Clinical Effects of the RBC Storage Lesion

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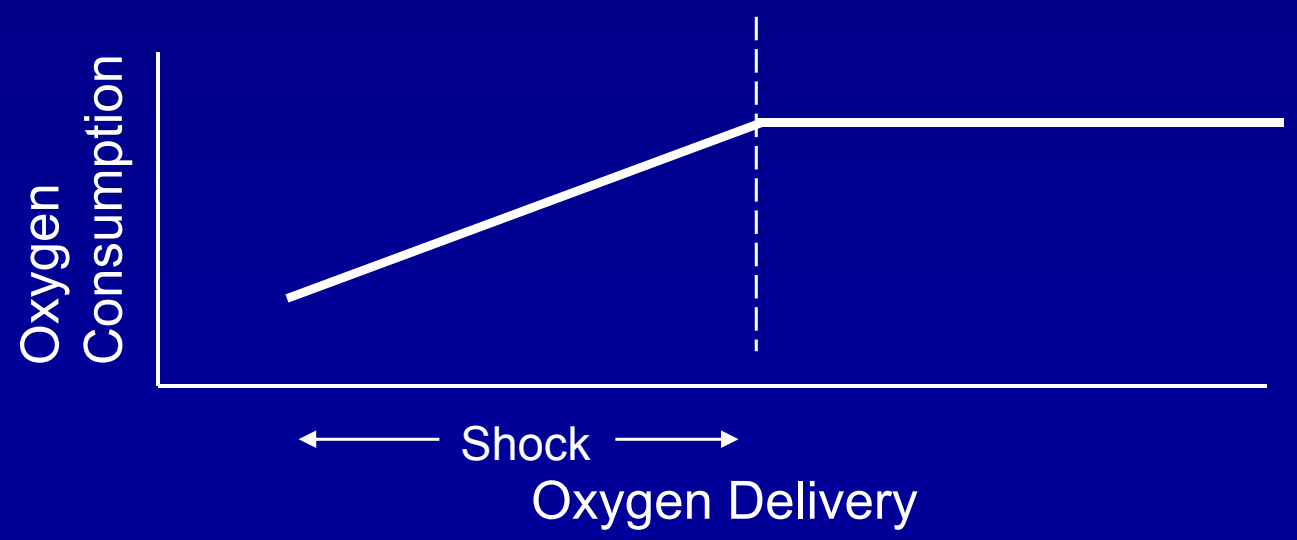
Why Transfuse RBCs?

- Increase 02 delivery to microvasculature to reverse shock state (oxygen debt)
  - Volume expansion & increased cardiac output
  - Hemoglobin delivery oxygen to microvasculature
- Evidenced by increased 02 consumption for patient in oxygen debt or shock



FDA requirements for RBC storage solutions

- Determined by RBC cell membrane viability
  - 75% viability, 24 hrs after transfusion
  - ATP, free hemoglobin, 2,3 DPG
- Approval Not Based On
  - Direct oxygen delivery or consumption ability
  - Inflammatory, immunological, vasoregulatory or coagulation side effects

RBC storage age

- Current solutions approved to 42 days
- Current storage solutions in development
  - Increase storage to 56 days
  - Not being tested for direct ability to deliver oxygen to microvasculature
  - Or for adverse inflammatory or immune effects
- FDA does not require this

Mean or Median RBC Storage Age Upon Transfusion

- US Civilian Hospitals
  - Mean RBC age: 17-21 days [1,2]
- US Trauma Centers (Denver Health Medical Center) [4]
  - Mean RBC age: 28 days,
  - 25% of RBCs > 35 days
- US Military Hospitals – Massive transfusion
  - 2004: Median RBC age: 33 days [3]
  - 2009: Mean RBC age: 29 days

1 Corwin, HL, Crit Care Med, 2004.
2 THE 2005 NATIONWIDE BLOOD COLLECTION AND UTILIZATION SURVEY REPORT
3 Spinella, PC, Crit Care Med, 2007.
4 Zallen G, Shock, 2000;13(1):29-33

RBC Distribution Pattern

- After collection
- Community Hospital first
- Then if not used: old RBCs are sent to trauma center or medical center
  - High rate of use at trauma medical center
  - Guarantees use of RBCs
  - Minimizes waste

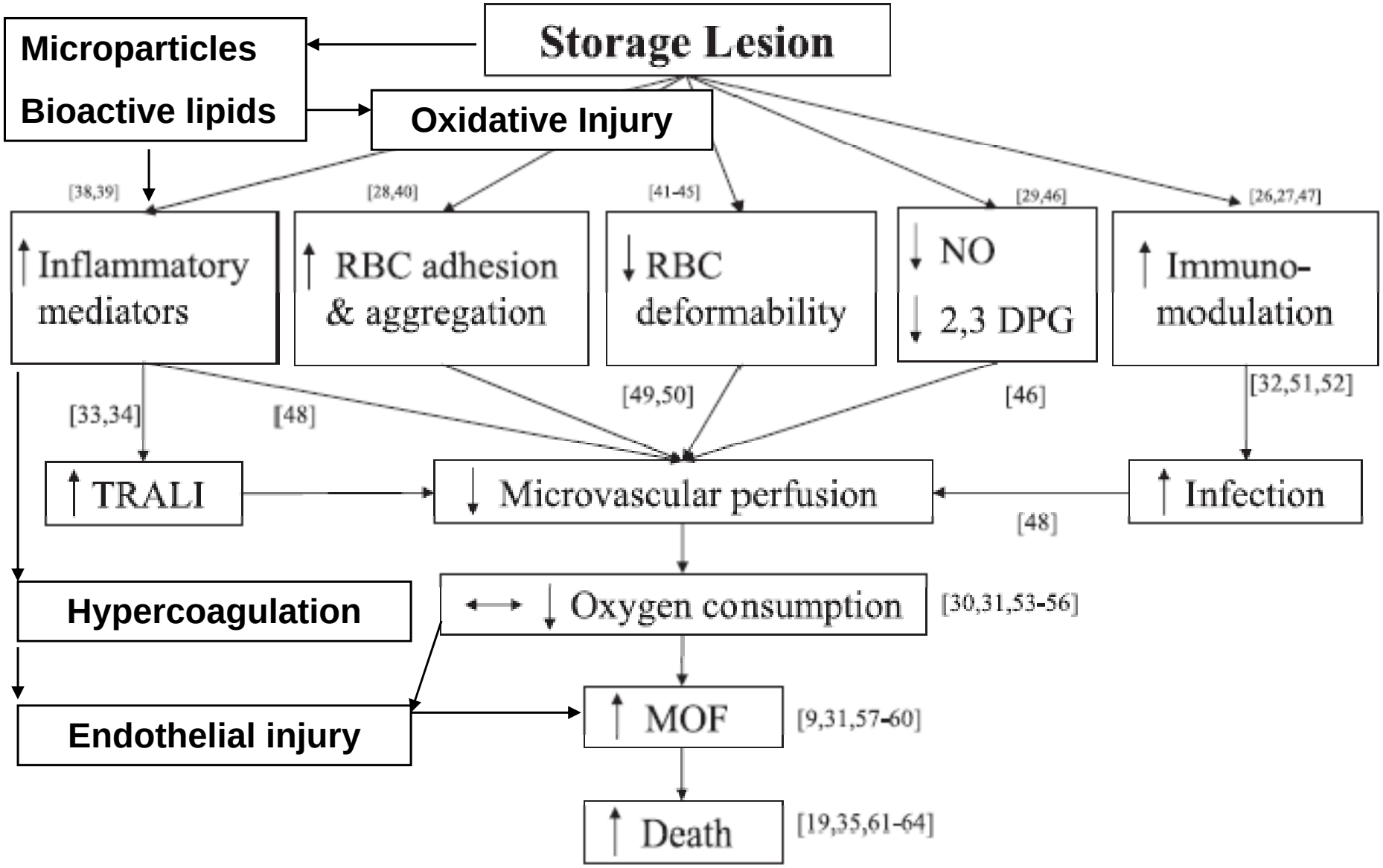
Scary Part

- No evidence that RBCs > 14 days
  - Increase oxygen consumption for patients in shock
  - RBC can be stored for up to 42 days
- Standard approach is to give the oldest to minimize waste
- Sickest patients get the oldest blood since they use the most

Risks associated with fresh whole blood and red blood cell transfusions in a combat support hospital

Philip C. Spinella, MD; Jeremy G. Perkins, MD; Kurt W. Grathwohl, MD; Thomas Repine, MD; Alec C. Beekley, MD; James Sebesta, MD; Donald Jenkins, MD; Kenneth Azarow, MD; John B. Holcomb, MD; 31st Combat Support Hospital Research

Crit Care Med 2007 Vol. 35, No. 11



Why Fresh RBCs for Shock?

- Improved function
  - 1 day RBCs have improved microcirculatory perfusion compared to 21 day RBCs
    - Arslan, E., Am J Surg, 2005. 190 (3): p. 456-62.
  - 3 day RBCs increase 02 consumption, 28 day do not
    - Fitzgerald RD. Crit Care Med. 1997; 25(5): 726-32

Why not Old RBCs for Shock?

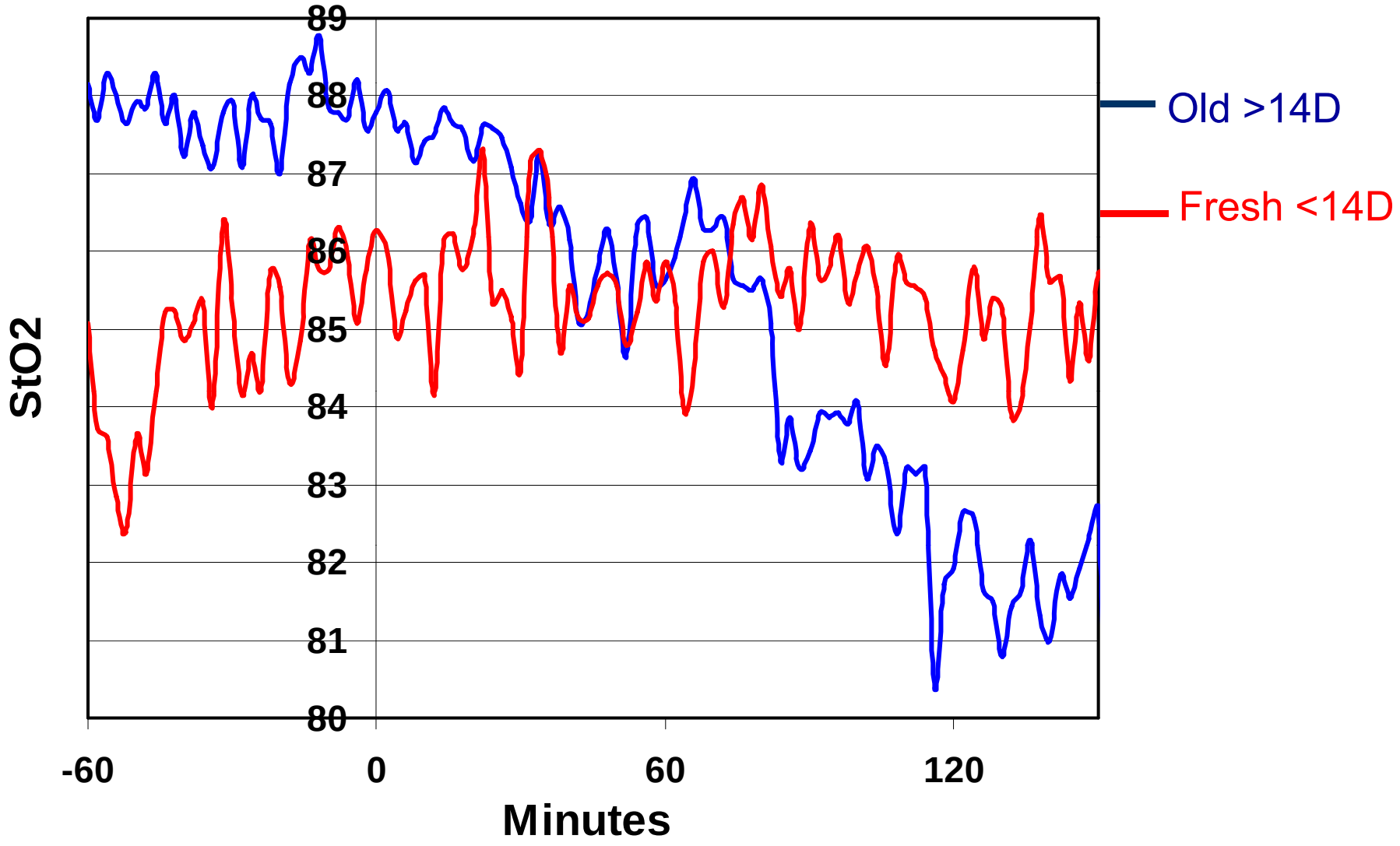
- Increased inflammation with old RBC[1]
  - Day 0 vs Day 21
- Increased oxidative injury with old RBC[2]
  - Day 0 vs Day 42
- Increased abnormal apoptosis with old RBC[2]
  - Day 0 vs Day 21
- Increased risk of hypercoagulation[3]
  - Day 4 vs Day 42

1 Zallen G. Shock. 2000. 13;1, p29-33

2 Biffl WL. J Trauma. 2001. 50;3, p426-432

3 Cardo LJ. Trans Apher Science. 2008.141-147

Comparison of Old and Fresh Blood in Trauma Patients



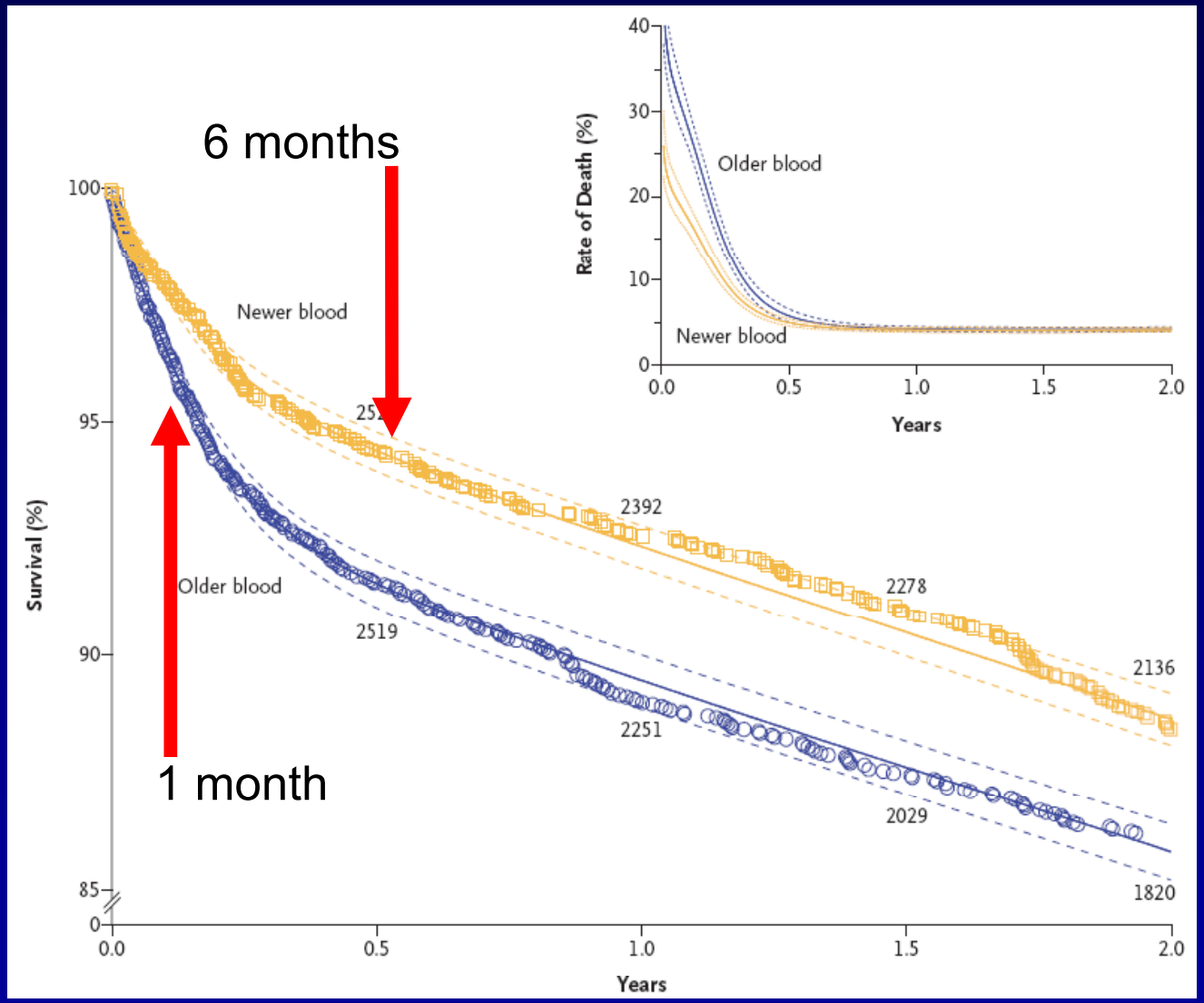
Kiraly LN, Schreiber MA. J Trauma. 2009 Jul;67(1):29-32.

>14 days of RBC storage

- Increased RBC aggregation [1]
- Decreased 2,3 DPG [2]
- Increased free hemoglobin, decreased NO [3]
- Increased inflammatory mediators [4]
- TRALI [4]
- Severe infection [5]
- Decreased O2 delivery[6,7]
- MOF [8,14,15]
- Mortality [9-13,16]

1 Hovav. Transfusion 1999 39(3) 277-81
2 Marik, P.E. JAMA. 1993. **269**(23): p. 3024-9
3 Nishiyama. Can J Anesthesia 200 47 (9):881-5
4 Silliman, C.C. J Lab Clin Med, 1994. **124**(5)
5 Offner PJ, Arch Surg 2002;137:711-71
6 Marik PE. JAMA. 1993; 269(23): 3024-29
7 Leal-Noval SR. Crit Care Med. 2008;36:1290-96
8 Zallen. Am J Surg. 1999; 178:570-72
9 Purdy FR. Can J Anaesth. 1997; 44:1256-61
10 Basran. Anesth Analg, 2006. **103**(1): p. 15-20
11 Weinberg JA. J Trauma, 2007 63(2):480
12 DeSimone N. Anesth Analg. 2005,100,SCA1-116
13 Koch CG. NEJM 2008. 35812;1229-39
14 Gauvin F. Submitted
15 Karam O. Submitted
16 Spinella. Submitted

How long does effect of old RBCs last ???



Koch CG. NEJM 2008. 35812;1229-39

6 Studies that report no difference between fresh and old RBCs

- Ventilator Days [1-3]
- Hosp LOS [2,3]
- Splanchnic ischemia[4]
- Mortality [5]
- Neurocognitive deficits [6]
- **Studies are underpowered to detect differences noted or are in non- critically ill populations**

1 Vamvakas Transfusion 1999;39:701-710
2 Vamvakas Transfusion 2000; 40:101-109
3 Leal-Noval Anesthes 2003 ;98:815-22
4 Walsh Crit Care Med 2004; 32(2):364 –371
5 Van Watering Transfusion 2006; 46:1712-1718
6 Weiskopf RB. Anesthesiology 2006;104(5):911-20

No Prospective level 1 evidence Old RBCs are harmful

- No studies of RBCs of >14 days indicate ability to increase:
  - 02 consumption or pO2 to microvascular bed in critically ill patients
  - True for any critically ill population

Criticism of data on RBC age and mortality

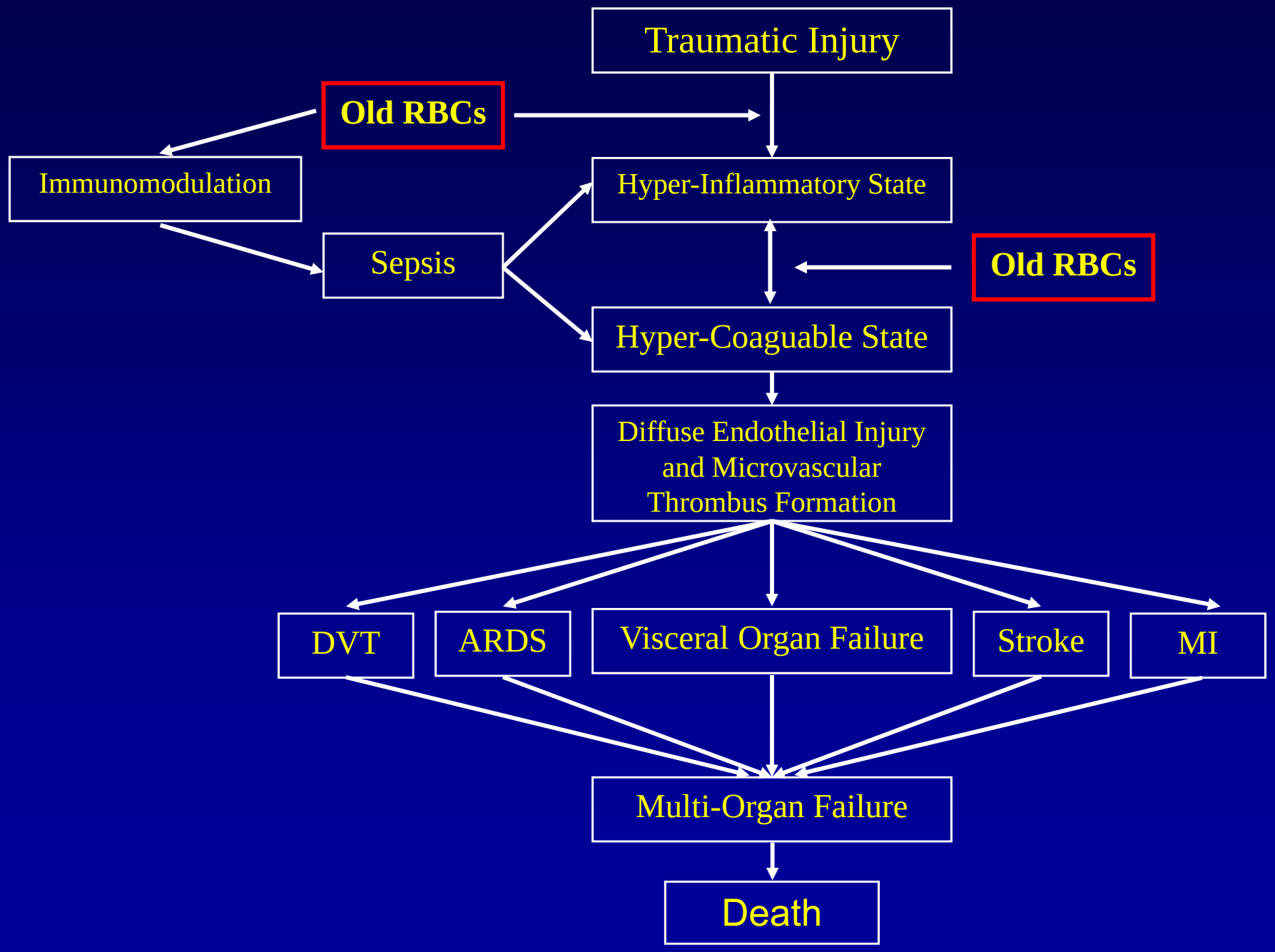
- Non leukoreduced cells in most clinical studies
  - Leukoreduction ability to improve survival controversial
  - Old LR RBCs: indep assoc with MOF and mortality[2-4]
- Patients with older RBCs also got more RBCs
  - Concern than regression cannot adjust for RBC volume
  - Interpretation: Old RBC group died more due to RBC volume and not age of RBCs

1 Phelan HA. J Surg Res. 2006
2 Weinberg JA, J Trauma Sept 08, 65(2): 279-284
3 Gauvin F. Submitted, Blood
4 Karam O. Submitted, Transfusion

Study of RBC age effect on DVT and mortality for patients matched by RBC amount

- Hypothesis
  - Older RBCs will increase risk of
    - DVT
    - In-hospital mortality
    - Increased death from multi-organ failure
  - In critically ill trauma patients
    - Transfused 5 or more units of RBCs
    - Matched by RBC amount

Theory


Traumatic Injury
Old RBCs
Immunomodulation
Hyper-Inflammatory State
Sepsis
Old RBCs
Hyper-Coaguable State
Diffuse Endothelial Injury and Microvascular Thrombus Formation
DVT
ARDS
Visceral Organ Failure
Stroke
MI
Multi-Organ Failure
Death

Stored packed red blood cells contain a procoagulant phospholipid reducible by leukodepletion filters and washing

Lisa J. Cardo *, Peter Hmel, Donna Wilder

L.J. Cardo et al. / Transfusion and Apheresis Science 38 (2008) 141–147

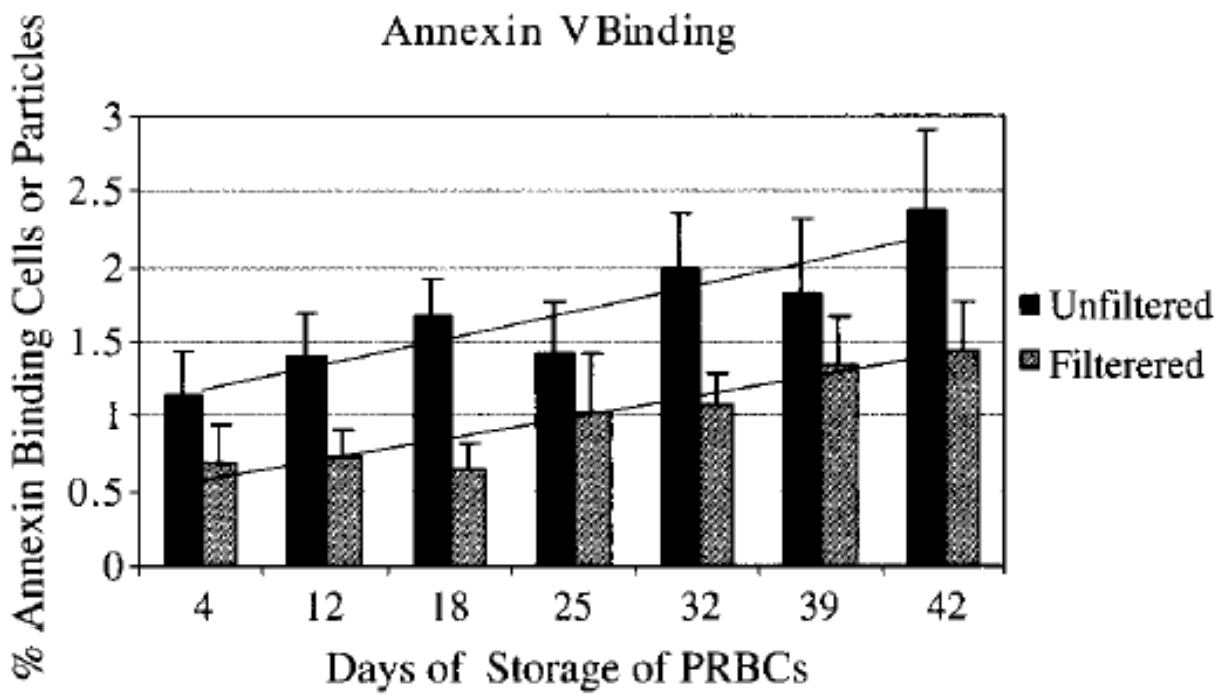


Fig. 2. Ten units of PRBC were divided into two aliquots producing 10 filtered and 10 unfiltered half units which were sampled at weekly intervals for 42 days. Binding of annexin V to cells and particles was measured by flow cytometry.

Methods

- Retrospective: Jan 2004 to Dec 2007
- Included:
  - Trauma patients ≥16 yrs of age
  - Transfused 5 or more units of RBCs
  - RBCs were either non-reduced or leukocyte reduced
- Excluded
  - Died acutely in ED or OR prior to ICU admission

Methods

- Primary outcome
  - In hospital mortality
- Secondary outcome
  - DVT
    - Routinely screened for in all trauma patients
  - Cause of death
    - Determined by chart review from one investigator

Methods

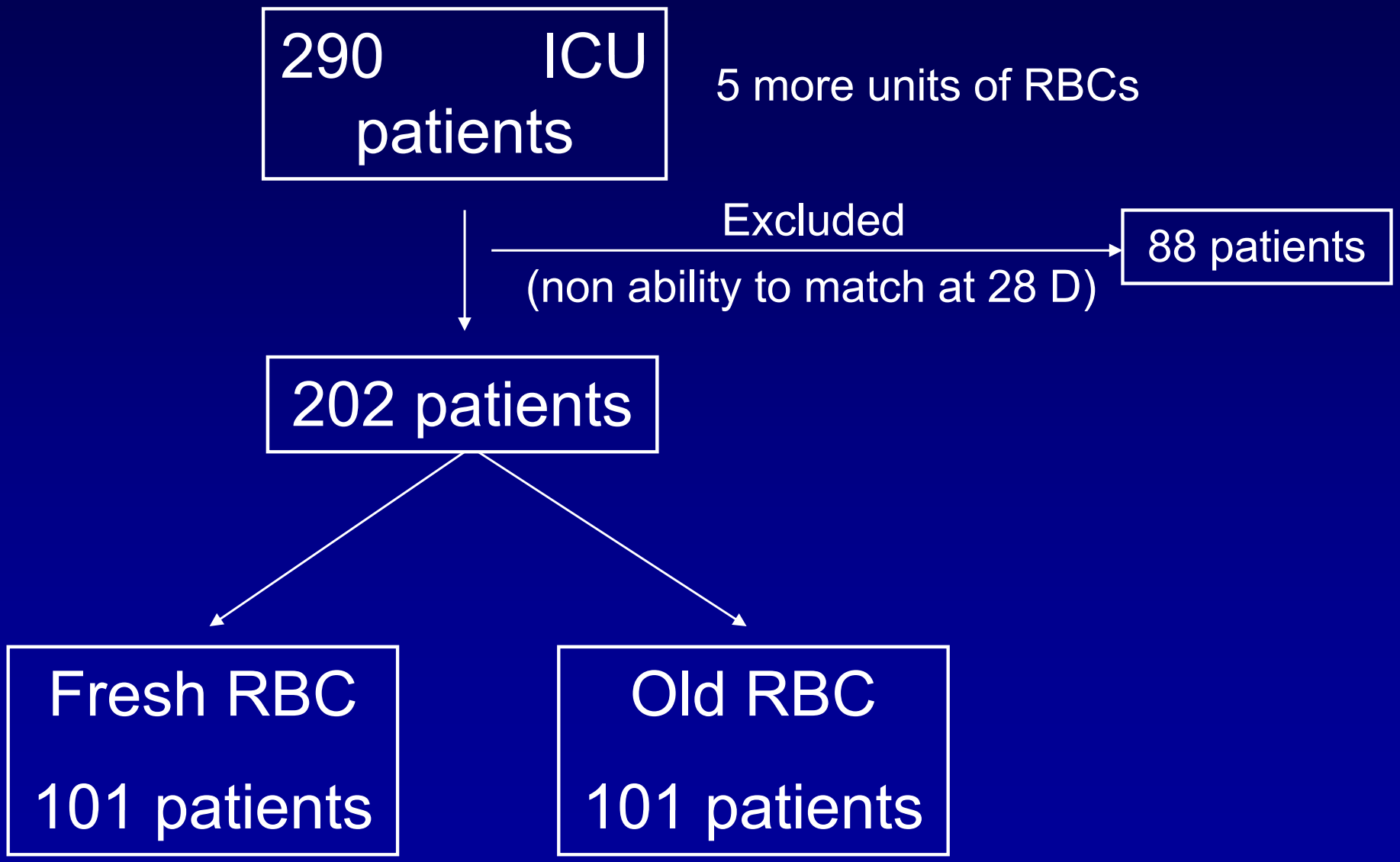
- Fresh vs Old RBC groups
  - Defined by maximum RBC age
    - 14 days
    - 21 days
    - 28 days

Outcome and max RBC age	*N =*	*Fresh RBC Group*	*Old RBC Group*	*Absolute Difference (%)*	*p-value*
DVT*					
± 14 days	50	12.0%	32.0%	20.0	0.09
± 21 days	159	17.1%	31.2%	14.1	0.04
± 28 days	183	16.7 %	34.5%	17.8	0.006
Mortality					
± 14 days	56	17.9%	21.4%	3.5	0.73
± 21 days	176	18.2%	25.0%	6.8	0.27
± 28 days	202	13.9%	26.7%	12.8	0.02

Statistical Analysis

- Patient groups were matched by RBC amount (+/- 1 unit)
- Logistic regression used to adjust for confounding and to determine independent associations with in hospital mortality
  - Backwards stepwise method
  - Best fit model according to the log likelihood between models

Results


290 ICU patients
5 more units of RBCs
Excluded
(non ability to match at 28 D)
88 patients
202 patients
Fresh RBC
101 patients
Old RBC
101 patients

Results

Comparison of admission variables between study groups

Variables	Fresh (n=101)	Old (n=101)	P-value
Age	48 (27-60.5)	45 (27-63)	0.83
Male %	78/101 (77.2%)	73/101 (72.3%)	0.42
GCS	14 (3-15)	14 (3-15)	0.48
SBP	126 (103-141)	123 (99.2-143)	0.57
Heart Rate	100 (80-120)	99 (79.5-120)	0.57
Temp (F)	96.5 (95.6-97.4)	96.5 (95.2-98.0)	0.75
Blunt injury	90/101 (89.1%)	97/101 (96.0%)	0.05

Results

Comparison of admission lab values between study groups

Variables	Fresh (n=101)	Old (n=101)	P value
HCO3	21 (19-23)	21 (19.2-23.8)	0.81
pH	7.3 (7.2-7.4)	7.3 (7.2-7.4)	0.37
PT	13.0 (12.2-14.5)	13.2 (12.2-14.2)	0.91
Hematocrit	36.9 (32.9-39.2)	36.1 (31.1-39.6)	0.32
ISS	24 (14-34)	24 (13.5-33.5)	0.82

Results

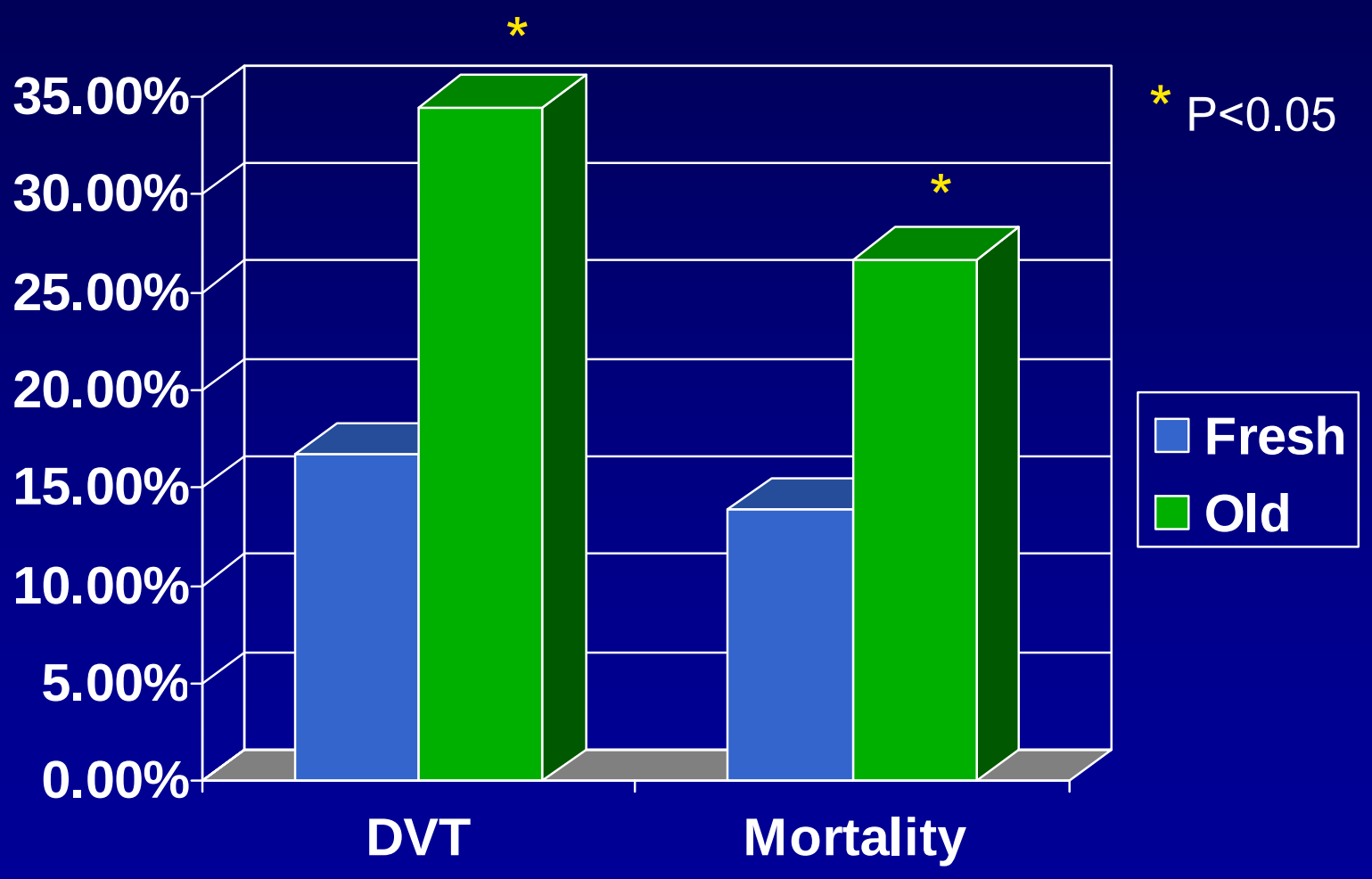
Variables	*Fresh (n=101)*	*Old (n=101)*	*P value*
Heparin IV (%)	13.9%	18.8%)	0.34
Heparin SC (%)	47.5%	50.1%)	0.67
Enoxaparin SC (%)	20.8%	24.8%	0.50
Pneumatic compression device (%)	78.2%	71.3%	0.26
Long bone fracture (%)	45.5%	47.5%	0.78
Spinal cord injury (%)	5.0%	9.9%	0.28

Results

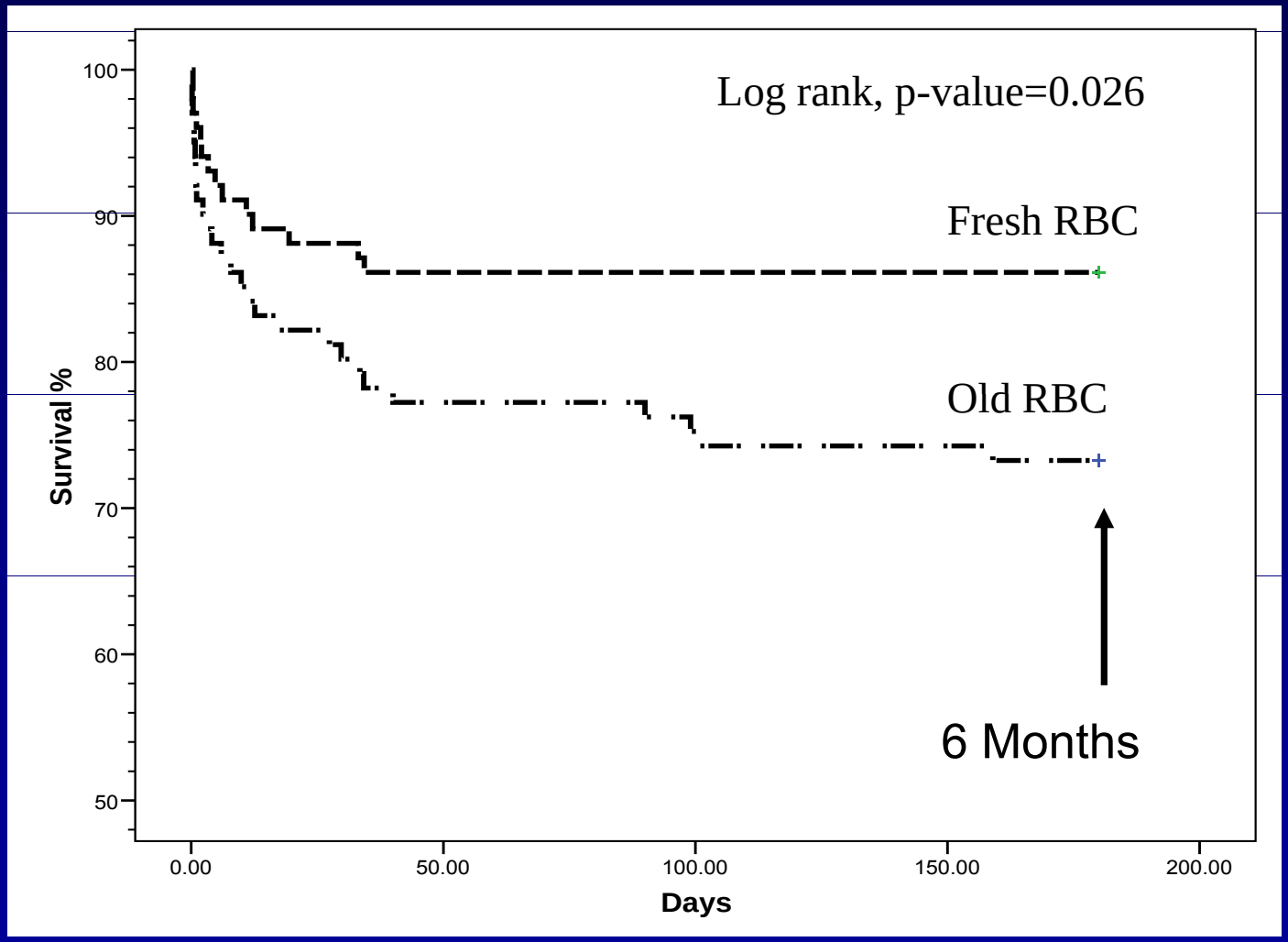
Comparison of blood products between study groups

Variables	Fresh (n=101)	Old (n=101)	P value
RBC amount	**9 (6-12.5)**	**9 (6-12)**	**0.95**
RBC maximum age	**19 (16-24)**	**34 (31-38)**	**<0.001**
RBC median age	**14 (11-17)**	**20.5 (15.5 - 26.0)**	**<0.001**
RBC leukoreduced %	50 (26 - 86)	62.5 (37 - 83)	0.49
FFP (U)	0 (0 - 4)	0 (0 - 4)	0.82
Apheresis PLT (U)	0 (0 - 0)	0 (0 - 0.5)	0.24
Cryoprecipitate (U)	0 (0 - 0)	0 (0 - 0)	0.44

Unadjusted Results



Kaplan Meier Curve over 180 days for patients transfused fresh and old RBCs



Comparison of cause of death between study groups

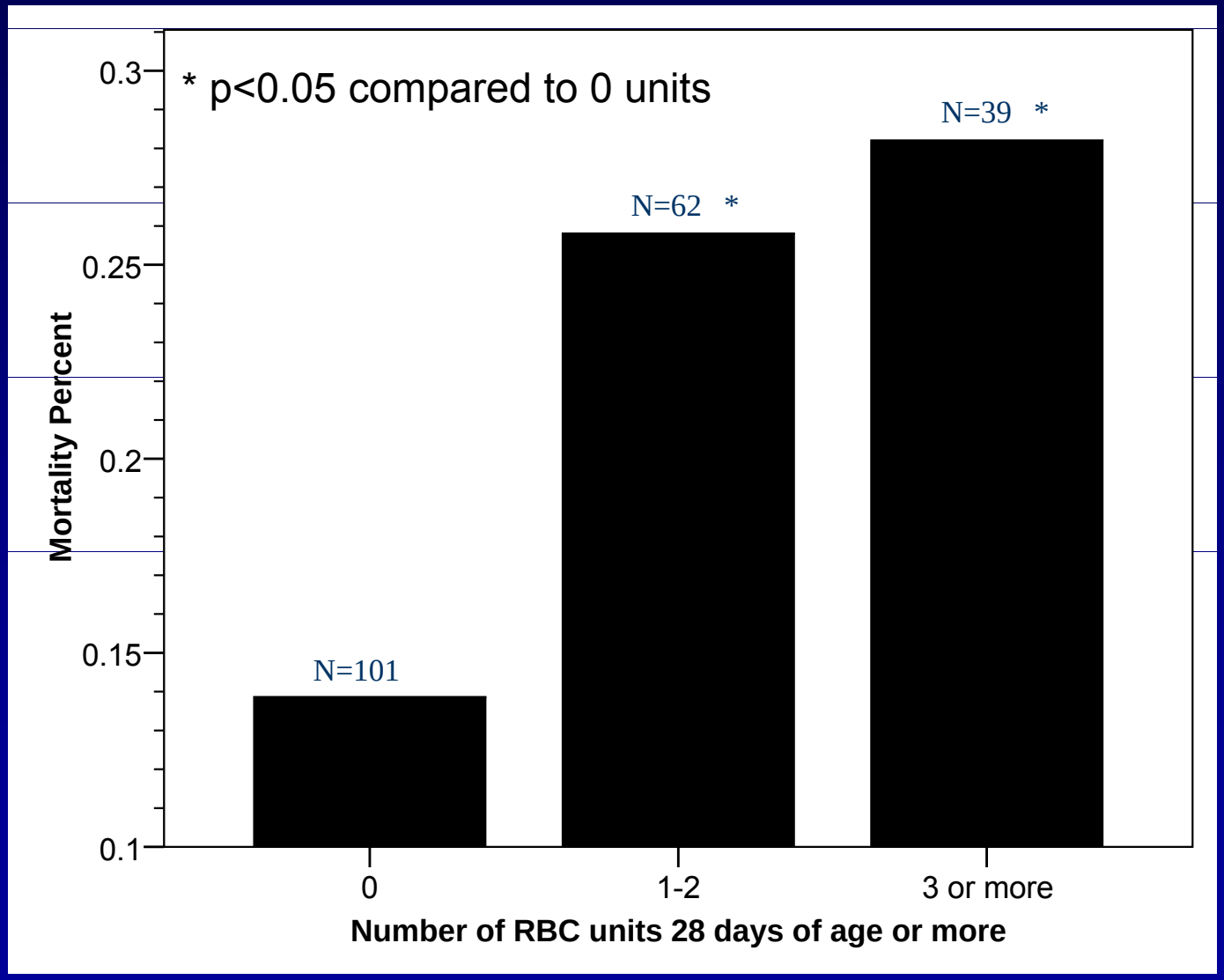
Cause of death	Fresh RBC (n=101)	Old RBC (n=101)	P value
Hemorrhage	1/101 (1%)	1/101 (1%)	1.0
CNS	6/101 (6%)	10/101 (10%)	0.21
Multi Organ Failure	**7/101 (7%)**	**16/101 (16%)**	**0.037**

Adjusted Mortality Results

- Old RBC group compared to Fresh group

  - OR 4.0 (1.34-11.61), (p=0.01)

Relationship between in-hospital mortality and the amount of RBC units transfused ≥ 28 days of storage for patients with ≥ 5 units of RBCs.



Subset Analysis

- For patients transfused ≥10 units of RBCs
- N=94

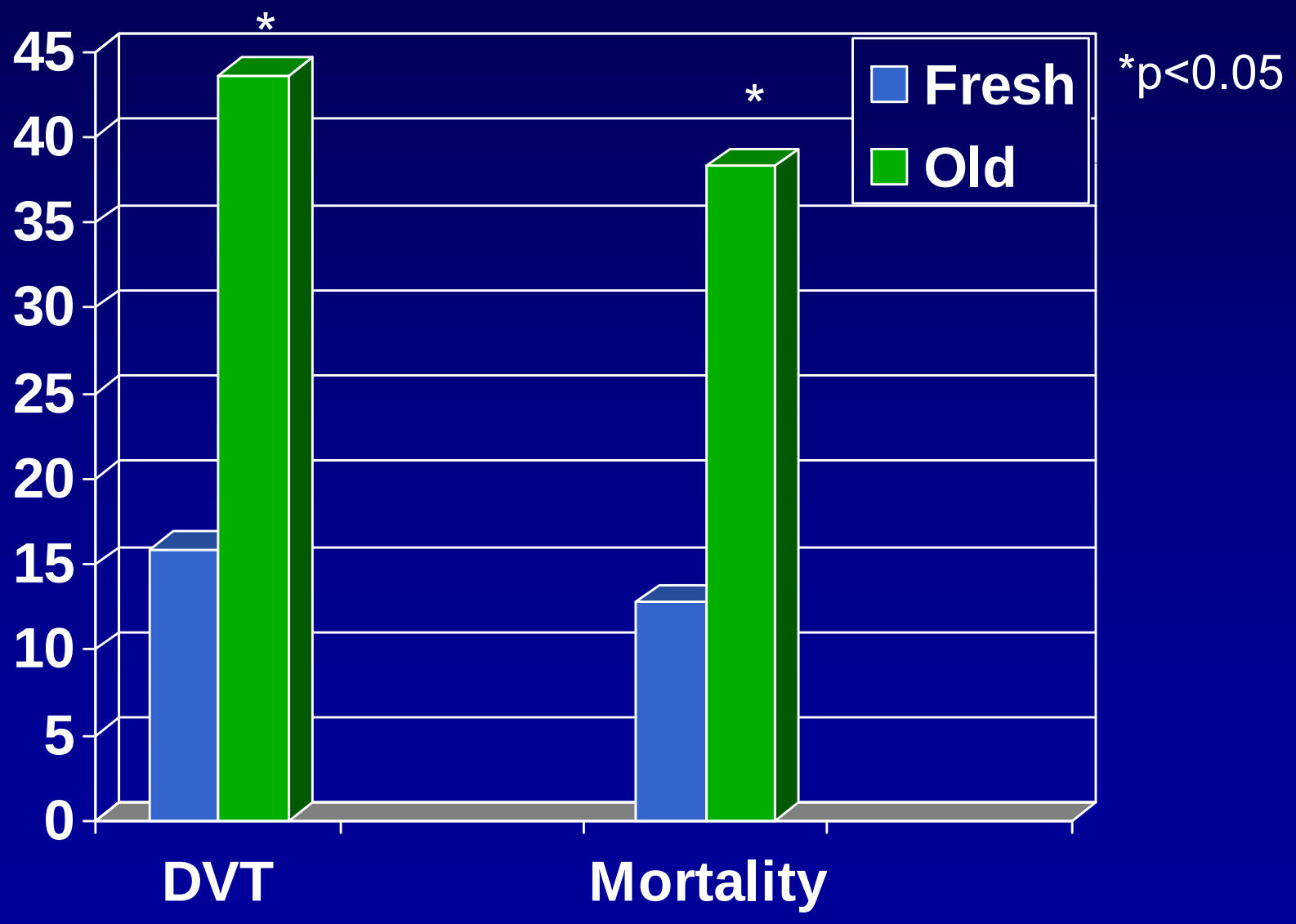
Comparison between patients who were transfused 10 or more RBCs

Variables	Fresh (n=47)	Old (n=47)	P value
Age	48 (27-580	50 (32-65)	0.23
Male %	36/47 (77%)	33/47 (70%)	0.48
Blunt injury	41/47 (87%)	45/47 (96%)	0.27
GCS	14 (3-15)	13 (3-15)	0.77
SBP	121 (98-134)	130 (99-147)	0.34
HR	101 (85-121)	89 (75-120)	0.13
Temp	96.6 (95.2-97.2)	96.1 (94.5-97.2)	0.32
HCO3	21 (19-23)	21 (18-23)	0.78
pH	7.27 (7.17-7.34)	7.22 (7.1-7.33)	0.13
PT	13.4 (12.6-15.4)	13.4 (12.3-14.4)	0.49
HCT	36.6 (33.3-39)	35 (29.2-40)	0.15
ISS	24 (14-34)	27 (17-34)	0.31

Comparison between patients who were transfused 10 or more RBCs

Variables	Fresh (n=47)	Old (n=47)	P value
RBC amount	13 (11-17)	13 (11-17)	0.85
RBC storage age max	**20 (18-24)**	**34 (31-38)**	**<0.001**
RBC storage age median	13 (11-17)	19 (15-24)	<0.001
RBC leukoreduced %	0.67 (0.41-0.86)	0.67 (0.43-0.81)	0.79
FFP	2 (0-7)	2 (0-6)	0.98
PLT	0 (0-4)	0 (0-2)	0.78
Cryo	0 (0-0)	0 (0-0)	0.38

Unadjusted Outcomes in patients transfused 10 or more RBCs (n=94)



*p<0.05

Conclusion

- First data set to reveal when patients matched exactly by RBC amount
  - And all other indices of severity of injury are similar
- DVT and mortality increased for group receiving old RBCs
- Independent association upon regression
- Death from Multi-organ Failure increased in old RBC group
- Only 1-2 units > 28 days needed to increase mortality

Questions not Answers

- At what point do we consider changing blood utilization practices ?
  - Send fresh RBCs to med centers?
  - Young RBC to sickest patients?
- This would increase waste and cost
  - Is cost / waste worth the potential unproven survival benefits?

Future Directions

- Prospective RCT very helpful
  - Mandatory to change practice ?
  - 4-6 years to complete
- Appropriate to continue current practice ?
  - There isn't data that old RBCs reverse shock
- At the very least need to record age of RBCs transfused in blood bank databases.
  - Only few places do this

RBC age and severity of illness

- Relationship between RBC age and outcomes with the degree of critical illness important to understand
  - Post-op ortho
  - Post-op cardiac
  - ARDS
  - Severe Trauma
  - Severe Sepsis

Increased risk of old RBC with increased critical illness

Future Research

- ABLE Trial
  - RCT of 2500 adult ICU patients
    - Expected to be ventilated > 48 hrs
    - Baseline mortality of 25%
  - Fresh (< 7 days) vs standard (mean 21 days)
  - Primary outcome is 60 day mortality
  - No mechanisms are being evaluated.

Future Research

- Collect samples over time in
  - 200 critically ill intubated patients
- In Fresh vs Old RBC groups
  - Inflammatory
  - Coagulation
  - Immune function
  - Microchimerism

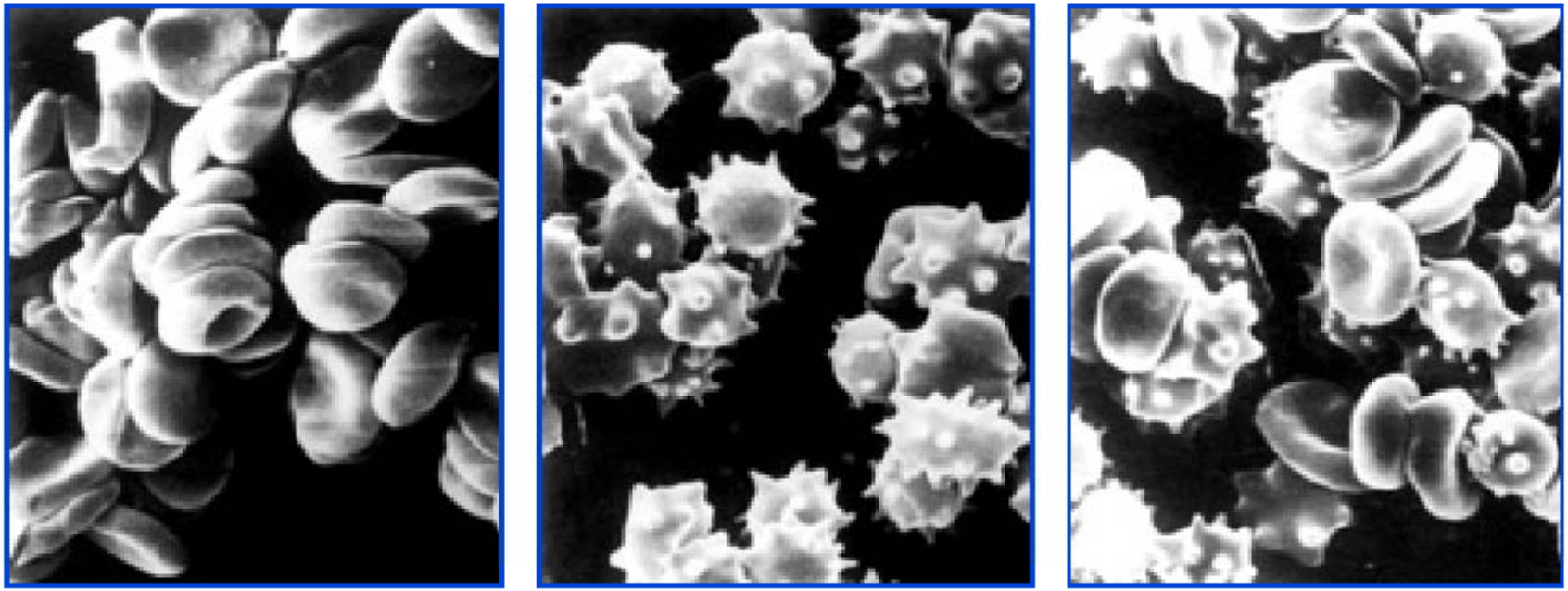
Timeline of Specimen Sampling

Time (days)	Specimen Source & volume	Day 0	Day 1	Day 3	Day 7	Day 28	Day 180
Cytokine & Coagulation	Plasma 5-7ml	x	x	x	x	x	x
Immune function & Microchimerism	Whole blood 7ml	x				x	x

RCT of RBC age in Trauma?

- Needed?
- Will positive results from general ICU population be applied in severe trauma?

Questions?

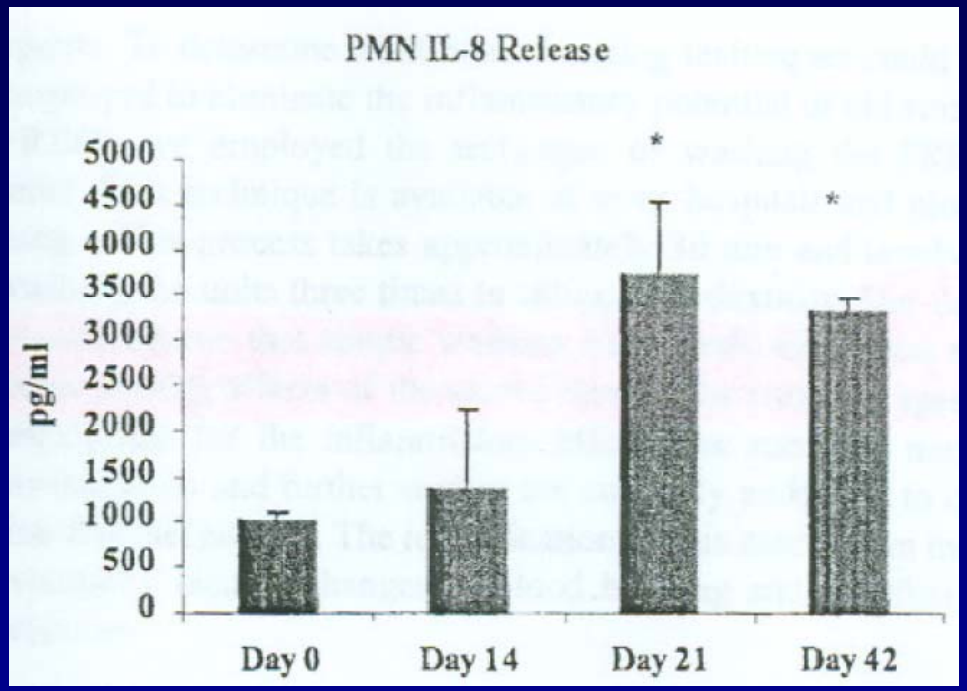


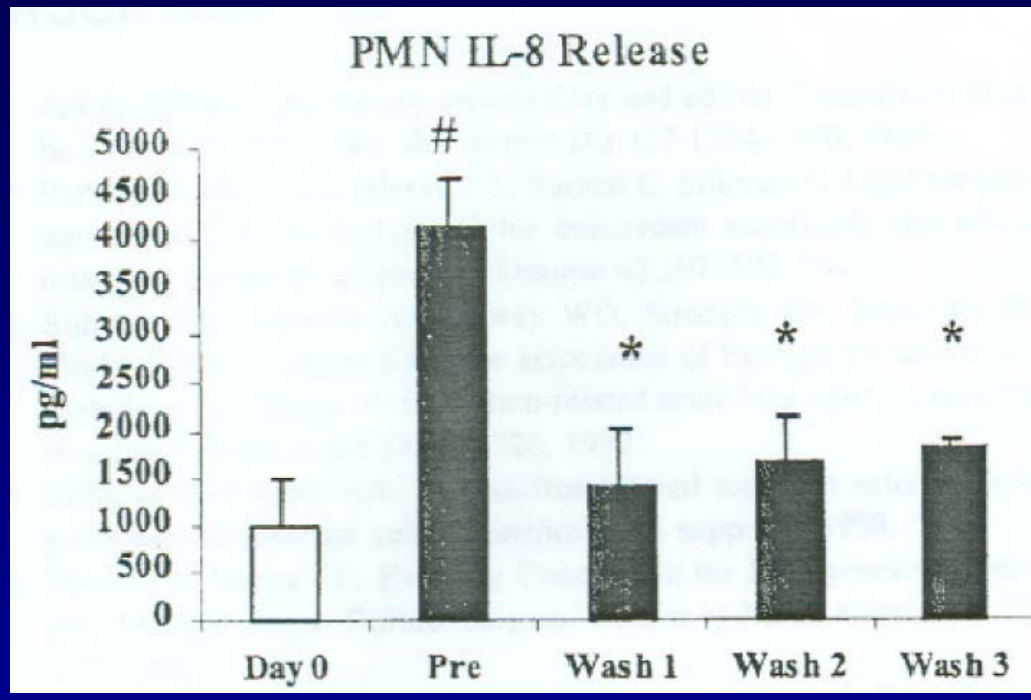
Day 1 Day 21 Day 35

Scanning electron micrographs of red blood cells isolated from stored blood on Day 1, Day 21, and Day 35. During storage, the shape of RBCs changed gradually from normal discoid to echinocytes (dented or shriveled red cells).

Reproduced with permission from: Hovav et al. *Transfusion*. 1999;39:277-281.

Wash RBCs ?





1 Zallen G. Shock. 2000: Vol 13;1, p29-33

Wash RBCs ?

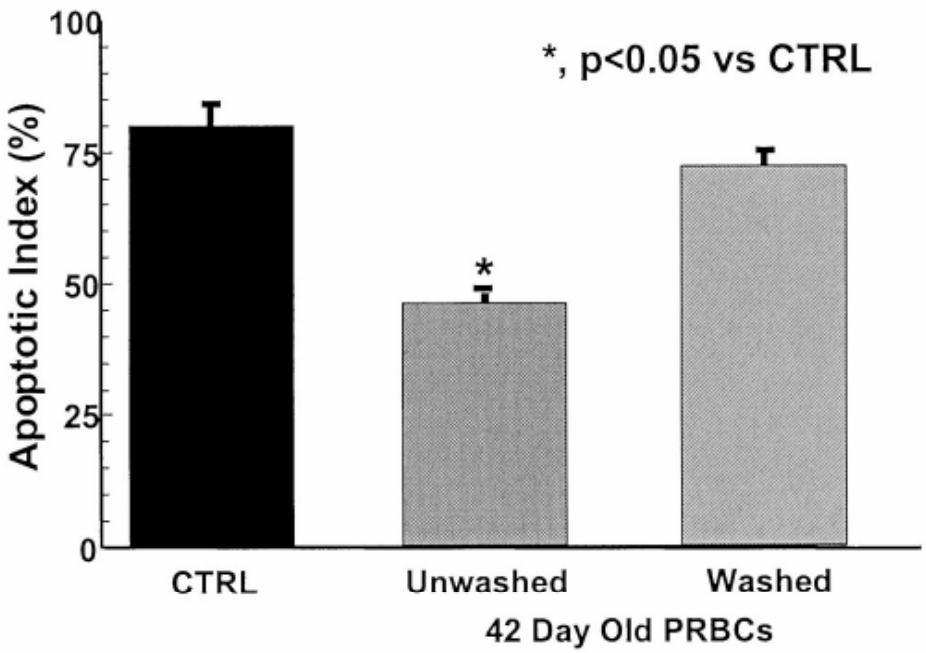


Fig. 3. *Apoptotic index of circulating PMNs harvested from healthy volunteers, after 24-hour incubation with fresh plasma from healthy volunteers (CTRL) or with the plasma fraction of stored RBCs. RBC*

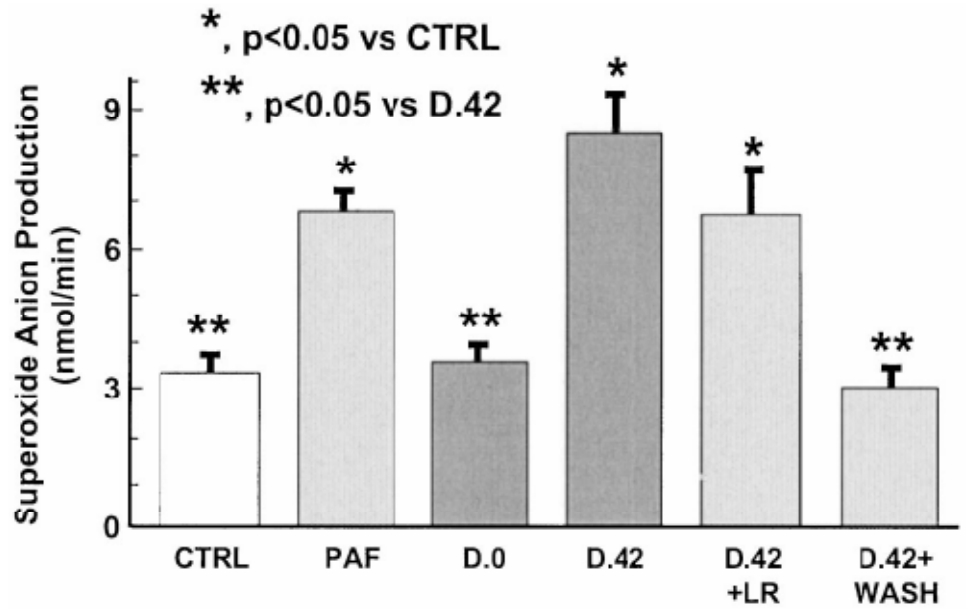


Fig. 4. *Priming of healthy donors' PMNs for O_2^- release, assessed by fMLP activation. Compared with albumin controls (CTRL), stored RBC plasma primes PMNs after 42 days in storage (D.42).*

2 Biffl WL. J Trauma. 2001: Vol50;3, p426-432

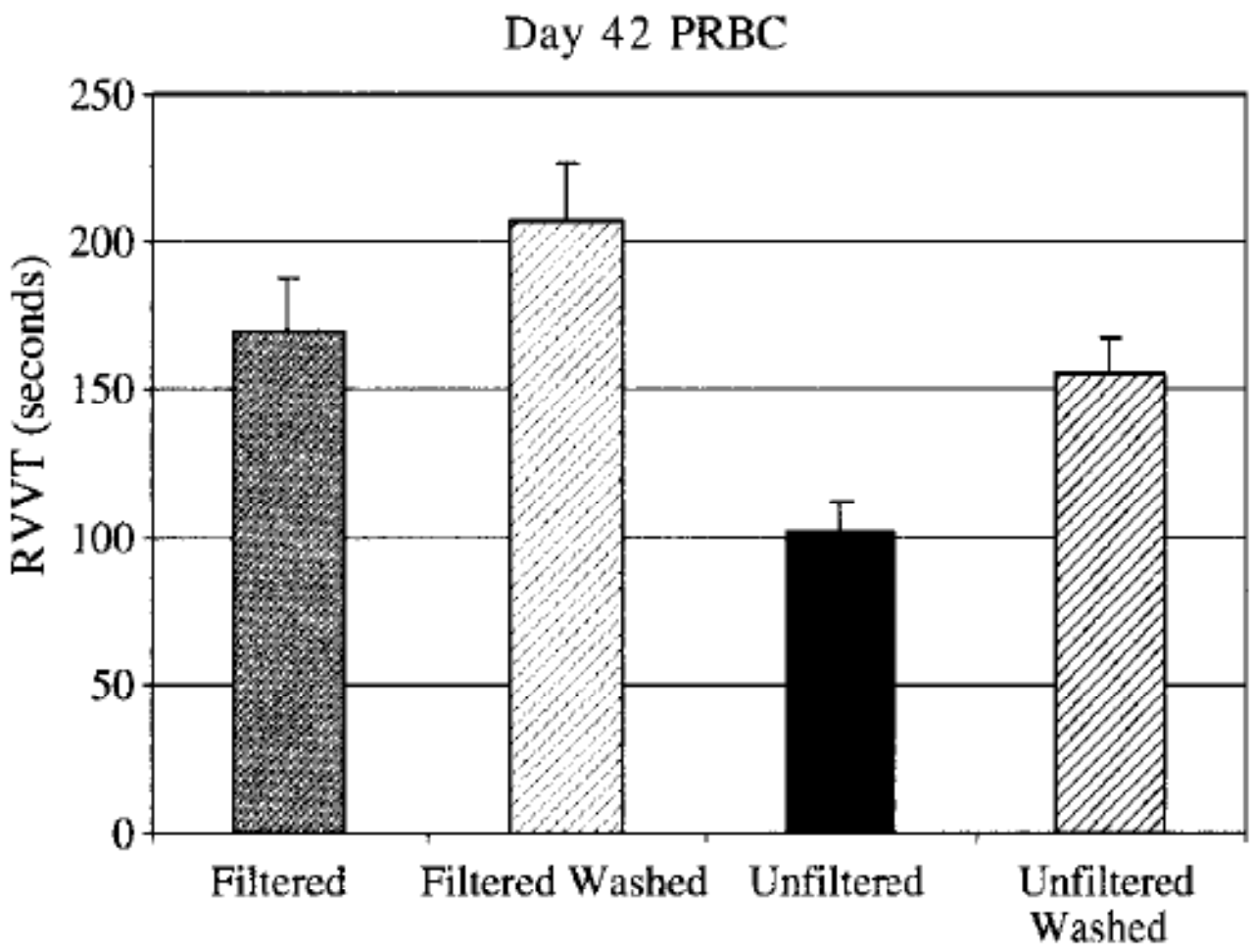


Fig. 3. RVVT was determined using PRBC from 42 day old units as the source of phospholipid in filtered and unfiltered units with and without washing of PRBC.

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RBC age and Infection

- 61 Trauma patients transfused >5 RBC U
- Major infections increased with
  - Amount of RBCs transfused
  - Number of RBC units > 14 days of storage
- Multivariate logistic regression
  - Number of RBC units > 14 days of storage independently associated with major infection
    - OR 1.12 (1.01-1.26), (p=0.03)

Offner. Arch Surg. 2002; 137:711-717

Mortality and RBC Age

- Retrospective study
  - 31 patients, severe sepsis
  - No difference in severity of illness or RBC amount between survivors and non-survivors
  - Median RBC age increased in non-survivors
    - Survivors, 17 d
    - Non-survivors, 25 d ($p<.001$)
  - Tx RBC > 16 days independently assoc with increased mortality

Purdy FR. Can J Anaesth. 1997; 44:1256-61

Independent Association of Mortality with Leukoreduced Old RBCs

- In 176 trauma patients transfused >5 units
- Old RBC: >14 days
- Adjustment for age, mech injury, ISS, RBC unit amount
- When 1-2 units of old RBCs an increased odds ratio of death
  - OR, 5.2 (1.4-12.7)
- When ≥3 units of old RBCs
  - OR, 7.8 (2.3-26)

Weinberg JA, J Trauma Sept 08, 65(2): 279-284

Prospective Pediatric RBC age data

- Prospective multicenter RCT of 455 transfused pediatric ICU pts
- Apriori planned secondary analysis of RBC age
- <u>All RBCs were leukoreduced</u>
- Maximum storage time of > 14 days was independently associated with
  - Increased MOF
  - (adj OR: 2.23; 95%, CI: 1.20, 4.15)

Gauvin F, Spinella PC. Submitted. Blood

Pediatric RBC age data

- Prospective multicenter observational study of 447 pediatric ICU patients
- Apriori planned secondary analysis of RBC age
  - 85% of RBCs prestorage leukoreduced
- Maximum storage time of > 14 days was independently associated with increased MOF
  - Adj OR, 1.87 (1.06;3.31), (p=0.03).

Karam O, Spinella PC. Submitted. CMAJ

Inflammation

- Luminex 100 IS system
- Compare Pro-and anti-inflammatory cytokines in patients transfused fresh or old RBCs

Hypercoagulation

- Compare concentrations of
  - Prothrombin Fragments 1+2,
  - Soluble Thrombomodulin
  - Protein C, PAI-1
  - Tissue Plasminogen Activator
  - Factors V, VII, VIII
  - D-Dimer, Antithrombin III
  - Soluble Endothelial Protein C Receptor

Immune Function

- Compare NK cell cytolytic ability
- Quantify the function of the adaptive immune system by stimulating PBMCs and measuring proliferation in a CFSE dilution flow cytometry assay.
- Determine the proportion of regulatory T cells and pro-inflammatory Th17 cells