Harmful effects of stored RBC transfusions: Bench ←→ Bedside

March 18, 2011

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Our Interests

Consequences of RBC clearance

Iron Status

Bacterial Infection
Malarial Infection
Co-infections

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Consequences of RBC clearance

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Bacterial Infection
Malarial Infection
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Outline

Background

Mouse model

Studies with healthy human volunteers

Unresolved questions

Conclusions & Future Directions

Holy Grail of Transfusion Medicine

Manipulate the composition of blood:

With complete control

Without adverse consequences

Transfusion Medicine

Transfusion of “products”:
RBC, Plt, WBC, PBSC, FFP

Infusion of recombinant proteins:
FVIII, FVIIa, ATIII

Prescription of “drugs”:
Epo, G-CSF, GM-CSF

Removal of “evil humors” (provide “good humors”):
Apheresis of cells and solutes

Holy Grail of Transfusion Therapy (A corollary)

Transfuse any unit of RBC into any recipient:

- **With perfect acquisition of the desired effect:**
  - **Normalizing Hct**
  - **Diminishing Hgb SS levels**
  - **Improving O_2 delivery**
- **Without adverse consequences:**
  - **Transfusion transmitted diseases (e.g. HIV)**
  - **Transfusion reactions**
  - **Missing the therapeutic target**
  - **Volume overload**

Holy Grail of Transfusion Therapy (Another corollary)

Blood products = Pharmaceuticals

White willow bark (Salix alba)

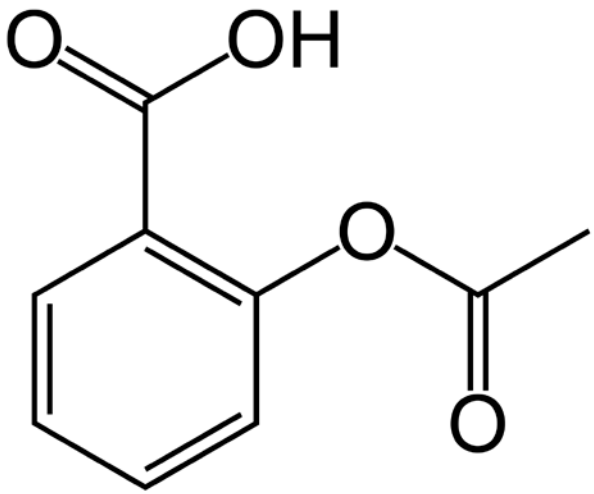
Preparing a hot infusion of white willow bark tea:

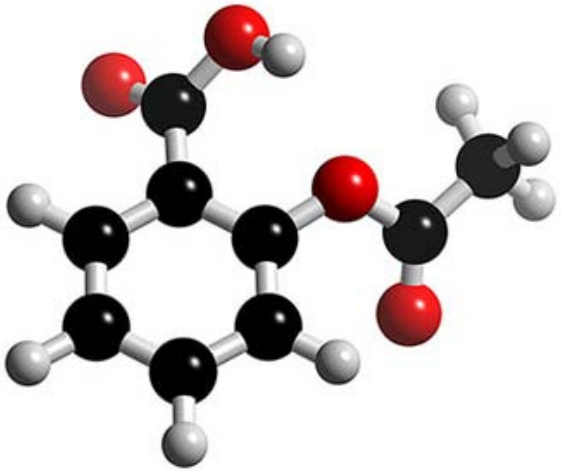
(1) Fill one tea infuser full of the white willow bark tea herbs
(2) Pour one cup of boiling water over the herbs
(3) Cover the cup to ensure all the volatile oils & aromas do not escape
(4) Allow the herbs to infuse for 3-5 minutes, then sip

Phytochemicals: Apigenin, beta-carotene, catechin, isoquercitrin, lignin, p-coumaric acid, quercitrin, rutin, salicin, salicylic acid, tannin

Nutrients: Calcium, iron, mangenese, magnesium, phosphorus, potassium, selenium, zinc, vitamins B1, B2, B3, and C.

Aspirin = Acetylsalicylic acid





“All aspirin is now chemically synthesized. It's not surprising, then, that white willow bark is often called ‘herbal aspirin.’”

Holy Grail of Transfusion Therapy (Another corollary)

Hemophilia A

Whole Blood

↓

Plasma

↓

Cryoprecipitate

↓

Purified FVIII

↓

Recombinant FVIII

The claims regarding RBC storage

Several **non-randomized, observational** studies suggest that transfusions of older, stored RBCs cause problems

The claims regarding RBC storage

Human studies suggest that transfusions of older, stored RBC products are associated with **increases** in:

- Sepsis
- Pneumonia
- Multi-organ failure
- Myocardial infarction
- Acute renal failure
- Thrombosis
- Length of stay
- Mortality

What is the evidence?

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Duration of Red-Cell Storage and Complications after Cardiac Surgery

Colleen Gorman Koch, M.D., Liang Li, Ph.D., Daniel I. Sessler, M.D., Priscilla Figueroa, M.D., Gerald A. Hoeltge, M.D., Tomislav Mihaljevic, M.D., and Eugene H. Blackstone, M.D.

N ENGL J MED 358;12 WWW.NEJM.ORG MARCH 20, 2008

ABSTRACT

CONCLUSIONS

In patients undergoing cardiac surgery, transfusion of red cells that had been stored for more than 2 weeks was associated with a significantly increased risk of postoperative complications as well as reduced short-term and long-term survival.

What is the evidence?

Table 2. Postoperative Complications, According to the Duration of Blood Storage.

Complication	Patients Receiving Newer Blood (N=2872)*	Patients Receiving Older Blood (N=3130)†	P Value‡
	no. (%)		
In-hospital death	49 (1.7)	88 (2.8)	0.004
Pulmonary			
Ventilation >72 hr	160 (5.6)	304 (9.7)	<0.001
Pneumonia	81 (2.8)	111 (3.5)	0.11
Pulmonary embolism	5 (0.2)	7 (0.2)	0.67
Respiratory insufficiency	177 (6.2)	278 (8.9)	<0.001
Renal			
Renal failure	45 (1.6)	84 (2.7)	0.003
Infectious			
Septicemia or sepsis	80 (2.8)	125 (4.0)	0.01
Deep sternal wound	25 (0.9)	25 (0.8)	0.76
Superficial sternal wound	44 (1.5)	62 (2.0)	0.19
Multiorgan failure	7 (0.2)	23 (0.7)	0.007
Peripheral vascular			
Iliac or femoral dissection	0	0	
Acute limb ischemia	7 (0.2)	18 (0.6)	0.05
Composite outcome§	642 (22.4)	810 (25.9)	0.001

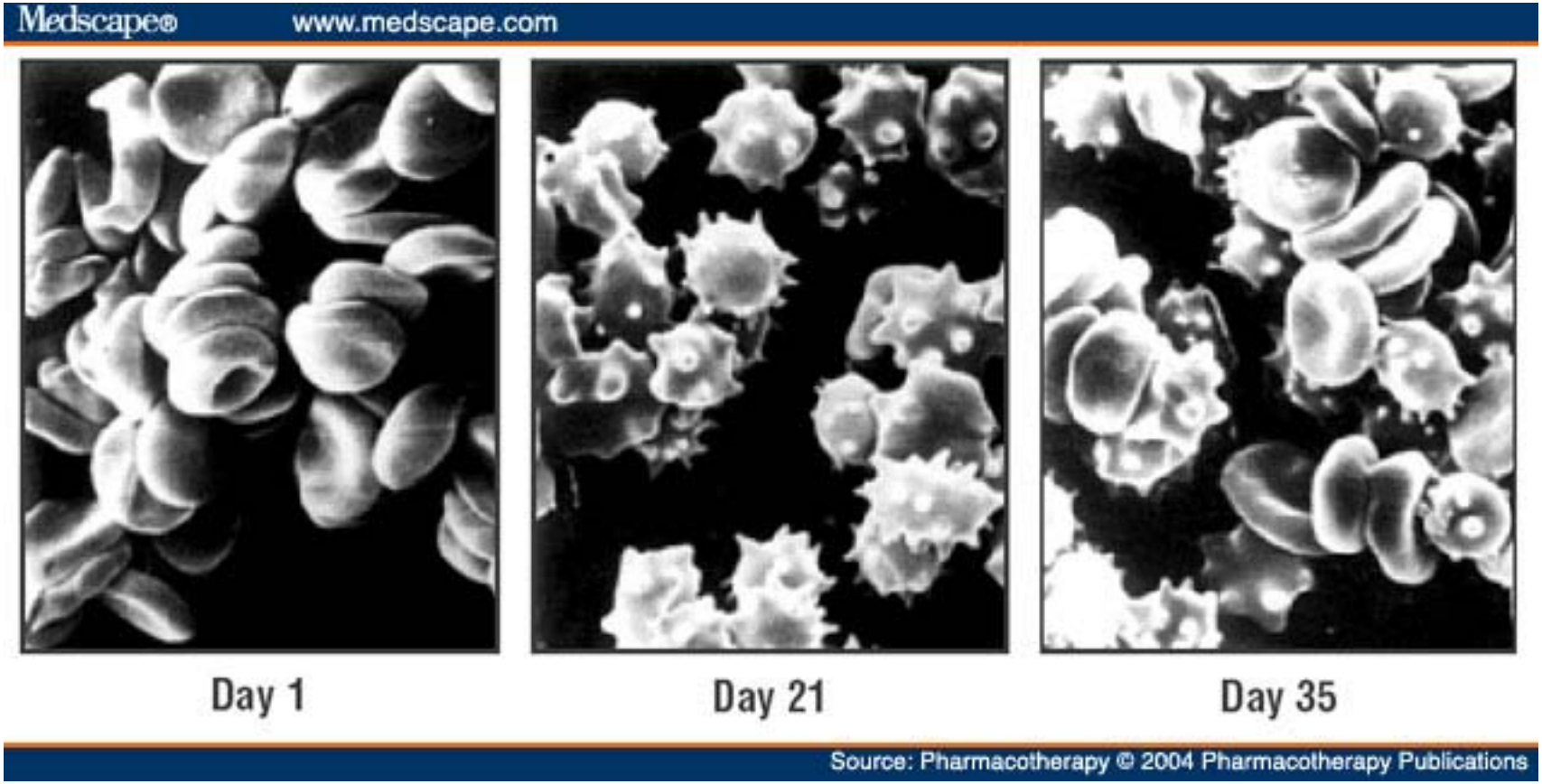
Critique of the Koch study

- Retrospective, non-randomized
- More Group O patients received fresh blood (51% vs. 31%)
- Older RBC group had more abnormal left ventricular function, mitral regurgitation, and peripheral vascular disease
- More leukoreduction in older RBC group
- More large dose transfusions in older RBC group

Dzik. Transfusion Medicine 18:260-5, 2008.

What happens to RBCs during storage?

The RBC storage lesion



The RBC storage lesion

- Decreased 2,3-DPG and ATP
- Vesiculation and membrane loss
- Increased lysophosphatidylcholine species
- Decreased nitric oxide
- Decreased deformability (~30% irreversibly deformed at 42 days of storage)
- Decreased CD47
- Hemolysis
- Protein oxidation
- Lipid peroxidation of RBC membrane

↓

Decreased RBC survival *in vivo*

Tinmouth et al. Transfusion 2006

How did the FDA decide on the maximal allowable storage time?

- 1979: CPDA-1, FDA-allowed 35 days storage (based on 70% 24-hr survival)
- 70% was picked arbitrarily
- 1985: survival criteria raised to 75%, arbitrarily
- AS-1: FDA originally allowed 49 days storage

RBC Survival Study

5-15 mL

Centrifuge

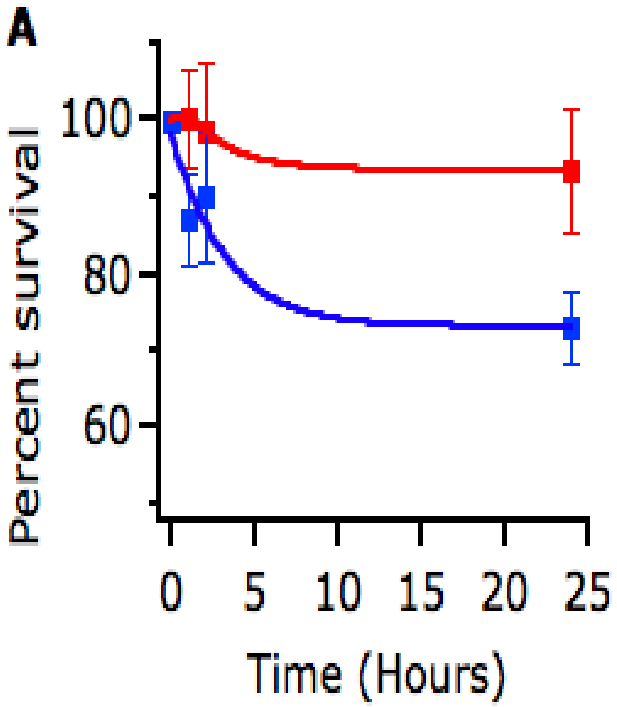
Wash

Incubate 51-Cr

Wash

IV push

Serial blood draws



FDA criteria regarding outdate approval

- 20 or more evaluable 24-hr RBC survivals
- Minimum of 2 laboratories
- Sample mean ≥75%
- Standard deviation ≤9%
- Hemolysis <1% at end of storage (95% of the time)

Variability of 24-hr RBC survival in healthy volunteers

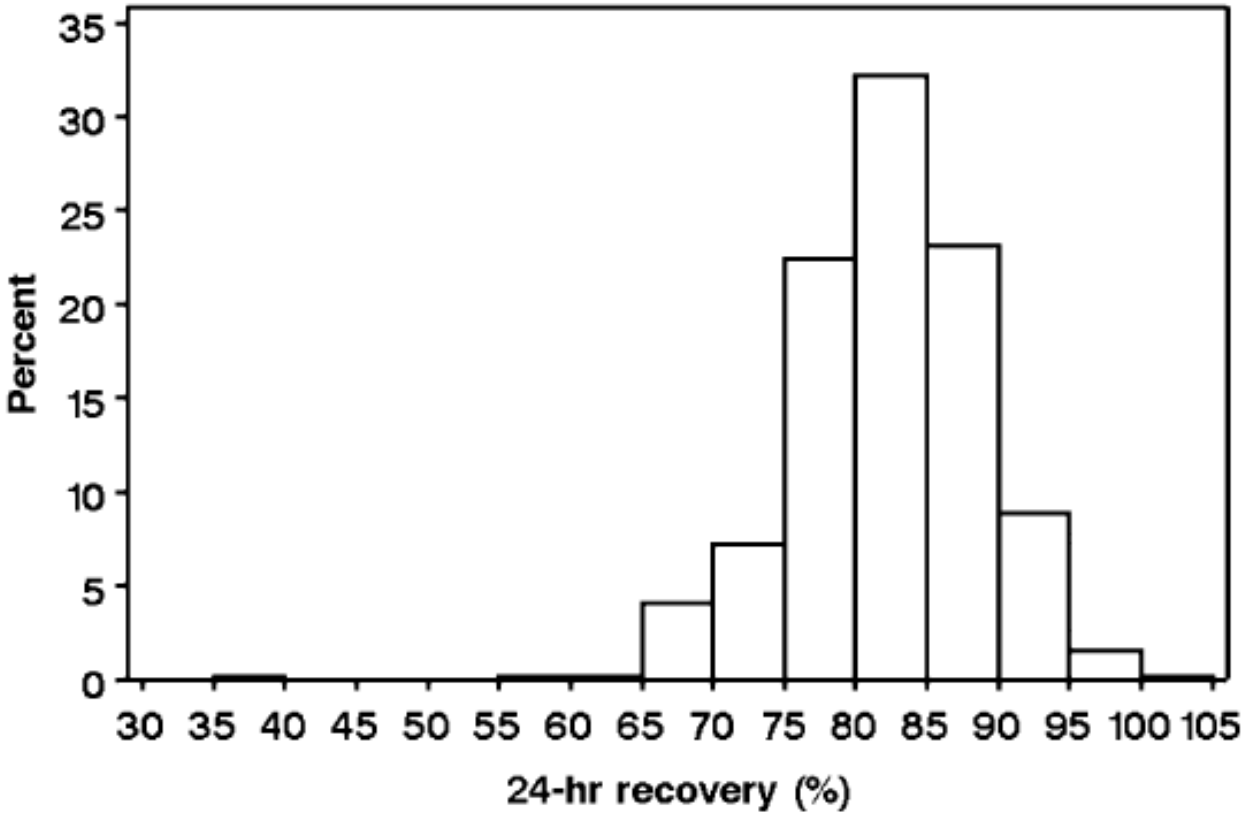


Fig. 1. Frequency distribution of 24-hour RBC recovery for RBCs stored for 42 days in AS. n = 641.

Dumont et al. Transfusion 48:1053-60, 2008.

Variability of RBC survival in patients (most clearance by 1 hour post-transfusion)

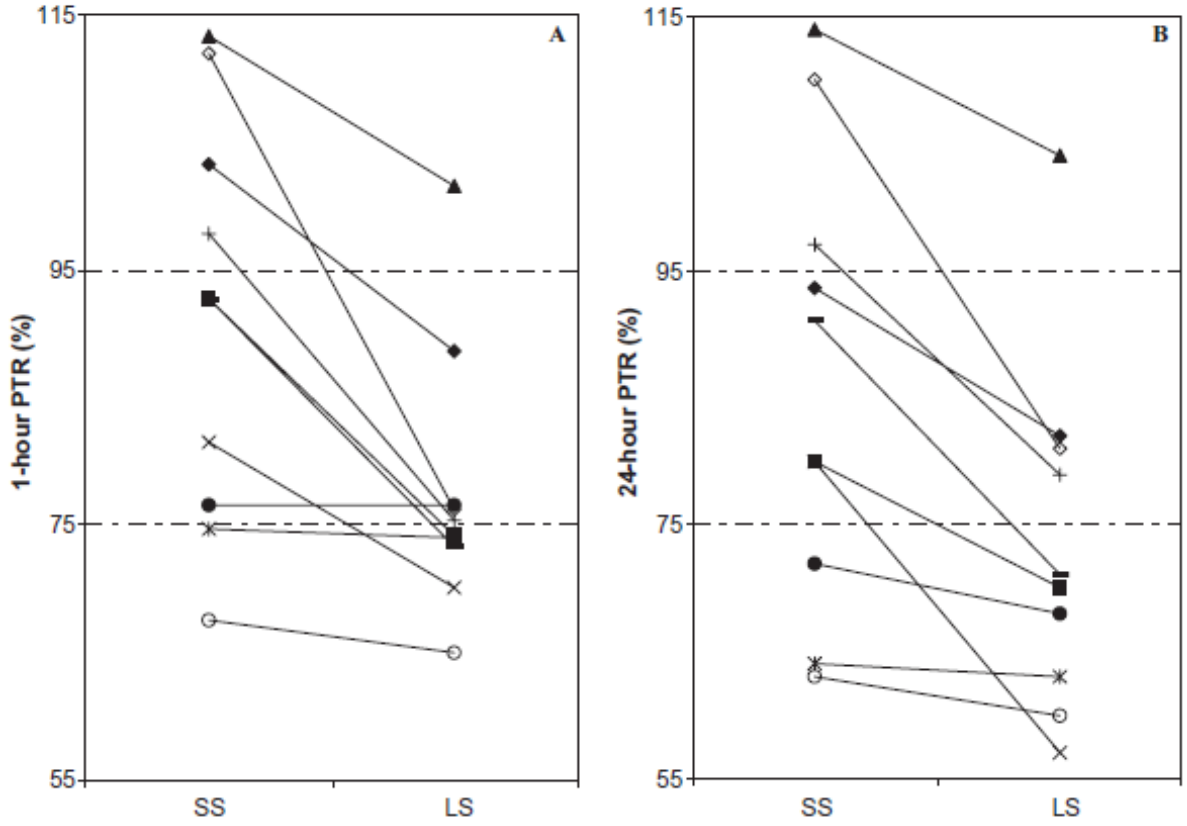


Fig. 1. Individual 1-hour PTR (A) and 24-hour PTR (B) of SS and LS RBCs. SS and LS RBCs that have been transfused into the same patient are connected to each other. Each symbol represents a patient.

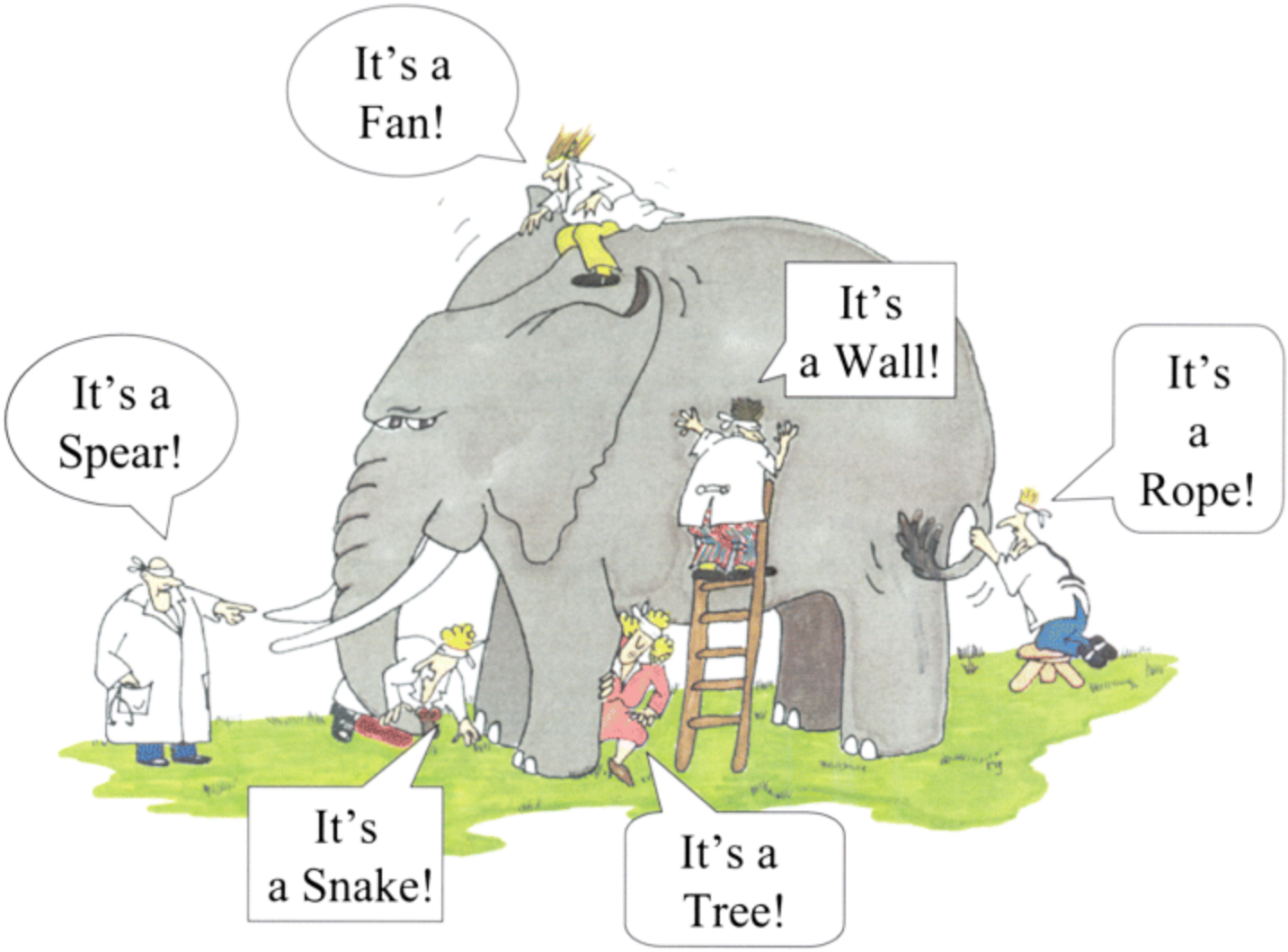
Luten et al. Transfusion 48:1478-85, 2008.

What are the consequences of this RBC clearance?

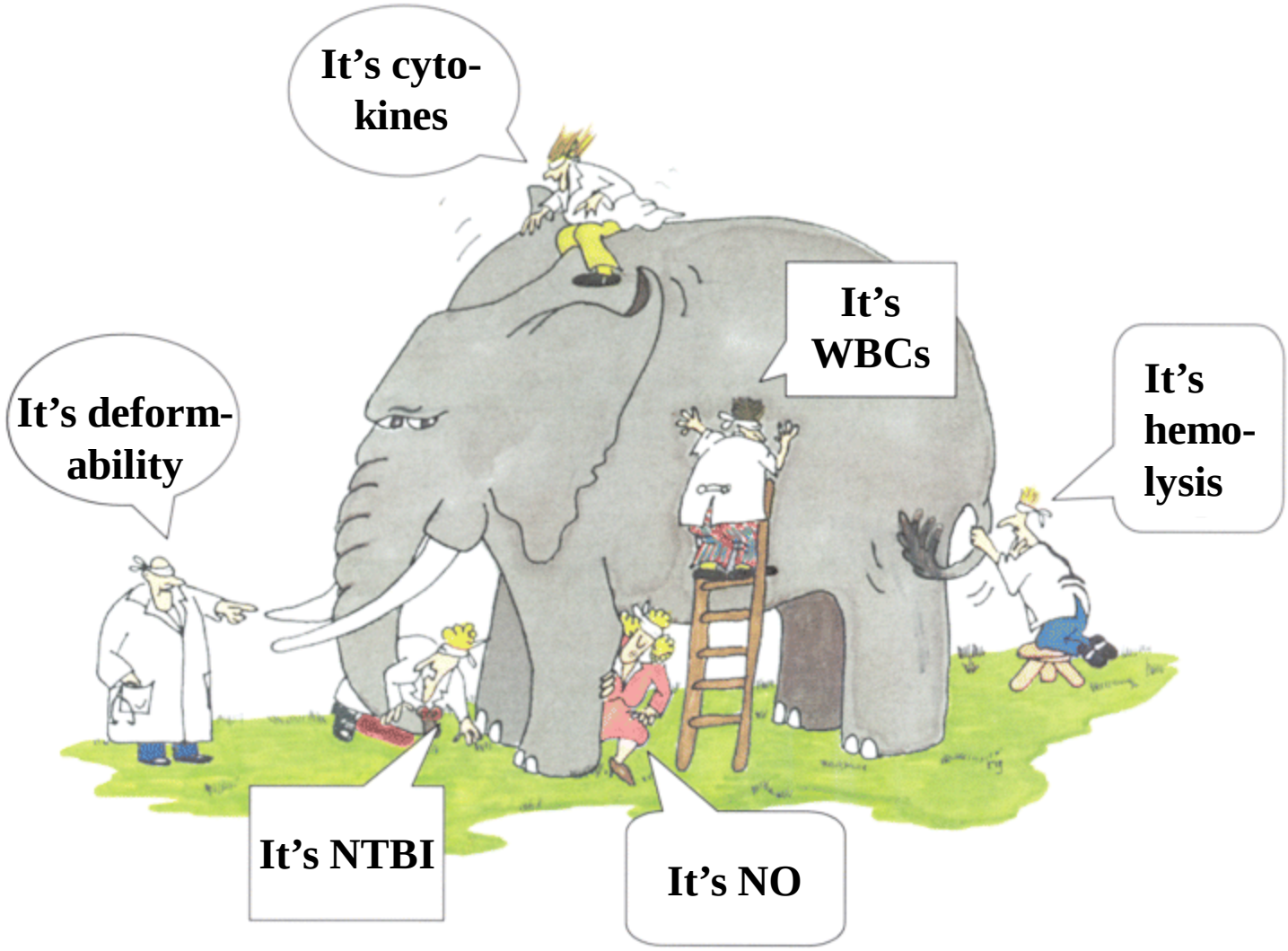
What are the consequences of this RBC clearance?

Are there any consequences of this RBC clearance?

The Blind Men and the Elephant



Consequences of the RBC Storage Lesion

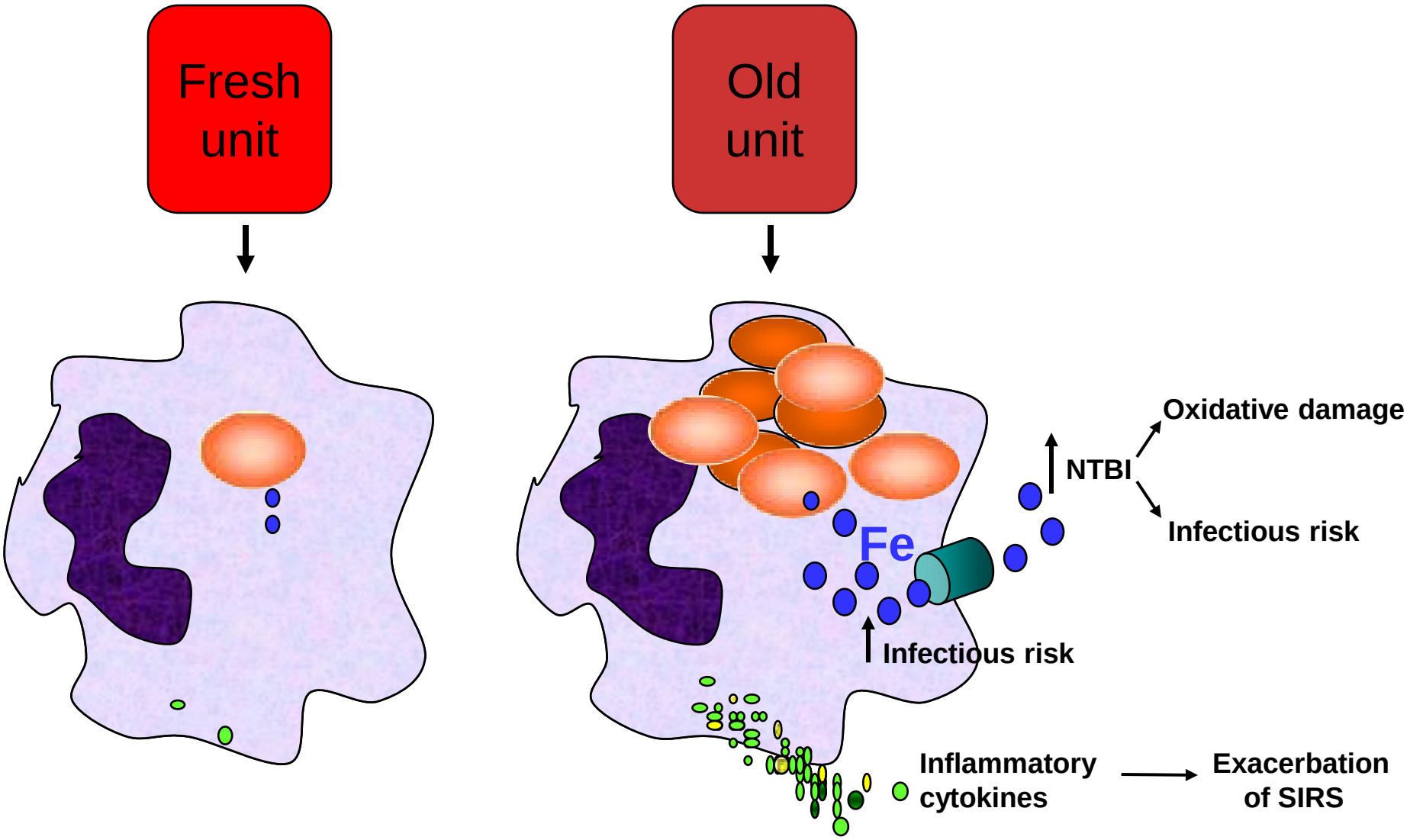


What are the consequences (if any) of the clearance of stored RBCs?

Hypothesis

Delivery of hemoglobin/iron to the monocyte-macrophage system by clearing a subpopulation of stored RBCs is responsible for the harmful effects of transfusion

Hypothesis


Fresh unit
Old unit
Fe
NTBI
Oxidative damage
Infectious risk
Infectious risk
Inflammatory cytokines
Exacerbation of SIRS

A little arithmetic

5 L total blood volume

RBC lifespan ~120 days

1/120th of RBCs gets cleared in 24 hr = 40 mL/24 hr

40 mL/24 hr x 50% Hematocrit = 20 mL/24 hr

~1 mL RBC/hour = ~$1x10^{10}$ RBC = ~1mg Fe

1 unit transfusion at outdate = 300 mL

25% cleared, most within 1 hour = 75 mL/hr

75 mL/hr x 70-80% hematocrit = ~60 mL RBC/hr

~60 ml RBC/hour = ~$6x10^{11}$ RBC = ~60mg Fe

A little arithmetic

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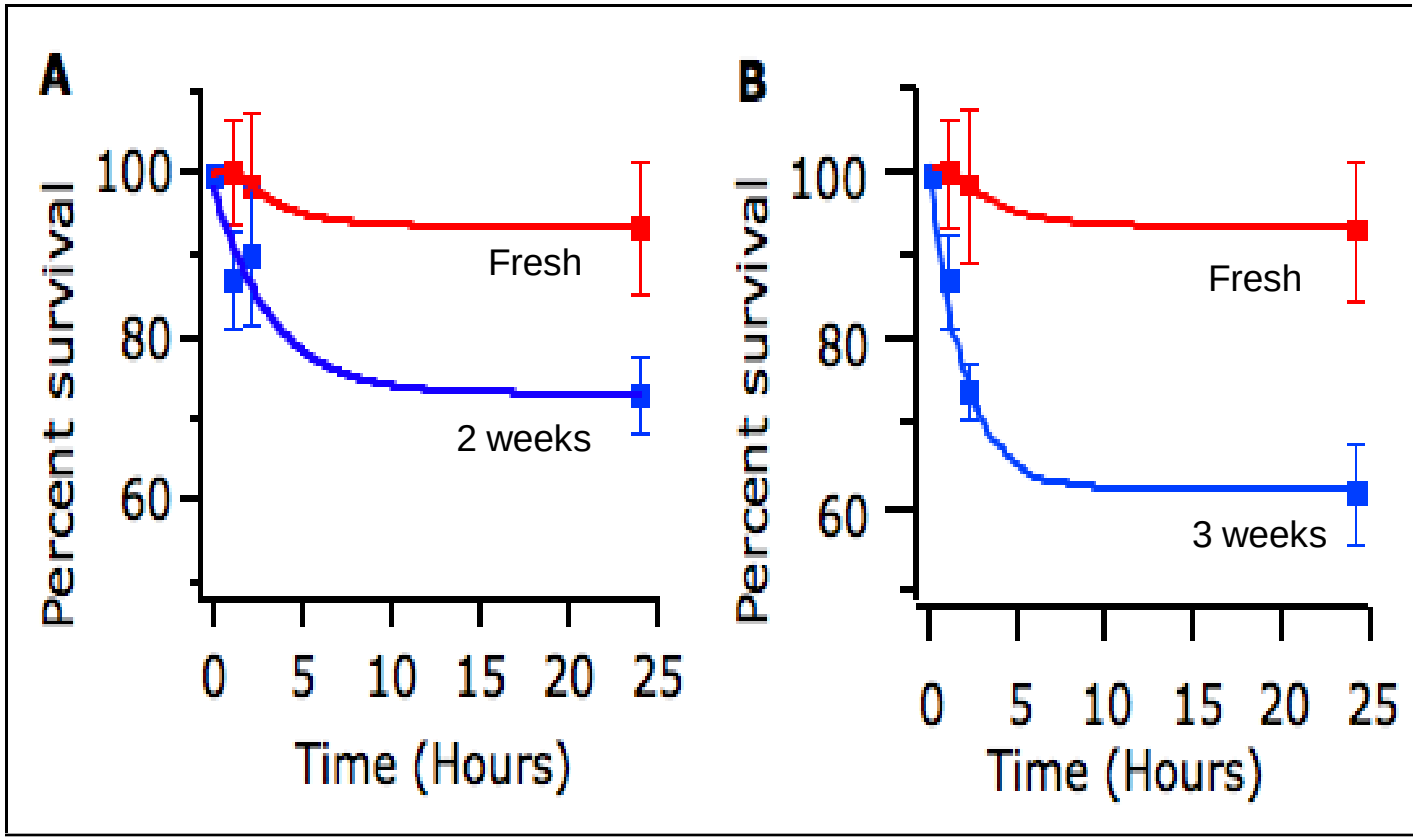
How are we studying this issue?

Mouse Blood Bank model

- Collect blood by aseptic cardiac puncture
- Pre-storage leukoreduction (Pall filter)
- CPDA-1 as preservative
- 60-80% hematocrit at 1-6ºC
- Aerobic blood culture; monitored for 5 days
- Transfuse into recipient mice

Survival of stored mouse RBCs



Gilson et al. Transfusion 49:1546-53, 2009

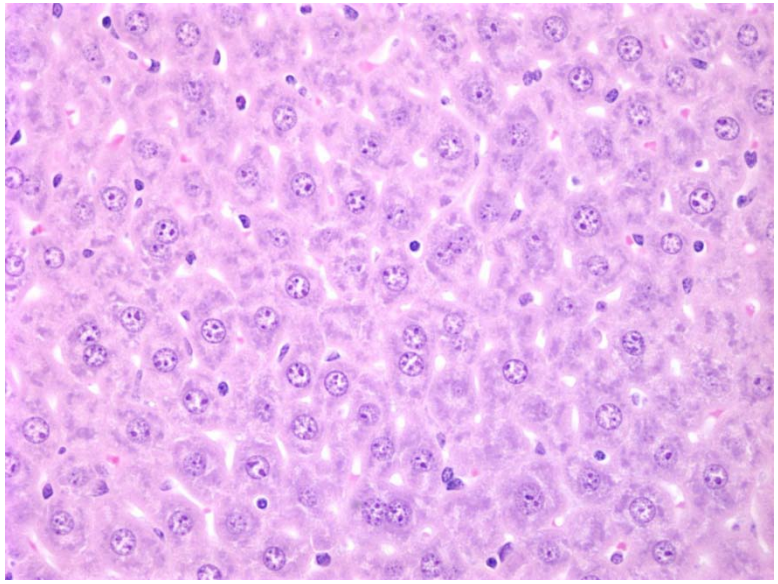
Where do the cleared RBCs go?

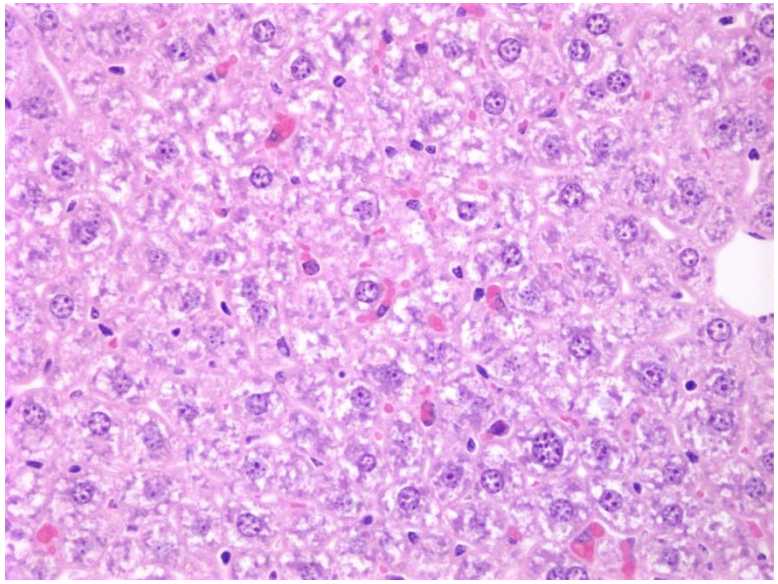
Liver

Fresh RBCs

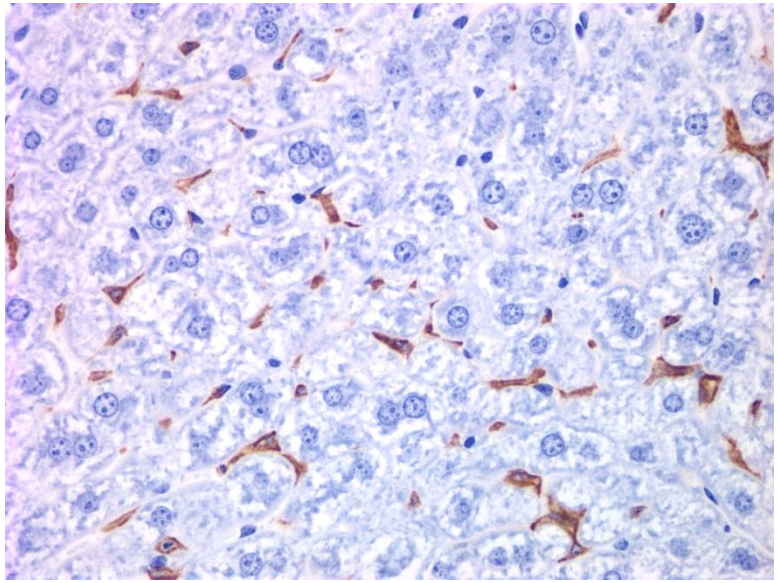
Stored RBCs

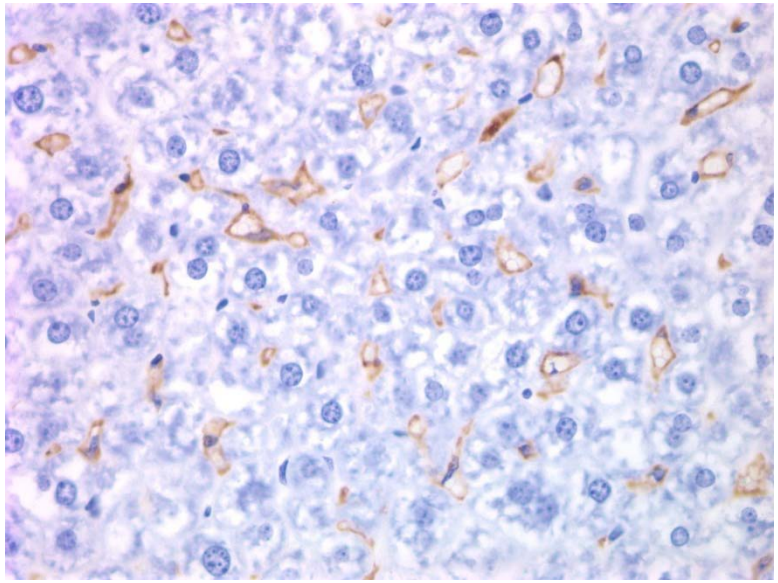
H&E





F4/80



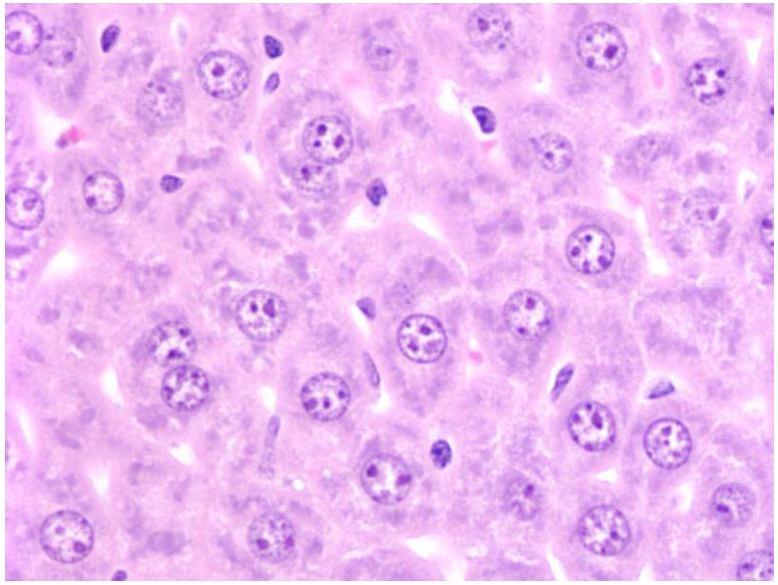


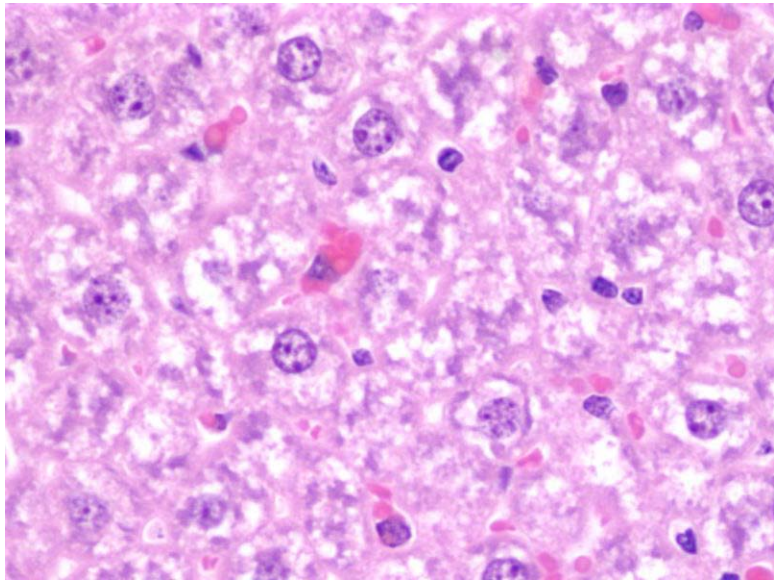
Liver

Fresh RBCs

Stored RBCs

H&E





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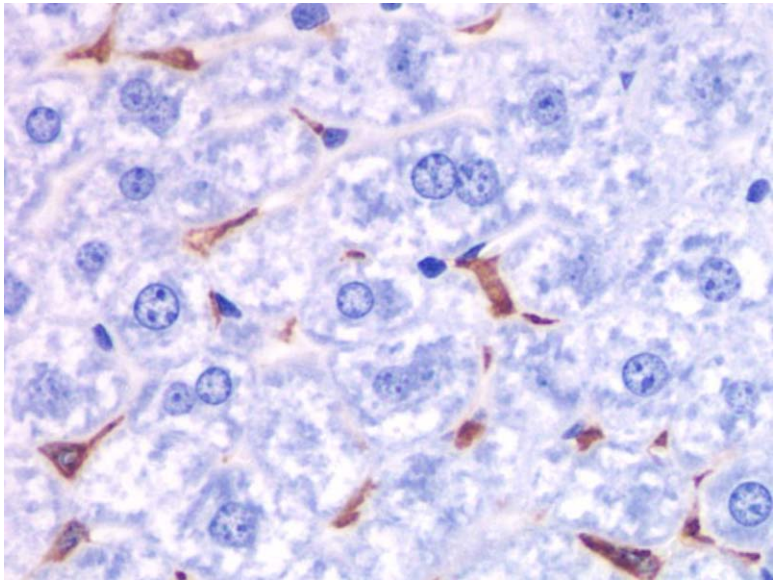
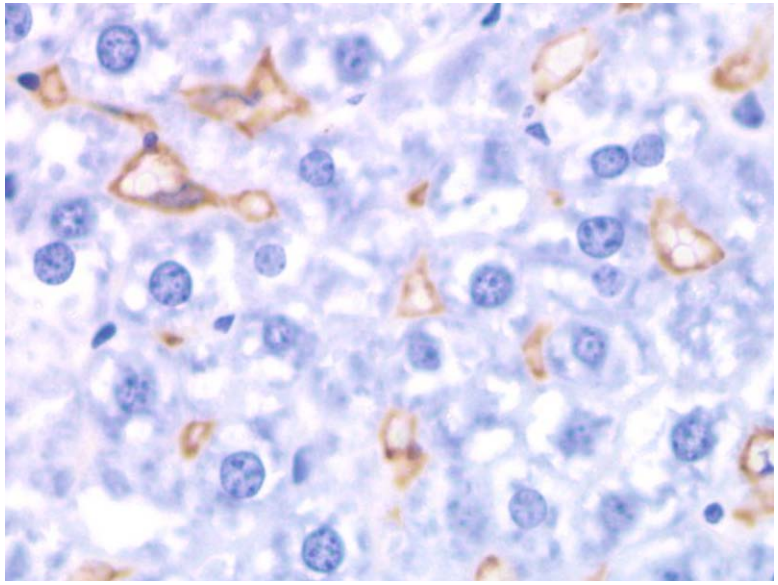
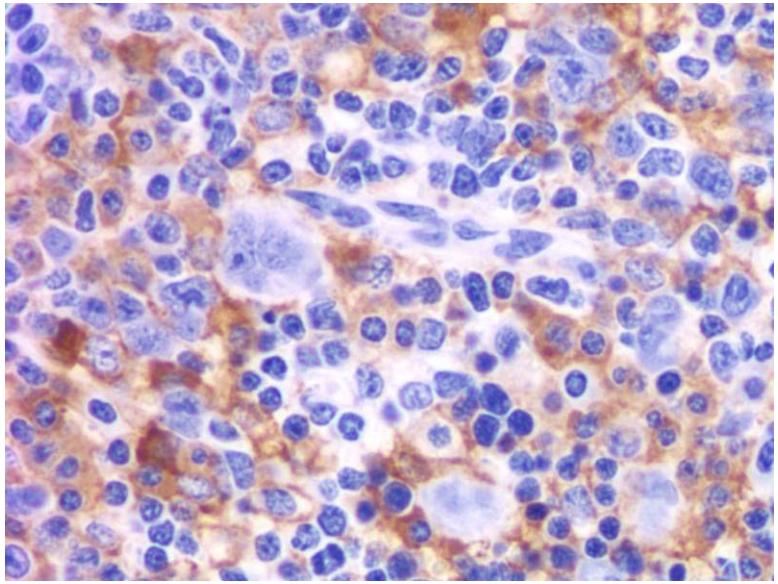
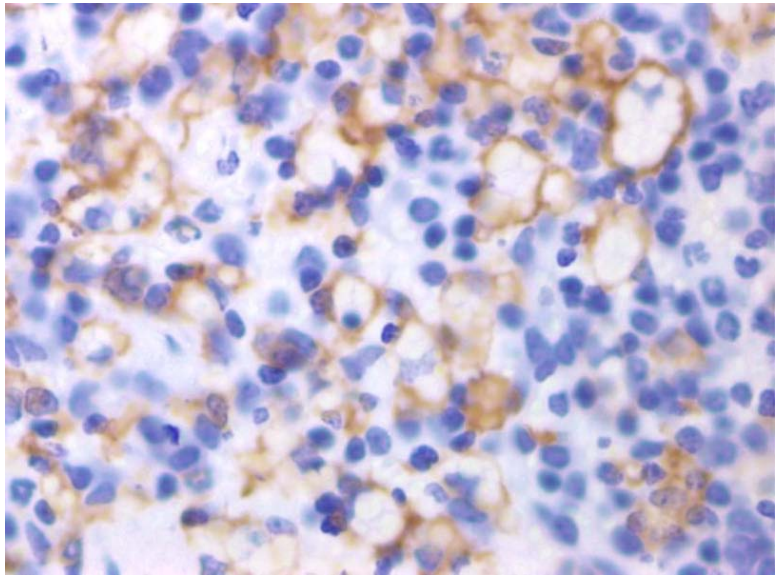
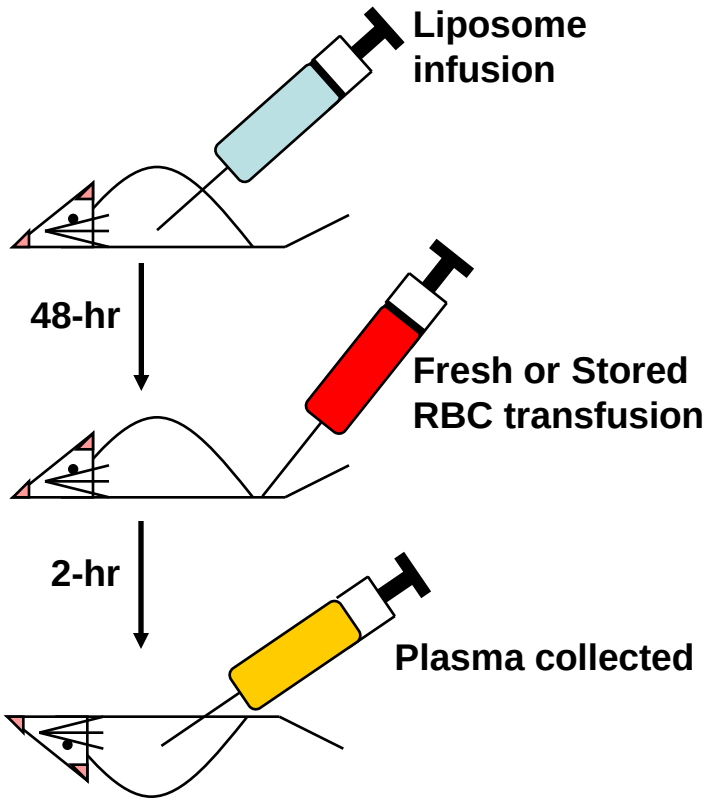




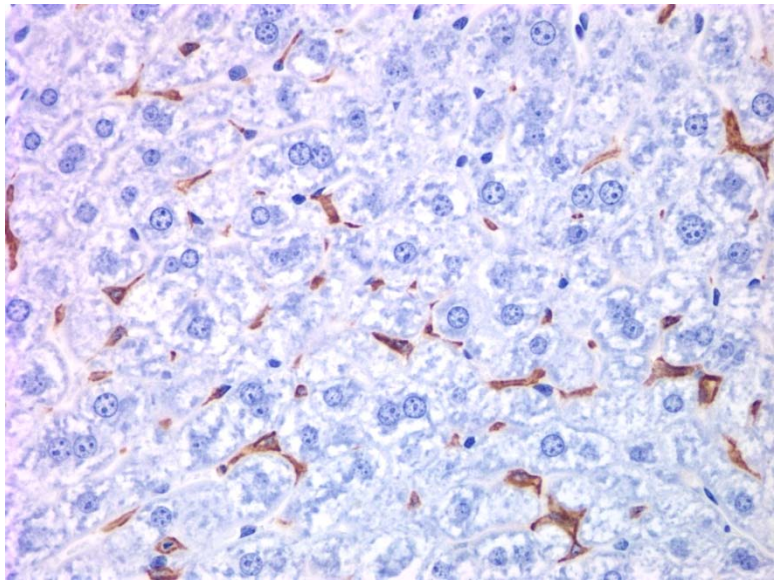
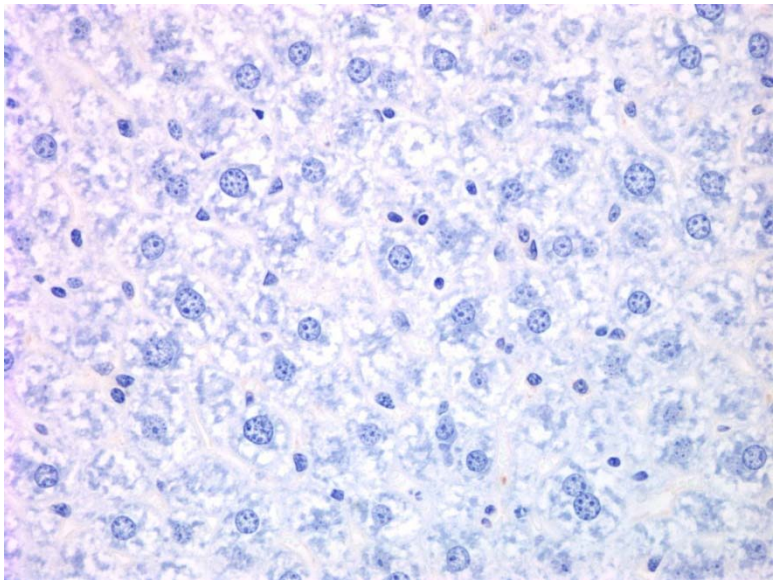
Transfused stored RBCs are cleared by splenic macrophages

	Fresh RBCs	Stored RBCs
F4/80		

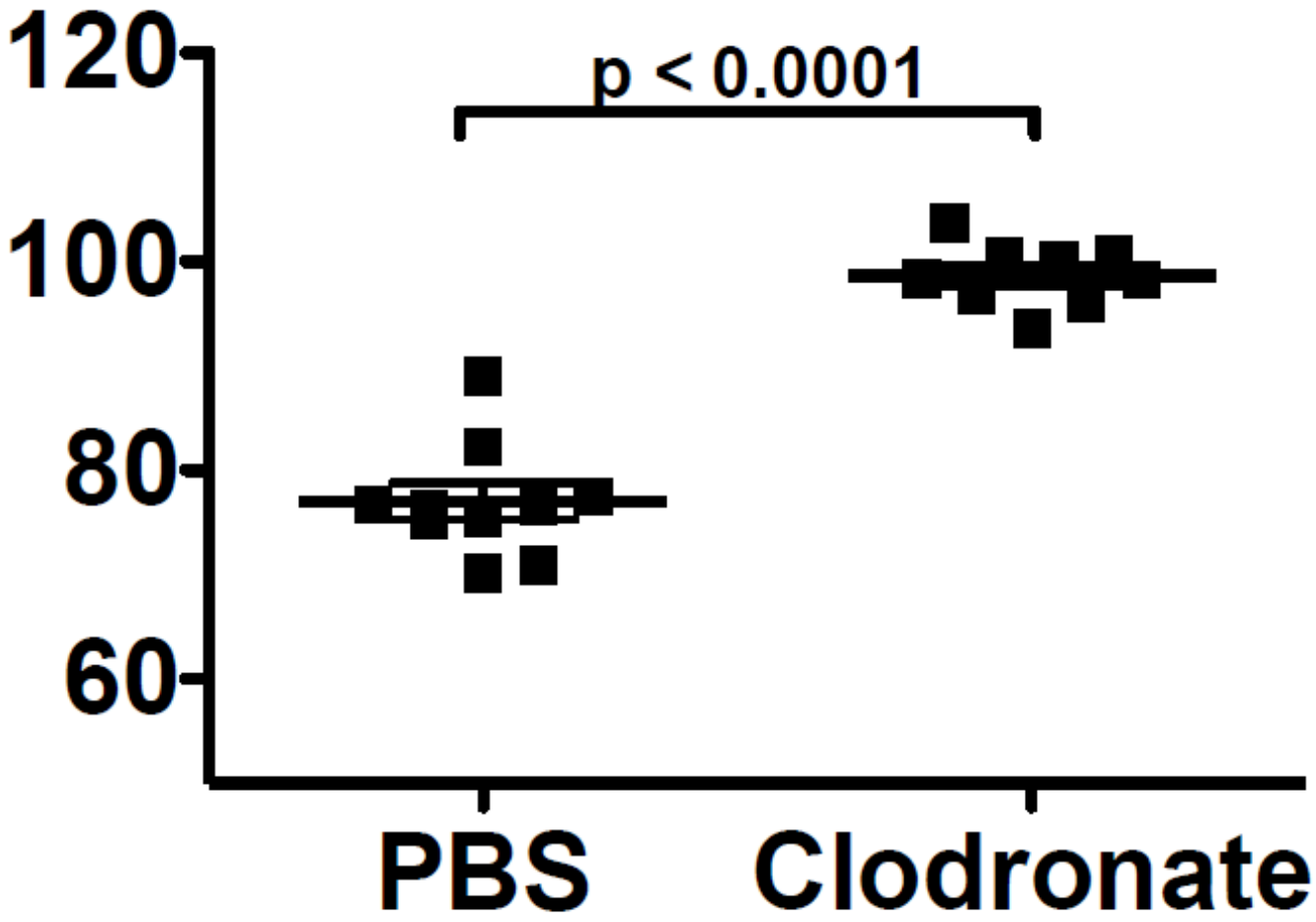
Liposomal clodronate infusions deplete hepatic and splenic macrophages



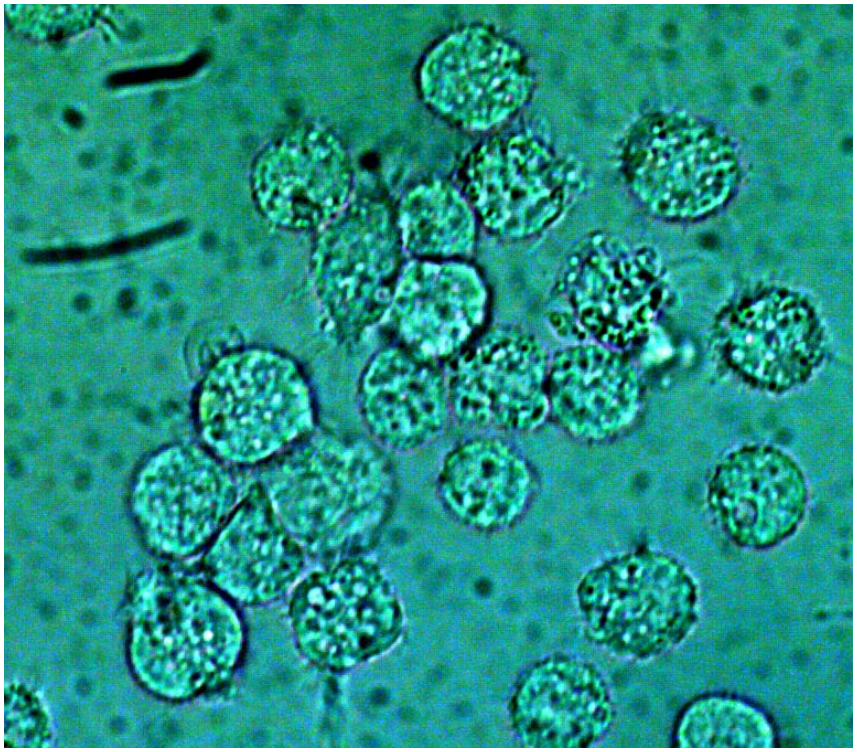
Liposomal clodronate infusions deplete hepatic and splenic macrophages

	No clodronate	Liposomal clodronate
F4/80		

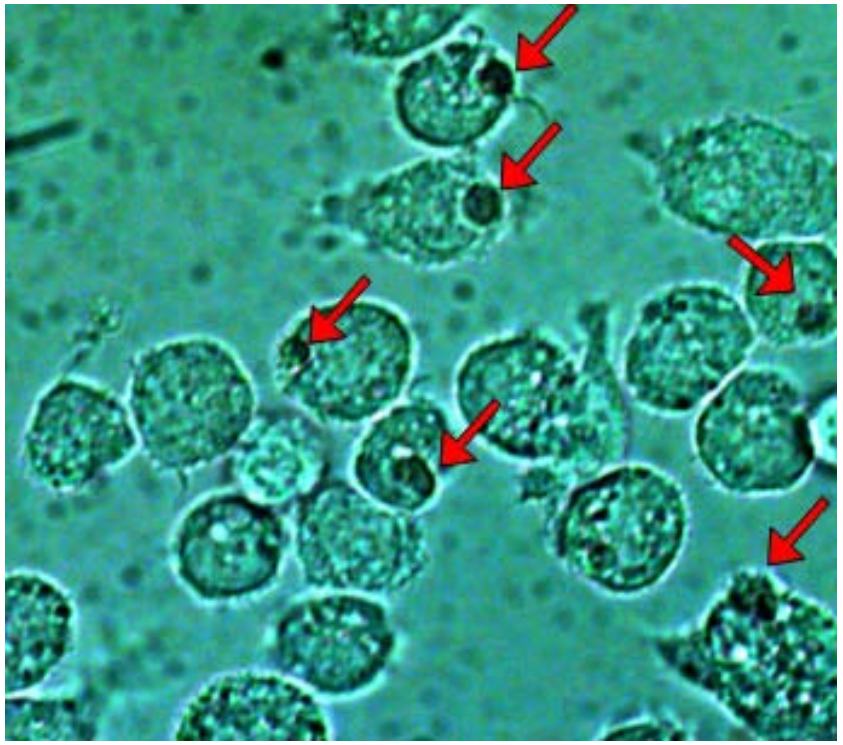
Macrophage depletion improves survival of transfused stored RBCs



Stored mouse RBCs are ingested by mouse macrophages *in vitro*



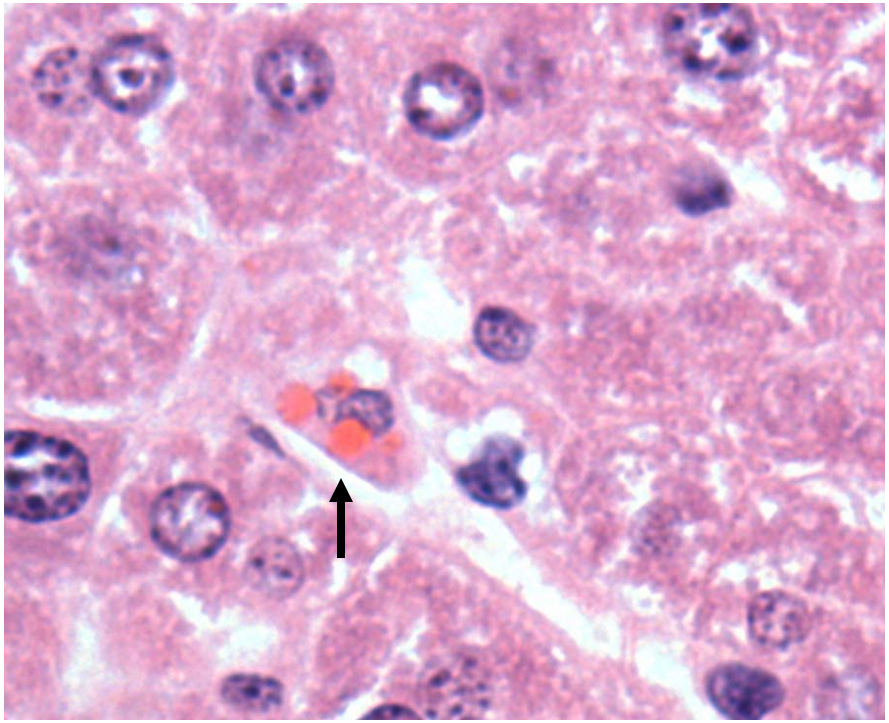
J774.1 cells + Fresh RBCs



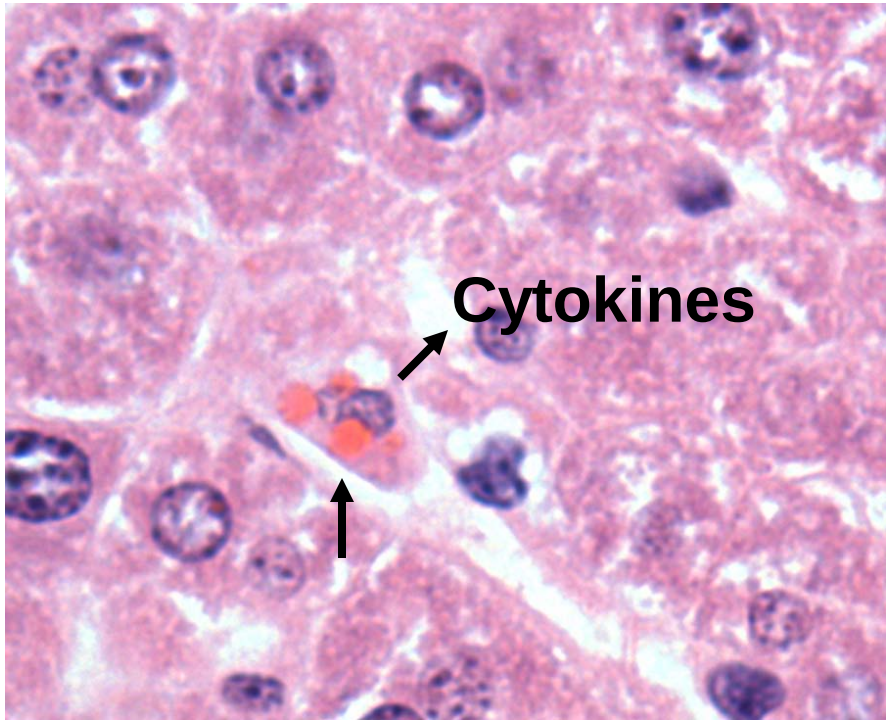
J774.1 cells + stored RBCs

What are the consequences of RBC clearance?

Hepatic macrophage (i.e. Kupffer cell) phagocytosis



Hepatic macrophage (i.e. Kupffer cell) phagocytosis and cytokine secretion

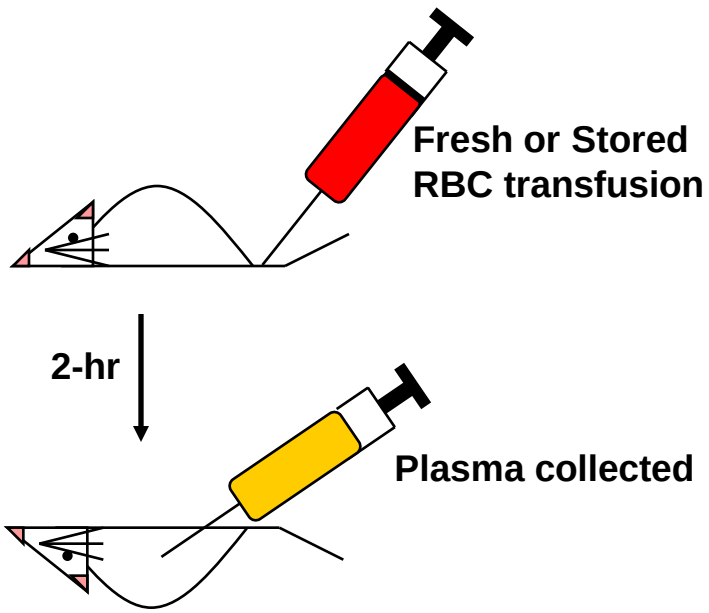


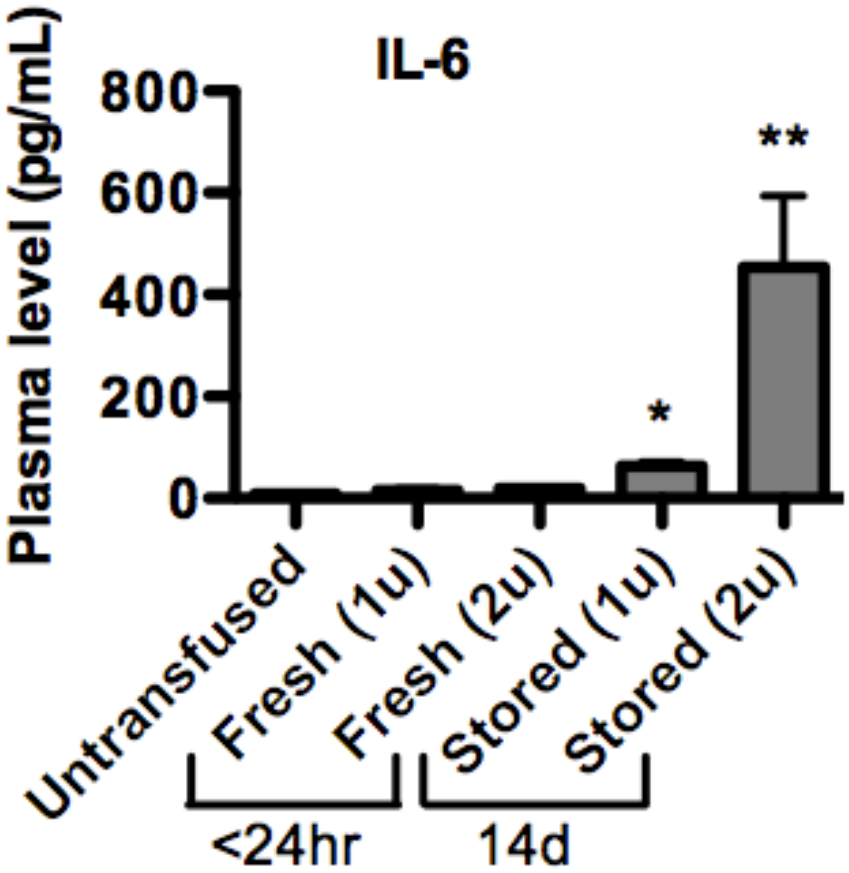
Cytokines

- Signaling molecules
- Often secreted by immune cells
  - Response to pathogens
- Interleukin (IL)-6 = pyrogen, acute phase reactant
- TNF-**α**, IL-1**β**, MCP-1, MIP-1**α**, etc.

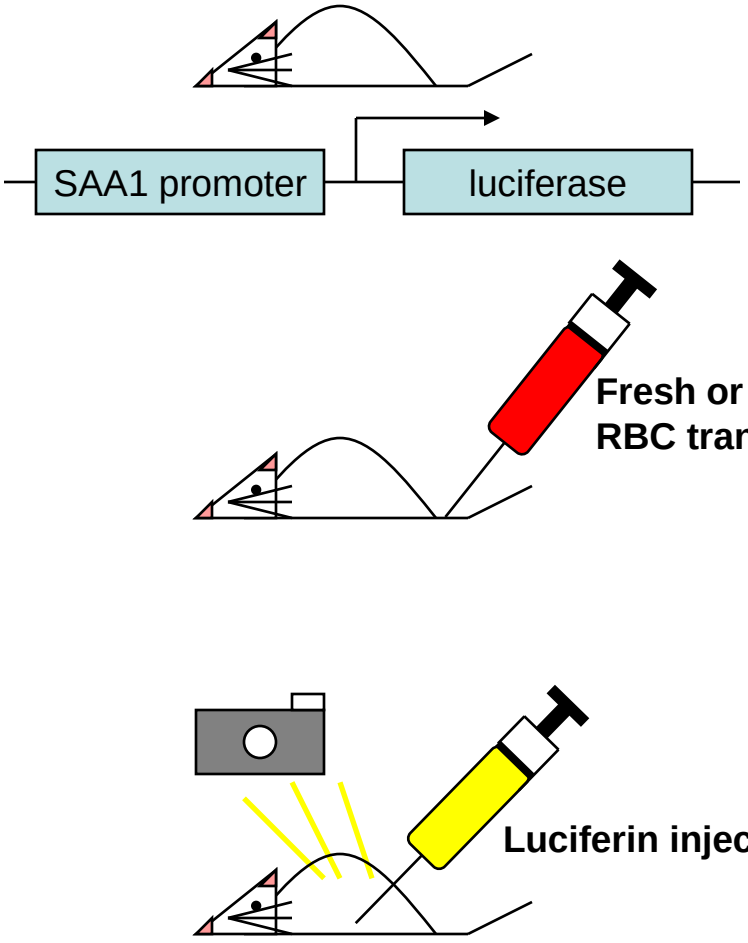
Does transfusion of older, stored RBCs induce a pro-inflammatory cytokine response in mice?

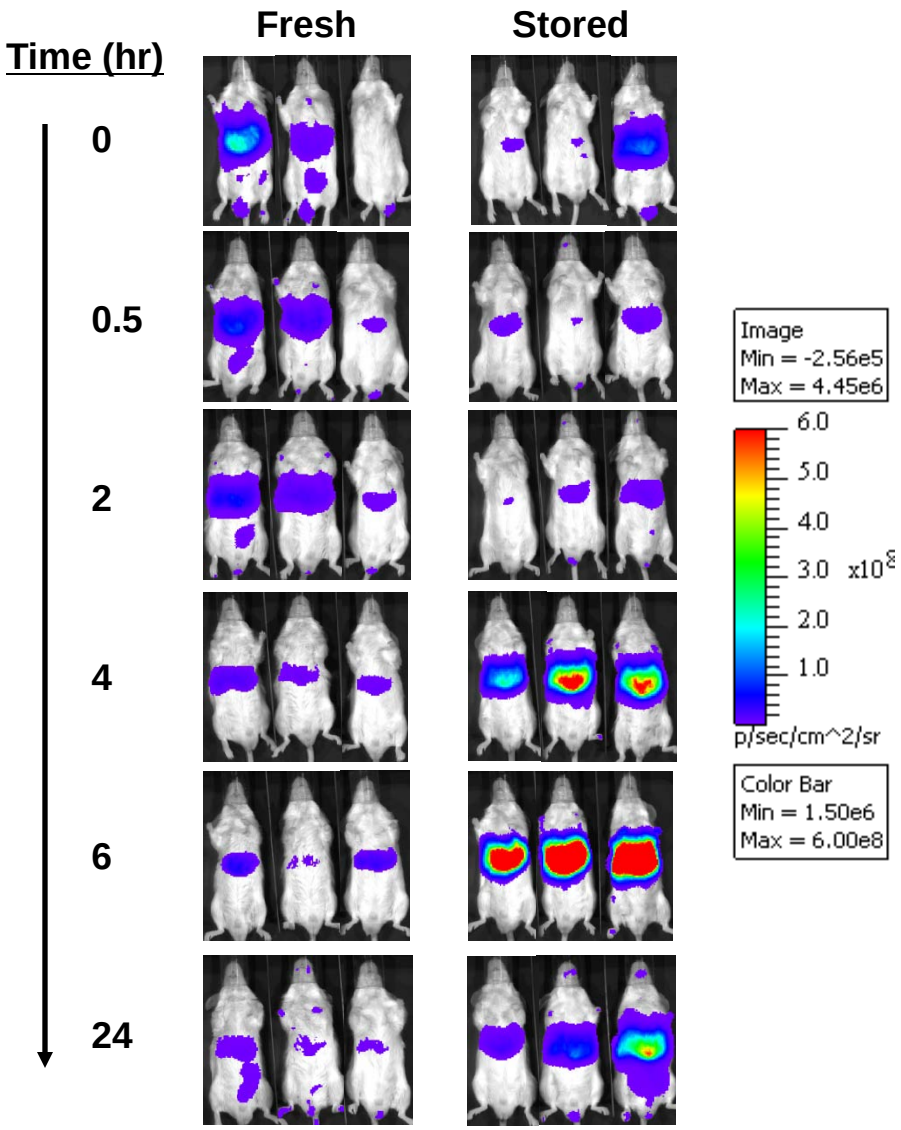
Transfusion of older, stored RBCs induces a pro-inflammatory cytokine response in mice





Transfusion of older, stored RBCs induces an acute phase response



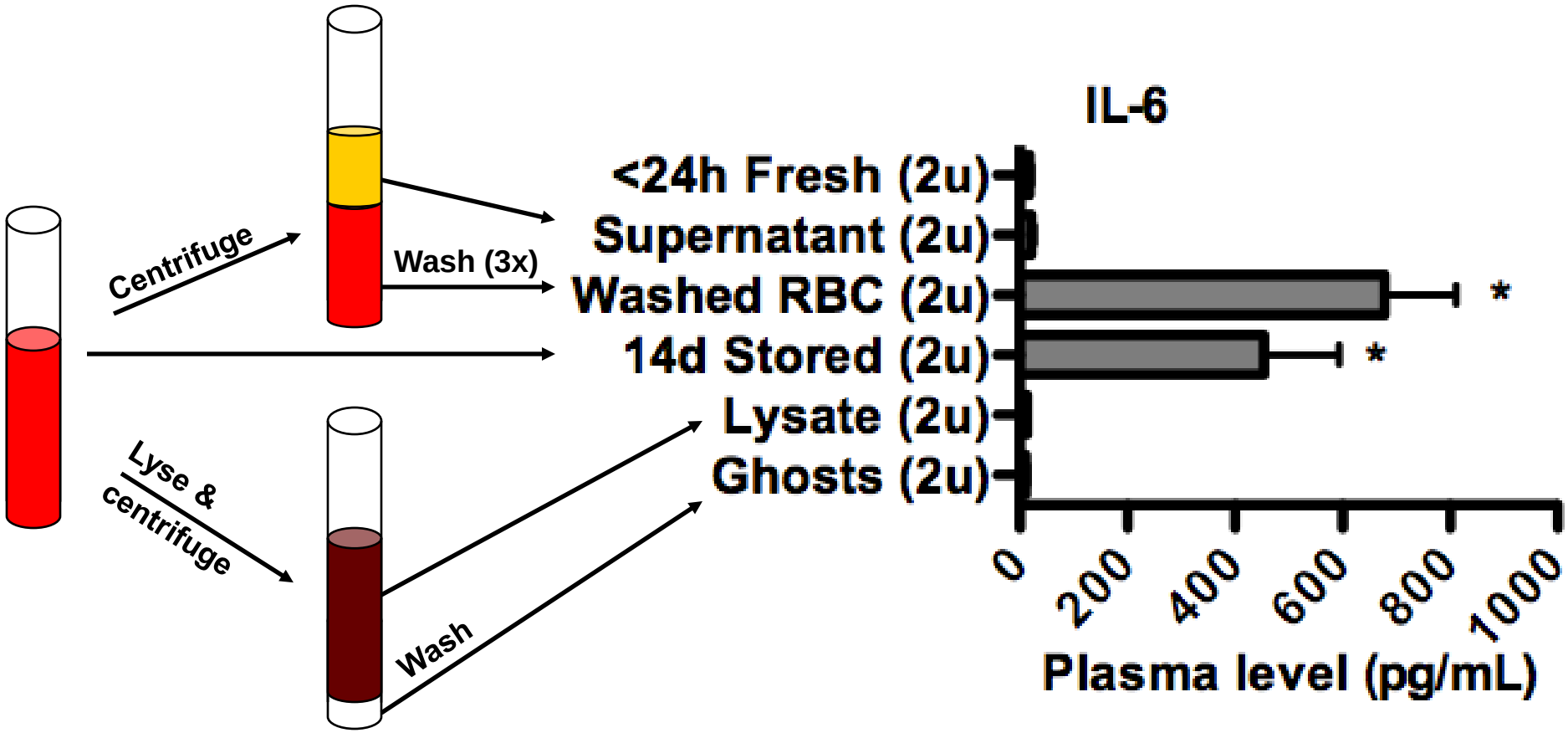


***Caliper*: Drs. Zhang, Ansaldi, & Francis**

What is responsible for the inflammation?

The RBCs or something else?

Only transfusion of washed stored RBCs induces the pro-inflammatory response



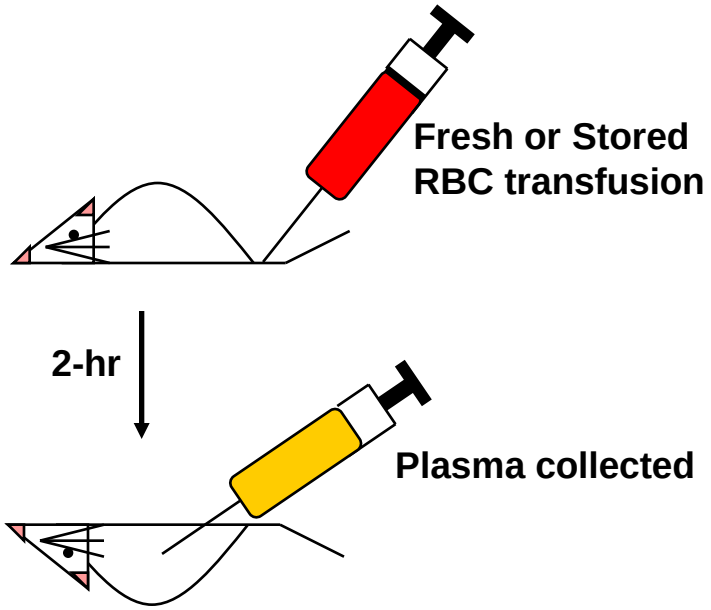
What is Non-Transferrin Bound Iron (NTBI)?

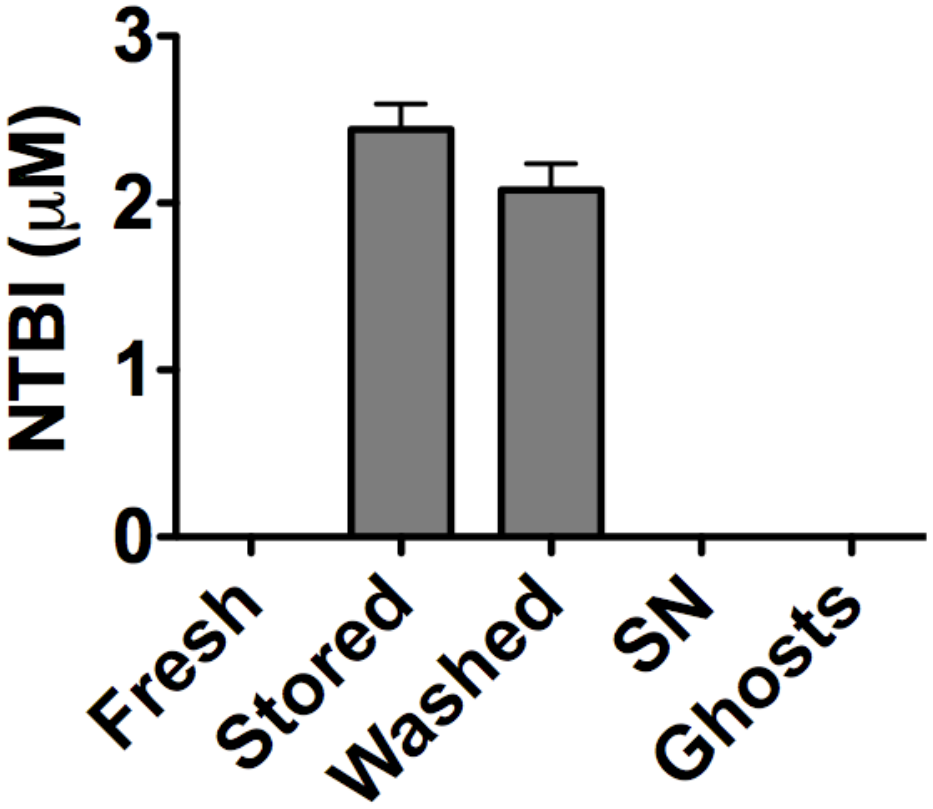
- Undetectable in healthy humans
- Oxidative damage

  Fenton chemistry:

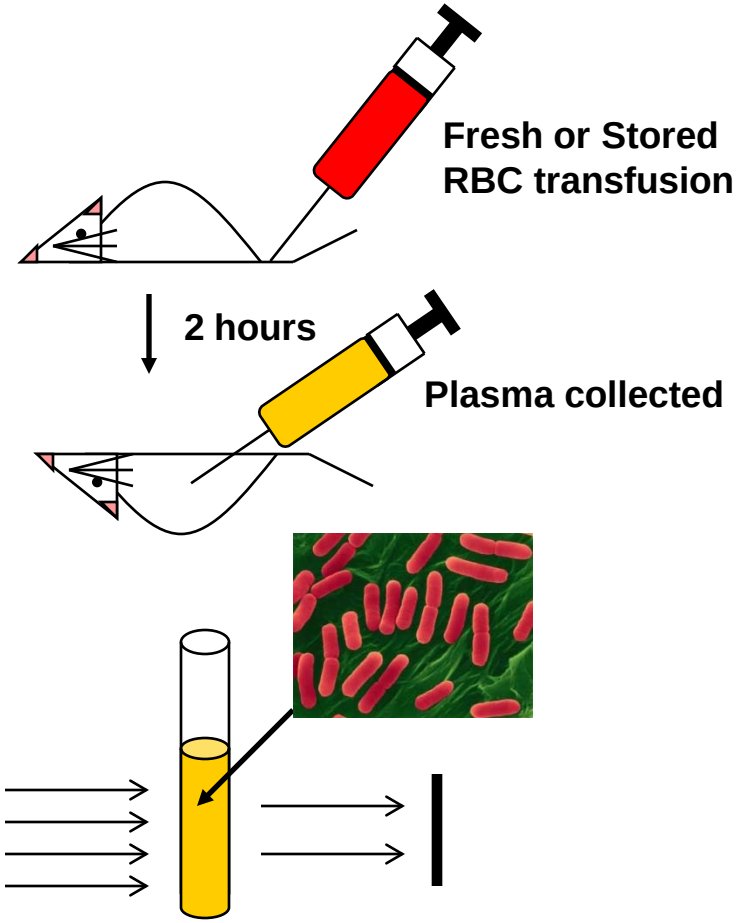
  $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$
- Cytotoxicity
- Enhanced endothelial expression of adhesion molecules
- Promotes pathogen growth

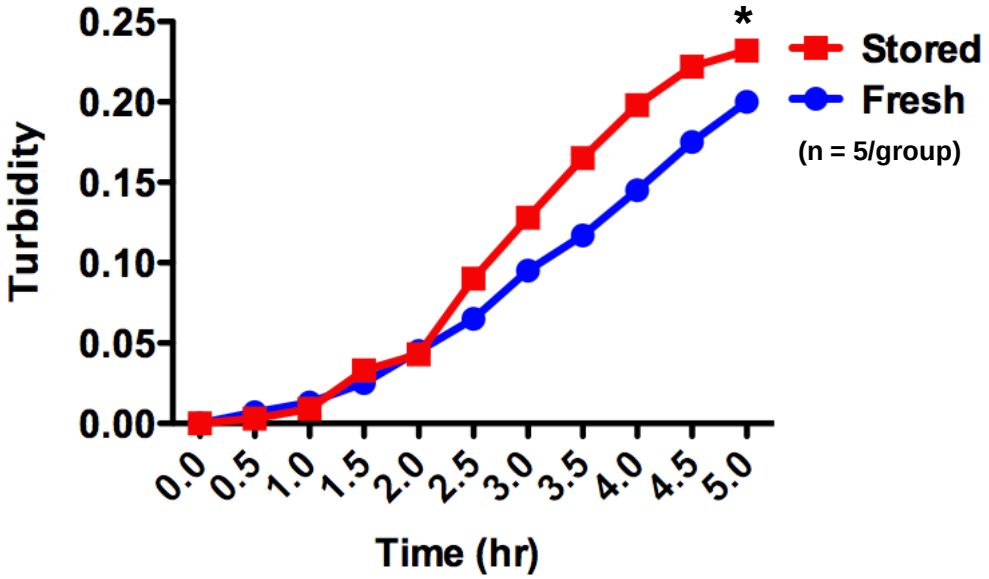
NTBI in transfused mice





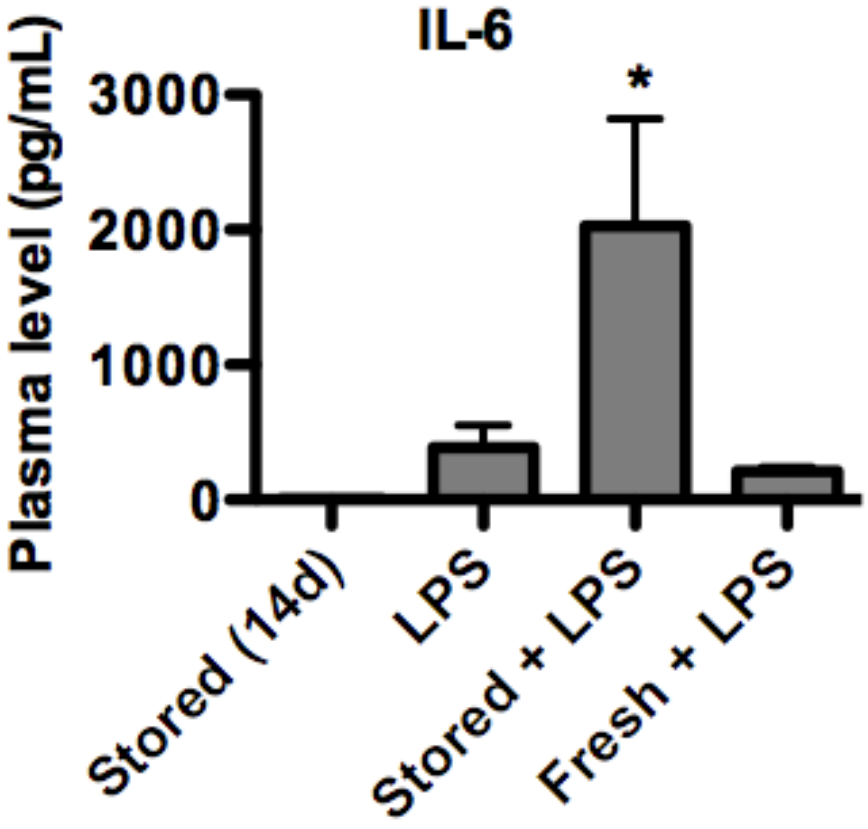
Plasma, after transfusion of older, stored RBCs, enhances bacterial growth



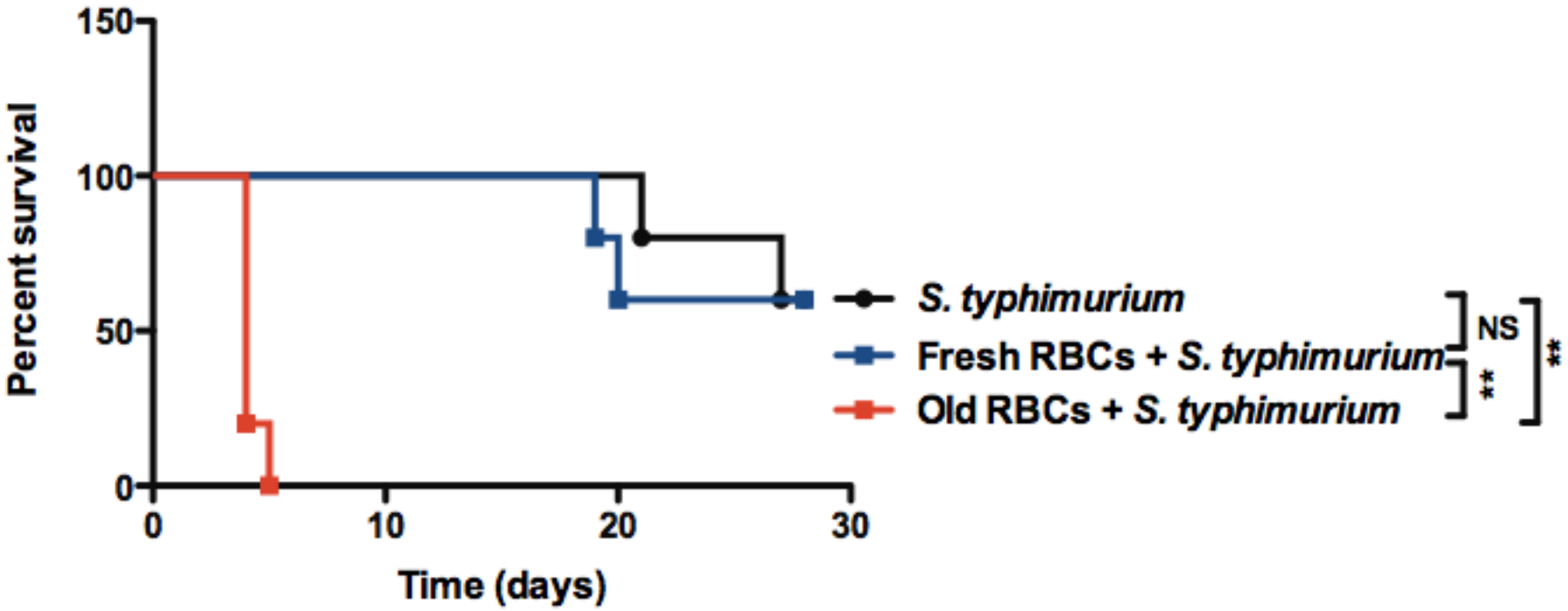


Are there clinical consequences to transfusion of older stored RBCs in mice?

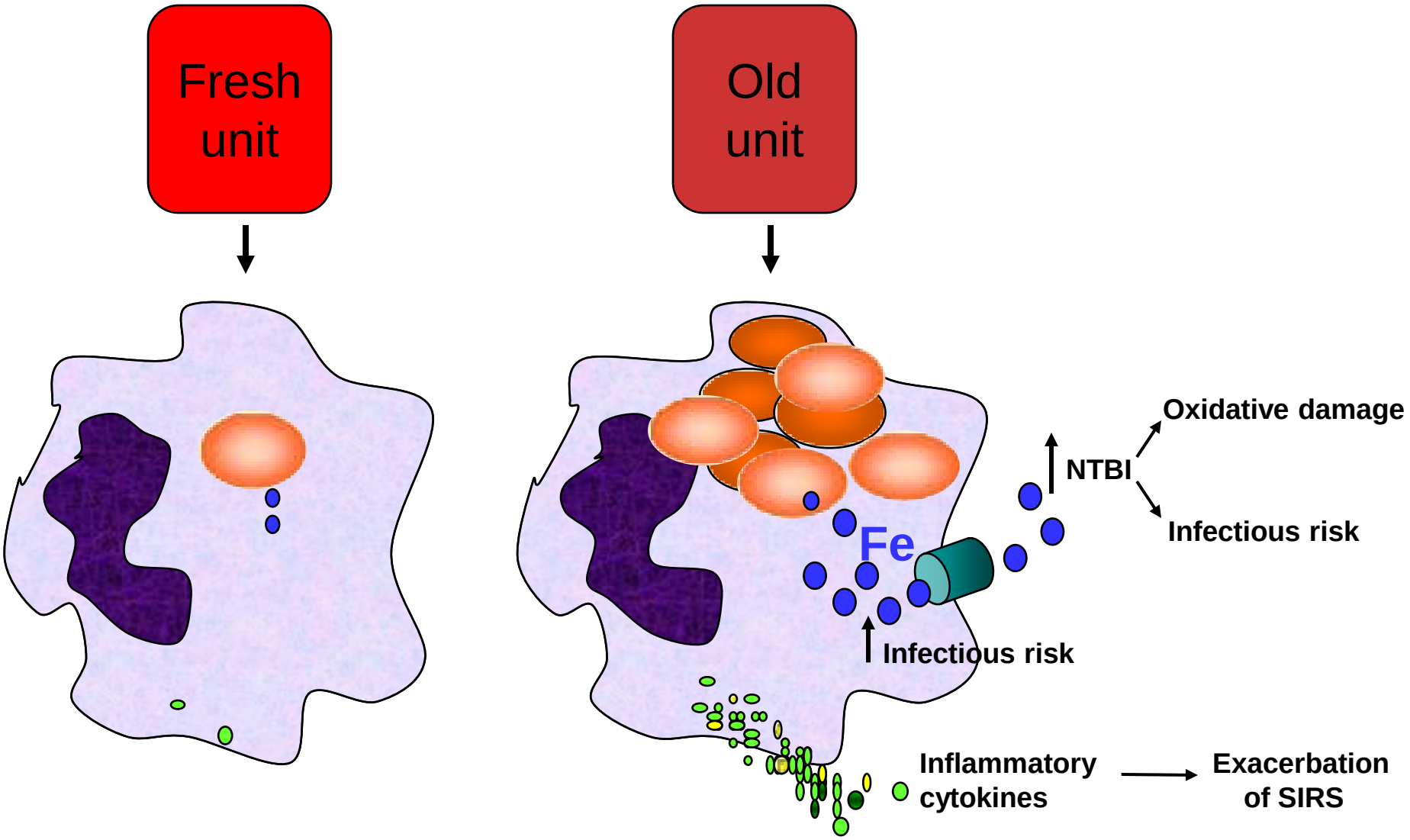
Transfusion of older, stored RBCs exacerbates LPS-induced inflammation (24 hrs)



Mice infected with *Salmonella* have shortened survival when transfused with old RBCs

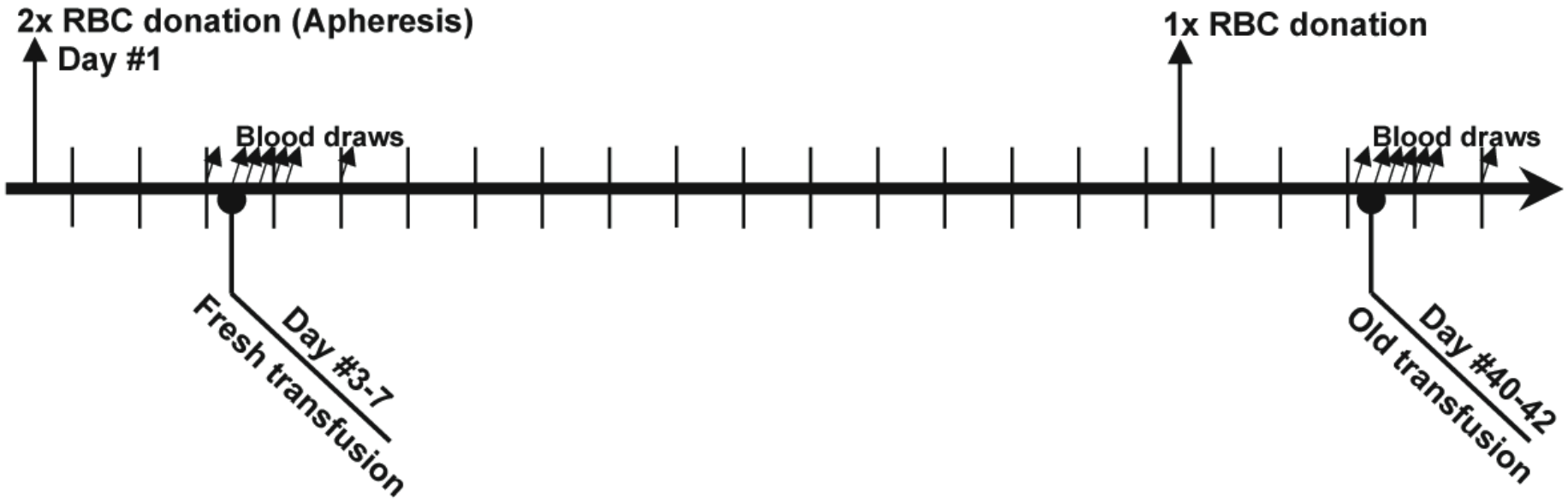


Hypothesis


Fresh unit
Old unit
Fe
NTBI
Oxidative damage
Infectious risk
Infectious risk
Inflammatory cytokines
Exacerbation of SIRS

But, mice aren’t human….

Protocol Schematic



Pre-storage leukoreduction, autologous

14 Volunteers: Baseline Characteristics

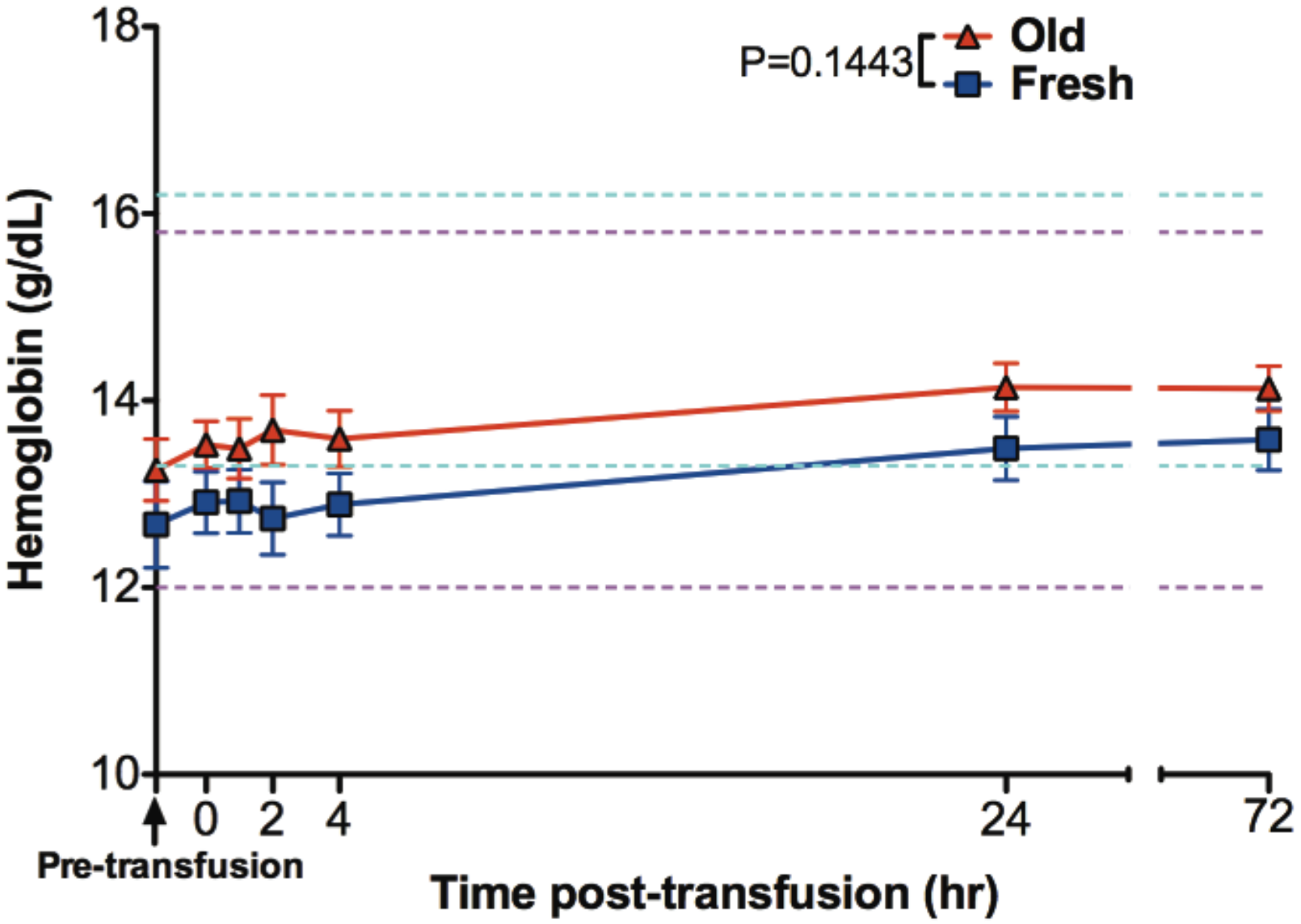
Age – yr (mean ± s.d.)	30.4 ± 9.1
Female – no.	4
Blood type: A, B, O, AB	6, 5, 3, 0
Race/ethnicity – no.	
White	9
Black	1
Asian	2
Hispanic	2
Height – inches (mean ± s.d.)	70.5 ± 3.7
Weight – pounds (mean ± s.d.)	193 ± 38
Baseline Hemoglobin – g/dL (mean ± s.d.)	
Male	15.3 ± 1.2
Female	14.2 ± 0.8

Transfusions were well tolerated

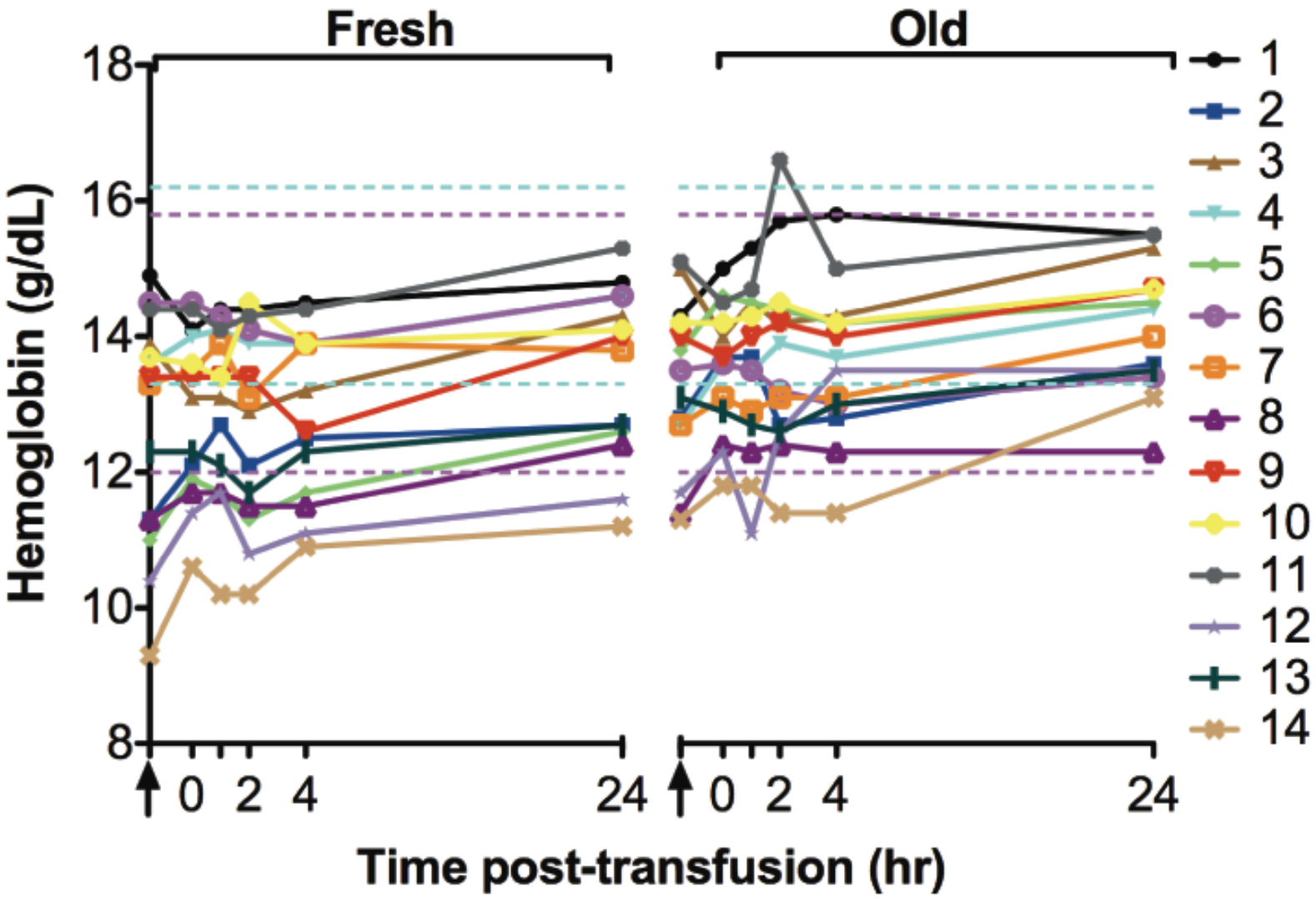
- No adverse events identified
- No deviations from protocol
- All volunteers remained afebrile
  & vital signs were stable throughout
- No transfusion reactions

Complete Blood Counts
“Therapeutic Effect”

“Therapeutic Effect”

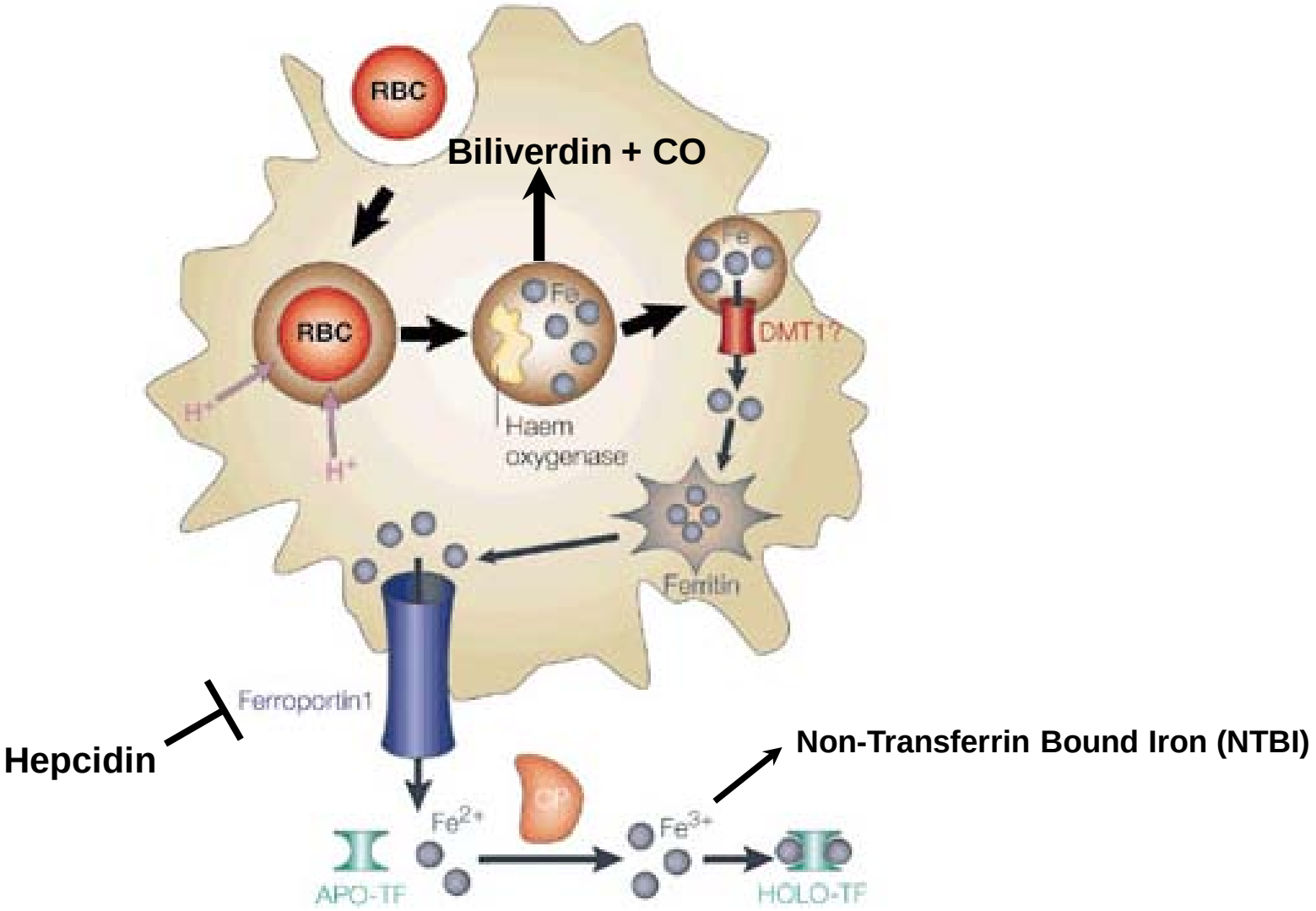


“Therapeutic Effect”



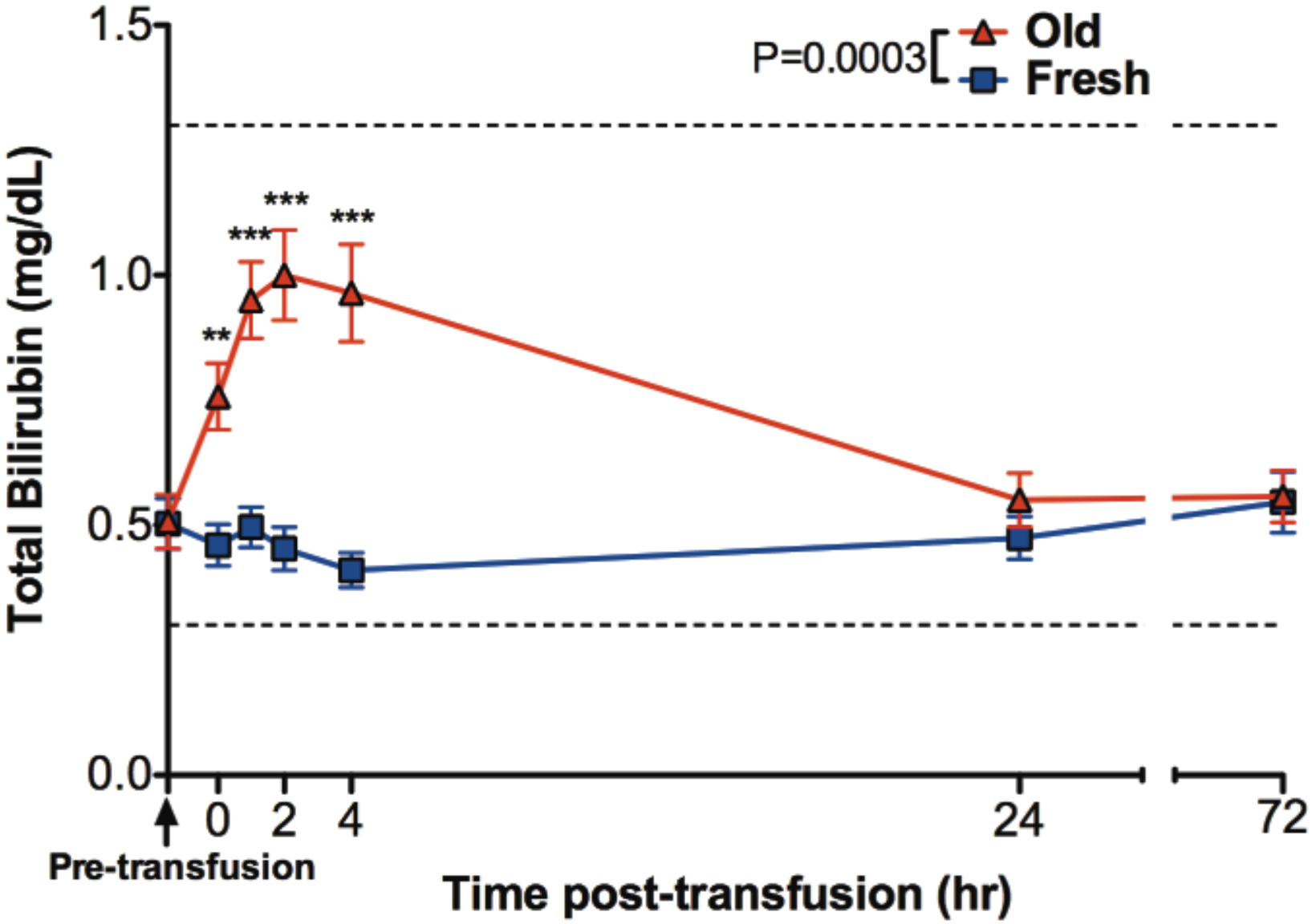
Markers of Hemolysis

What happens to cleared RBCs?

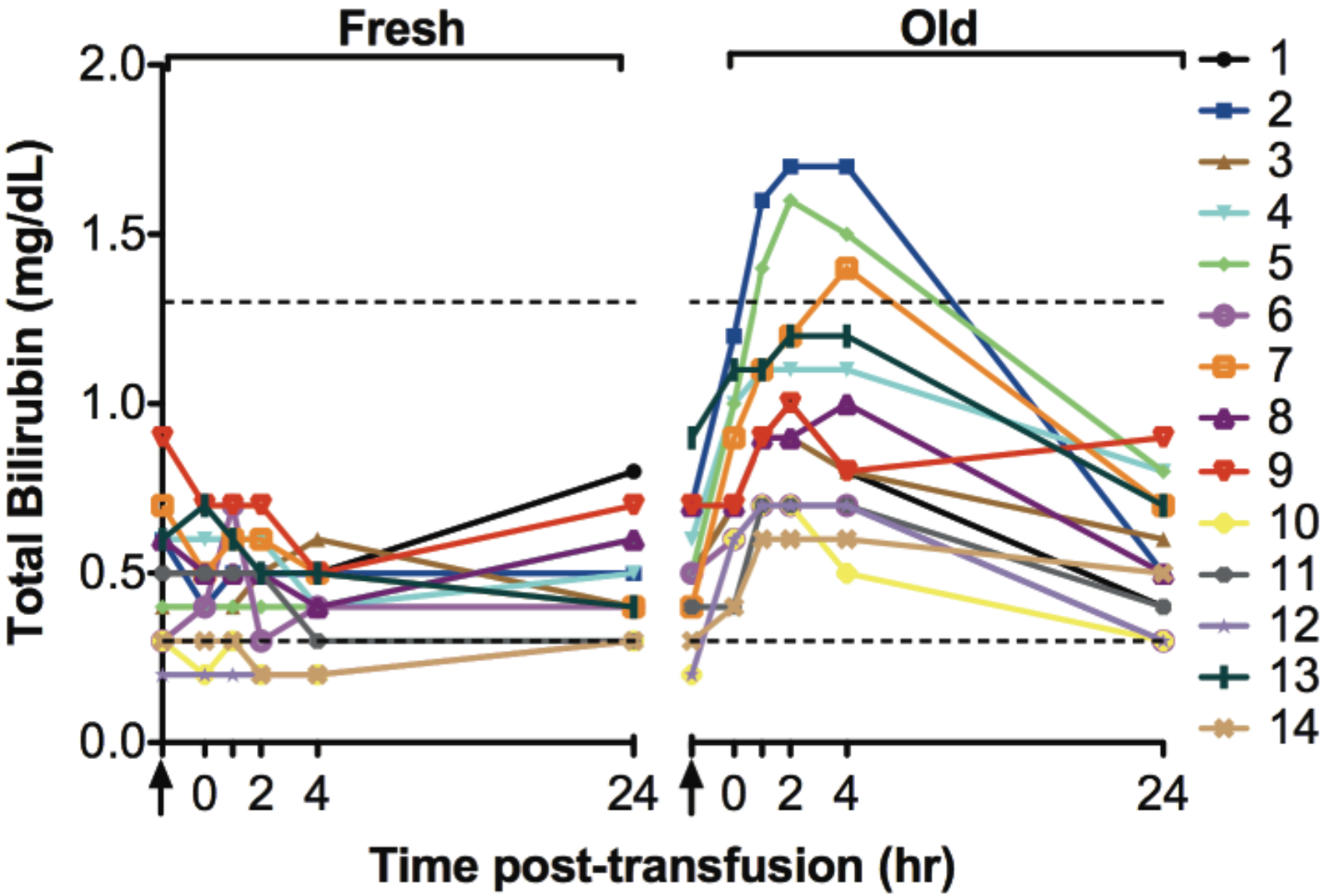


Andrews, NC. Nature Reviews Genetics, 2000

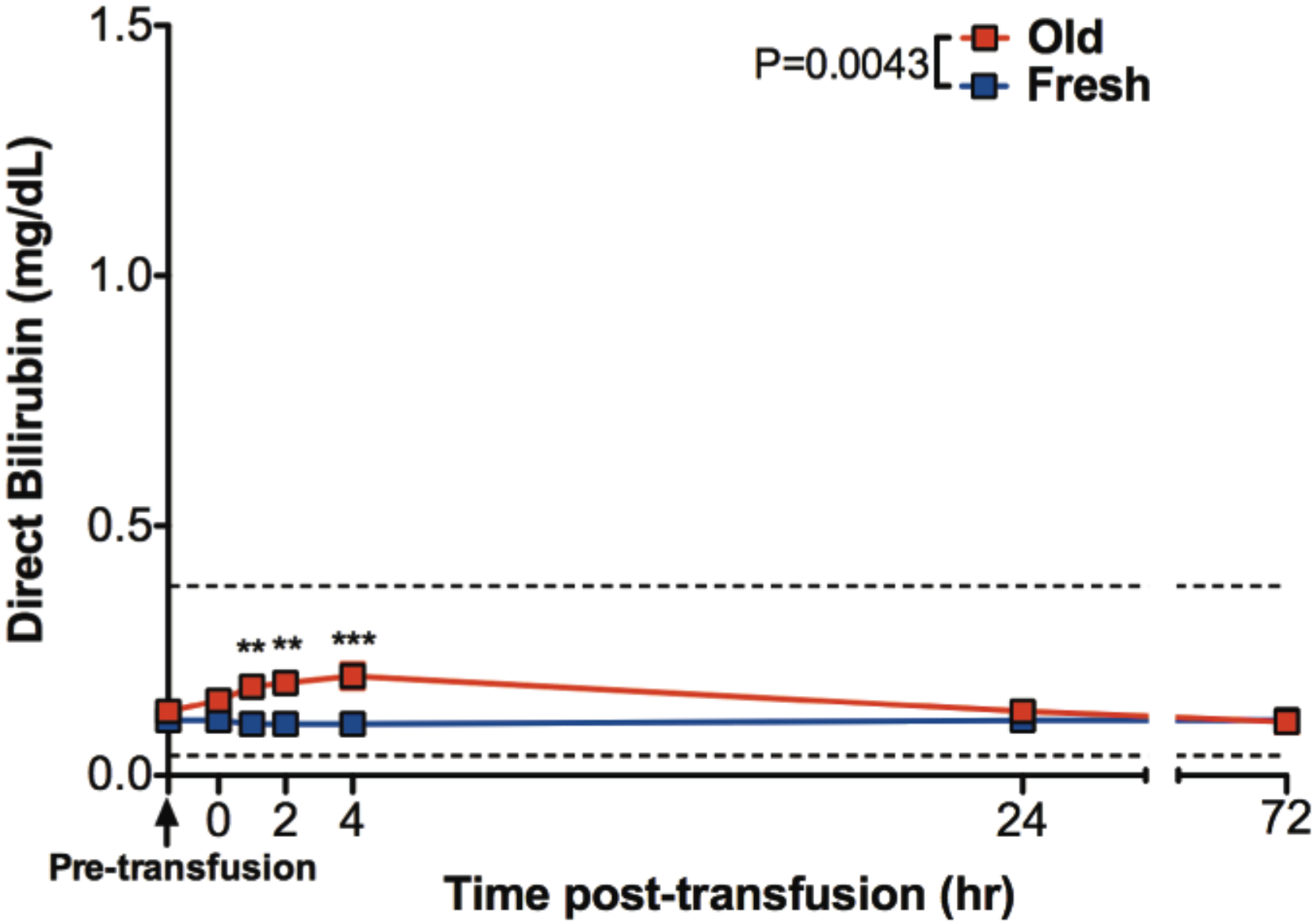
Total Bilirubin



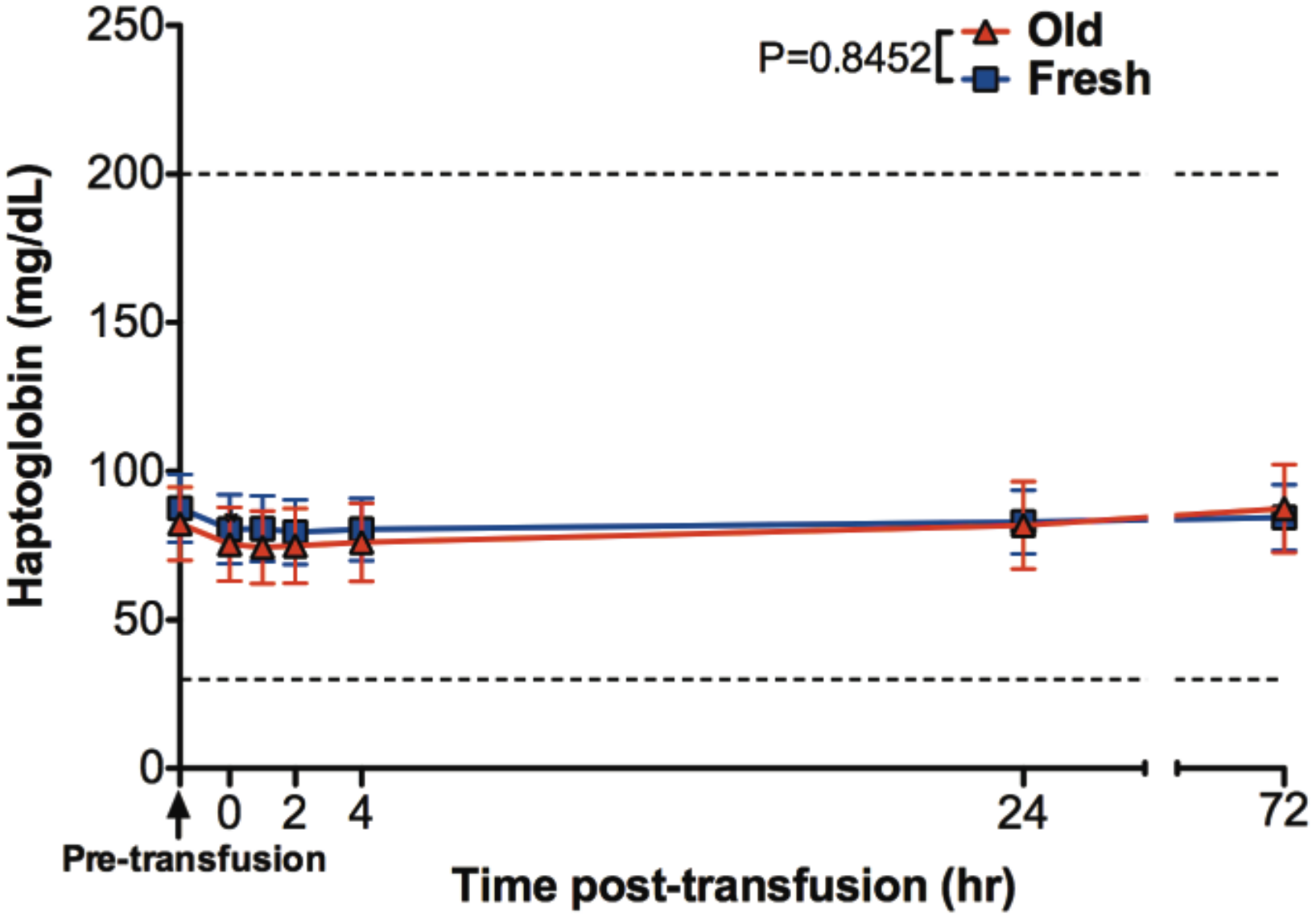
Total Bilirubin



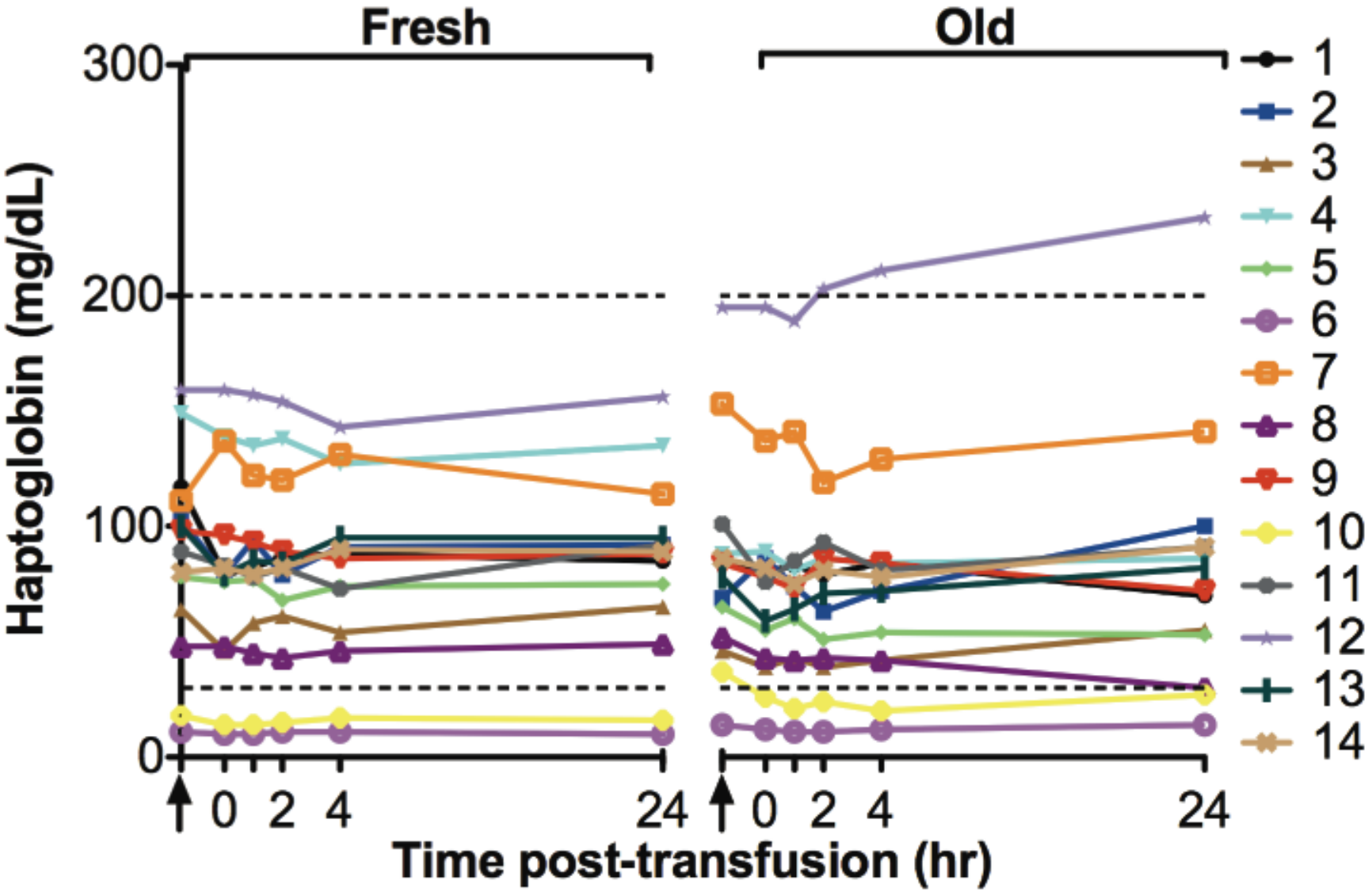
Direct Bilirubin



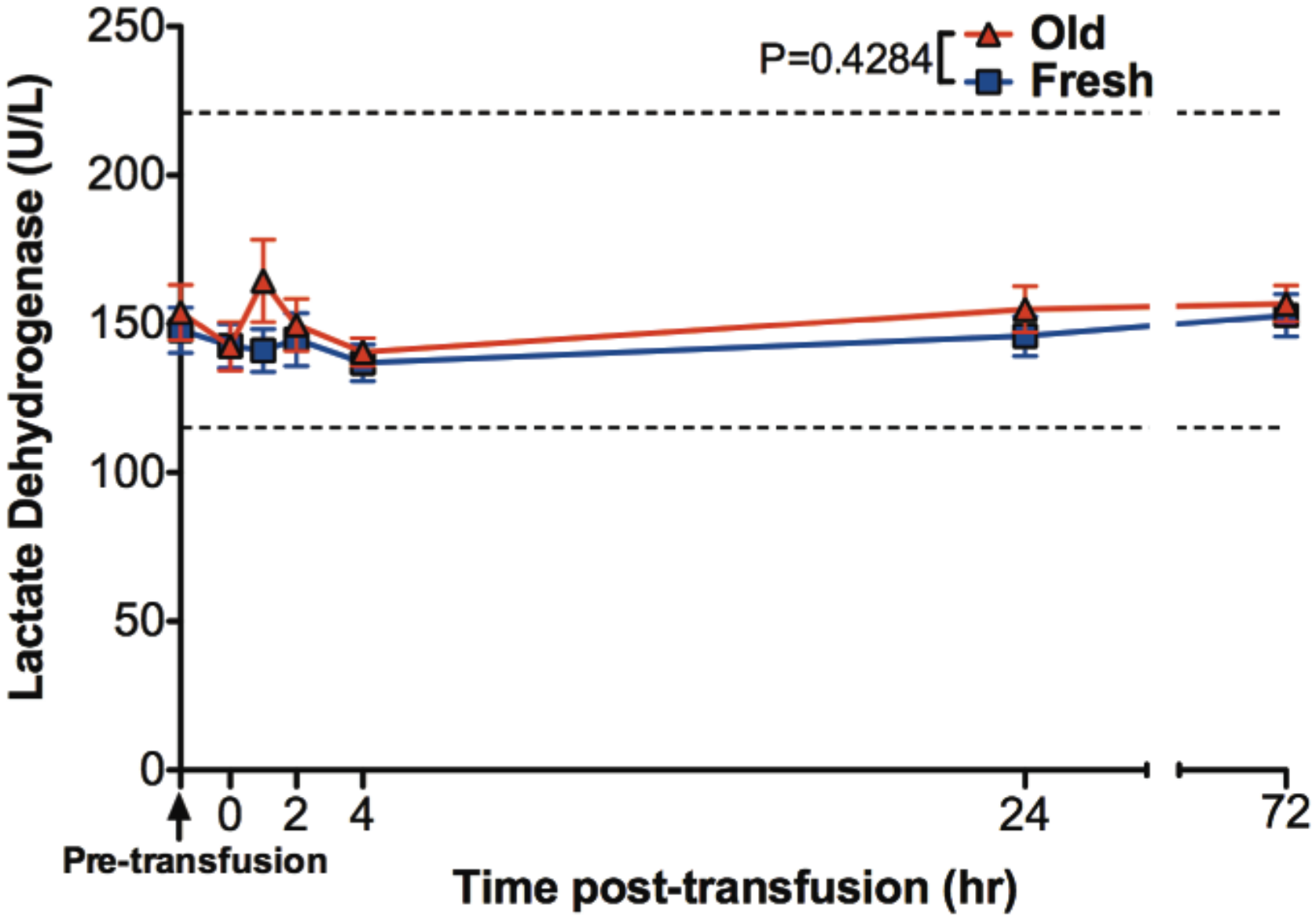
Haptoglobin



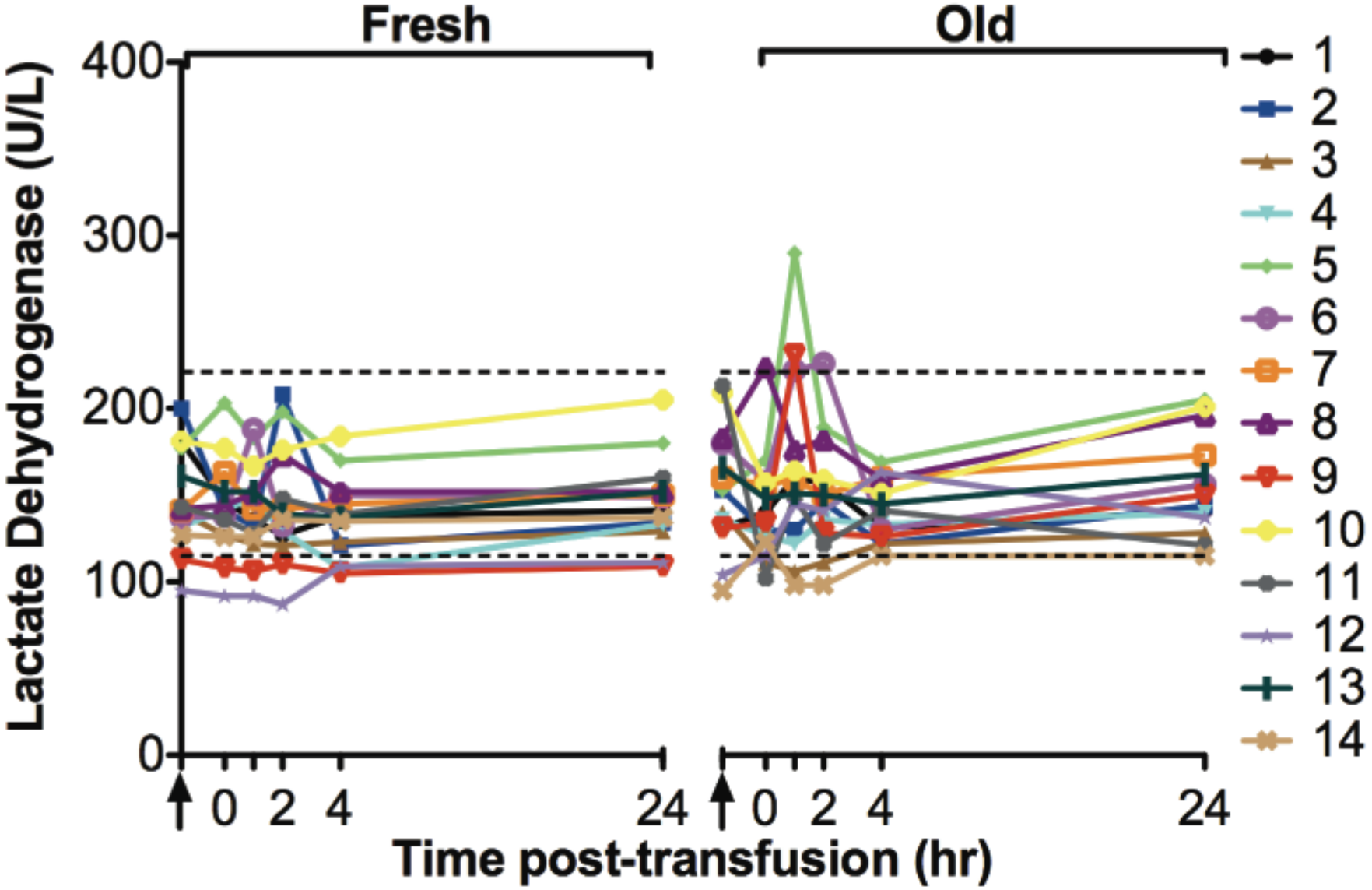
Haptoglobin



Lactate Dehydrogenase

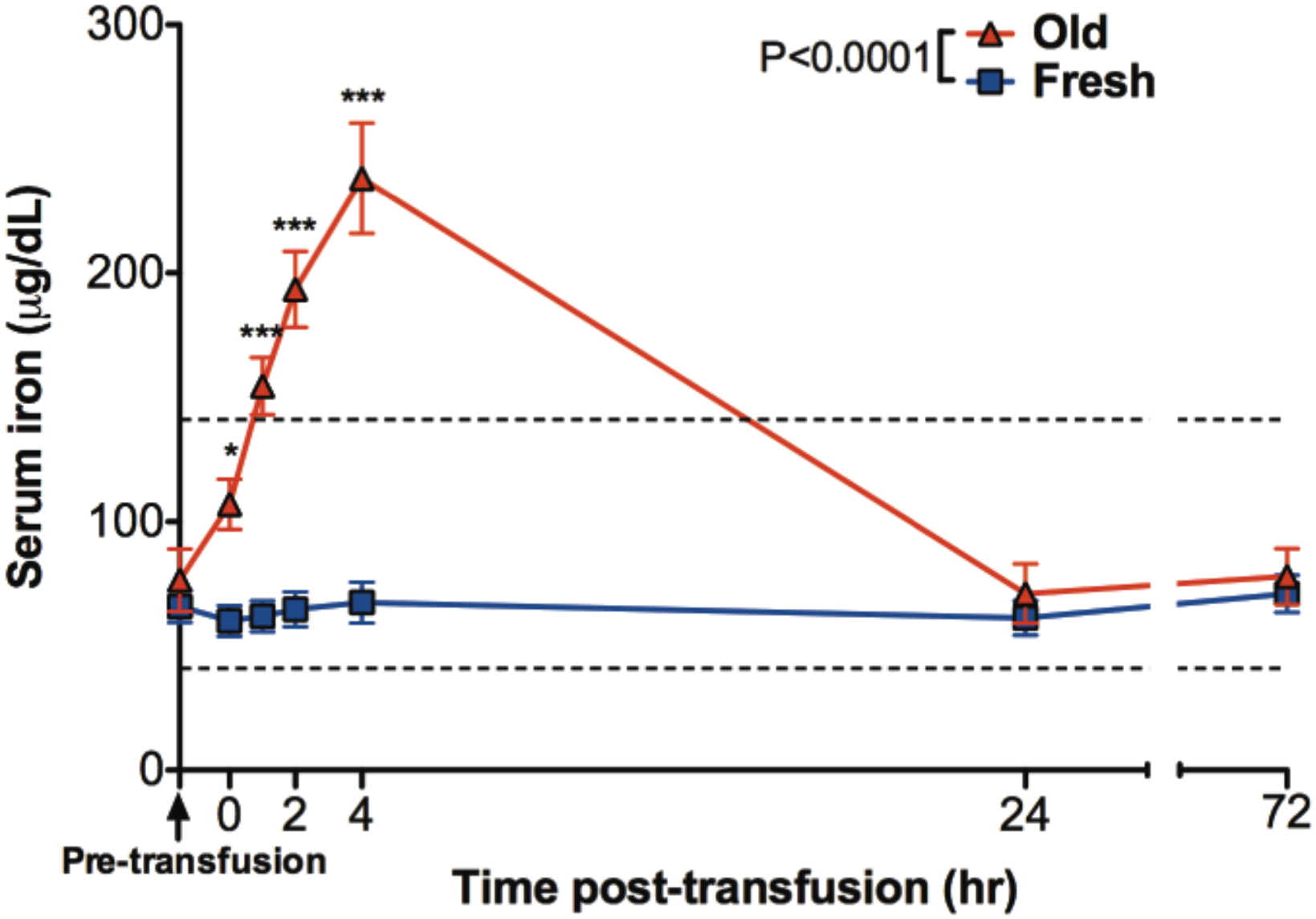


Lactate Dehydrogenase

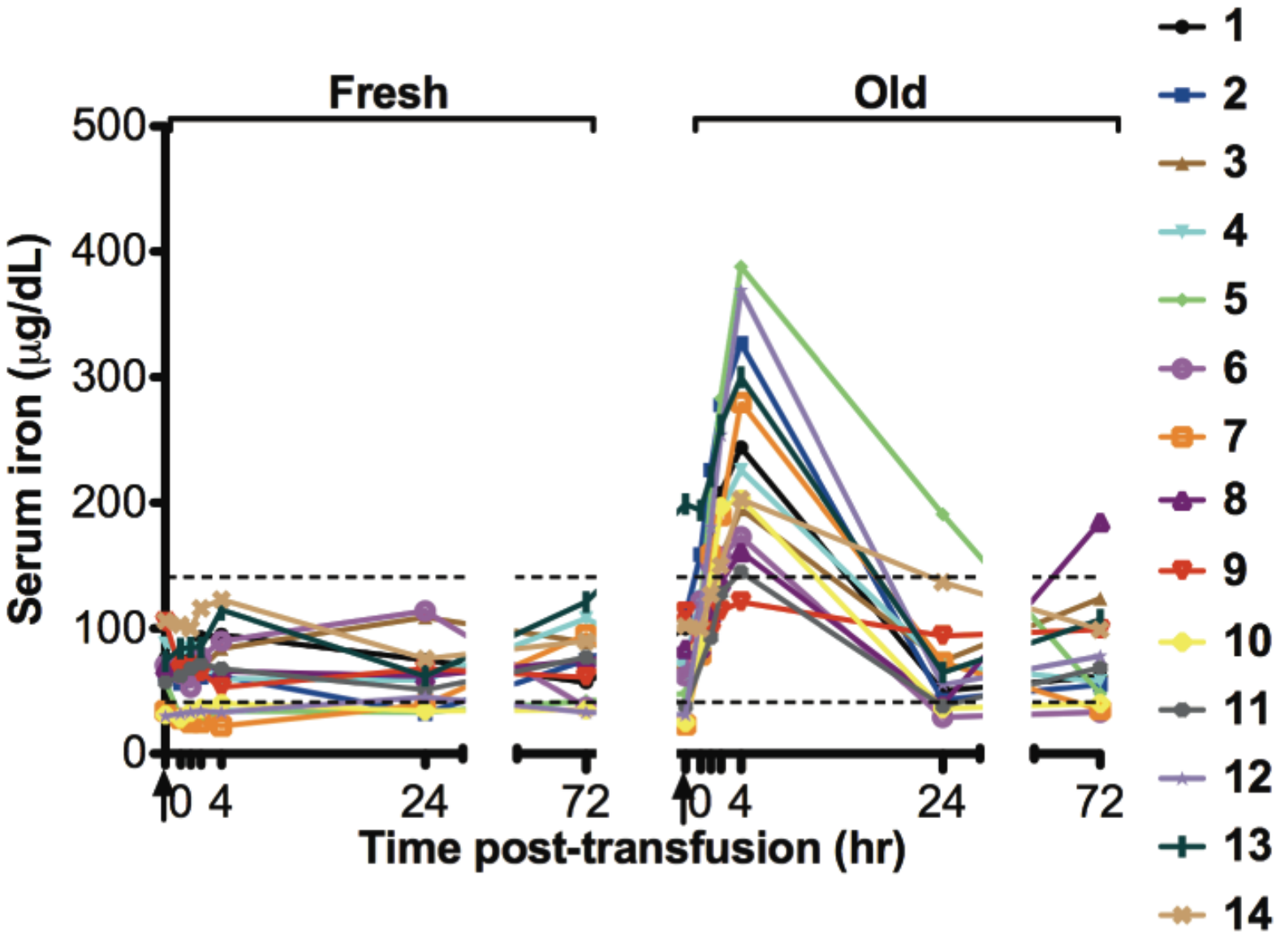


Iron Parameters

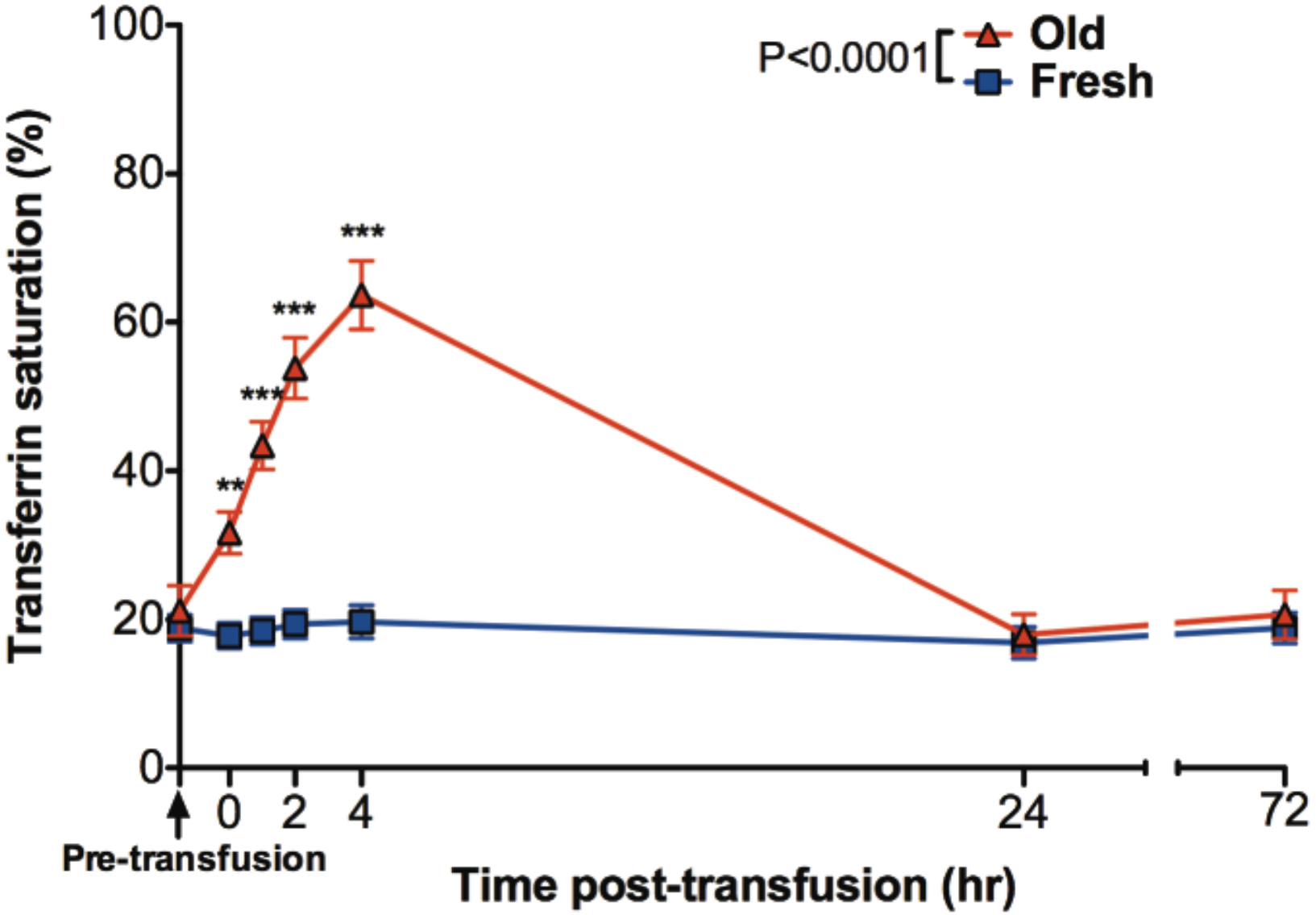
Serum Iron


300
200
100
0
Serum iron (μg/dL)
P<0.0001
Old
Fresh
***
***
***
*
0
2
4
24
72
Pre-transfusion
Time post-transfusion (hr)

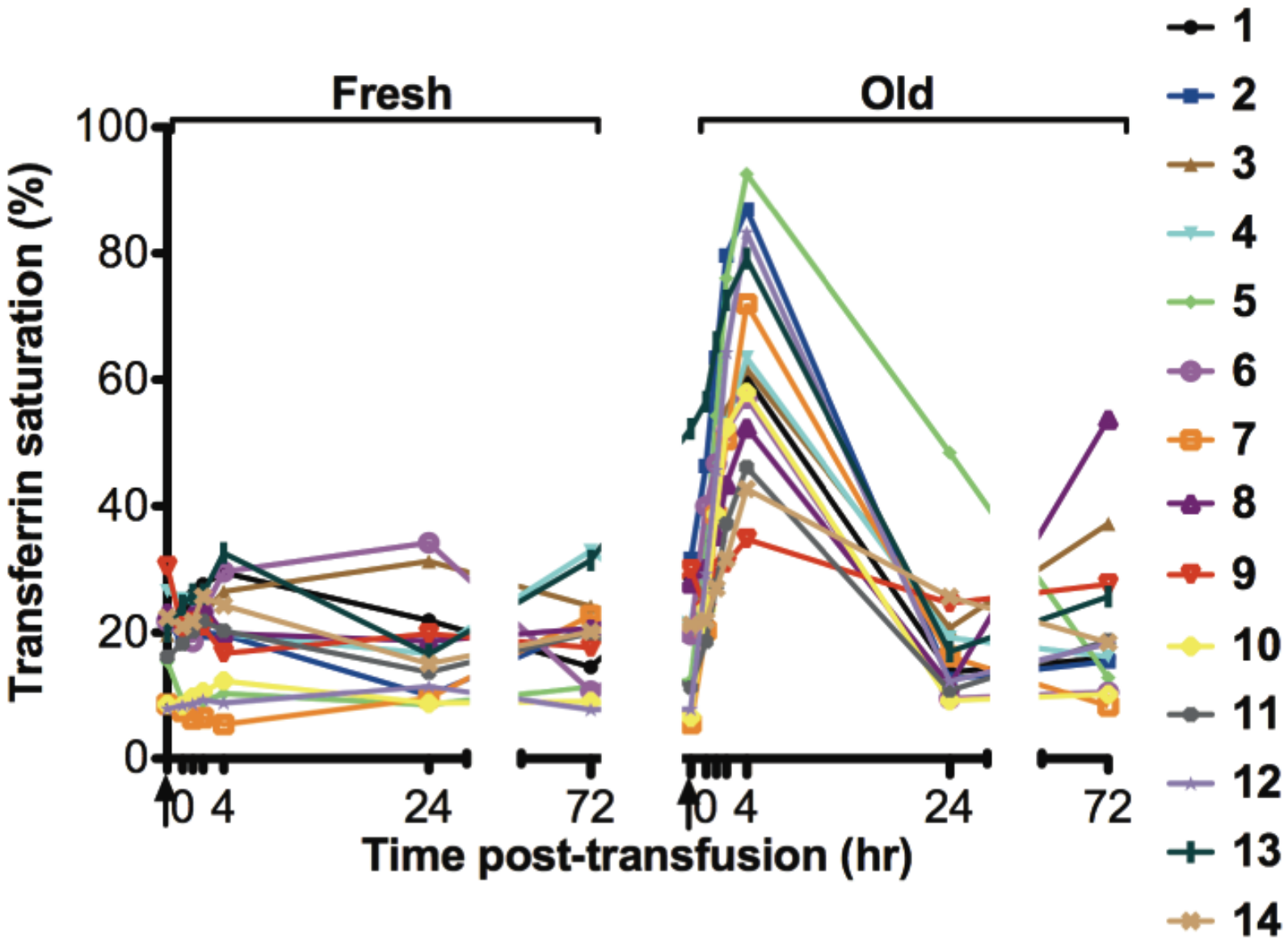
Serum Iron


Fresh
Old
500
400
300
200
100
0
Serum iron (µg/dL)
0
4
24
72
0
4
24
72
Time post-transfusion (hr)
1
2
3
4
5
6
7
8
9
10
11
12
13
14

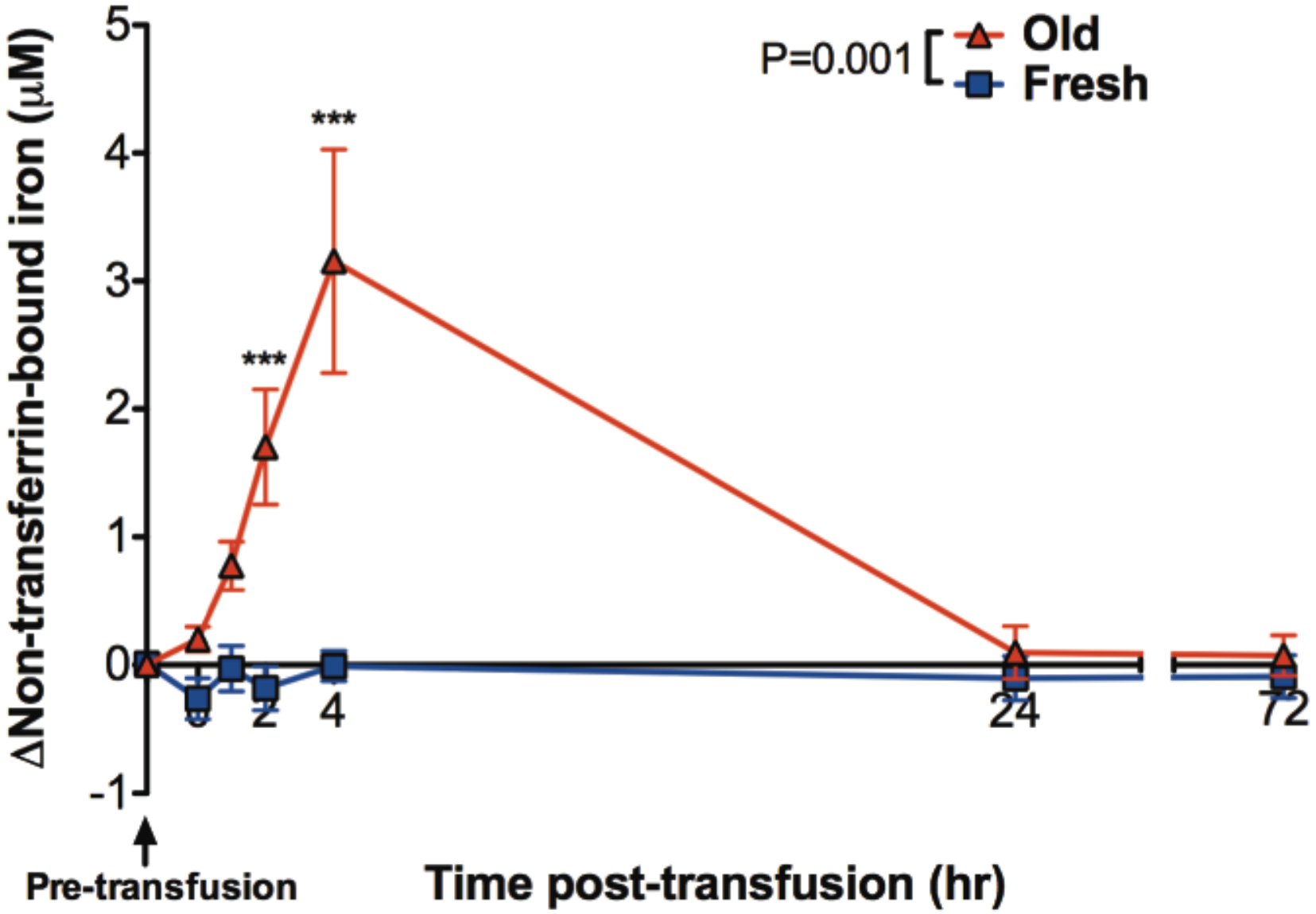
Transferrin Saturation



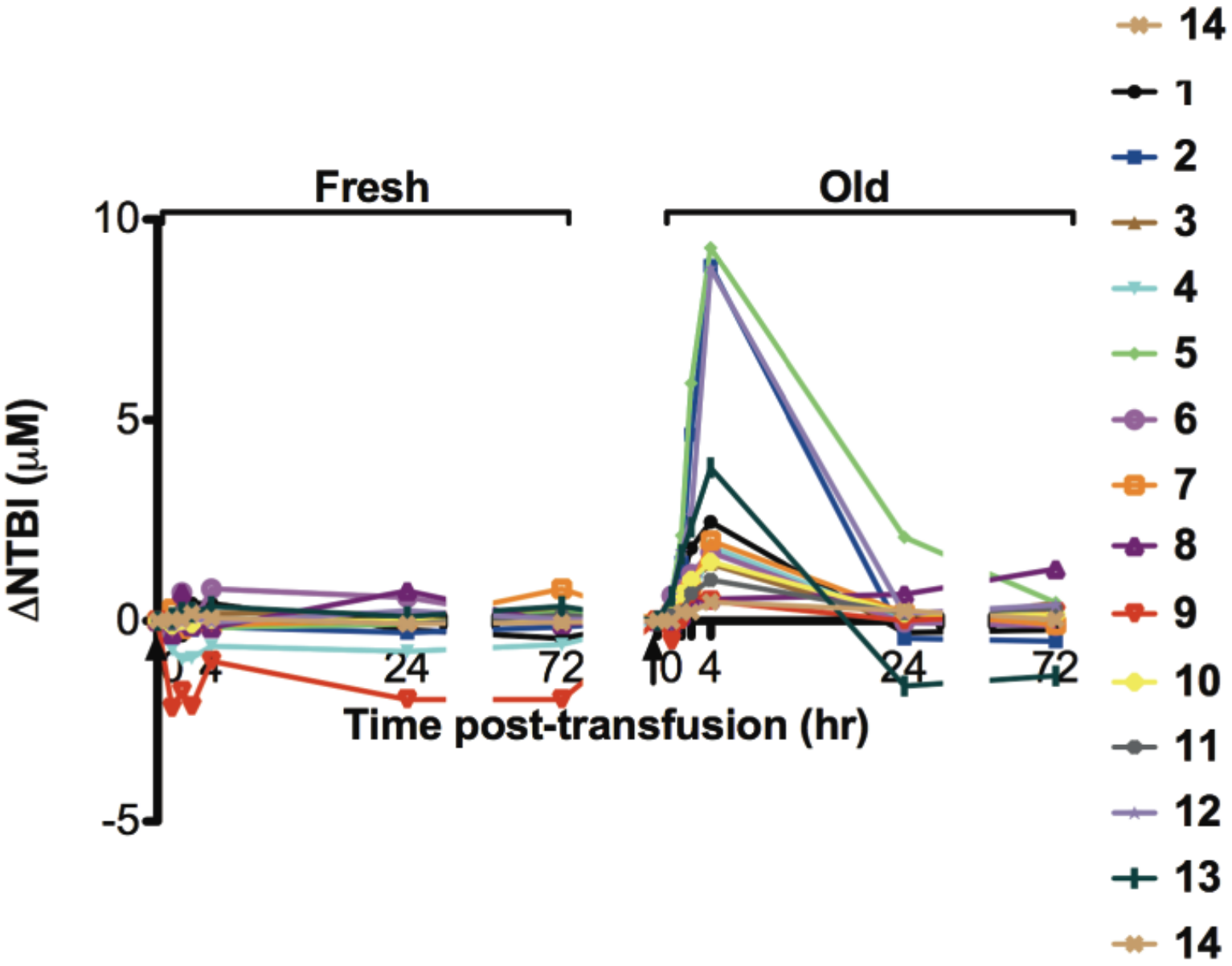
Transferrin Saturation



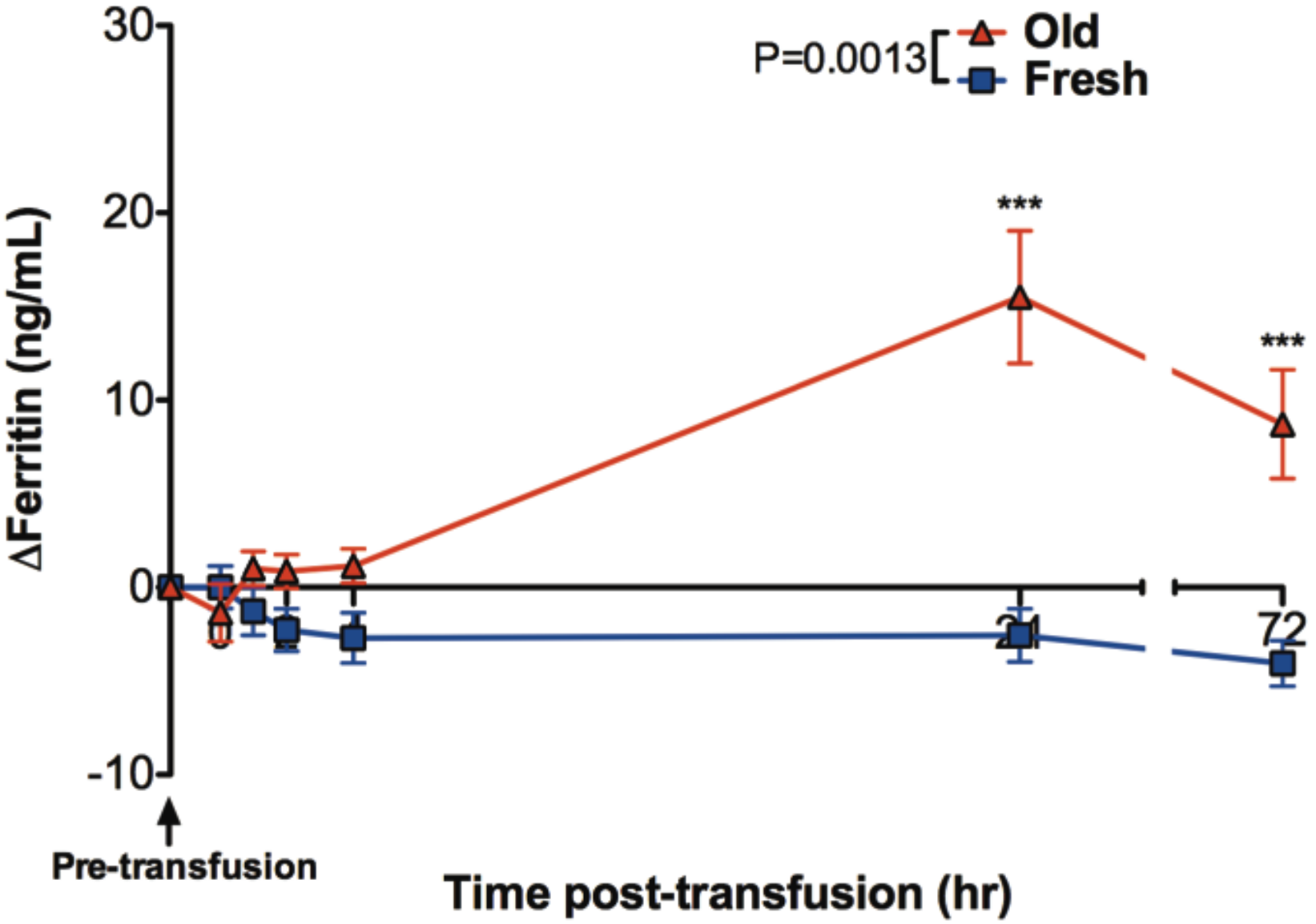
NTBI


P=0.001
Old
Fresh
ΔNon-transferrin-bound iron (μM)
5
4
3
2
1
0
-1
***
***
0
2
4
24
72
Pre-transfusion
Time post-transfusion (hr)

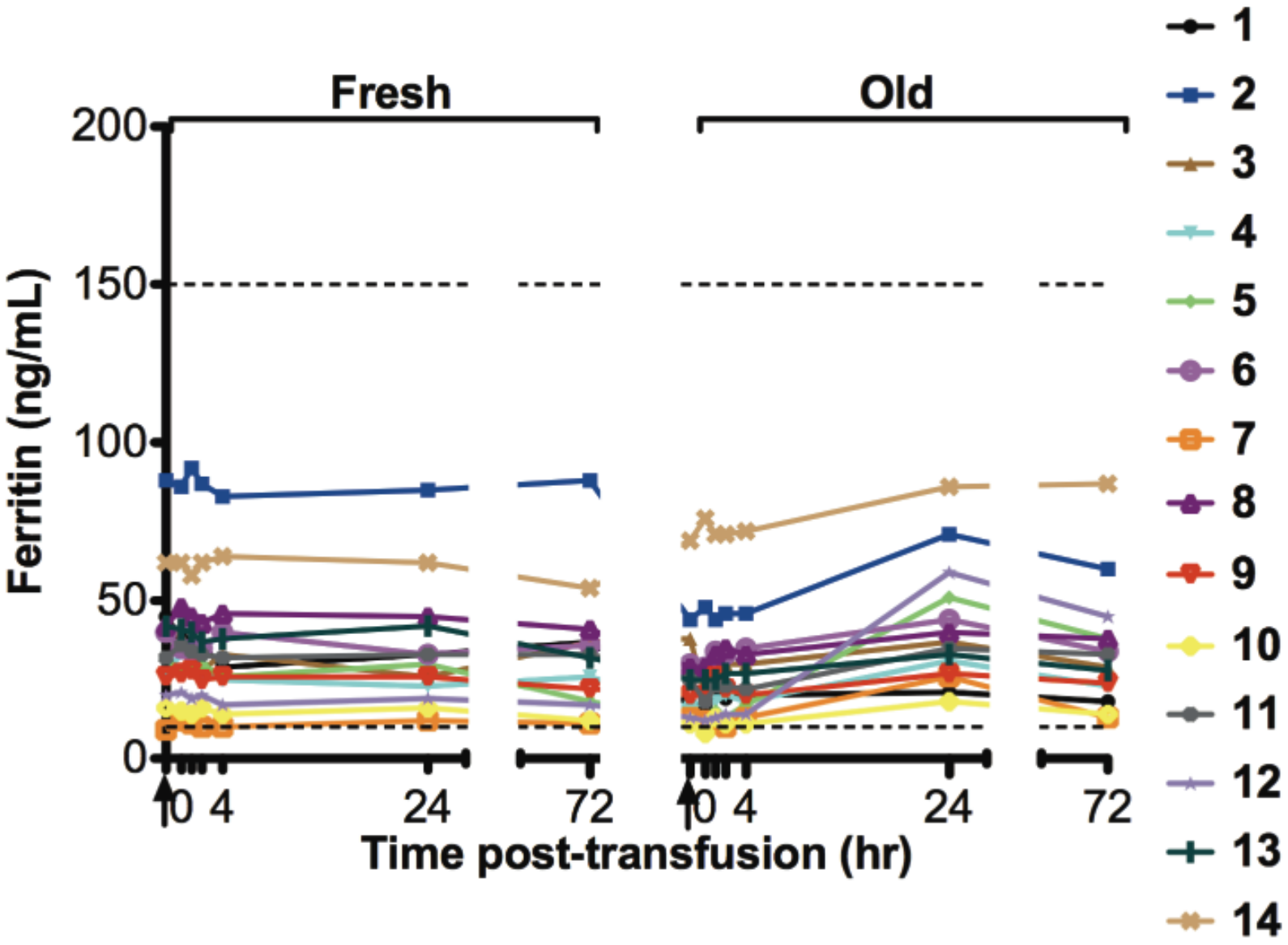
NTBI


Fresh
Old
10
5
0
-5
ΔNTBI (μM)
0
4
24
72
0
4
24
72
Time post-transfusion (hr)
14
1
2
3
4
5
6
7
8
9
10
11
12
13
14

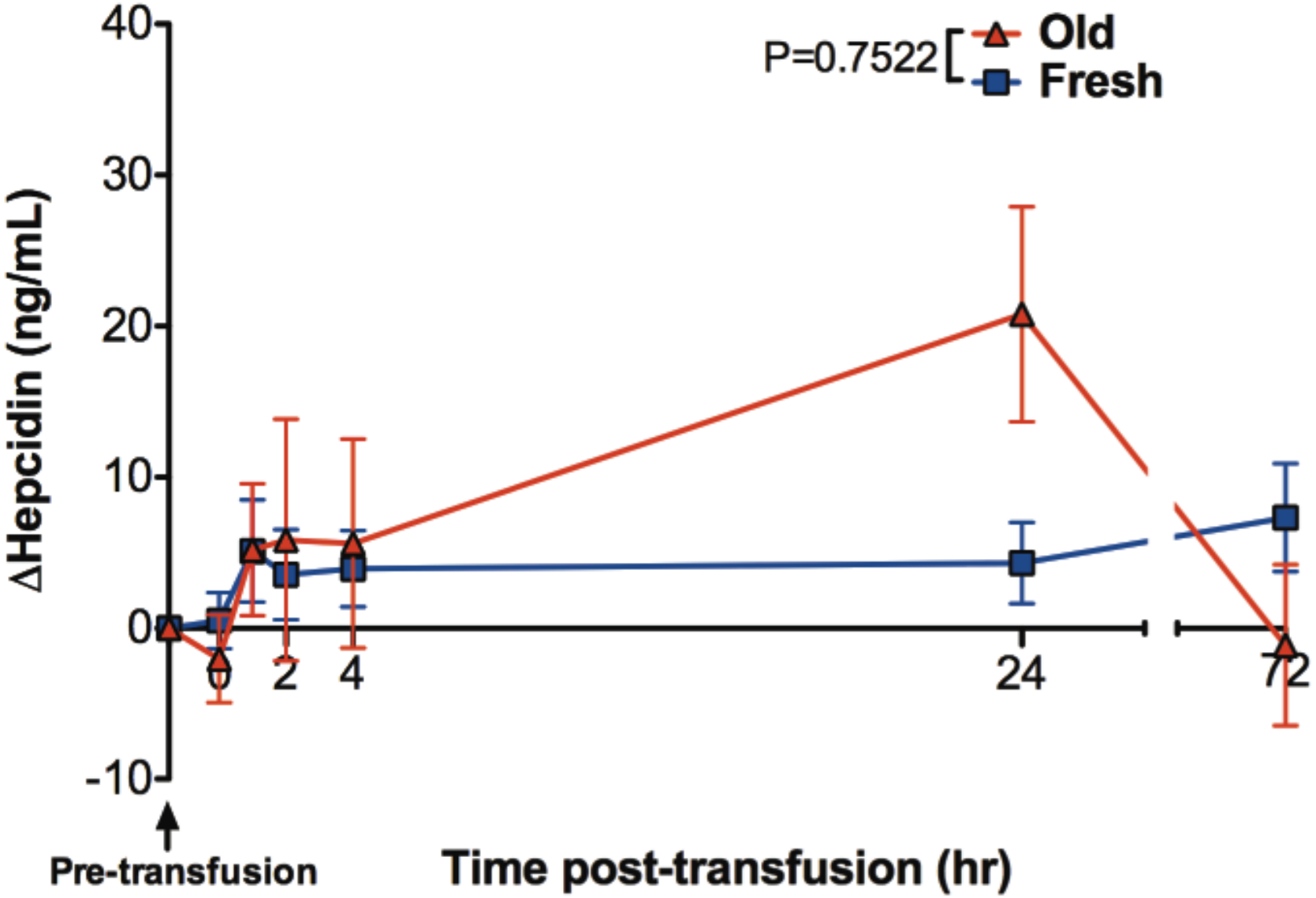
Ferritin


30
20
10
0
-10
ΔFerritin (ng/mL)
P=0.0013
Old
Fresh
***
***
72
Pre-transfusion
Time post-transfusion (hr)

Ferritin

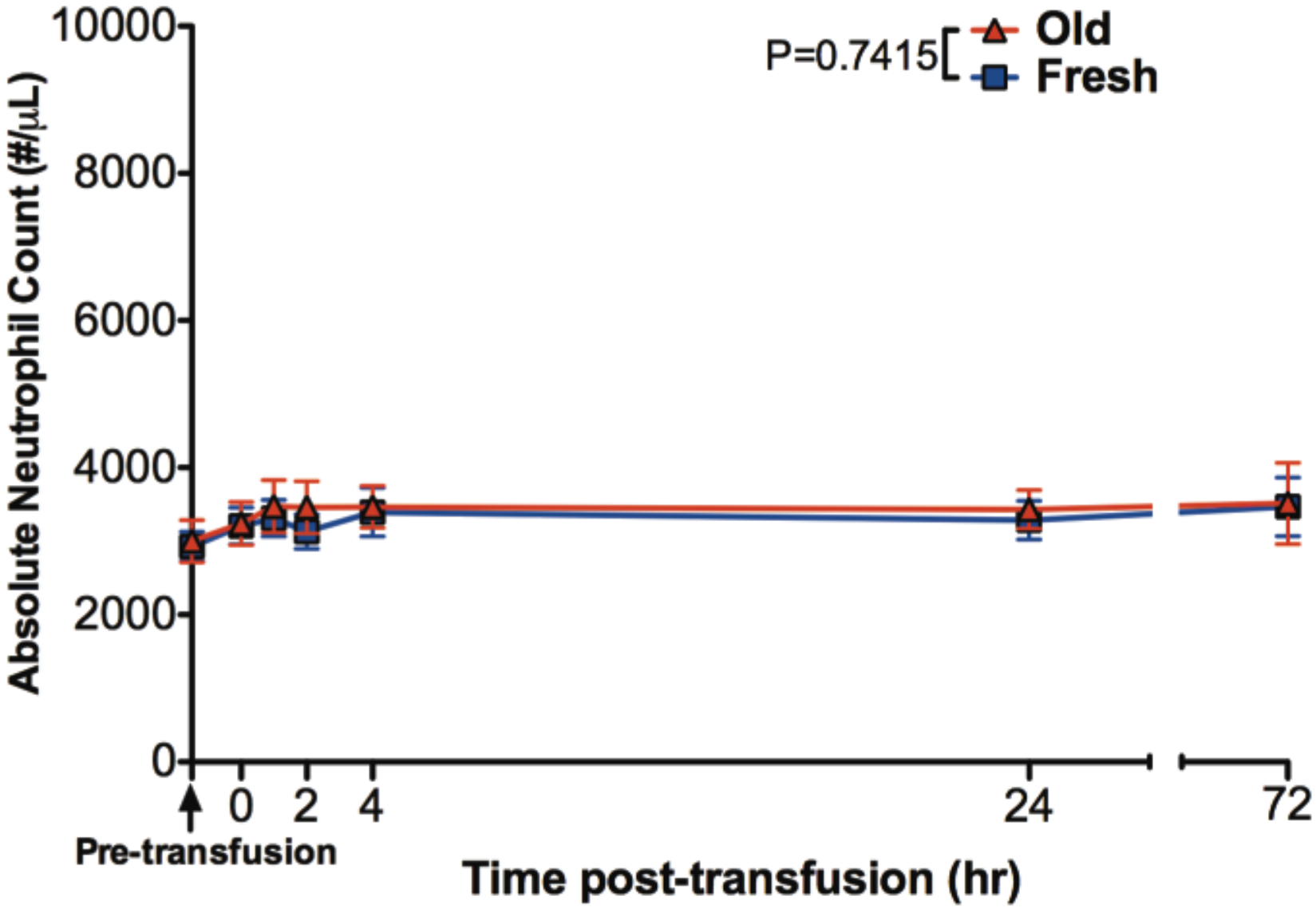

Fresh
Old
200
150
100
50
0
Ferritin (ng/mL)
0 4 24 72
0 4 24 72
Time post-transfusion (hr)
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Hepcidin

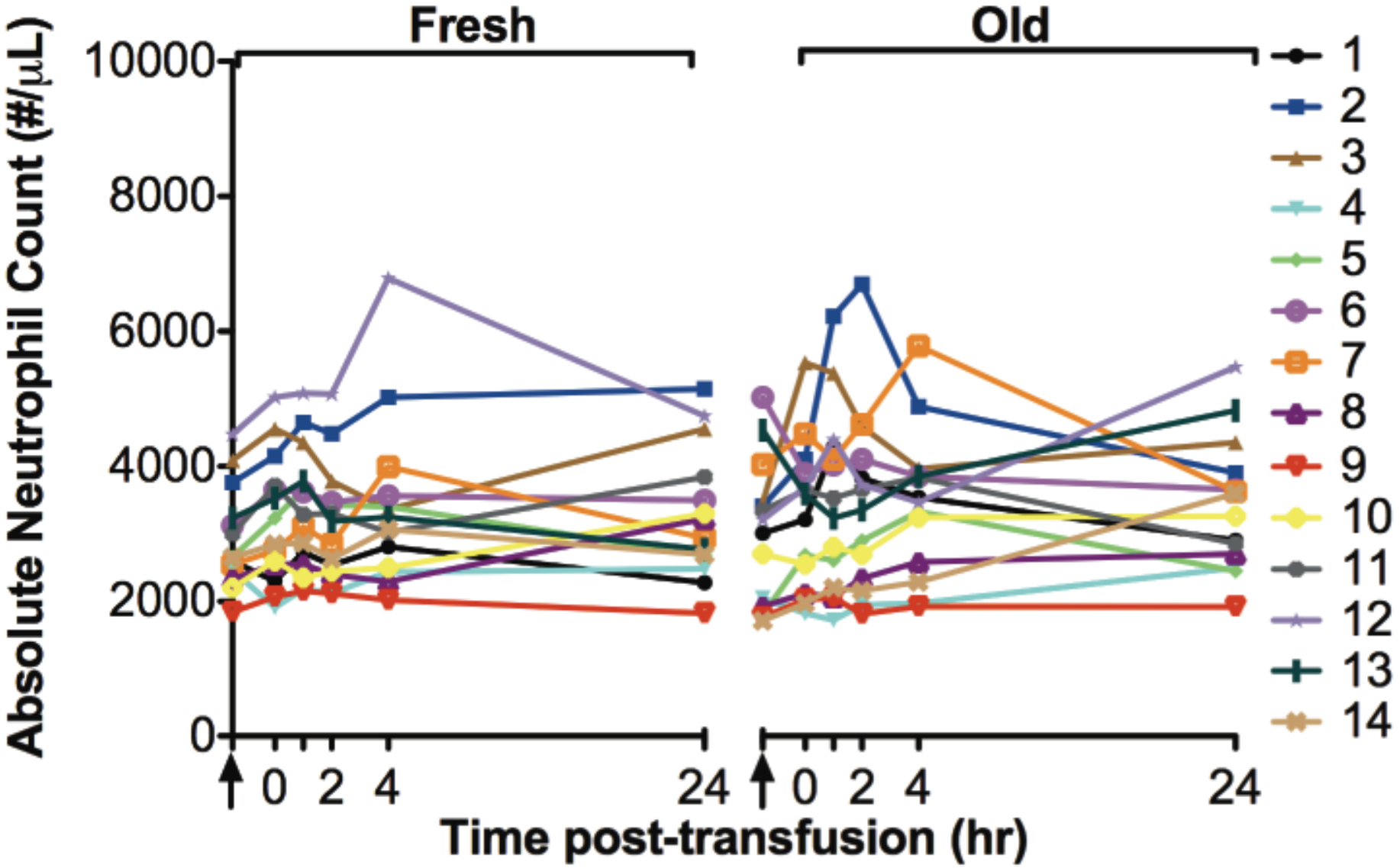

P=0.7522
Old
Fresh
ΔHepcidin (ng/mL)
40
30
20
10
0
-10
0
2
4
24
72
Pre-transfusion
Time post-transfusion (hr)

Markers of inflammation

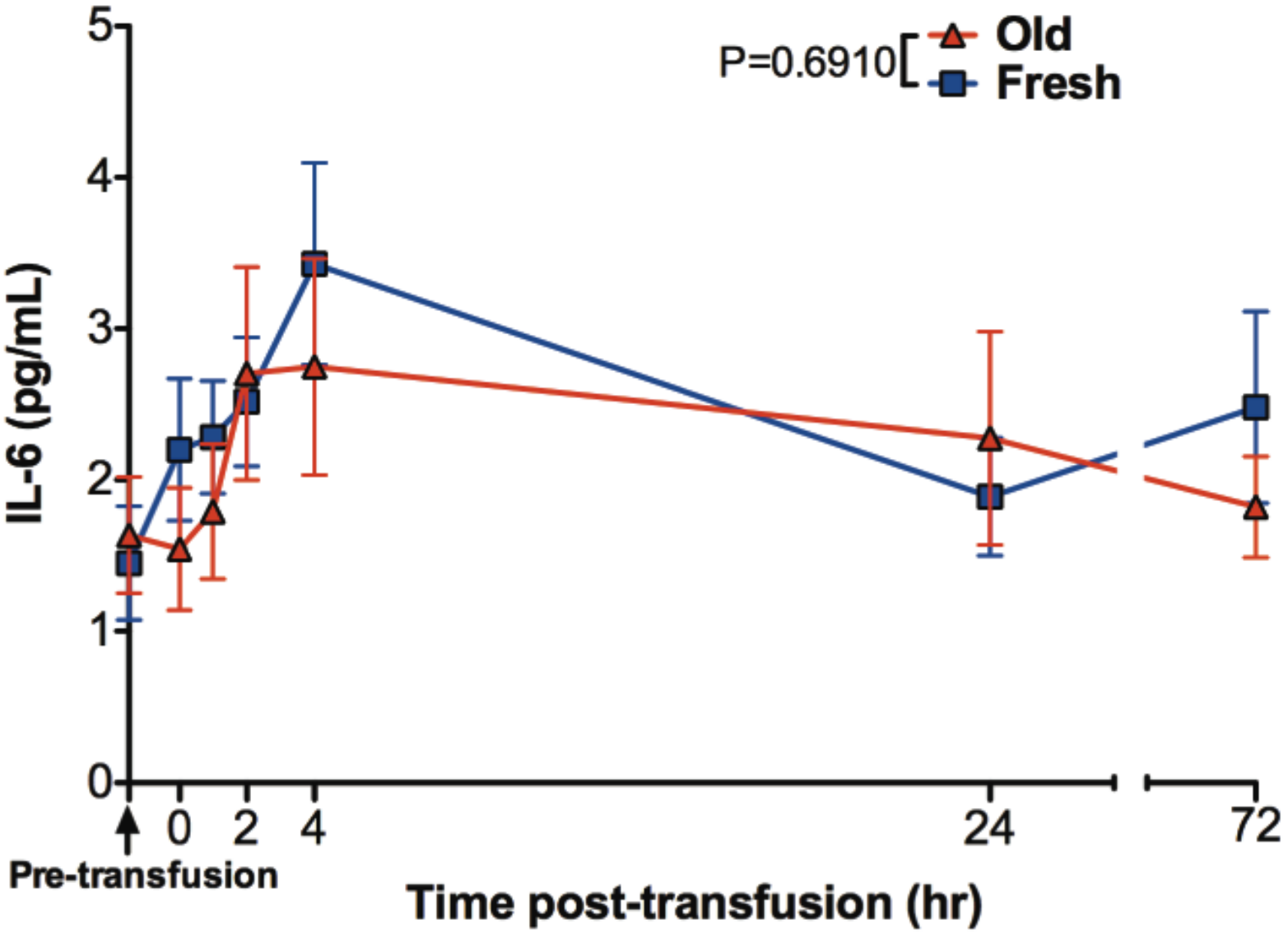
Absolute Neutrophil Count



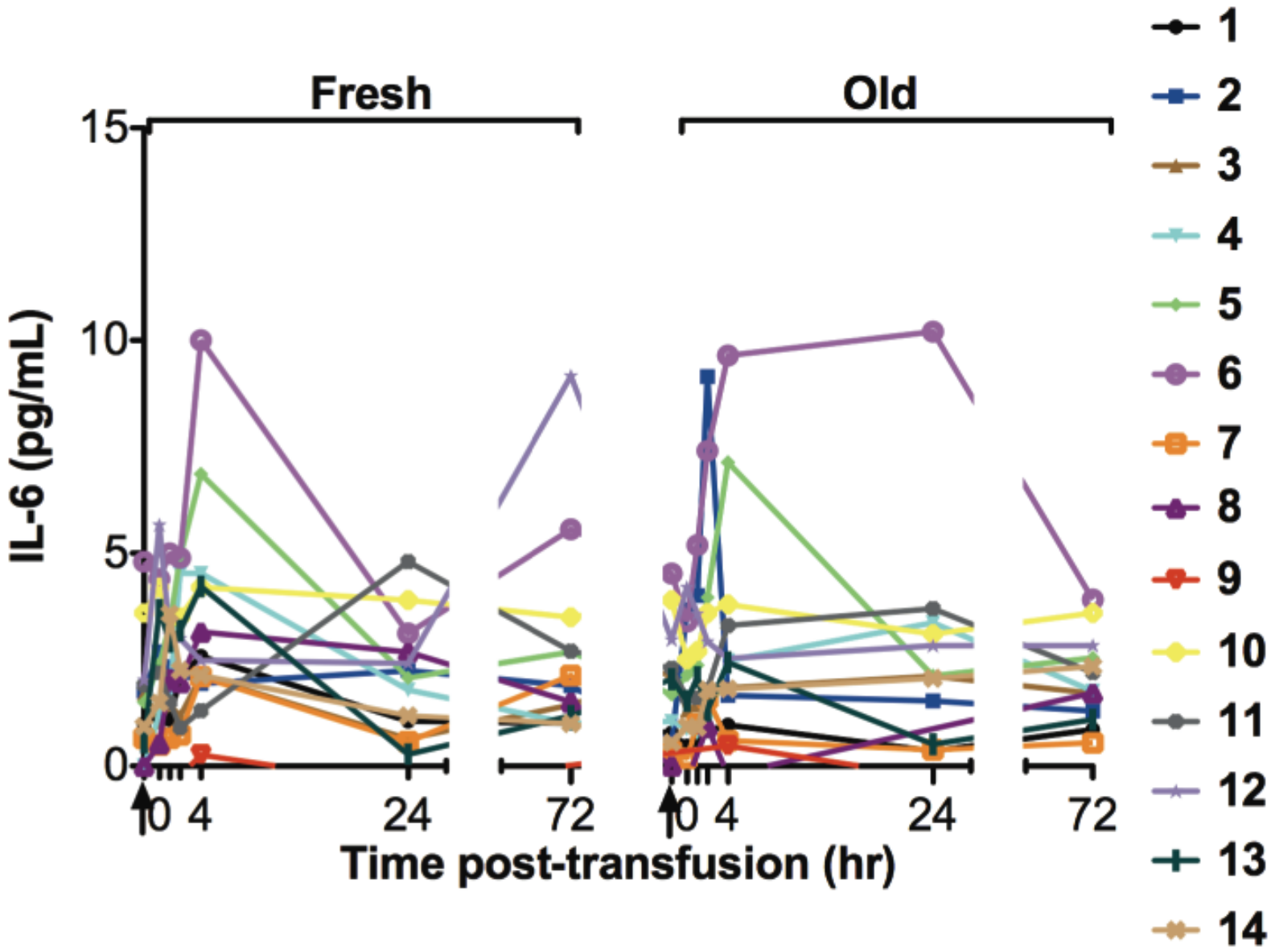
Absolute Neutrophil Count



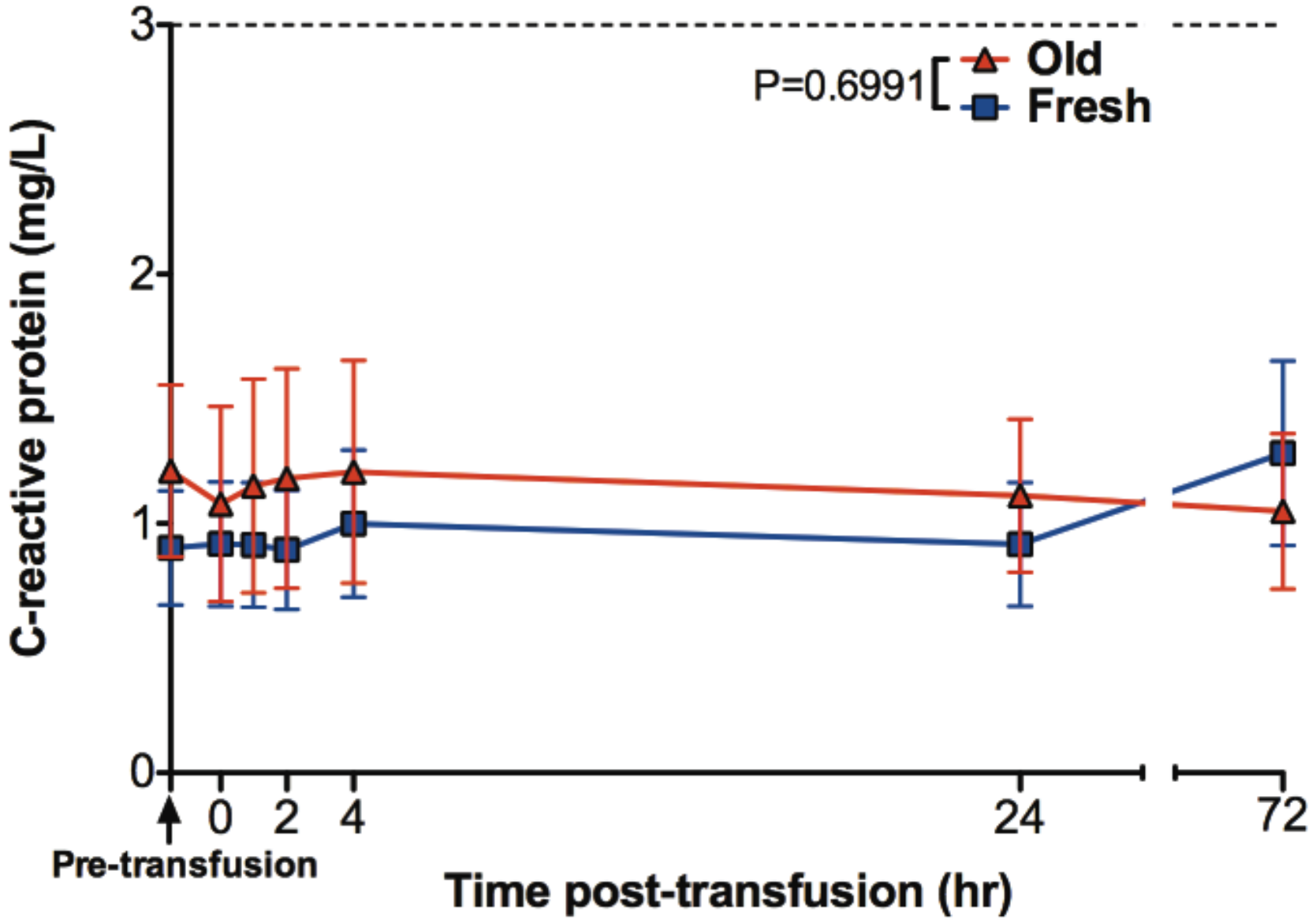
IL-6


P=0.6910
Old
Fresh
IL-6 (pg/mL)
5
4
3
2
1
0
Pre-transfusion
0
2
4
24
72
Time post-transfusion (hr)

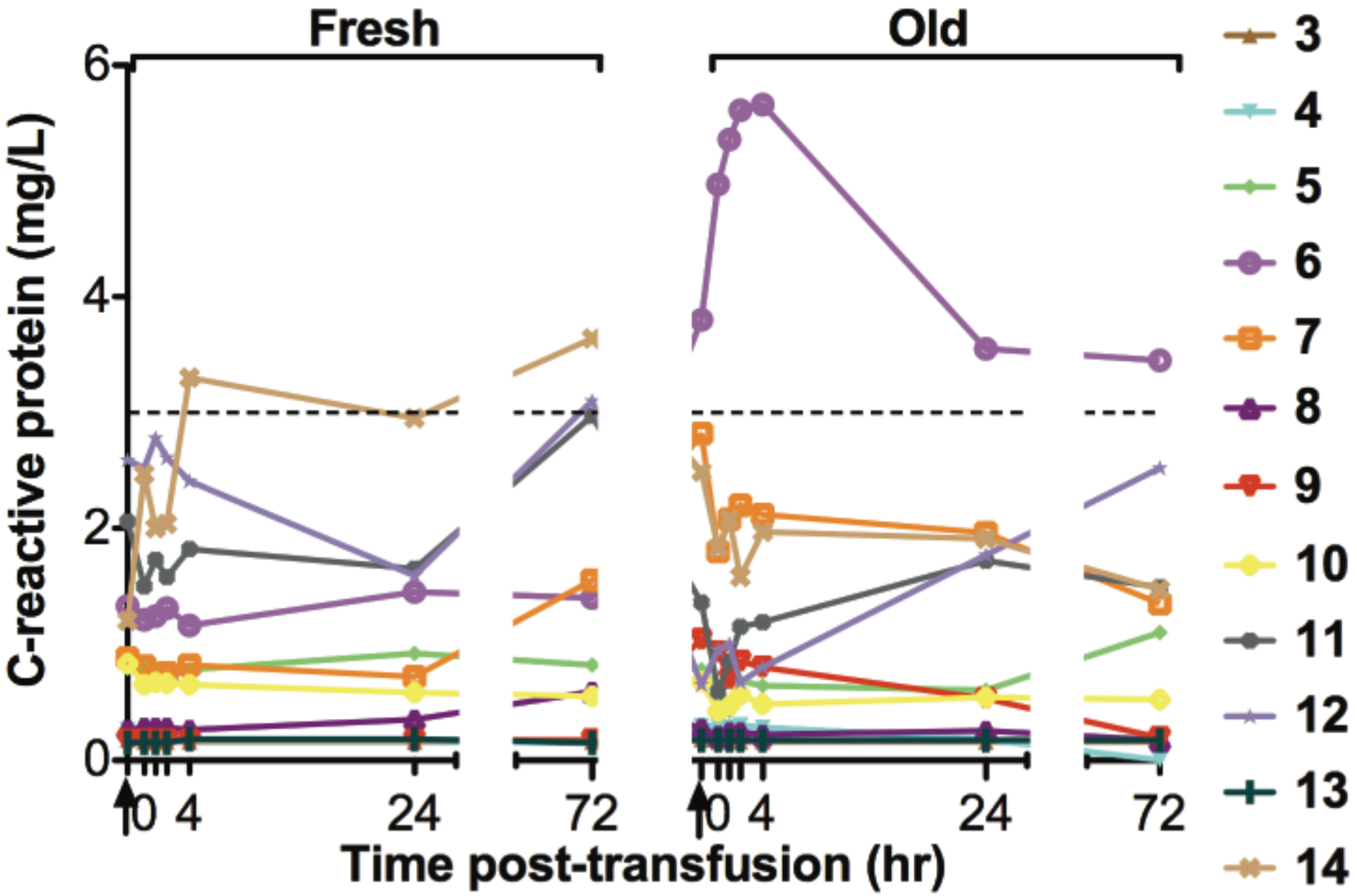
IL-6


Fresh
Old
15
10
5
0
IL-6 (pg/mL)
0
4
24
72
0
4
24
72
Time post-transfusion (hr)
1
2
3
4
5
6
7
8
9
10
11
12
13
14

CRP


C-reactive protein (mg/L)
3
2
1
0
P=0.6991
Old
Fresh
Pre-transfusion
0
2
4
24
72
Time post-transfusion (hr)

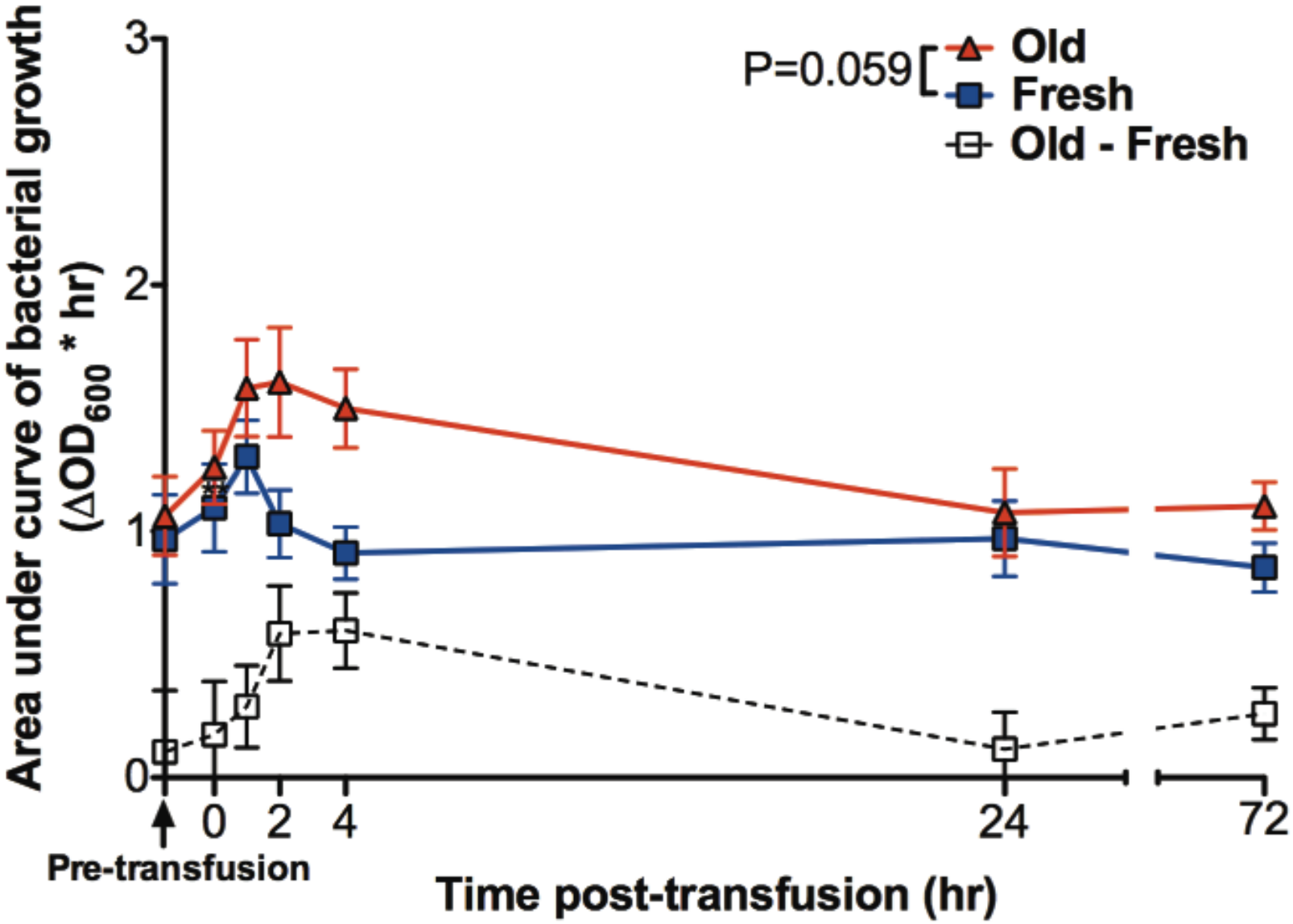
CRP


Fresh
Old
C-reactive protein (mg/L)
6
4
2
0
0
4
24
72
Time post-transfusion (hr)
3
4
5
6
7
8
9
10
11
12
13
14

Conclusions from studies with human volunteers

- **Responses to stored and fresh RBC transfusions differ**
- **Stored RBC transfusions are associated with significant rises in:**
  - **Total bilirubin**
  - **Serum iron**
  - **Transferrin saturation**
  - **Non-Transferrin Bound Iron (NTBI)**
  - **Serum ferritin**
- **With possible exceptions, transfusions of 1 unit of stored RBCs do NOT induce an inflammatory response**

Bacterial growth *in vitro*

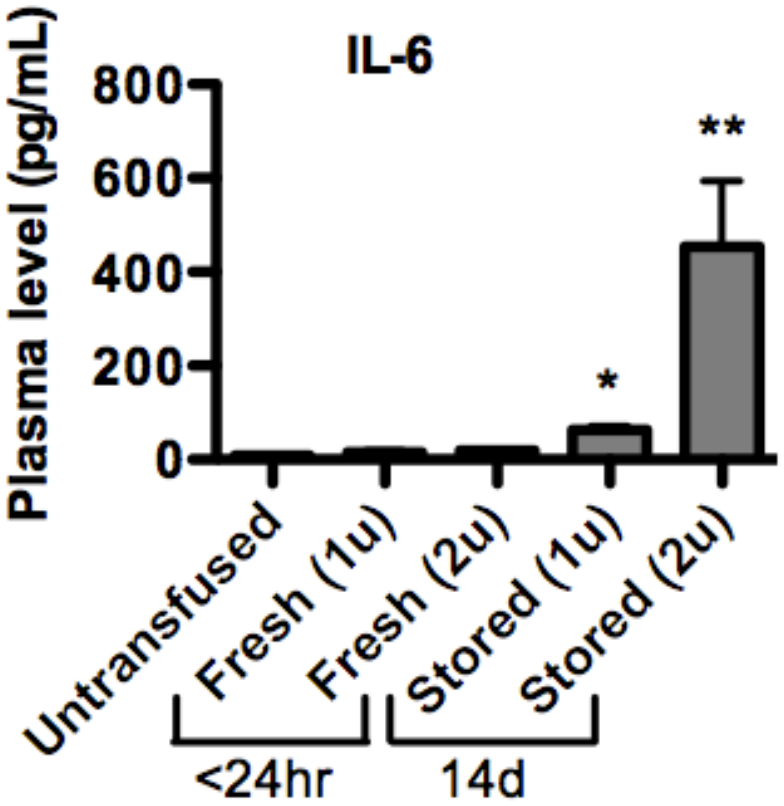


Potential explanations for muted pro-inflammatory response

- Humans are not mice

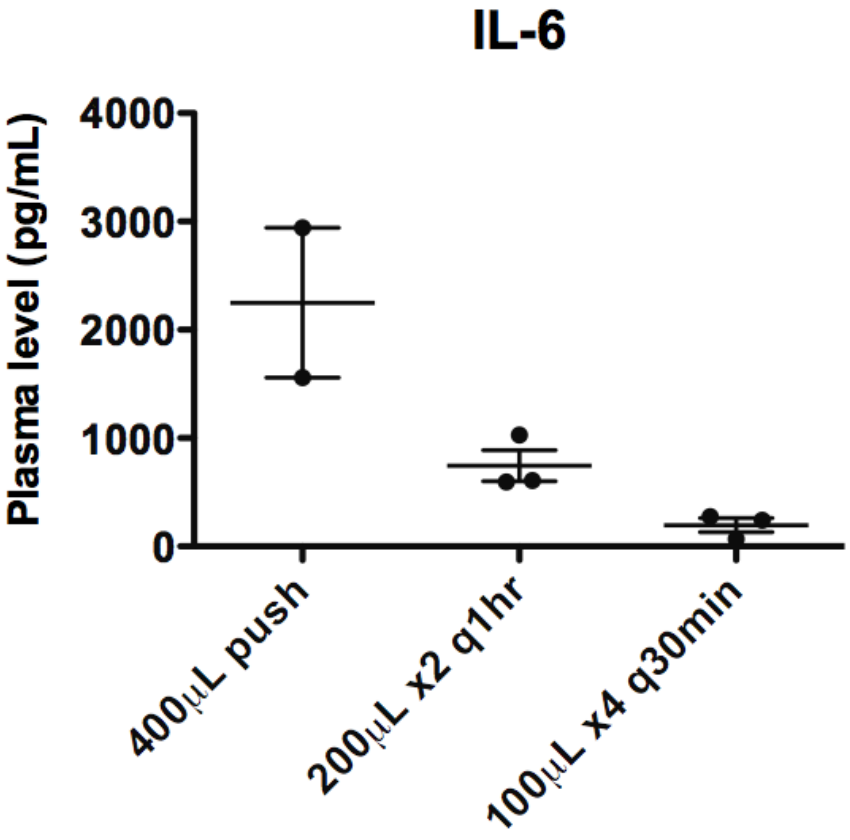
Potential explanations for muted pro-inflammatory response

- Humans are not mice
- Dose effect



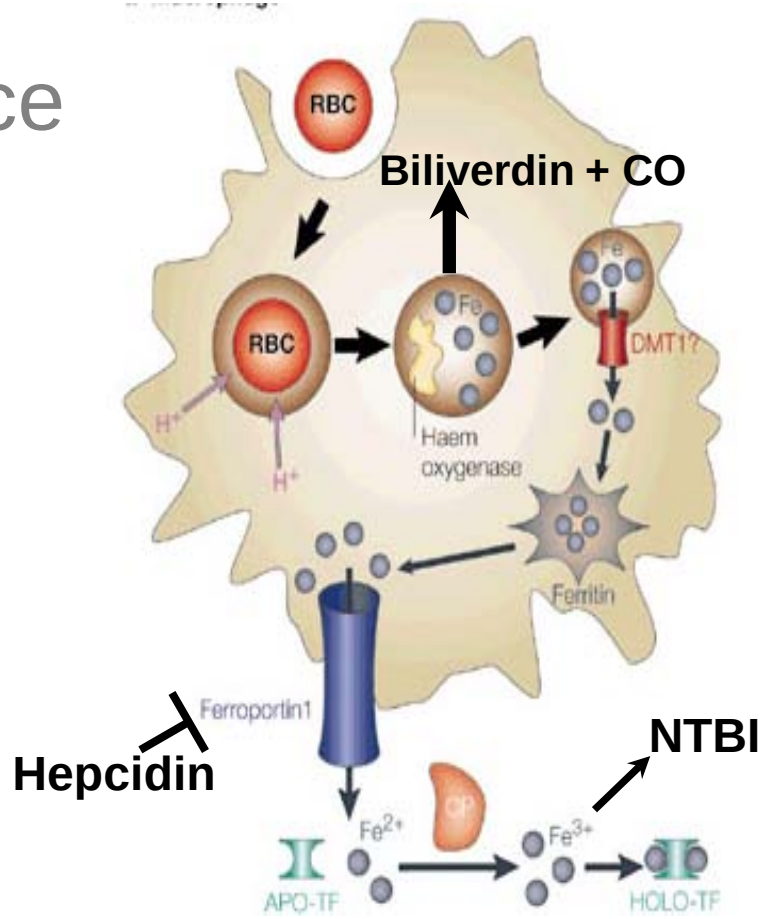
Potential explanations for muted pro-inflammatory response

- Humans are not mice
- Dose effect
- Rate effect



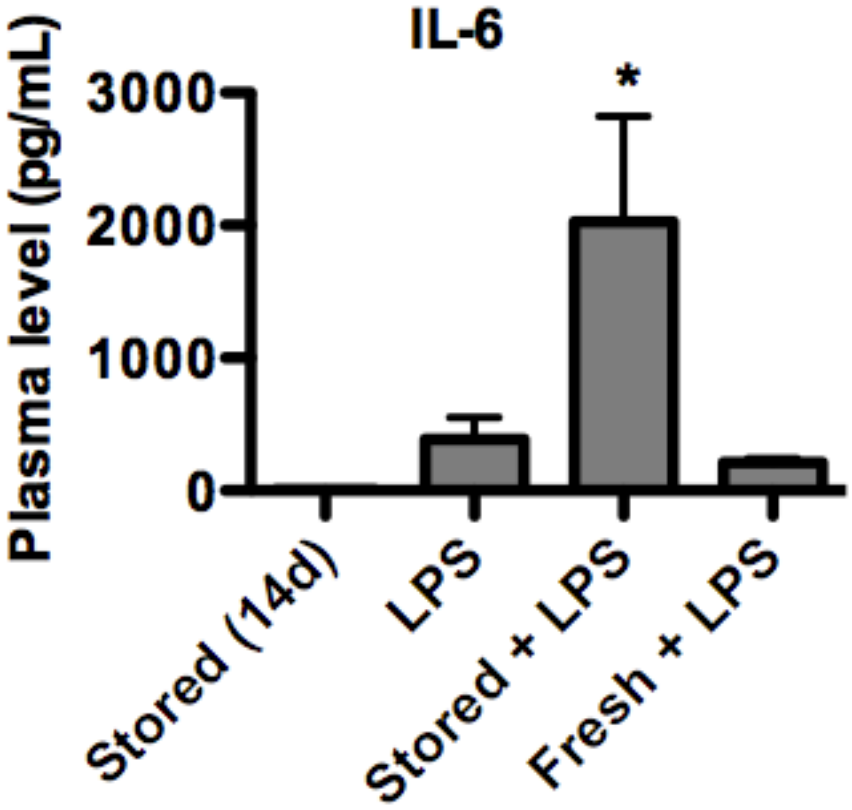
Potential explanations for muted pro-inflammatory response

- Humans are not mice
- Dose effect
- Timing effect
- Hepcidin effect



Potential explanations for muted pro-inflammatory response

- Humans are not mice
- Dose effect
- Timing effect
- Hepcidin effect
- Need to be ill



Other weaknesses of human study

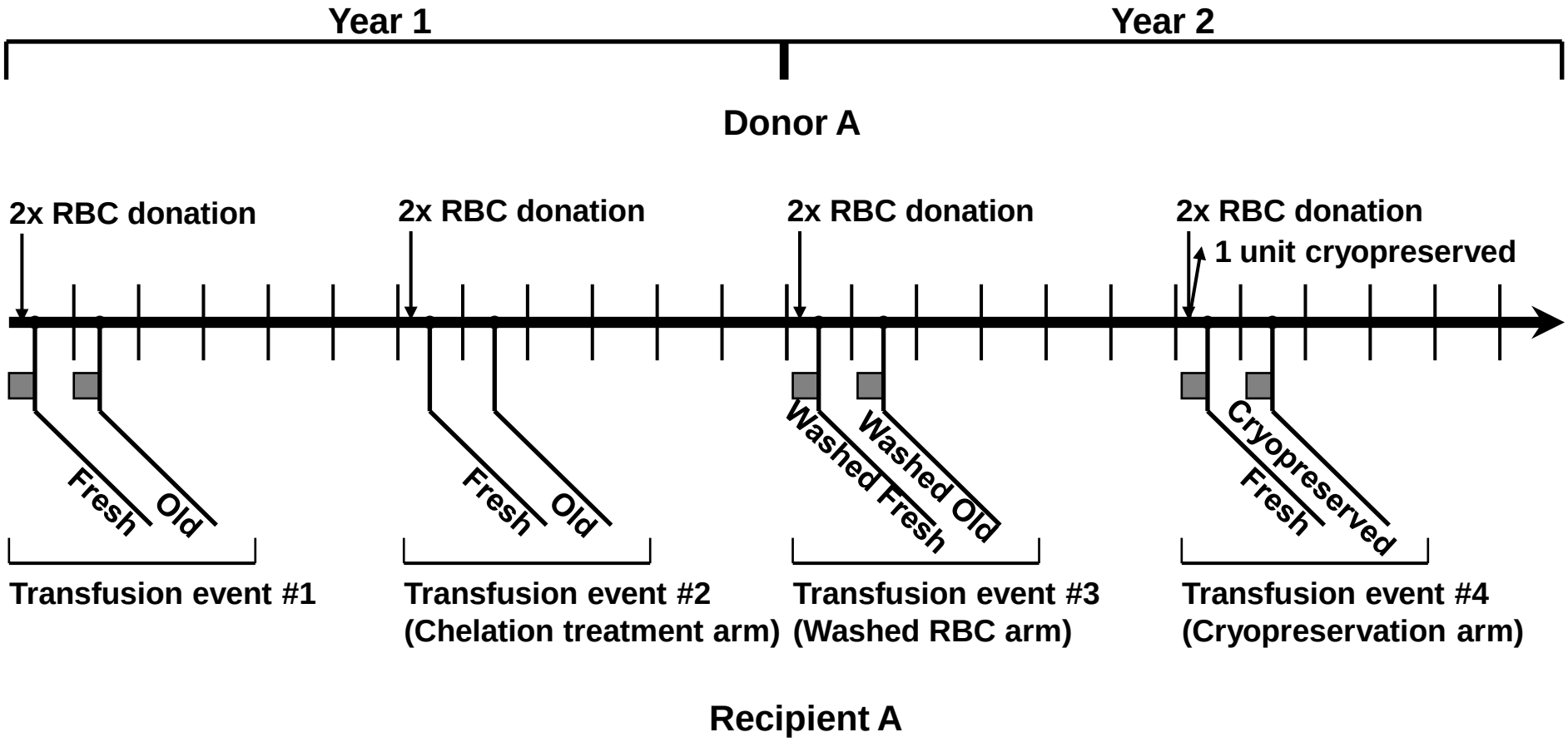
Didn’t measure RBC recovery

Didn’t measure non-protein inflammatory mediators

Probably missed hepcidin peak

Future Directions (in humans)

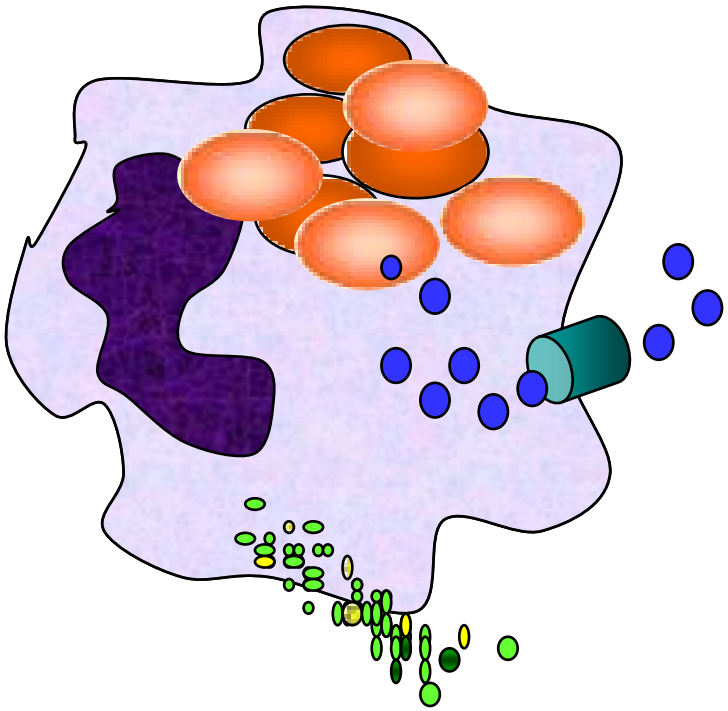
Sickle cell disease & β-thalassemia



Final thought

- 56 yo M, no PMHx, here for “elective” transfusion
- 4 hours after transfusion labs are drawn:
- Hb = 12.8 ➔ 12.8 g/dL
- WBC = 5.5 ➔ 7.4 x10^9/L
- T. bilirubin = 0.7 ➔ 1.7 mg/dL
- Haptoglobin = 69 ➔ 72 mg/dL
- Iron = 110 ➔ 327 μg/dL
- Transferrin sat. = 37 ➔ 87%
- NTBI = 0 ➔ ~8 μM

Conclusions



Older RBC transfusions:

- **Are harmful in mice**
- **Have side-effects in humans**
- **Can mimic a hemolytic transfusion reaction**
- **May result in unnecessary testing**
- **May lead to transfusion delays**

Does iron exacerbate infectious risk?

Do risks outweigh benefits?

We have frozen aliquoted samples…

Columbia
Eldad Hod, M.D.
Boguslaw Wojczyk, Ph.D.
Richard Francis, M.D., Ph.D.

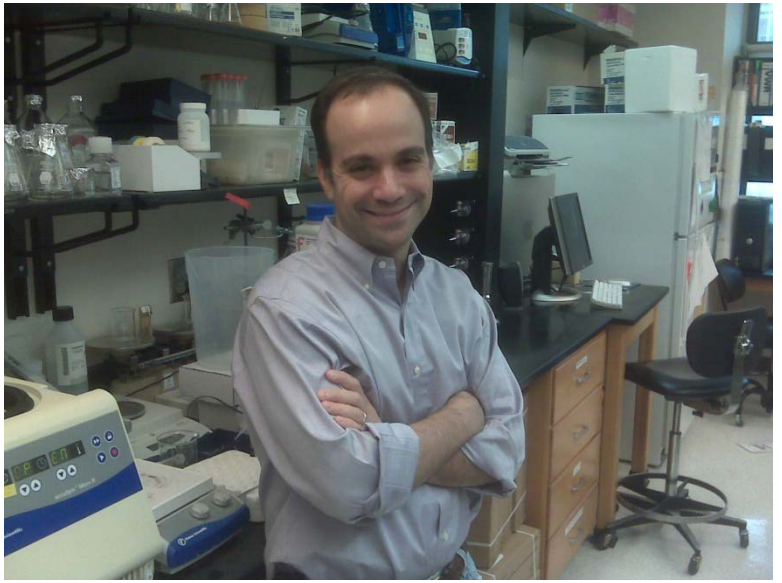
Gary Brittenham, M.D.
Sujit Sheth, M.D.
Genia Billotte, R.N.
Phyllis Della-Latta, Ph.D.
Susan Whittier, Ph.D.

Emory
James Zimring, M.D., Ph.D.
Jeanne Hendrickson, M.D.

Yale
Stephanie Eisenbarth, M.D., Ph.D.

New York Blood Center
Yelena Ginzburg, M.D.

Eldad Hod



Jim Zimring