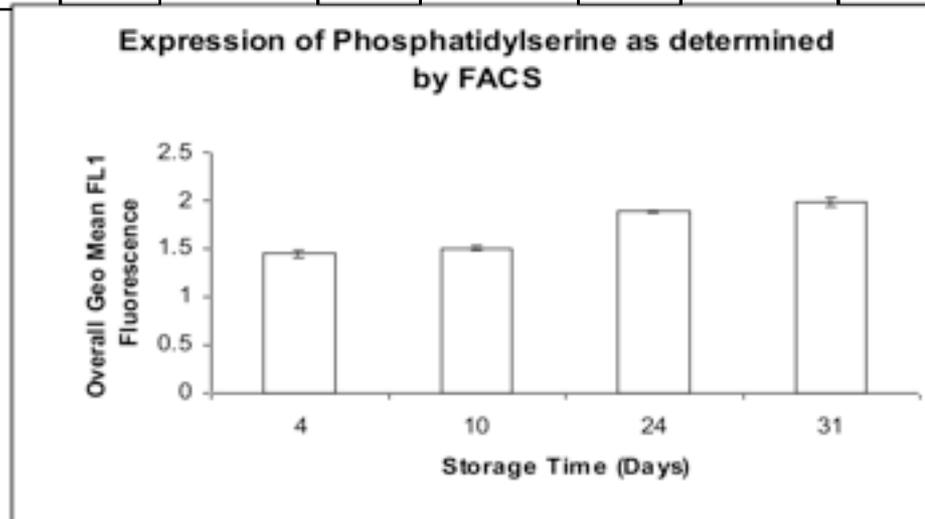
Fresher is Better in Vivo (*Spinella et al*) and in Vitro (*Bessos et al*)

• A



CD 47 decline,
on washed
stored LR-
RCCs

• B



Parallel
Increase in
PS
exposure/
apoptosis

Impacts of Anemia on Coagulation

- In non-thrombocytopenic animals the microvascular BT varied inversely with the haematocrit ($r = -0.799$). [*Blajchman MA et al; Br J Haematol 86(2):347-50, 1994*].
- In normal human subjects, the withdrawal of 2 u red cells produced a 60–percent increase in the Bleeding Time associated with a 15–percent reduction in the peripheral venous Hct. Reinfusion of these 2 units of RBCs restored the BT. Valeri RC et al; *Transfusion 41: 977–983, 2001*
- The low haematocrit is the main determining factor of the prolonged bleeding time in uraemic haemodialysed patients. Following red cell therapy, the bleeding time is normalized when the post-transfusion haematocrit was over 26%, with no change in platelet function tests or FVIII:vWF levels. [*Fernandez F et al; Br J Haematol 59(1):139-48, 1985*]

4. Characteristics of Platelets used in Massive Transfusion

- Platelet Concentrates: are produced from a single unit of blood or a pool of buffy coat concentrates or several type of cellular apheresis, with or without WBC filters.
- One transfusion dose = 3×10^{11} platelets. contains allogeneic platelets between 1 – 7 days old further aged in vitro up to another 5- 7 days in different storage media.
- PCs may also contain <5 million WBC; variable levels of PLT/RBC/WBC-derived microvesicules, cytokines & other BRMs , causing febrile reactions.
- Flourishing microbes (incidence of severe septic episodes is probably of the order of 1 per 50,000 platelet units transfused; *Blajchman, MA and Goldman M; Semin Hematol;38(4) Suppl 11:20-6, 2001.*

Impacts of Platelets on Coagulation in Massive Transfusion

- **Functionality of Stored Platelets (?):** rapid reduction occurs during storage in aggregation response except with multiple agonists; 20-30% plts lose most of the surface GPIb and are thus less adhesive; reduced thromboxane production and responsiveness; extensive vesiculation/fragmentation and release of procoagulant MP; decreased mean platelet volume; aberrant morphology (*Seghatchian [ed.]; Platelet Therapy. Amsterdam: Elsevier, 2000*).
- **supposed rejuvenation function in transfused stored platelets after 24 hr circulation** *Stein Holme and others*

Impacts of Platelets on Bleeding in MT

- **Professional Consensus:** prophylactic platelet infusion is not recommended beyond a loss of two blood volumes, in less than 24 hr & to reverse bleeding, platelet precede plasma infusion. [*Samama CM et al; Can J Anaesth; 52(1):30-7, 2005*]
- **Inference from outcomes:** In 466 MT trauma patients transported from the scene to 16 level, the 30-day survival was increased(20%) in patients with high platelet:RBC ratio ($\geq 1:2$) versus low ratio ($< 1:2$).
The combination of high platelet and plasma to RBC ratios increases 30-day survival, with no change in multiple organ failure death.
Infusion of platelets is intuitive, supported by broad measures. [*Holcomb JB et al; Ann Surg 248: 447–458, 2008*].

Are all PC Preparations Equivalent?: In terms of Cellular Lesion; BRMs; Immunomodulation!

- collection, processing and storage time/containers -induced increase in the rates of PS exposure & apoptosis; Changes in membrane surface antigens upon activation and development of soluble & MV- bound antigens(P-Selectin, Glycocalcin) & Release of cytosolic / granular content (Conventional and new generation marker of PSL, i.e. cytosolic soluble Annexin-V, TGF/RANTES, BRMs) leading to untoward events and immunomodulation.

Comparative Analysis of Selected Biomarkers on Various Types of Platelets

1. Complement

Products	C3a (ng/mL)	
	Day 1	Day 5
Non-LD BC	641 (362-1022)	3665 (1608-8747)
Autostop (-)	217 (84-458)	3269 (2509-4298)
PLX5 (+)	1107 (428-2070)	3758 (1861-8922)
Imugard II (N)	629 (339-933)	2673 (1480-6143)
Cobe LRS	472 (179-664)	2214 (1156-2865)
MCS+ LDP (-)	348 (121-533)	2045 (1437-3125)
Amicus	693 (469-1252)	1812 (891-2896)

C3a lowest in -ve filters, highest in +ve filter

2. WBC/ PLT-derived cytokines and soluble antigens / PS exposure in PCs

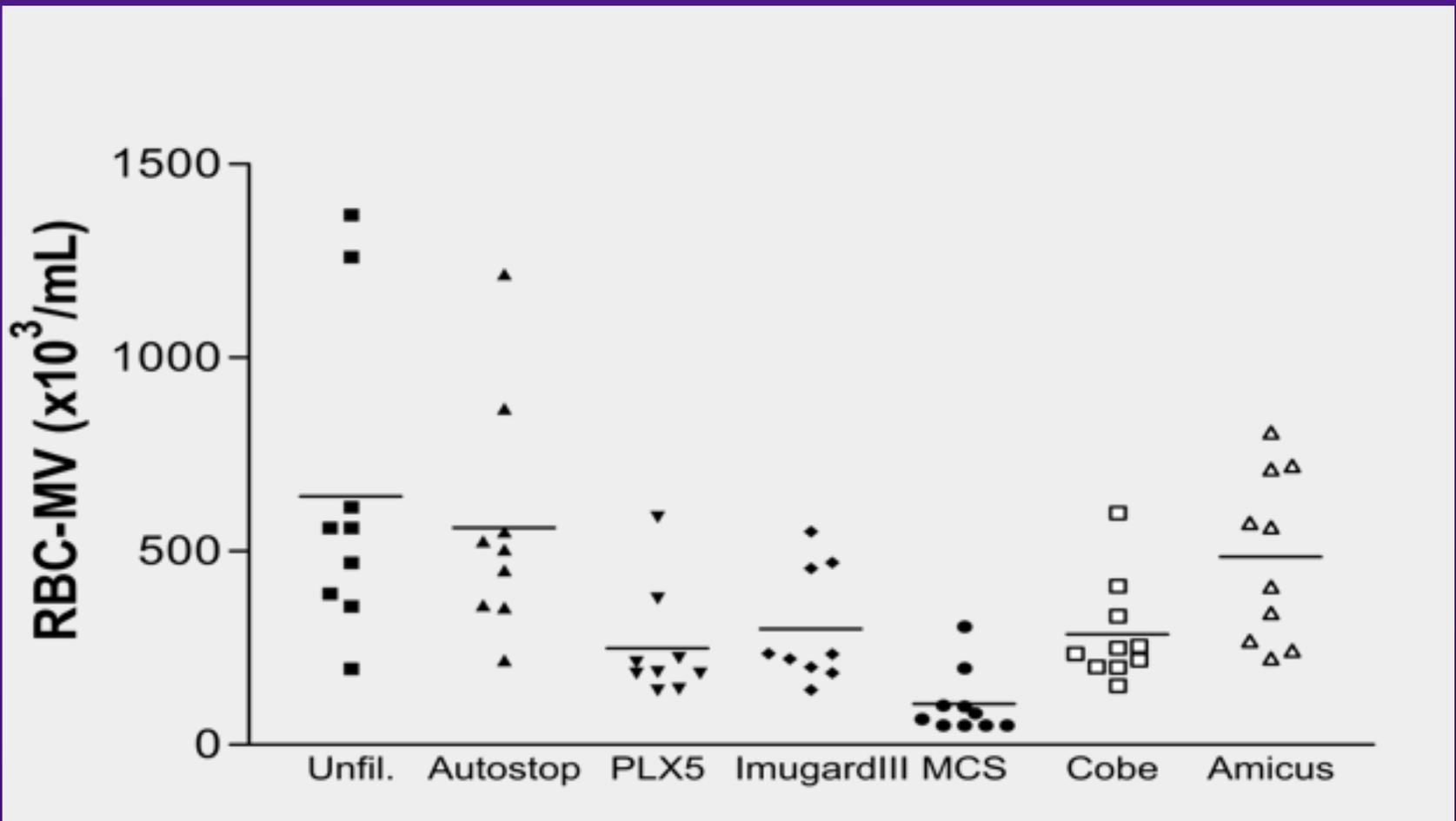
✿ IL8 is 4X higher in non – LR at day 5, lowest in Amicus. Leucofiltration almost abrogate IL-8.

✿ PLT-Derived RANTES & TGFB increase significantly at day 5, apheresis higher than BC-PC.

✿ Soluble HLA: the rate increased higher in LRS-PC than filtered- PCs (0.12 versus 0.05 ug/ml/day).

✿ Good correlation between PS exposure and MV ($r=0.8723$, $p<0.0001$) & release of soluble cytosolic Annexin -V, a new marker of cellular storage lesion.

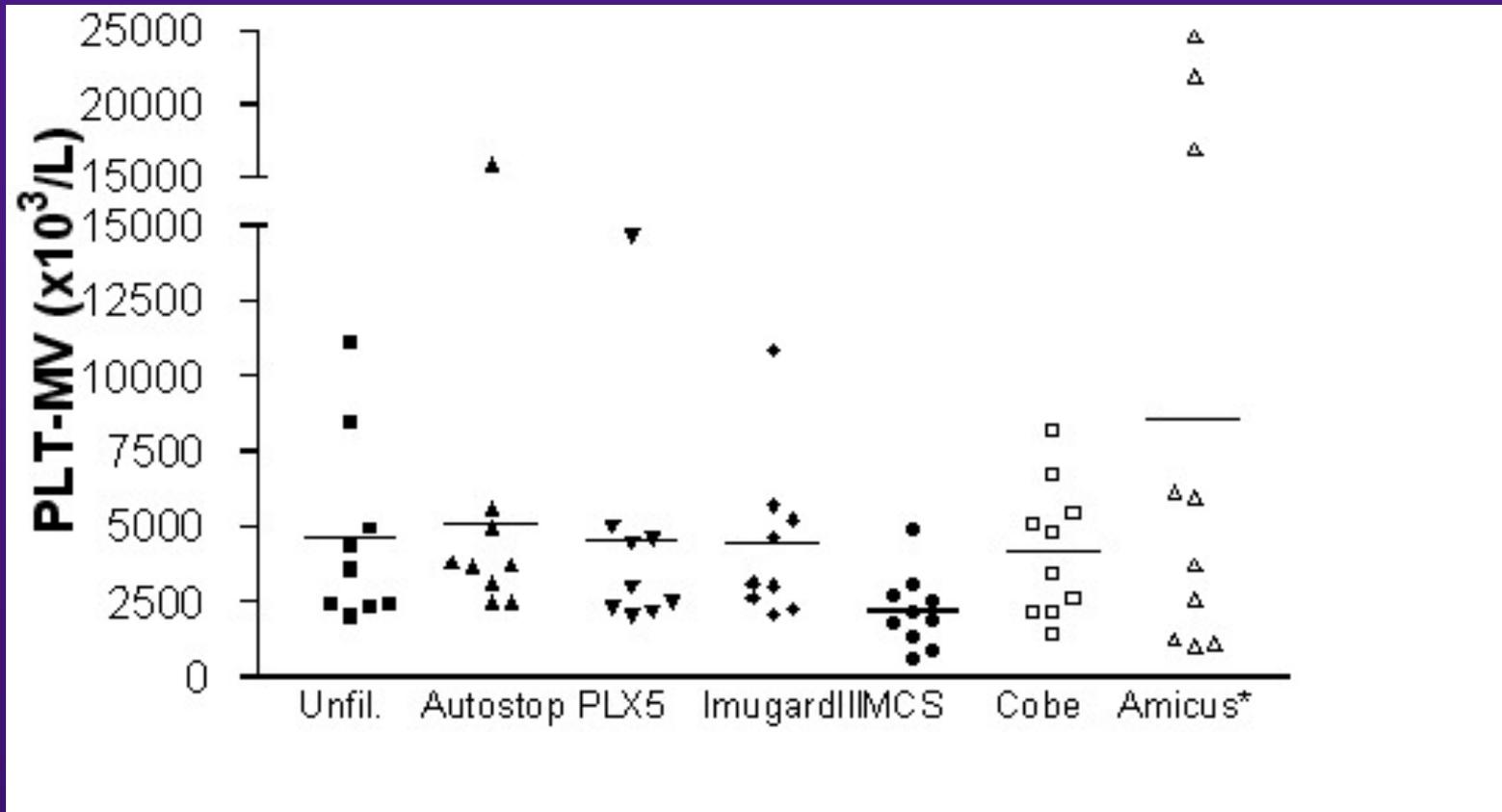
3. RBC - derived Microvesicles in PCs



Significant difference ($P < 0.0001$) exists between groups.

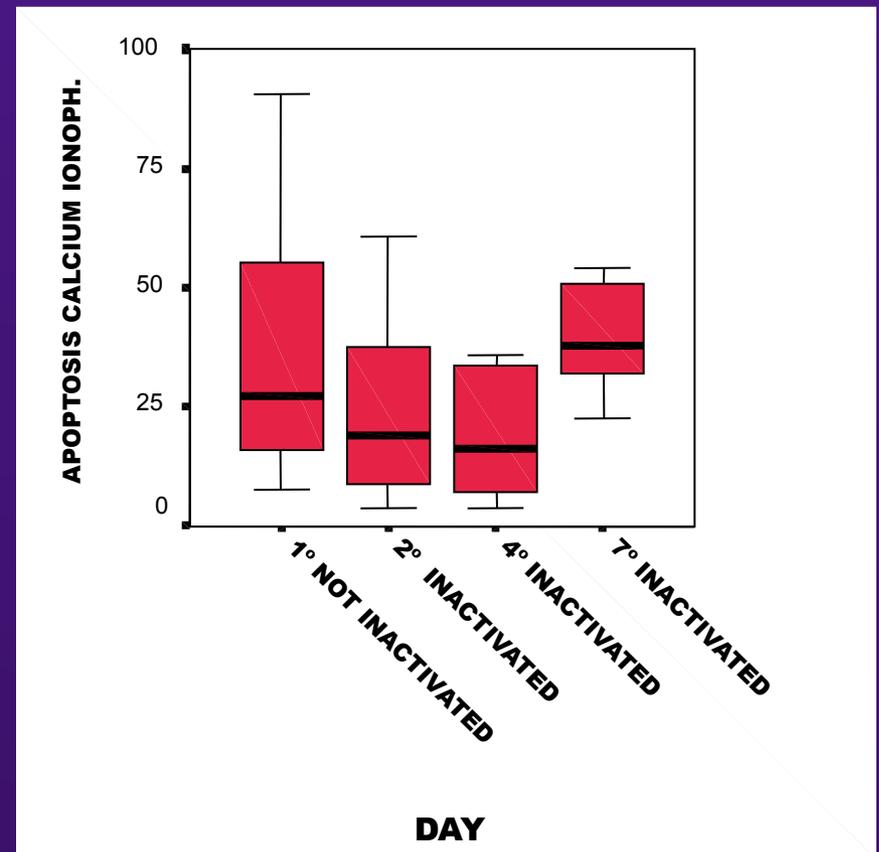
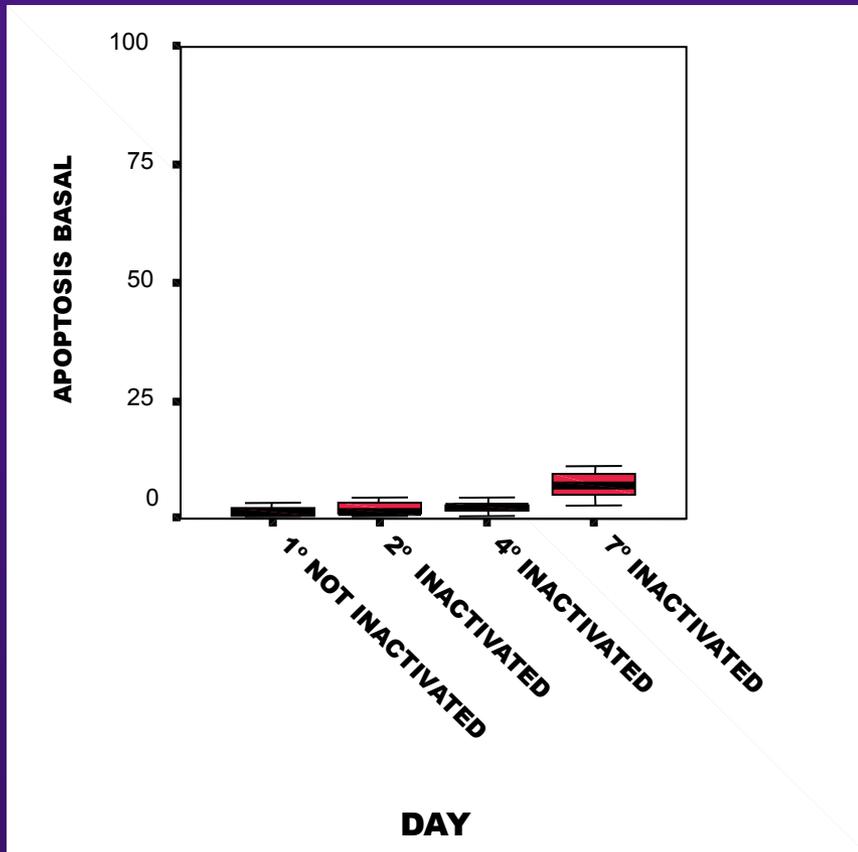
MCS+LDP the lowest.

4. PLT- derived Microvesicles in PCs



Significant differences exist between seven groups, with BC method lower than Apheresis, except MCS+LDP.

Effect of Intercept-PRT on platelet PS exposure/ apoptosis(Basal) and after stimulation Ca-ionophore :



Similar patterns of changes seen in Mirasol and emerging UVC treated platelets

PS- Exposure, Microvesiculation, Apoptosis TGF Beta Release: *are they useful markers of PSL& immunomodulation?*

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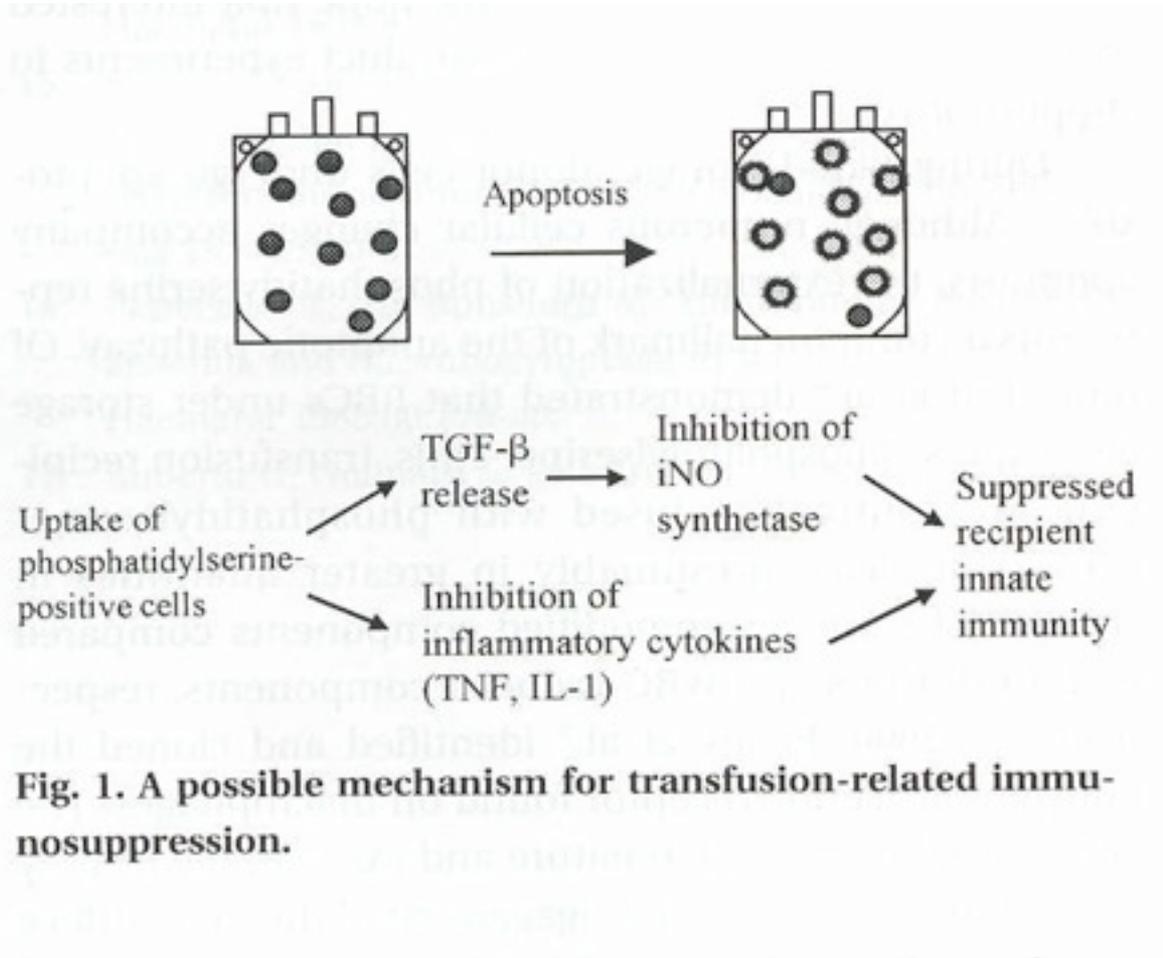
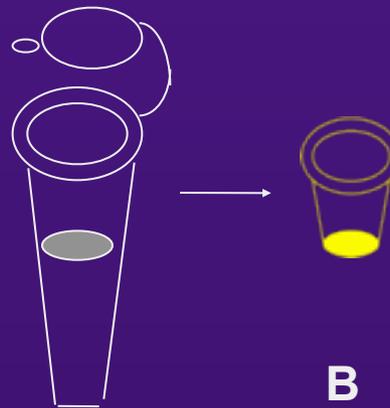


Fig. 1. A possible mechanism for transfusion-related immunosuppression.

Pro-coagulant / Anti-Coagulant activities of cell-free / plasma-free microvesicles: A novel practical approach, based on micro centrifugation & ultrafiltration

Pro-

FXa 0.05 U/mL
FVa 0.20 U/mL
Prothrombin 0.10 U/mL
CaCl₂ 2 mM



A

Anti-

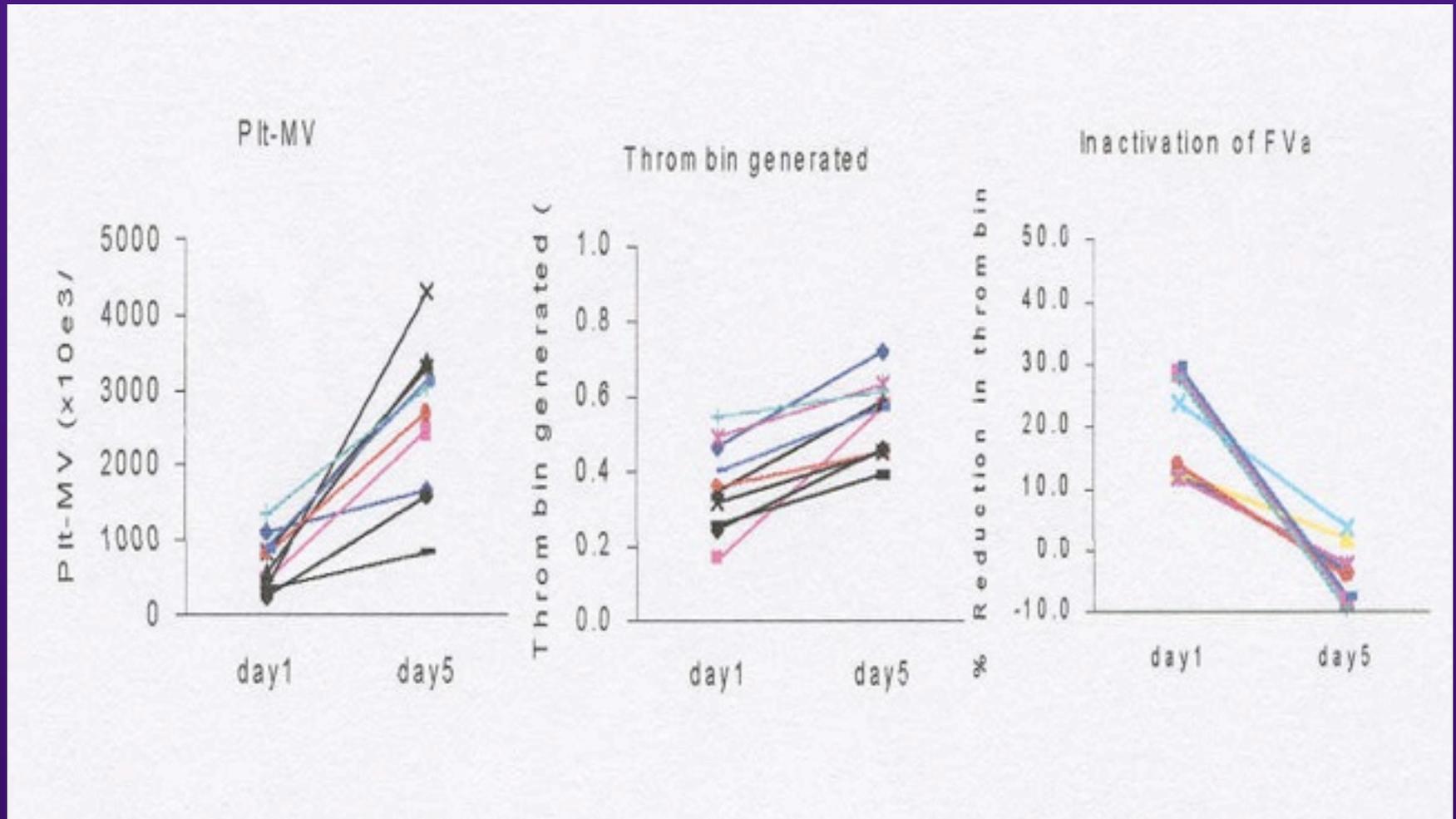
APC 0.16 nM
FXa 0.05 U/mL
FVa 0.20 U/mL
Prothrombin 0.10 U/mL
CaCl₂ 2 mM

B

Chromogenic substrate S2238 0.25 mM

A centrifugal ultrafiltration unit (A) containing a receptacle (B) with 0.1 μ filter in the bottom

Storage-induced increased platelet microvesicle (Plt – MV) with concomitant increase in pro- and decrease in anti-coagulant levels : LRS -PC



5. Characteristics of Plasma used in MT

Massive Transfusion

- **Fresh Frozen Plasma (FFP):** 200–250 mL plasma separated within 8 hours from a unit of citrated whole blood or collected by an apheresis procedures, and placed in a freezer within 8 hours.
- Variants include “recovered plasma” if frozen after 8 hours but before 24 hours after collection, and “thawed plasma” if FFP bag is thawed and kept at 1 to 6°C for up to 5 days for use.
- Supposed to carry 1u/mL each coag factor and regulator, actually quite variable since from individual donors & moderately low in FV and FVIII especially “thawed plasma”, –dose of 10mL/kg should raise clotting factor levels by 15% (one unit increases factors by 5%).
- predisposes for TRALI and TACO, contains enough citrate to need countering with 10mg/kg CaCl₂ or risk of hypotension, often not pathogen-reduced.

Impacts of FFP in Massive Transfusion & Dilutional Coagulopathy

- FFP is used in preoperative or bleeding patients who require multiple coagulation factors (eg, liver disease, DIC) or undergoing MT with significant coagulation deficiencies (not approved for strictly volume replacement).
- In the prospective observational cohort study of trauma patients in U.K. Blood was drawn before and after each 4-U PRBC interval for prothrombin time and analysis by rotational thromboelastometry.
- All clotting factors deteriorated with low FFP:PRBC ratios (<1:2) & Maximal haemostatic effect was observed in the 1:2 to 3:4 group of patients with coagulopathy. [*Davenport R et al; J Trauma;70: 90–96, 2011*]. This is supported by German trauma survival study [*Borgman MA et al Vox Sang 2011*] & Army Surgical Records [*Holcomb JB et al; Ann Surg, 2008*].

Impacts of FFP on Endothelium

- Endothelial cell culture(EC) permeability was induced by hypoxia and assessed by the passage of 70-kDa Dextran between monolayers. Thawed FFP reversed EC permeability by 10.2-fold Protective effects diminish (to 2.5-fold) by day 5.
- Fresh(day 0) FFP is also superior to day 5 FFP in better maintaining mean arterial pressure.
- FFP has also beneficial effects on endothelial vascular stability, and resuscitation after HS, independent of haemostasis. (*Pati S et al: J Trauma-Injury Infection & Critical Care 69 (1): S55-S63, 2010*)

6. Impacts of Fresh Warm Whole Blood (FWWB) on Massive Transfusion

- Fresh warm (non-refrigerated) whole blood is the best combination of blood elements for massive transfusion.
- After cardiopulmonary bypass, one unit of FWWB restored platelet aggregation on extracellular matrix to preoperative status (3.0 ± 1.0), whereas eight platelet units were needed for the same result (3.2 ± 0.8).
- One unit of FWWB increased platelet count in a manner similar to that achieved by six platelet units and mean platelet volume increased to a level higher than that achieved by 10 platelet units.
- Overall the effect of one unit of FWWB, on platelet aggregation after cardiopulmonary bypass, found to be equal/superior, to the effect of 8 to 10 platelet units.

.(Lavee J et al; J T C S Vol 97: 204-212, 1989).

Summary and Scope for Panel Debate

1. In most trauma cases death occurs within 24-48 h (Deaths: 5 million/yr). *Uncontrolled haemorrhage is challenging but preventable.*
2. Massive fluid infusion leads to lethal triad of hypothermia, acidosis and acute traumatic hypocoagulopathy & increased mortality due multi-organ failure. *What IS the benefit?*
3. Tx-circulatory overload, in intense care units, occurs in [1:350]. *Was it all necessary and based on clinical evidence?*
4. Life saving blood is not a “benign substance”, having adverse effects mitigating its benefit. *But you can't live without it !*

5. Inflammatory events and lung injury are the most frequent types of serious adverse reactions of aged components [1:1000- 1:5000]. *Is this due to BRMs?*
6. Increased ratios of PC/ FFP to RBC, independently improve survival, while the amount of transfused RBCs decrease survival. *More is better? Or is it balance?*
7. Warm Fresh WB, with limited storage lesion, is more efficient than older cold stored or reconstituted 1:1:1 blood. *But is it available & the optimal ratio?*
8. The evidence-based “ Quality Surveillance” in Massive Transfusion is essential for better understanding both patient safety & the potential cause of adverse events.

For Massive Transfusion, do we go back to Fresh WB?

Is this the best we can do?



Warm
Fresh
Whole
Blood?

Or what is the future?

Dried Plasma



Platelet farms



HBOCs

Stem cells?