



Potential mechanisms of the RBC storage lesion

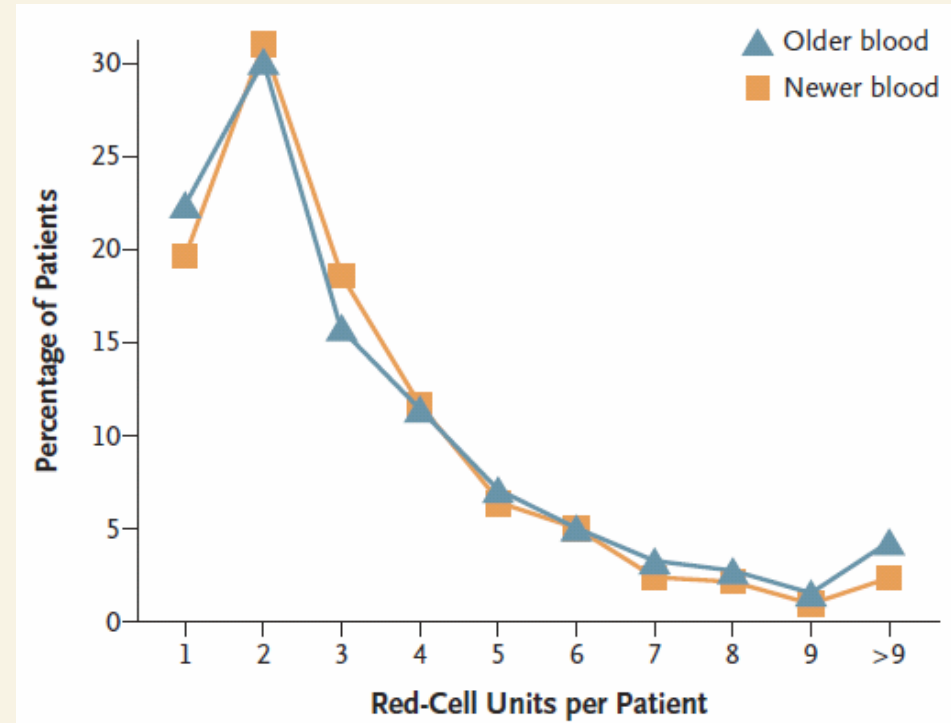
Philip Norris

Does the RBC storage lesion exist?

- Epidemiological studies suggest older RBC units associated with:
 - Increased risk of pneumonia
 - Longer length of stay
 - Increased mortality
 - Decreased recurrence of colorectal cancer
 - Decreased alloimmunization

Age of blood and mortality

- Compared cardiac surgery patients who received only blood ≤ 14 days (“fresh”) vs. only blood stored >14 days.
- Older blood 2.8% mortality vs. 1.7% in “fresh” blood



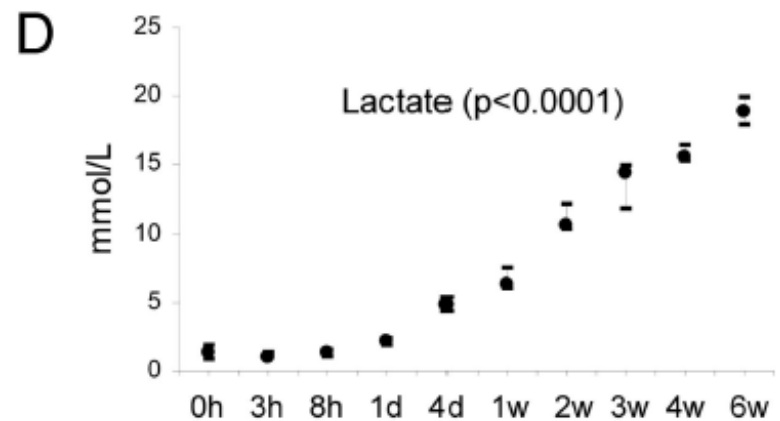
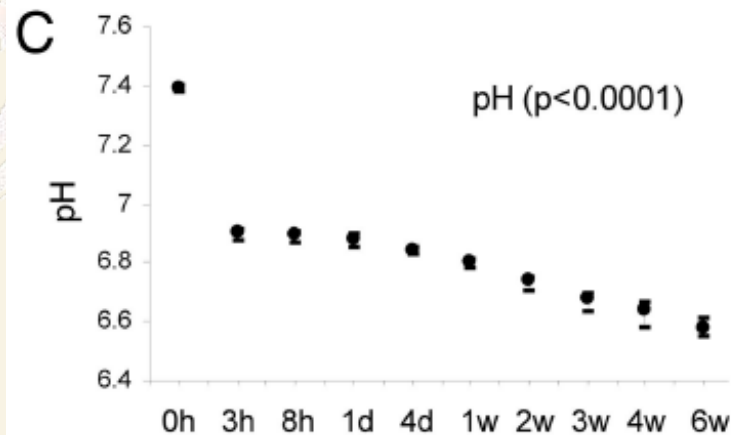
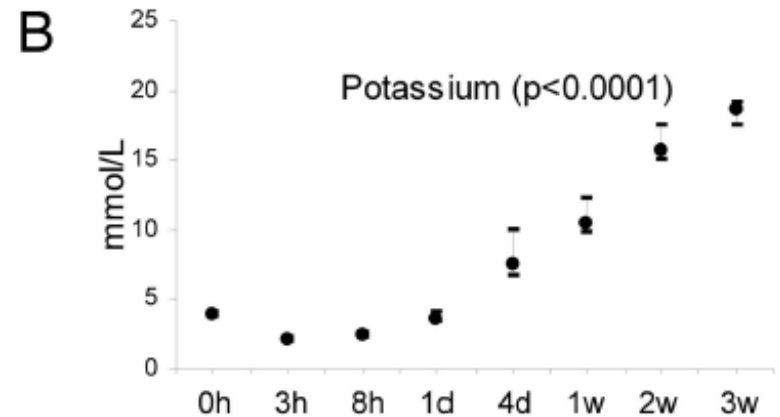
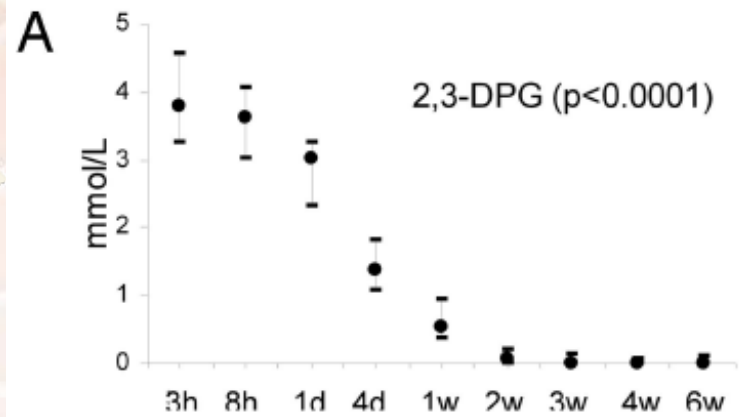
RBC changes with storage

- Decreased 2,3-DPG, pH
- Increased lactate, potassium
- Signs of RBC damage
- Morphological changes
- Increased adhesion to endothelial cells
- Decreased SNO

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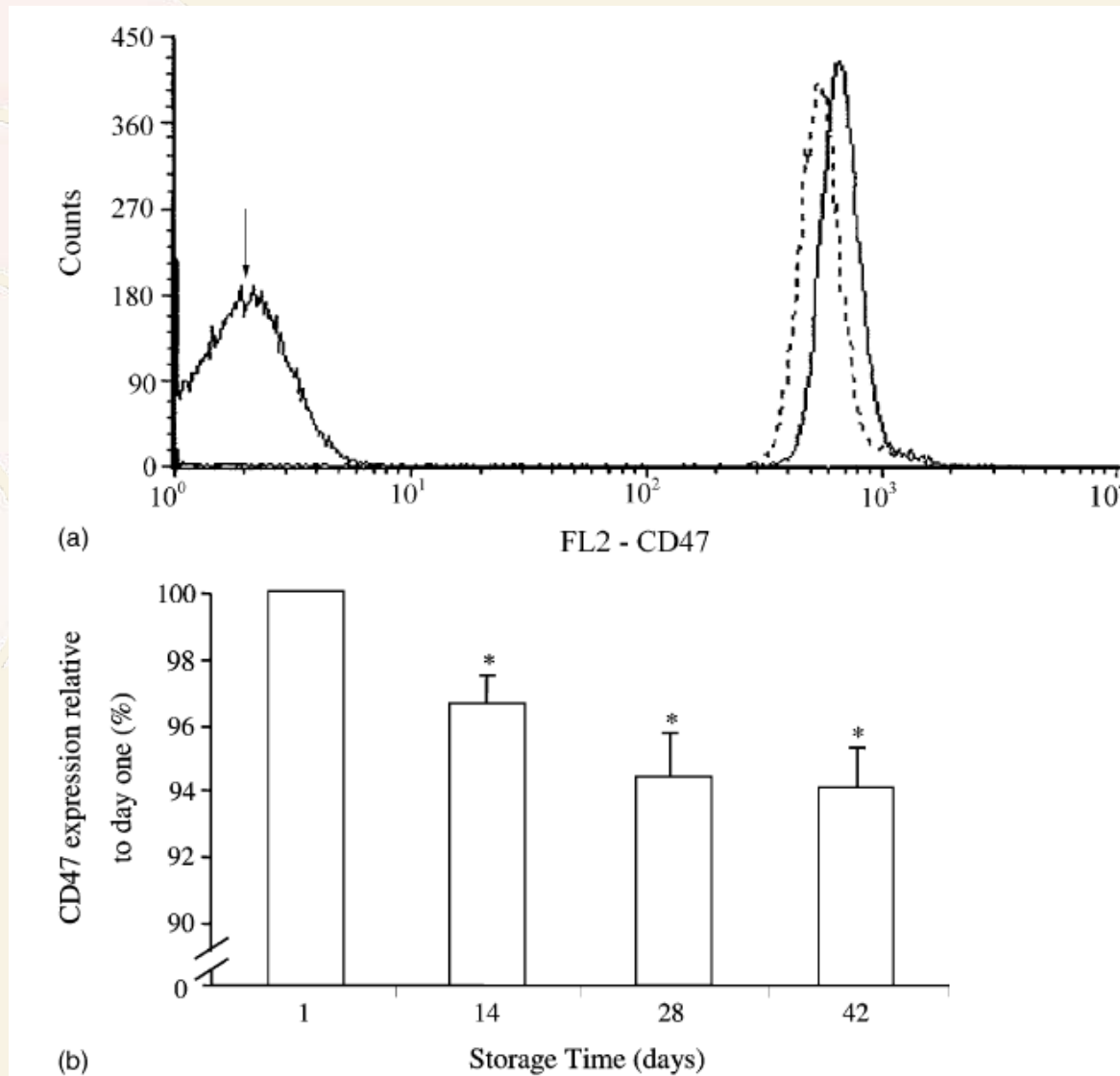
Electrolyte changes with storage



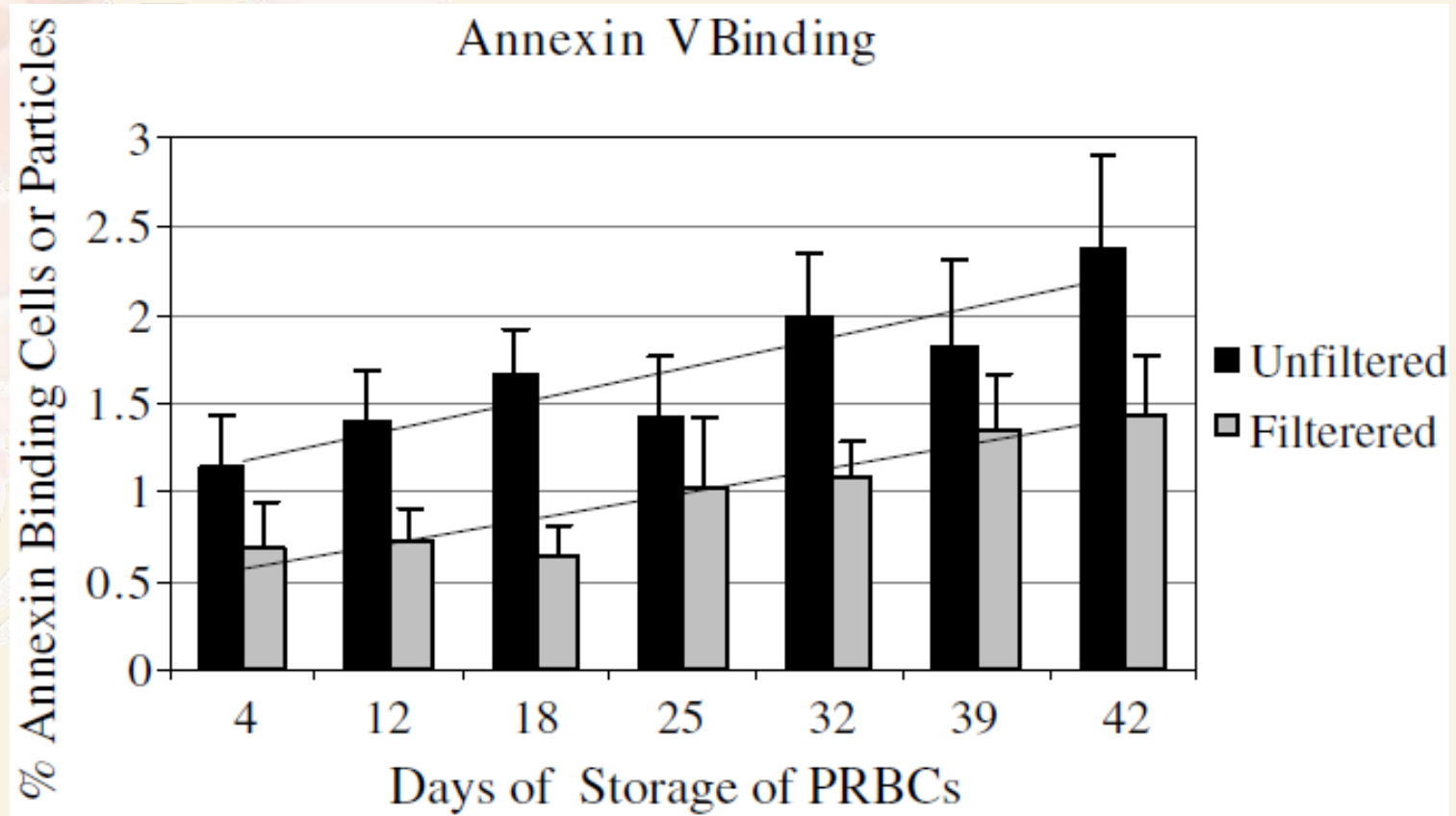
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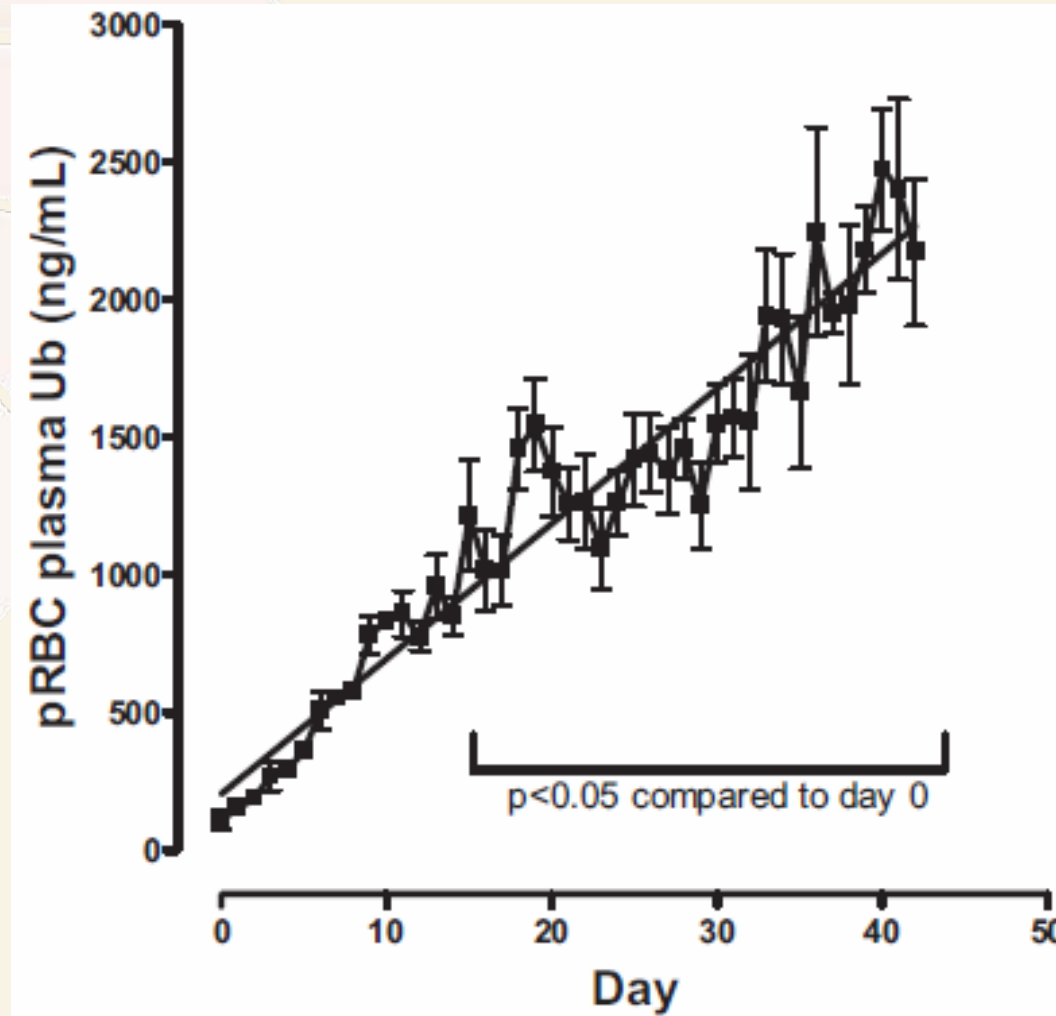
Decreased RBC CD47 at 42 days



Increased Annexin V Binding



Ubiquitin levels rise in stored RBCs



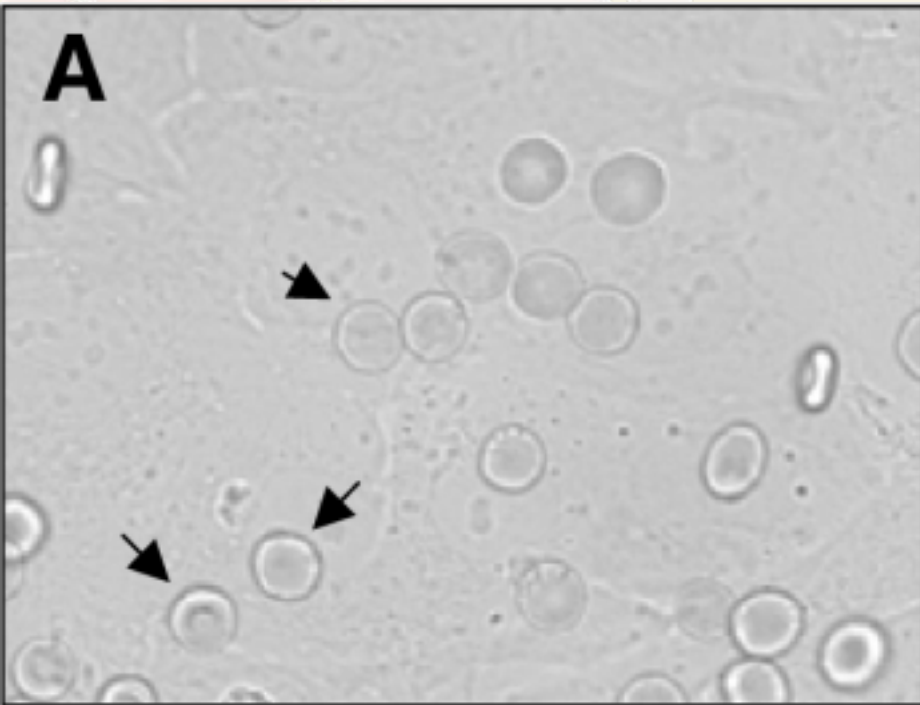
Patel et al., J Surg Res 2006

RBC changes with storage

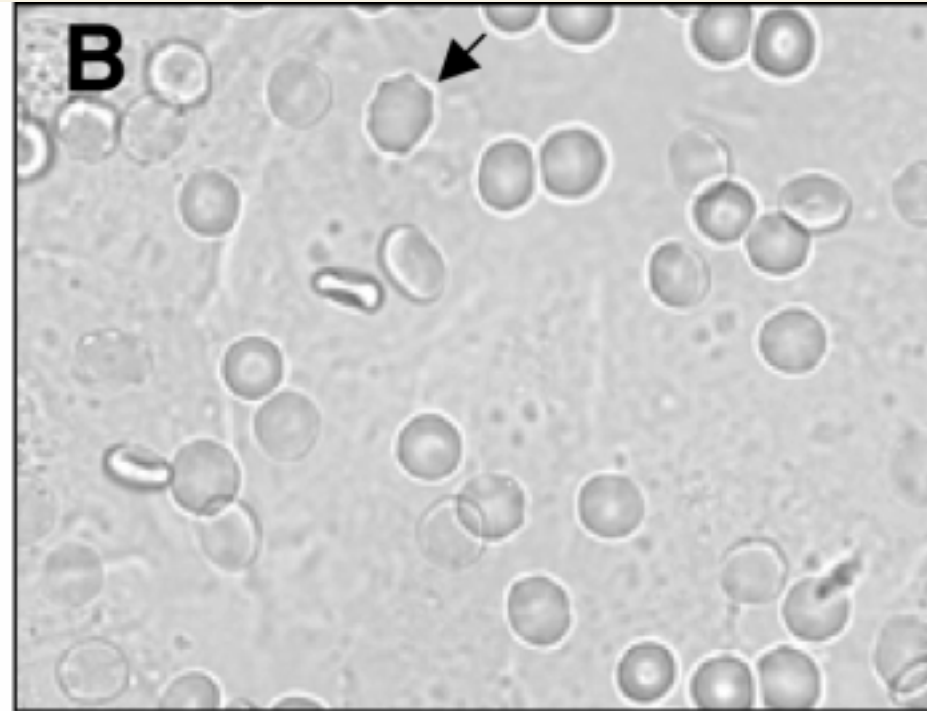
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RBC morphology changes with storage time

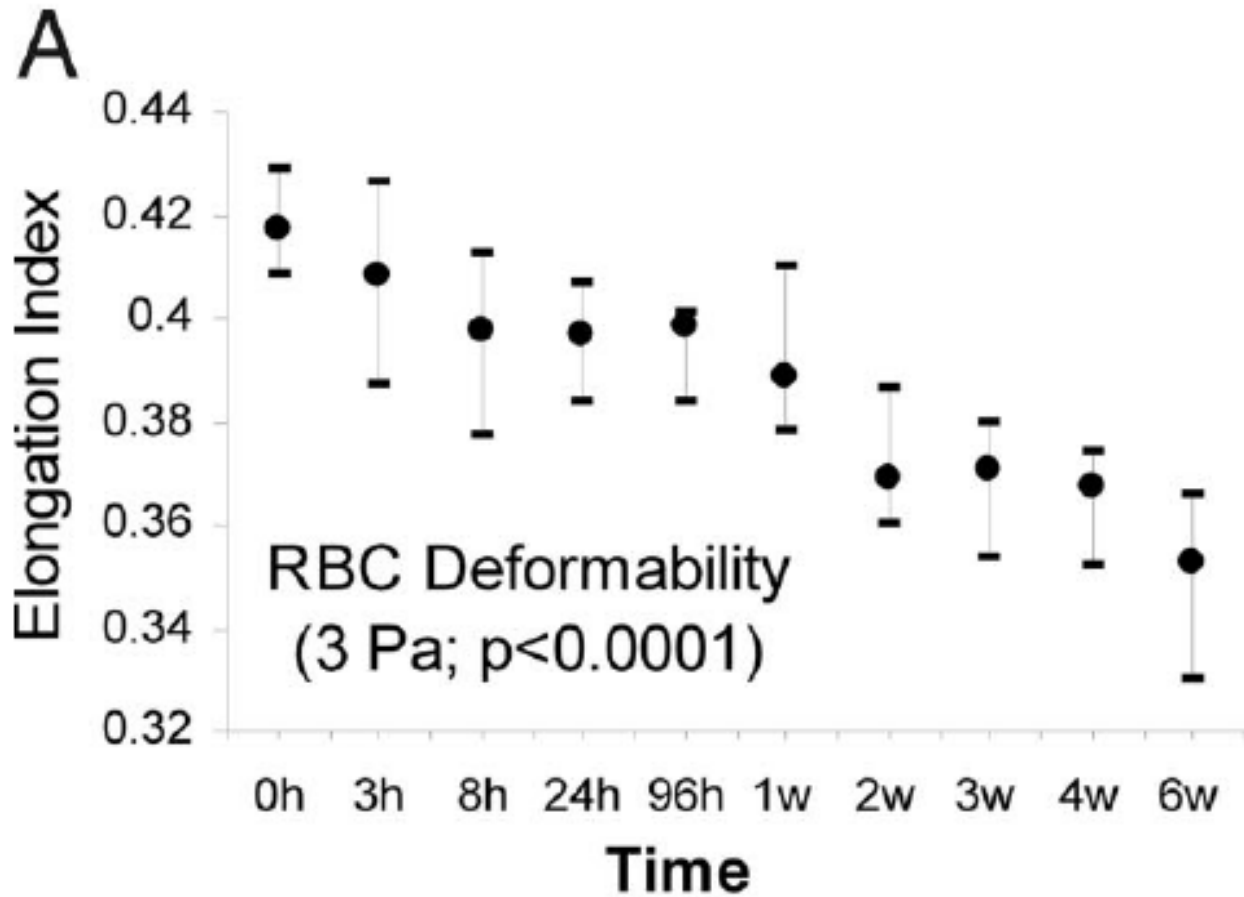
Day 1



Day 21



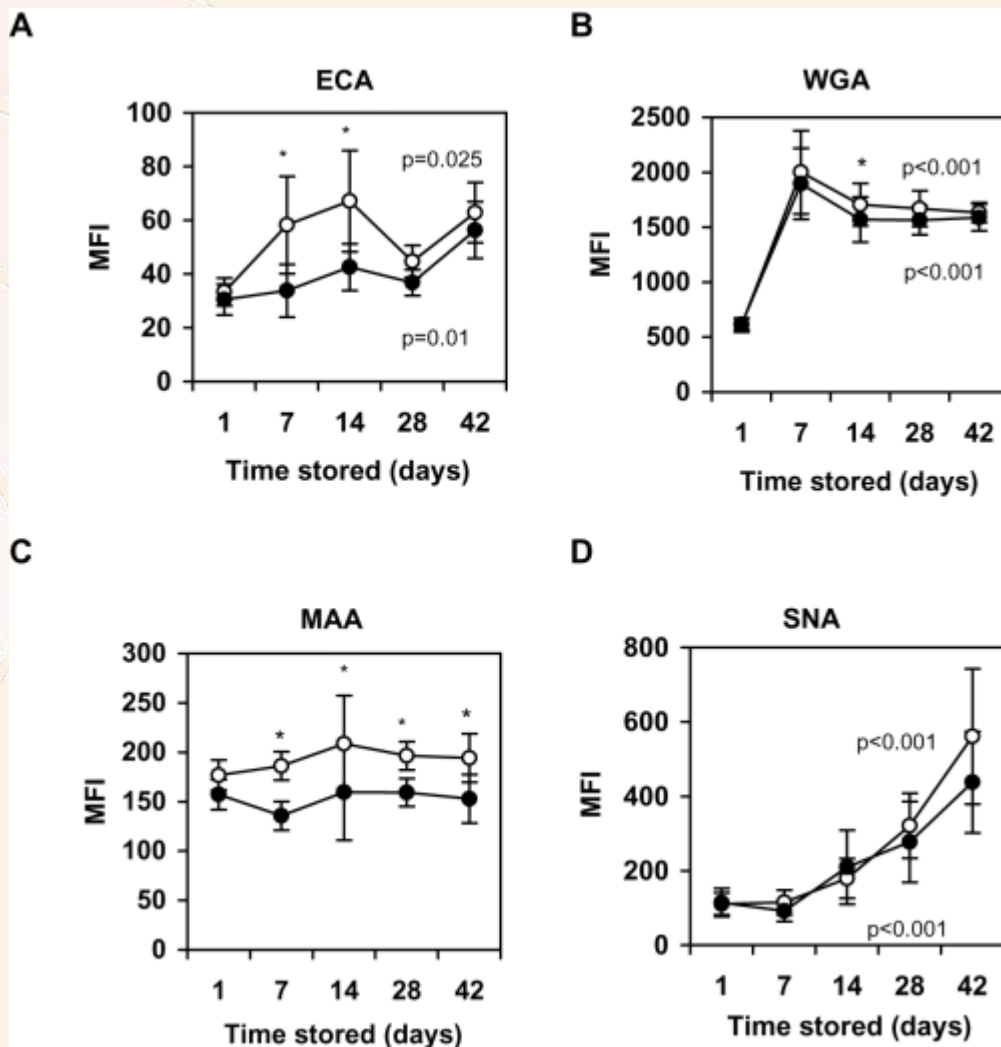
RBC deformability decreases



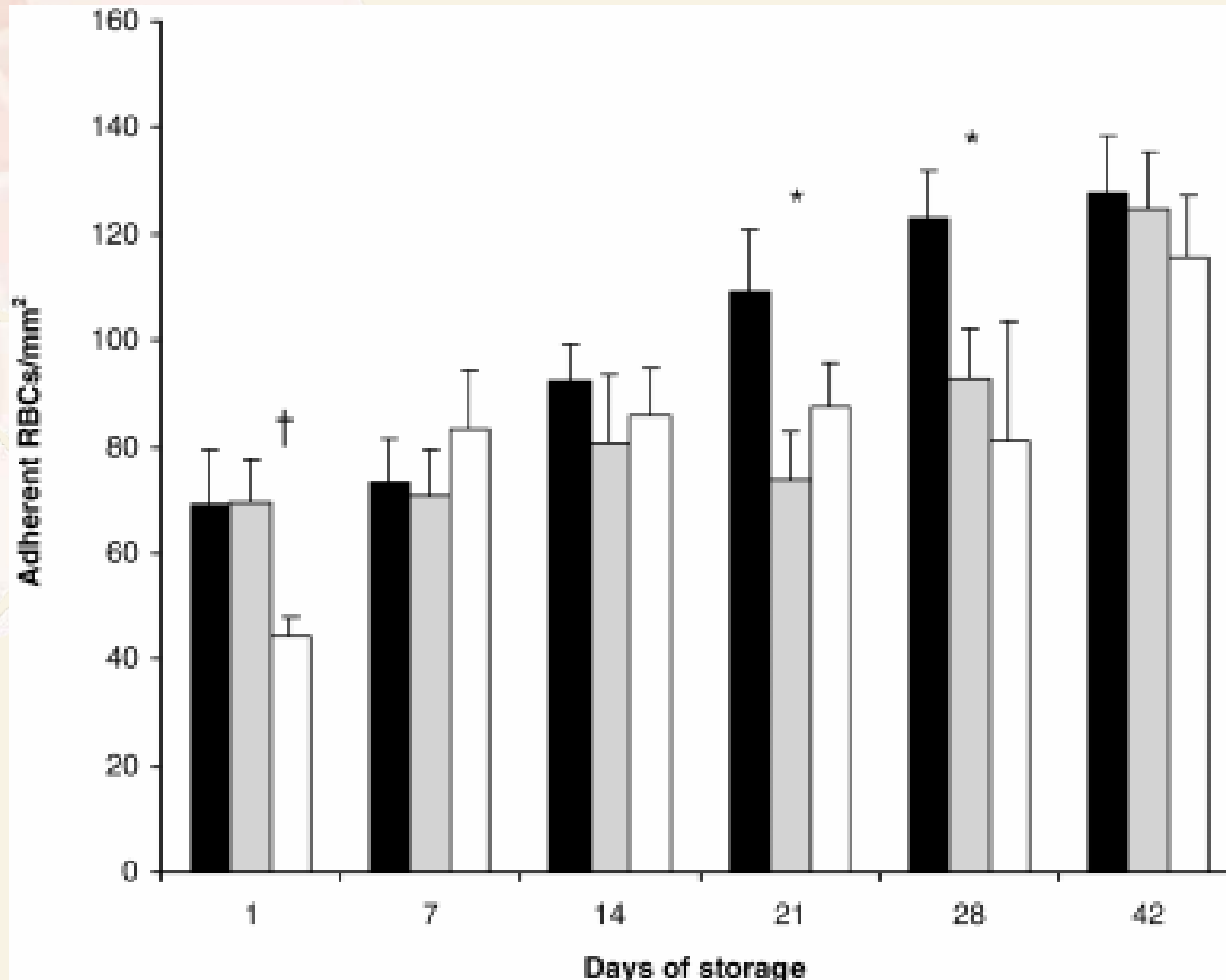
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Lectin binding changes with RBC storage



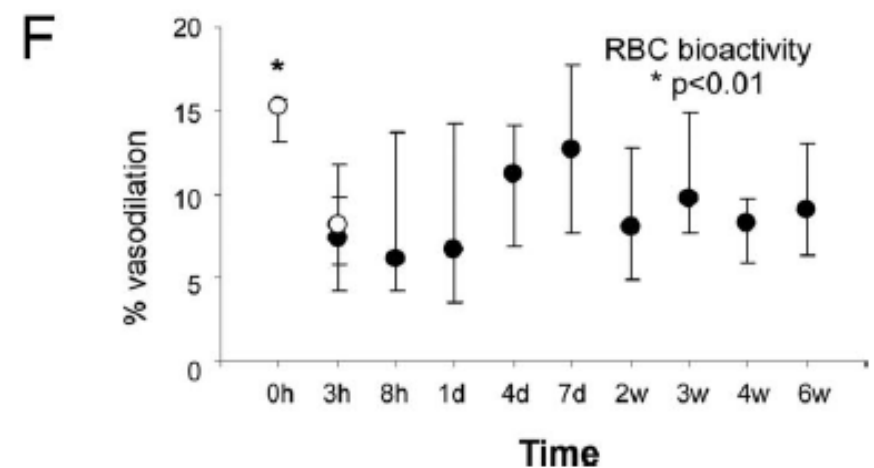
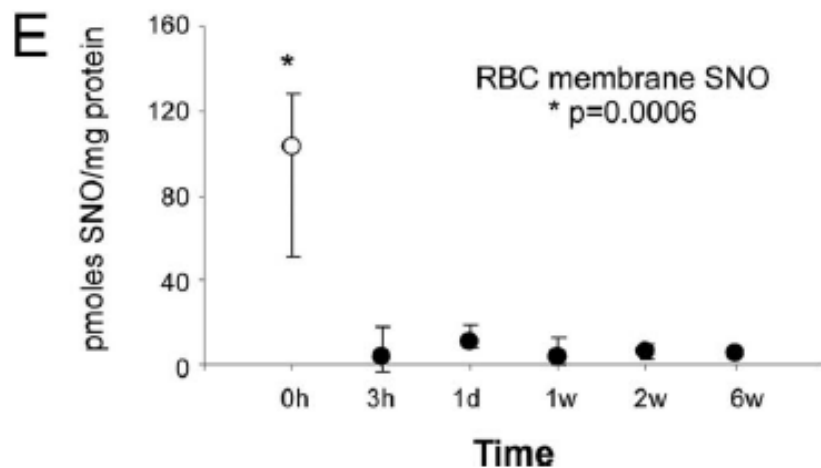
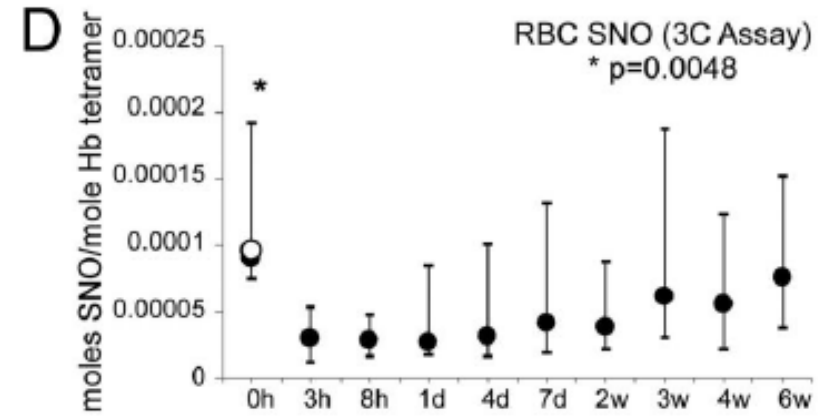
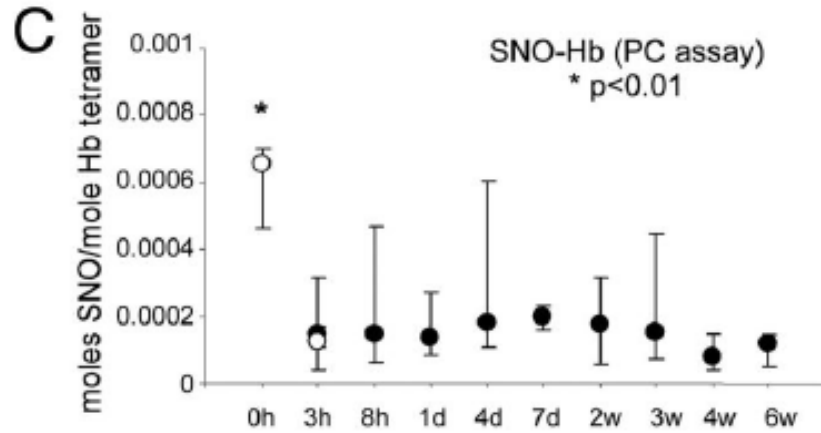
RBC-EC adhesion increases with storage time



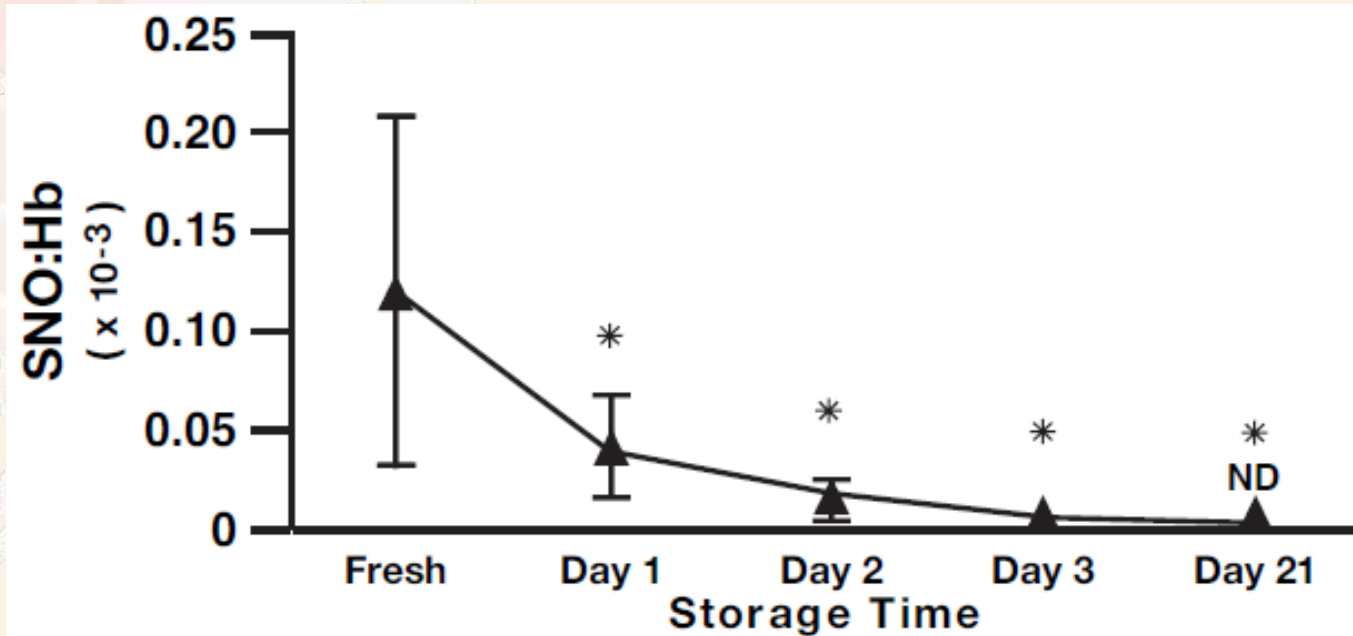
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Decreased SNO



Decreased SNO



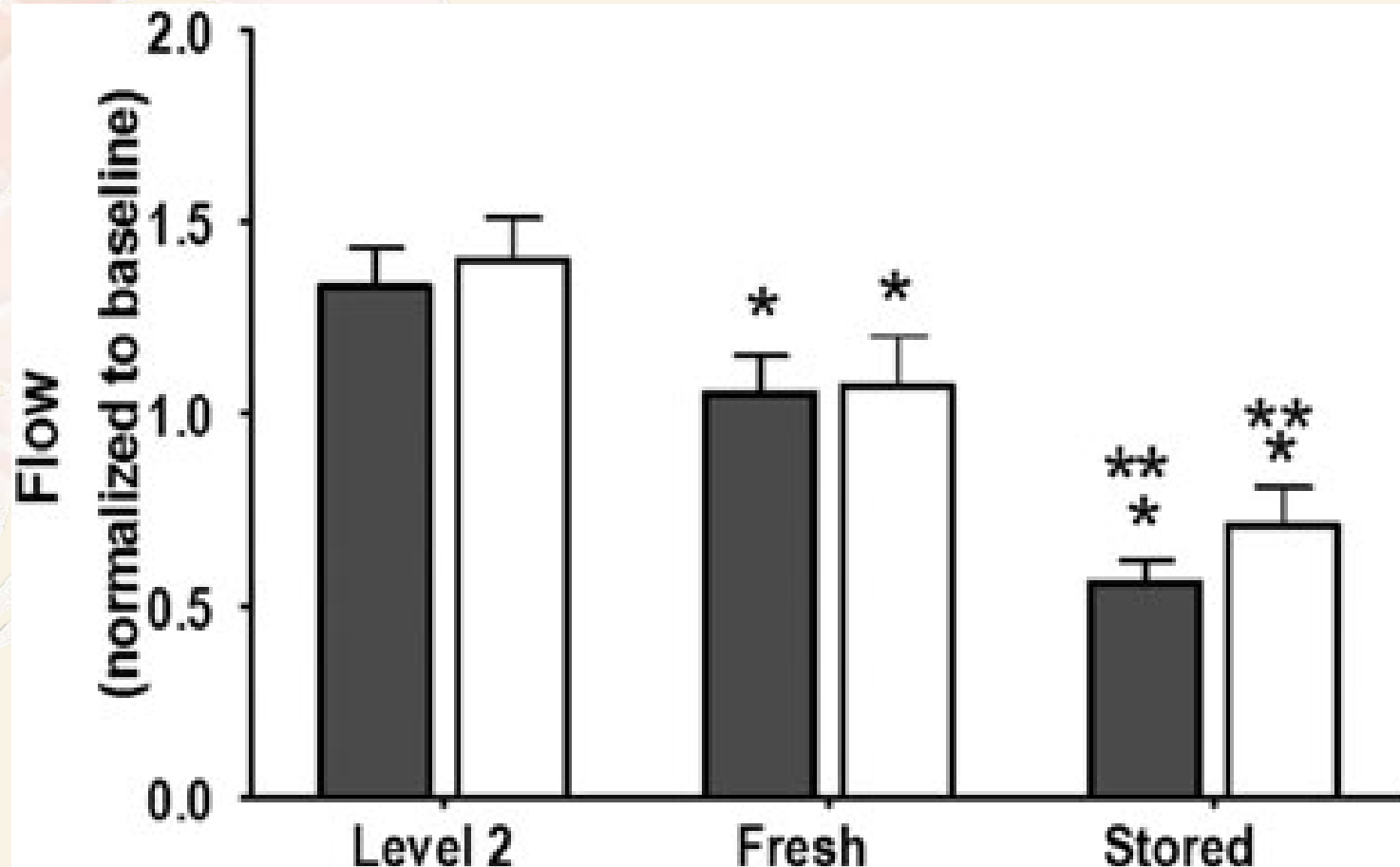
Recipient effects of RBC storage

- Blood flow in transfused tissues
- Effects on the clotting cascade
- Activation of recipient immune system
- Interaction of donor and recipient WBCs

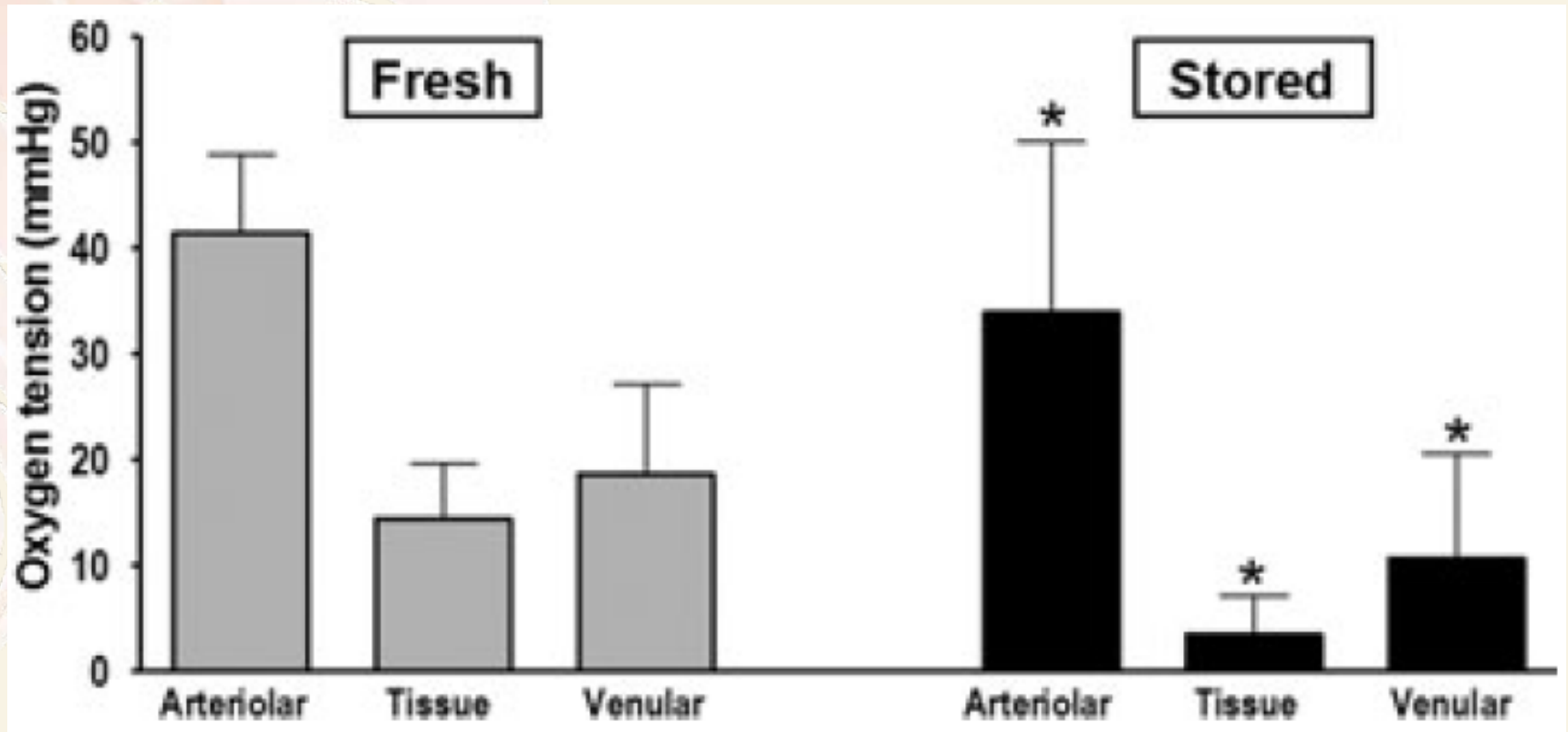
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Decreased vascular flow with stored blood transfusion

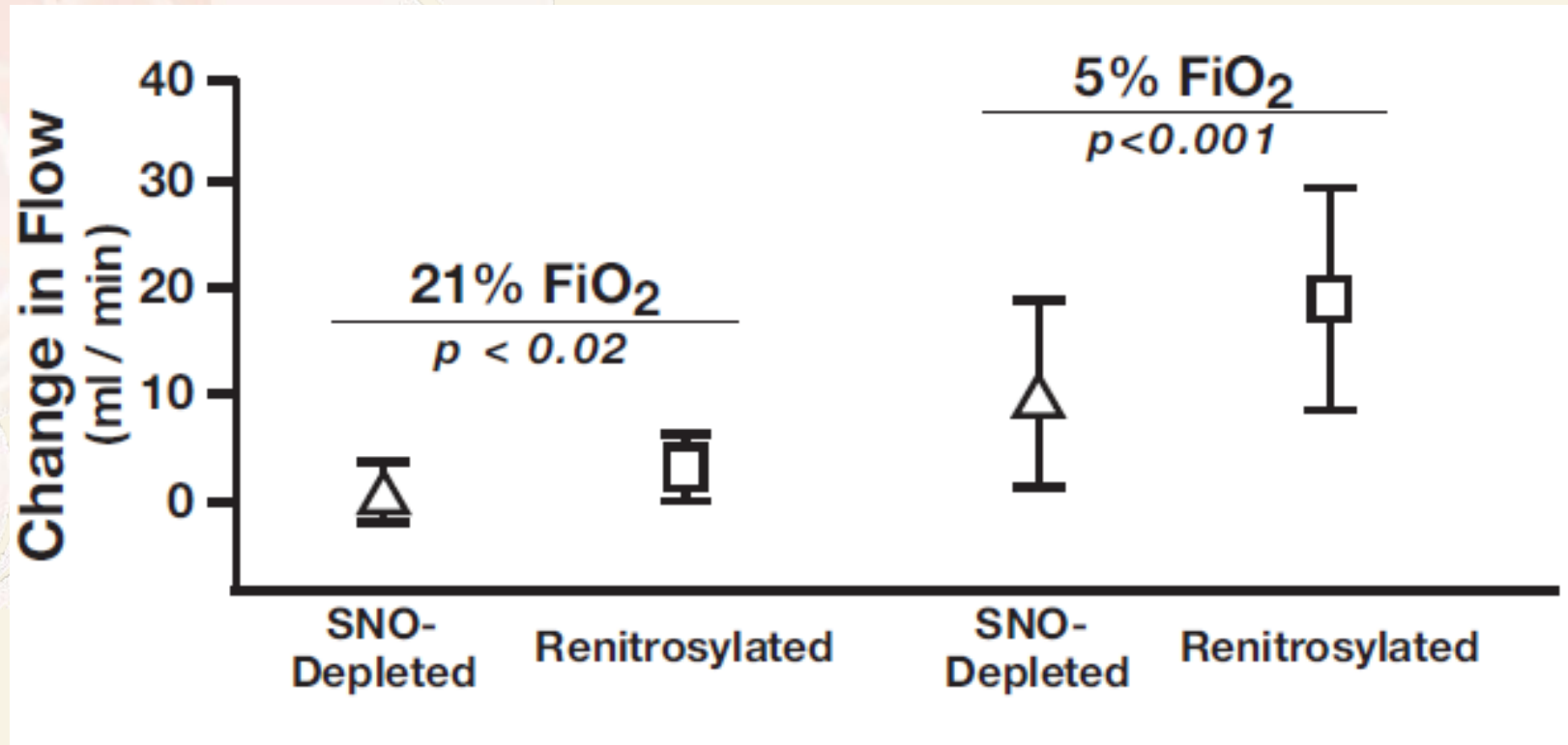


Lower tissue oxygenation with stored blood



Tsai et al., Transfusion 2004

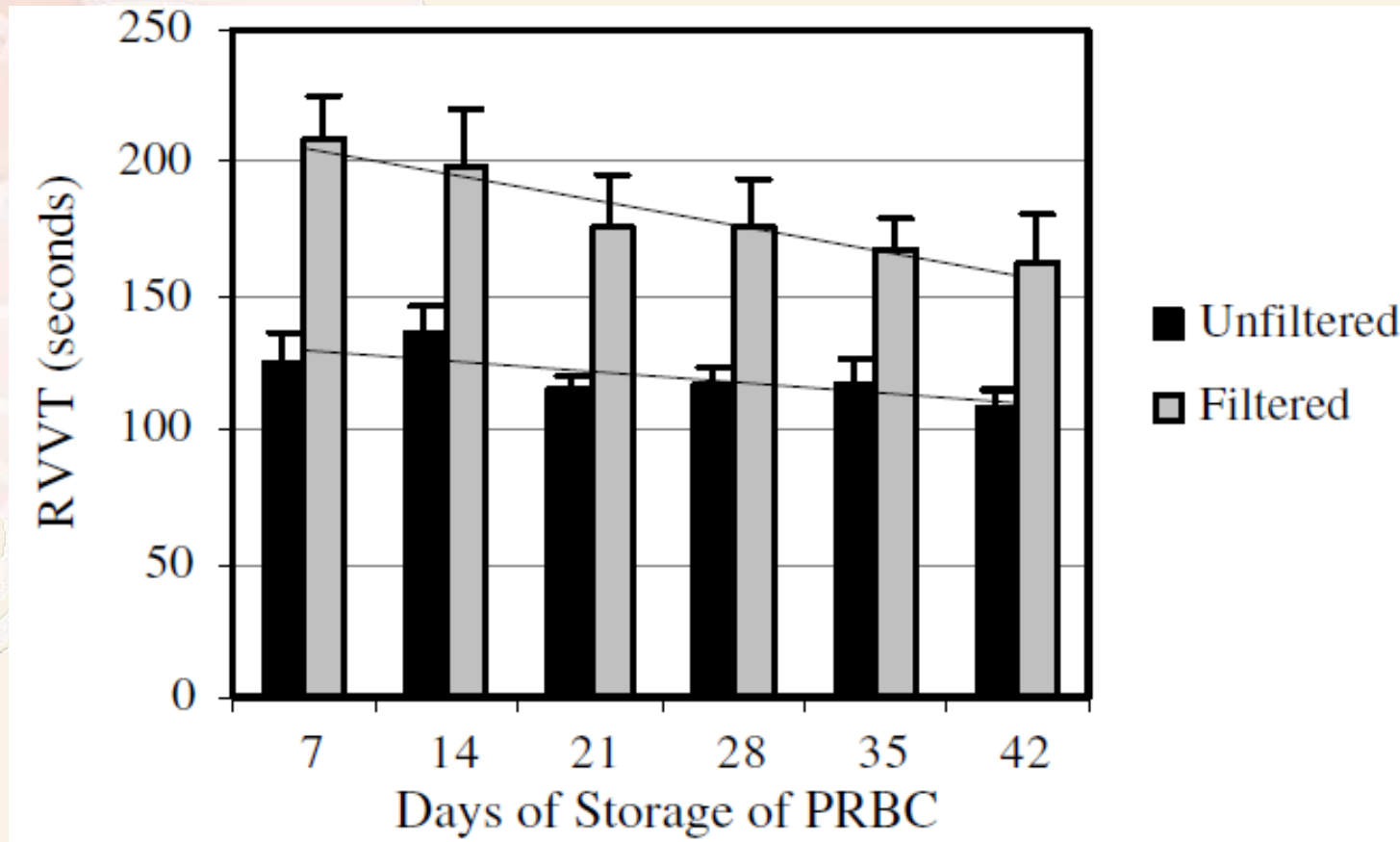
Vasodilation rescued by SNO repletion



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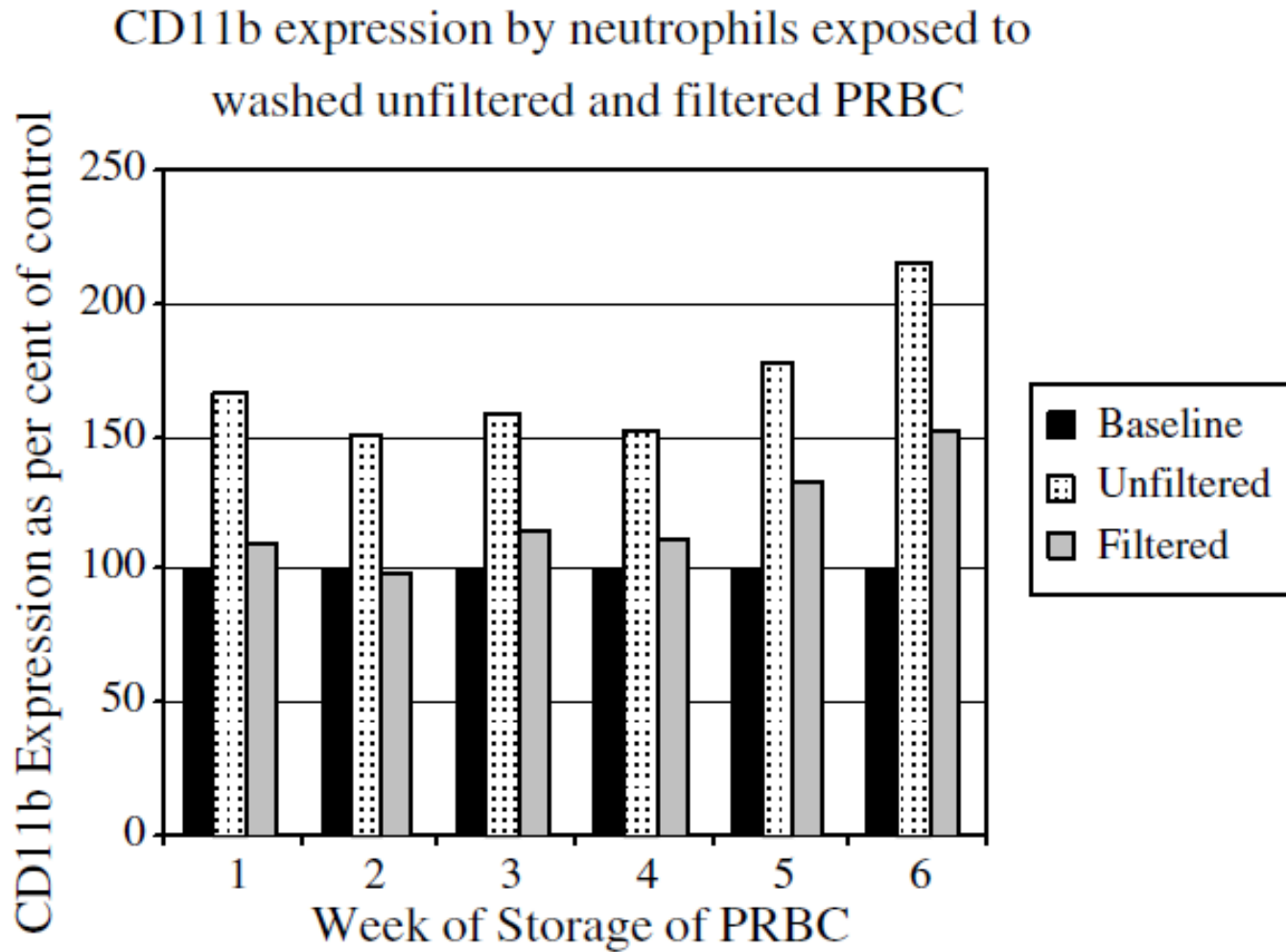
Russel's viper venom time decreases



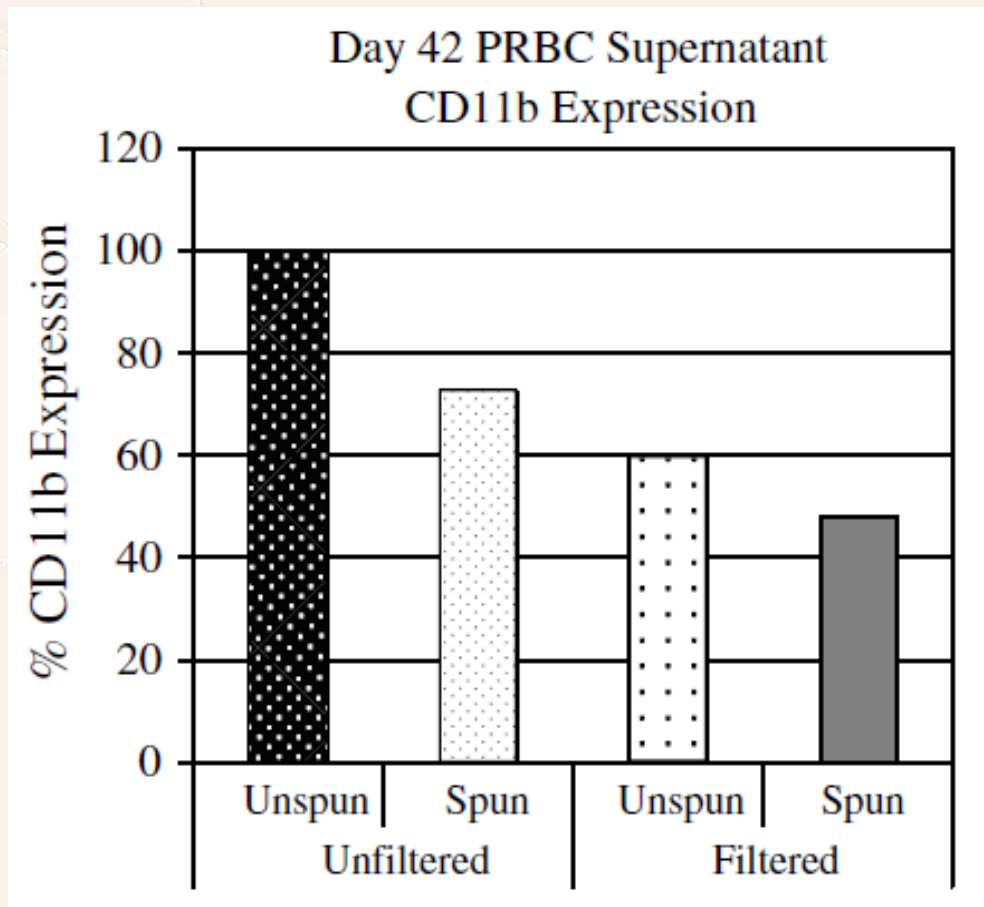
Recipient effects of RBC storage

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- Effects on the clotting cascade
- **Activation of recipient immune system**
- Interaction of donor and recipient WBCs

Neutrophil priming increases



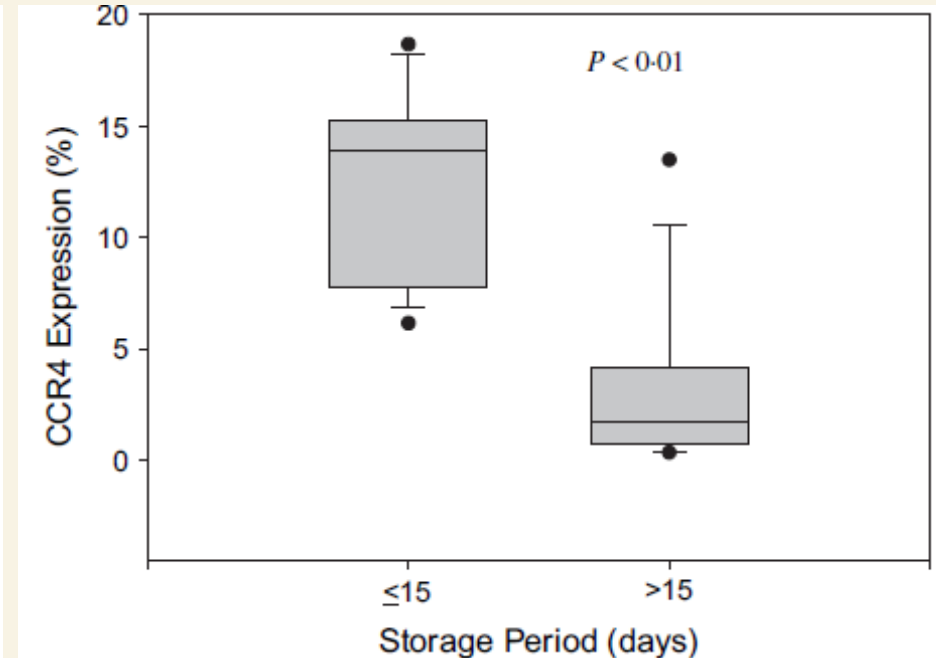
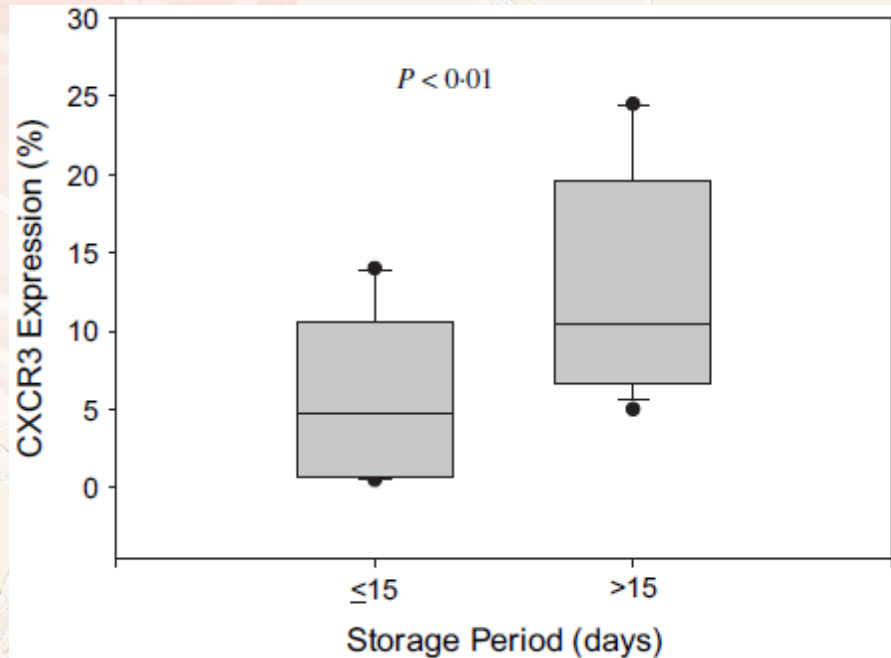
Priming activity in RBC supernatant



Recipient effects of RBC storage

- Blood flow in transfused tissues
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- Activation of recipient immune system
- Interaction of donor and recipient WBCs

Chemokine receptor changes in WBCs after storage



New directions

- Characterize microparticle mediators of inflammation in fresh and stored RBC units.
- Measure the immune modulation of polymorphonuclear cells (PMNs) and peripheral blood mononuclear cells (PBMCs) exposed to fresh and stored RBC units.
- Determine if novel storage solutions or pathogen reduction can ameliorate the “storage lesion”.

New directions

- (A) Measure the frequency and strength of RBC-EC adhesion using fresh and stored RBCs.
- (B) Test the dependence of RBC-EC interaction on RBC surface membrane carbohydrate receptors.
- (C) Determine if pretreatment of ECs with an “insult” increases RBC-EC adhesion with stored RBCs.
- (D) Determine if novel storage solutions or pathogen reduction can ameliorate the “storage lesion”.

New directions

- (A) Characterize the profile of soluble mediators of inflammation in recipients of fresh vs. stored RBCs.
- (B) Measure effector and regulatory T cells, Th17 and NK cells in recipients of fresh vs. stored RBCs.



Preliminary data from a mouse model of traumatic blood loss and transfusion

Why a Mouse Model?

- Control over experimental conditions
- Access to samples prior to event and at any time points desired
- Ability to collect diverse sample types (beyond peripheral blood)
- Easy tracking of transferred cells
- Availability of mice with defined genetic backgrounds
- Availability of genetically manipulated mice

Setting up a mouse model

Transfusion versus blood loss

Context of loss & transfusion

Stress

Wound type

Innate immune activators

Product transfused



Minimal requirements for establishment of microchimerism

Tracking of transferred cells



Donor mice



B6 mice

1. Collect whole blood via orbital enucleation into CPDA-1 (14%)
2. Spin down and remove supernatant to hematocrit of ~75%
3. Setup transfusion “units” of 100 μ l blood + 400 μ l normal saline



Recipient mice



Untreated



Transfused



~30% blood volume removed



~30% blood volume removed,
Wait 1 hour, transfused



Sacrifice at 4 hrs or
2 weeks later

Collect blood
sample and spleen
for cellular and
cytokine analysis

Sample Processing

Whole blood



Serum



Cytokine
Analysis

+

Screen for Allo
Abs.

Spleen



Culture cells +/- B6 cells

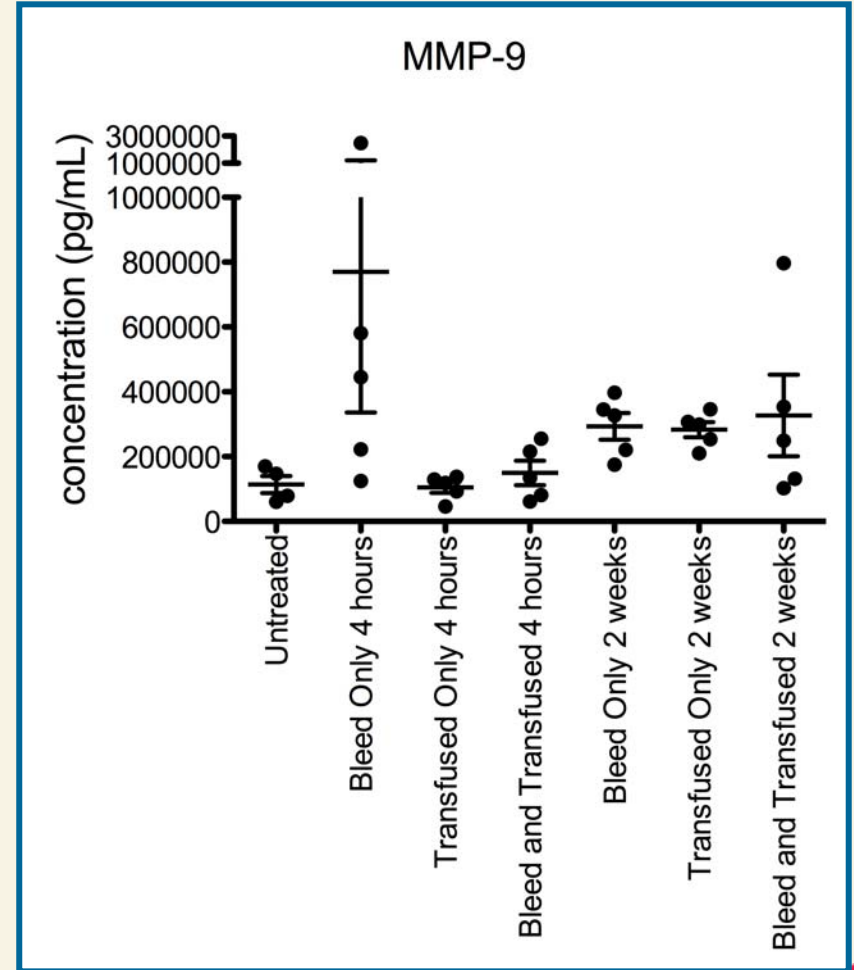
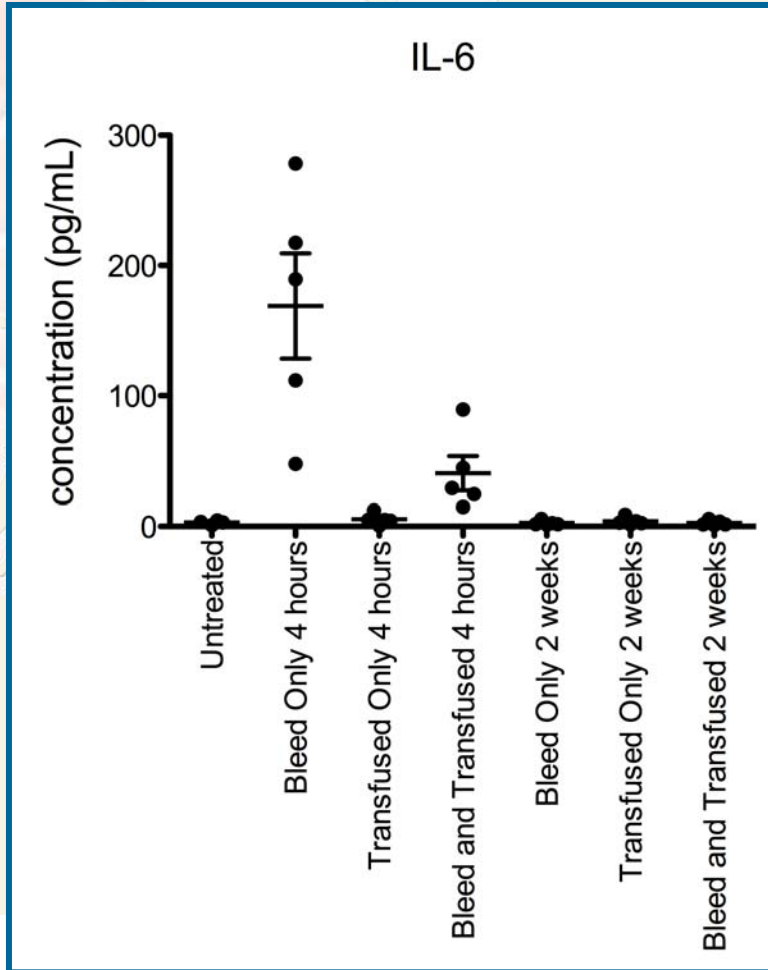


Cytokine
Analysis

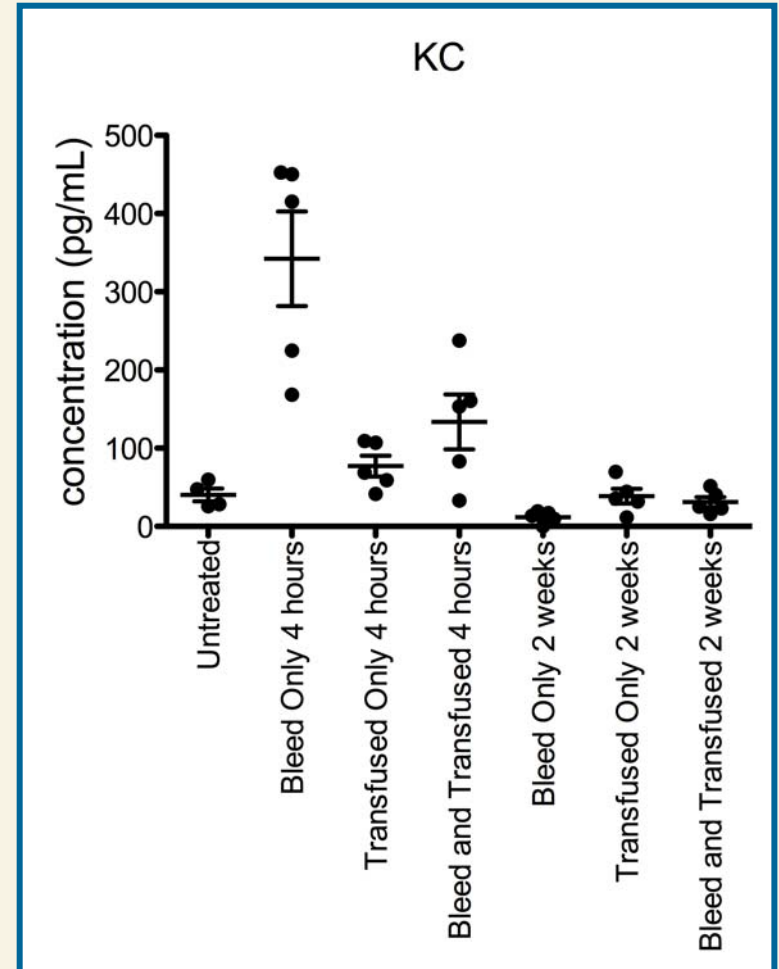
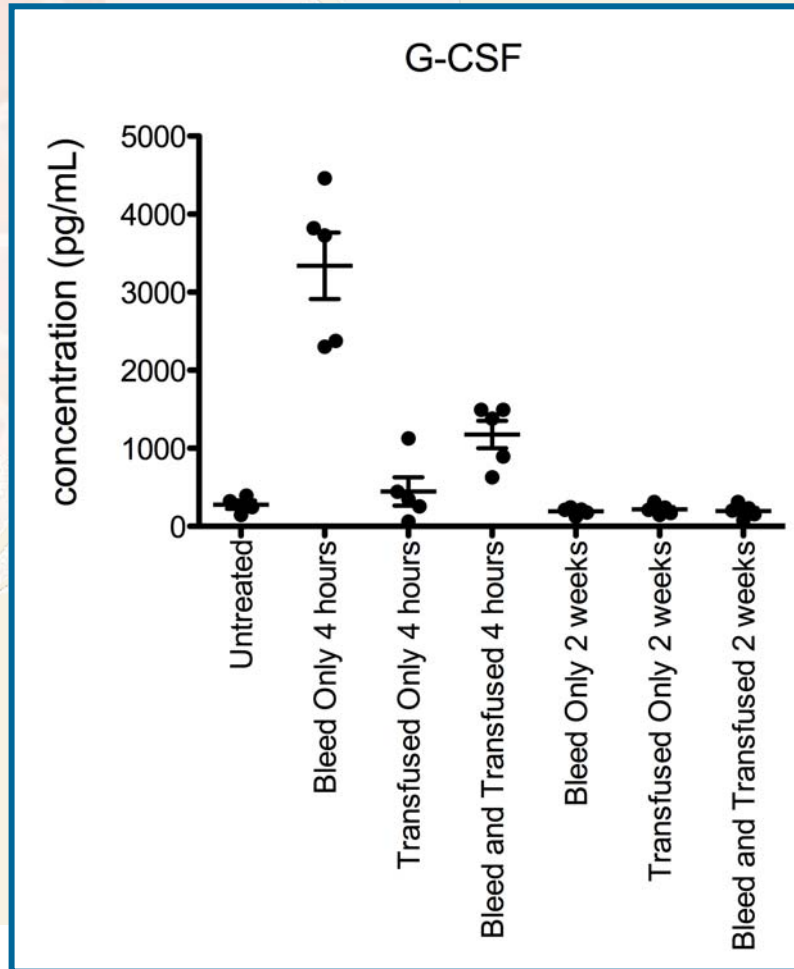
+

MLRs, Screen
for MC

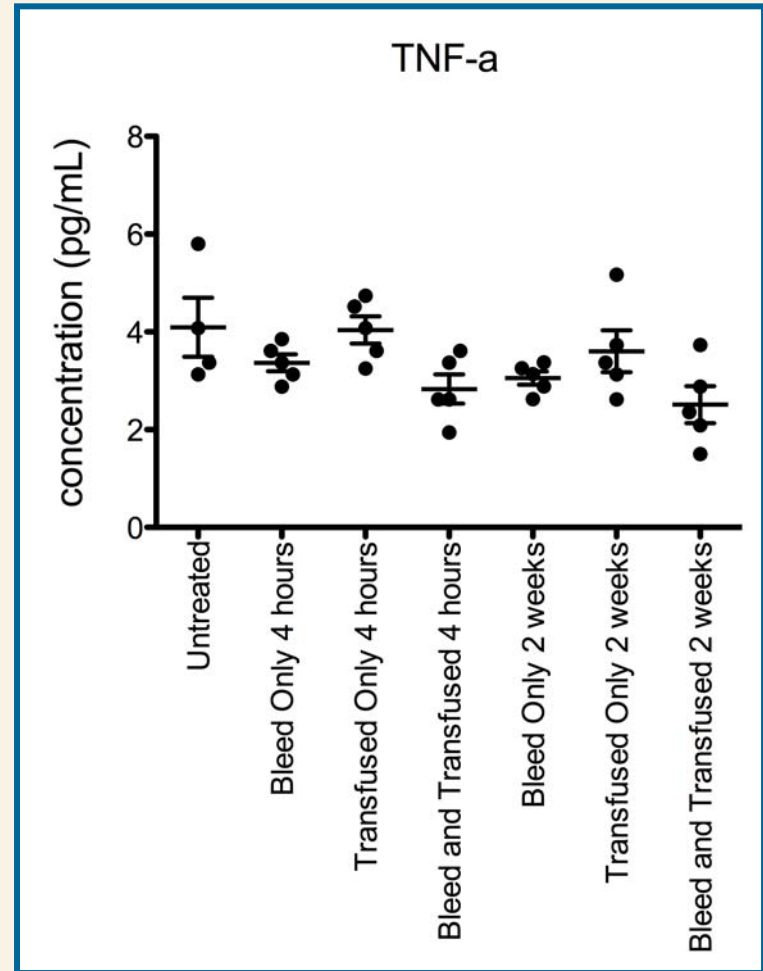
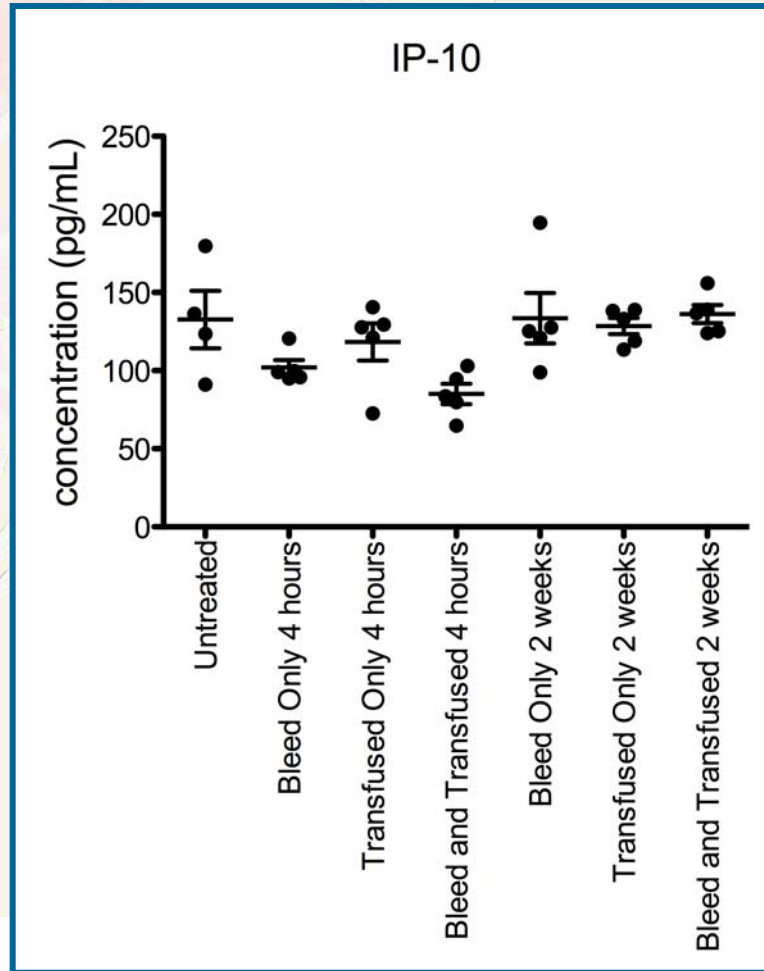
Serum Cytokines: Response to Traumatic Blood Loss



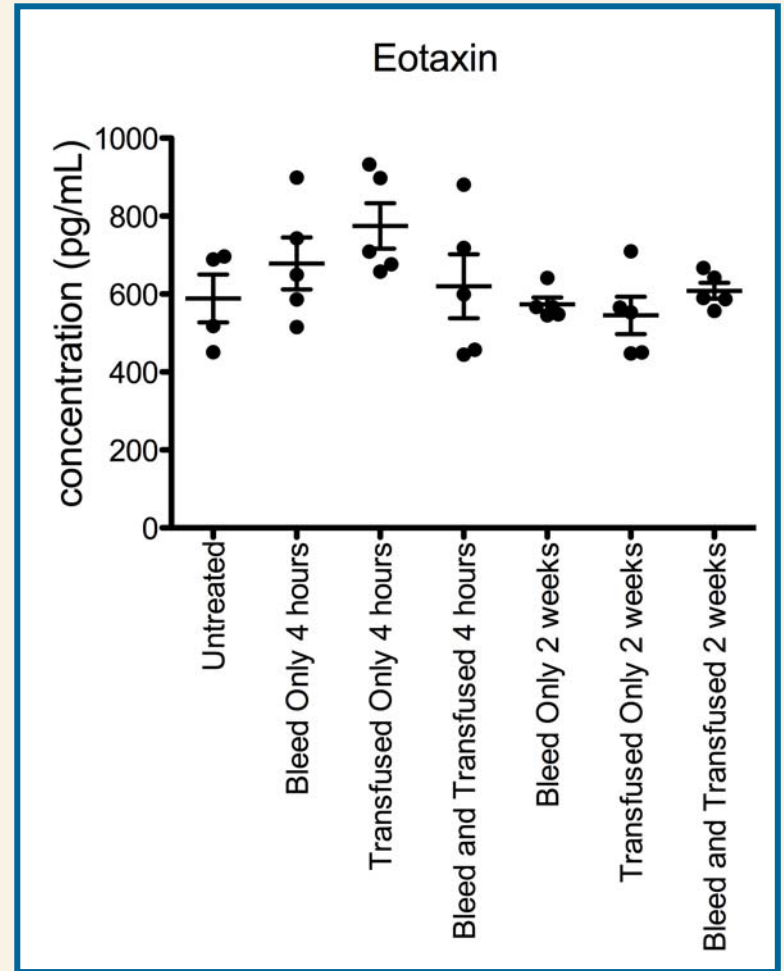
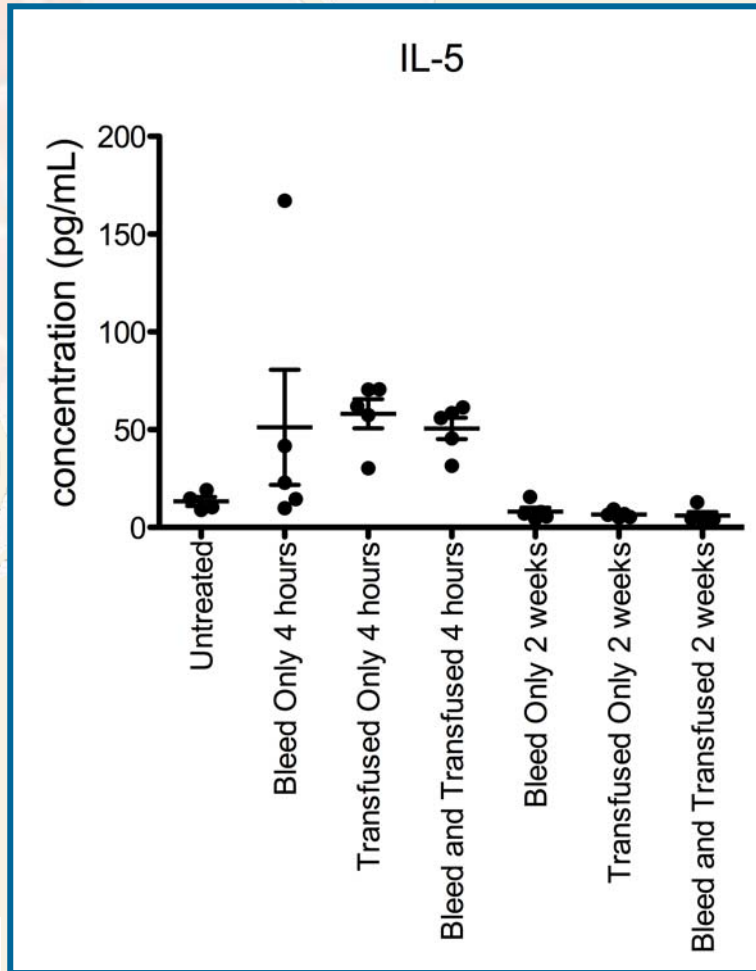
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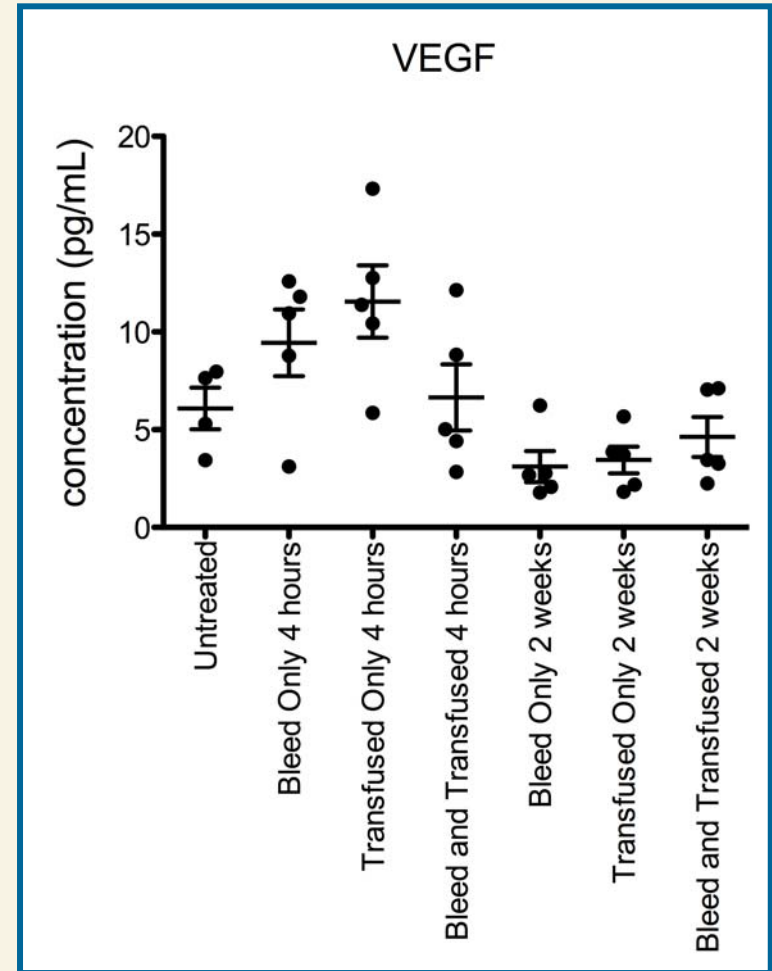
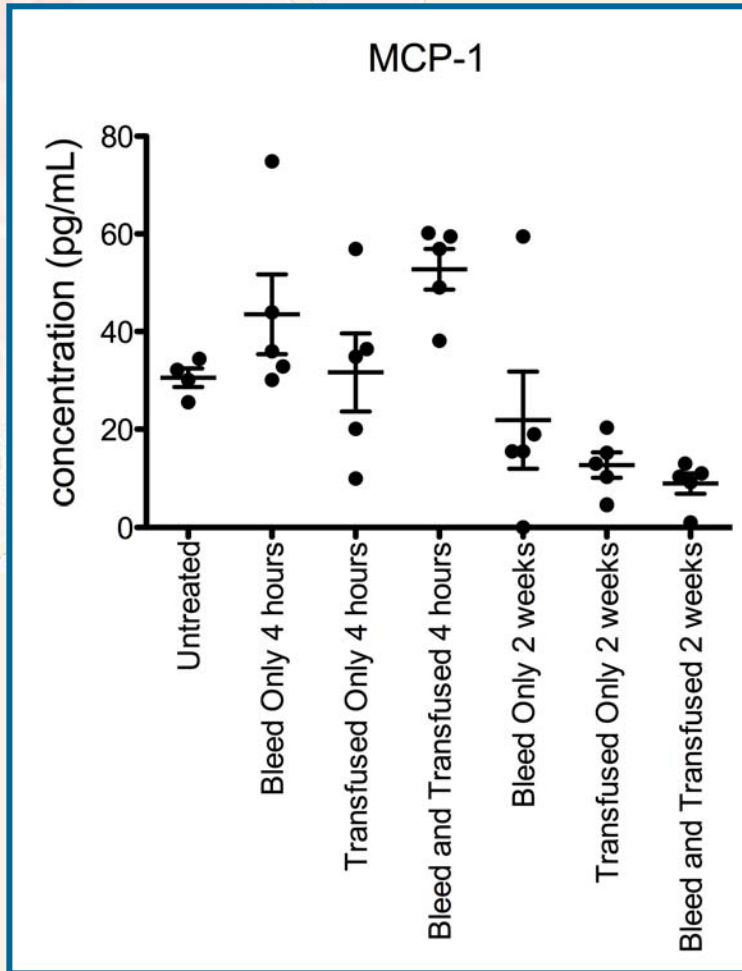
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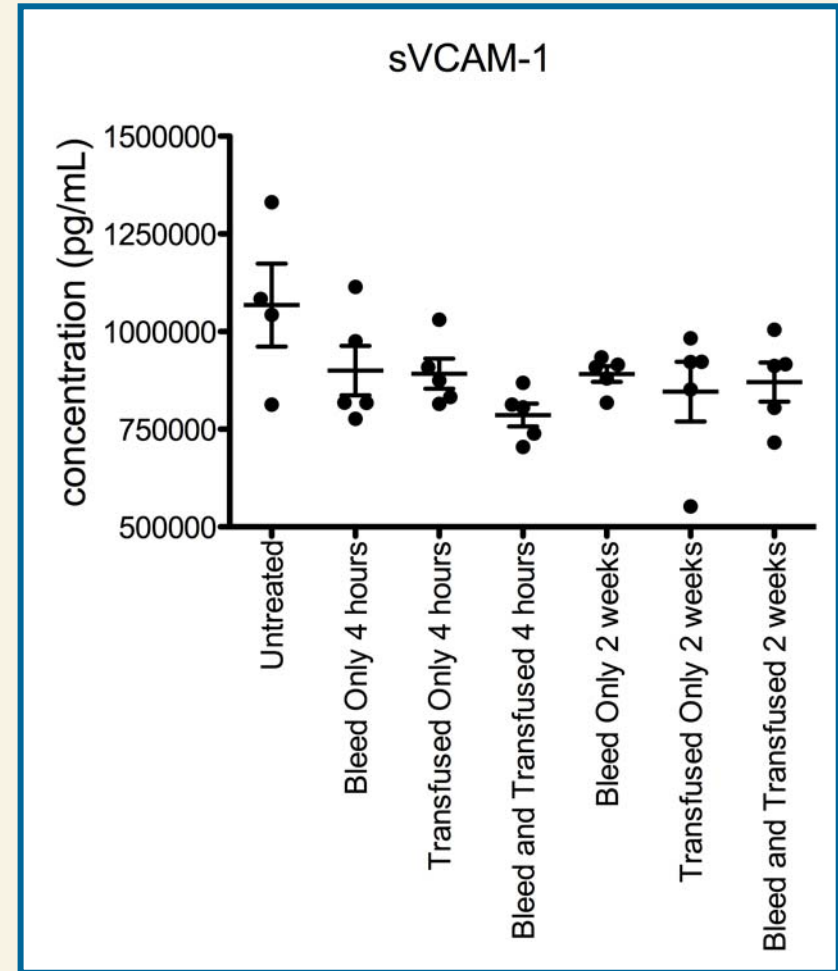
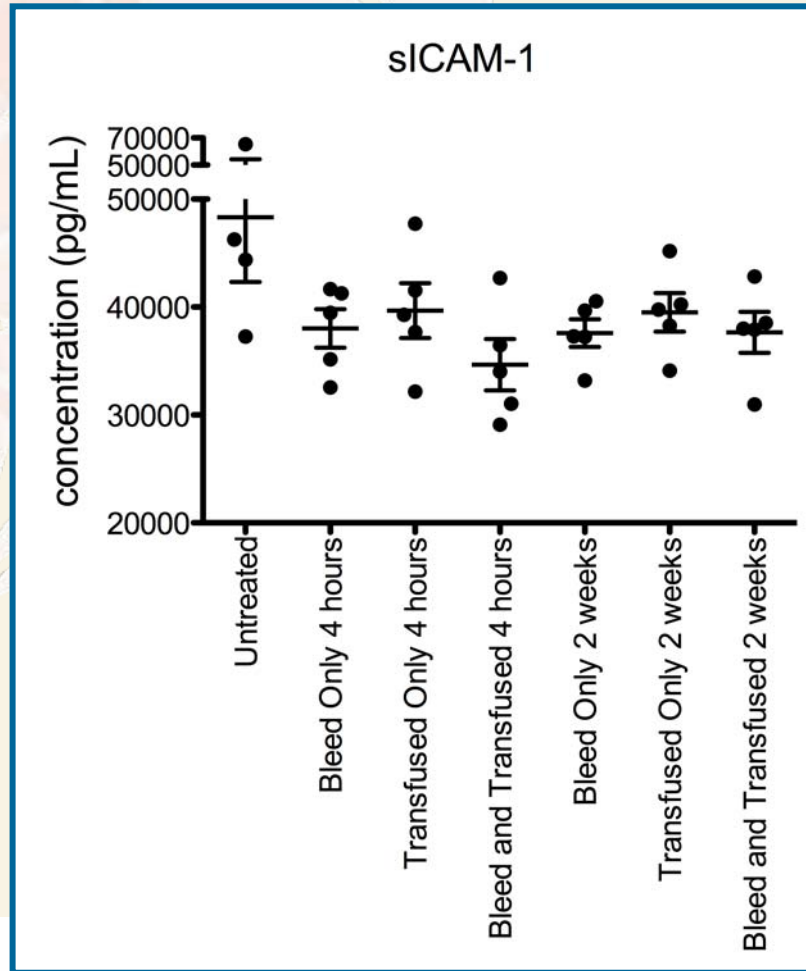
Serum Cytokines: Response to Transfusion



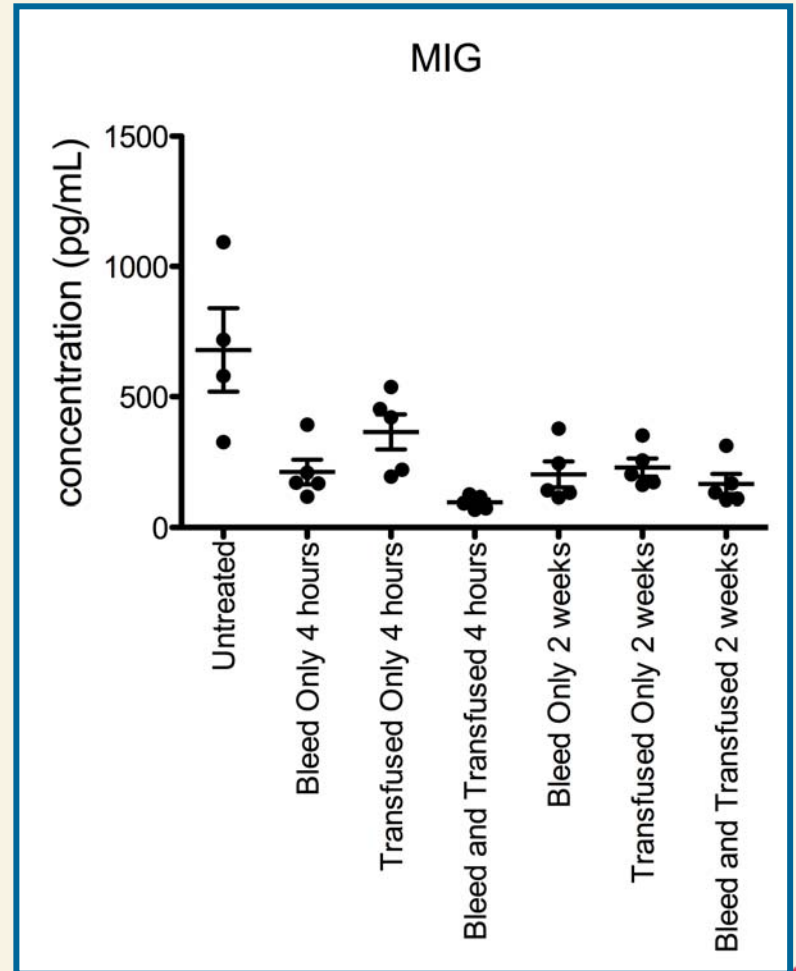
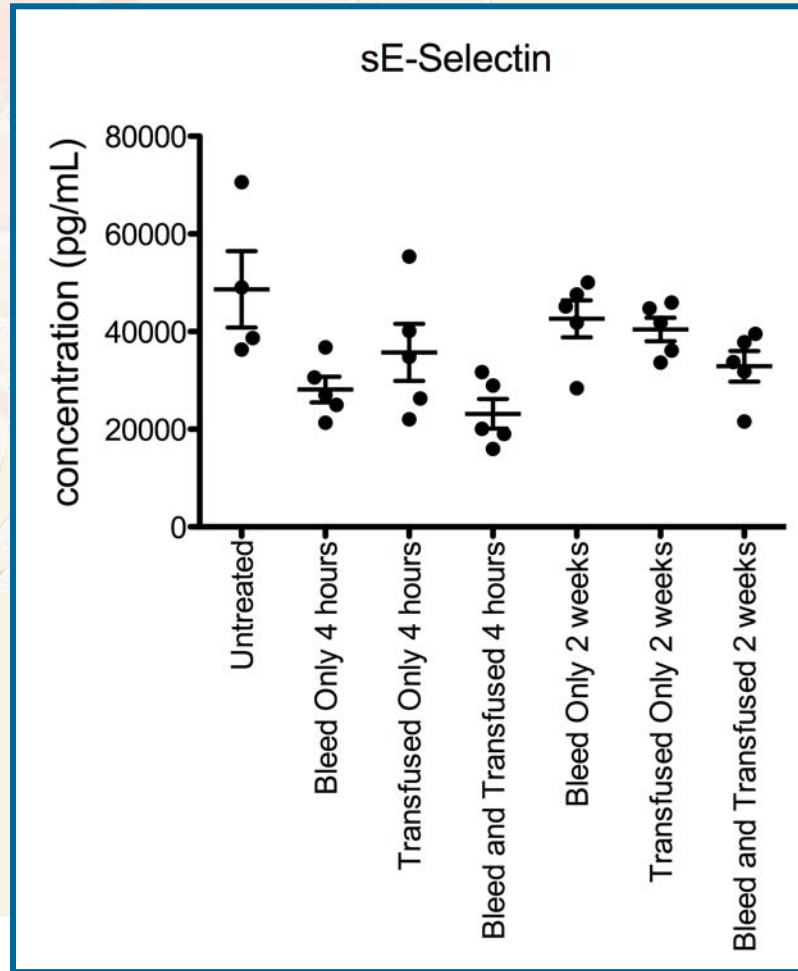
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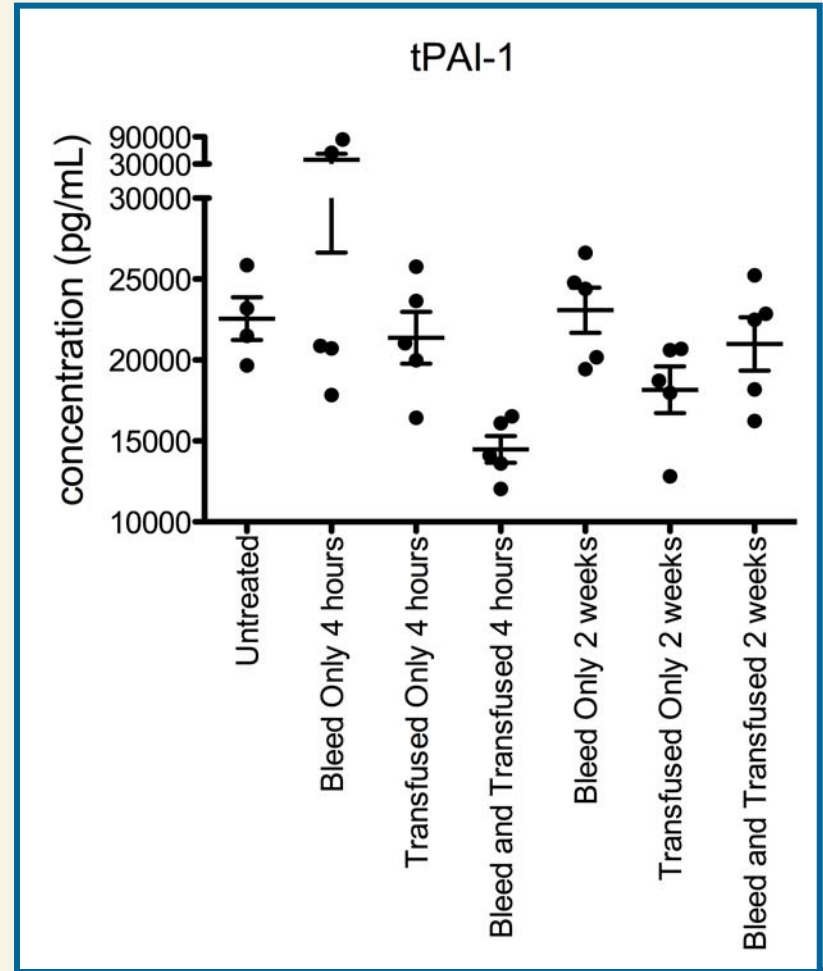
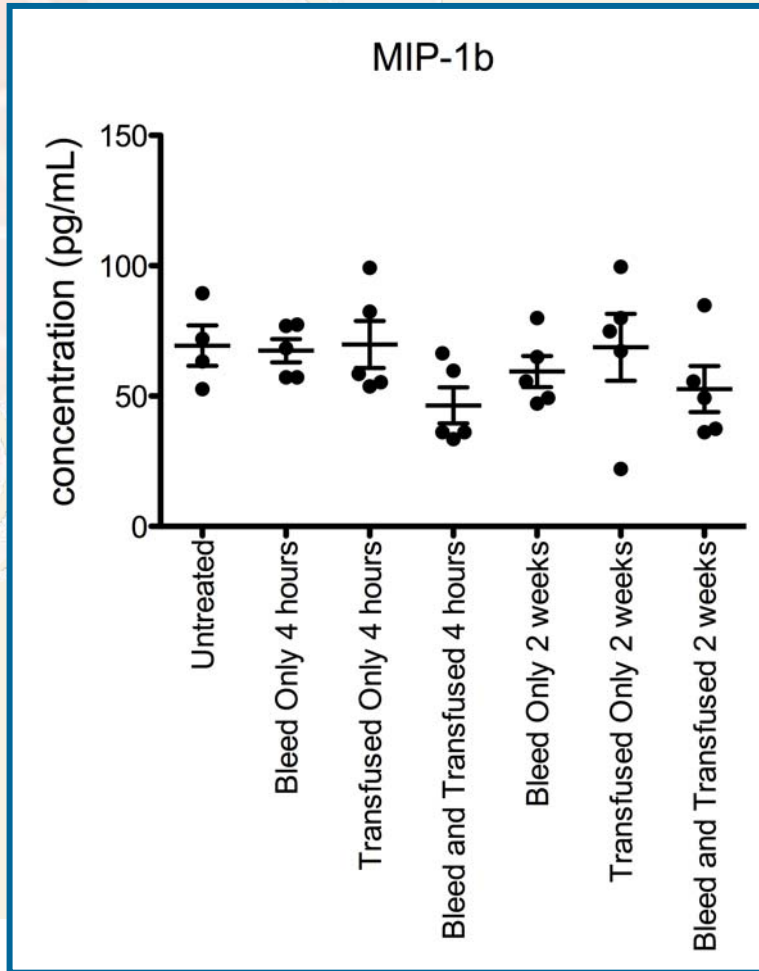
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Acknowledgments

BSRI

- Dale Hirschhorn
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- Ryan Rivers

ARCBS

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