Clinical presentation of DHTR and hyperhemolysis in Sickle Cell Disease

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Disclosures

- No relevant conflicts

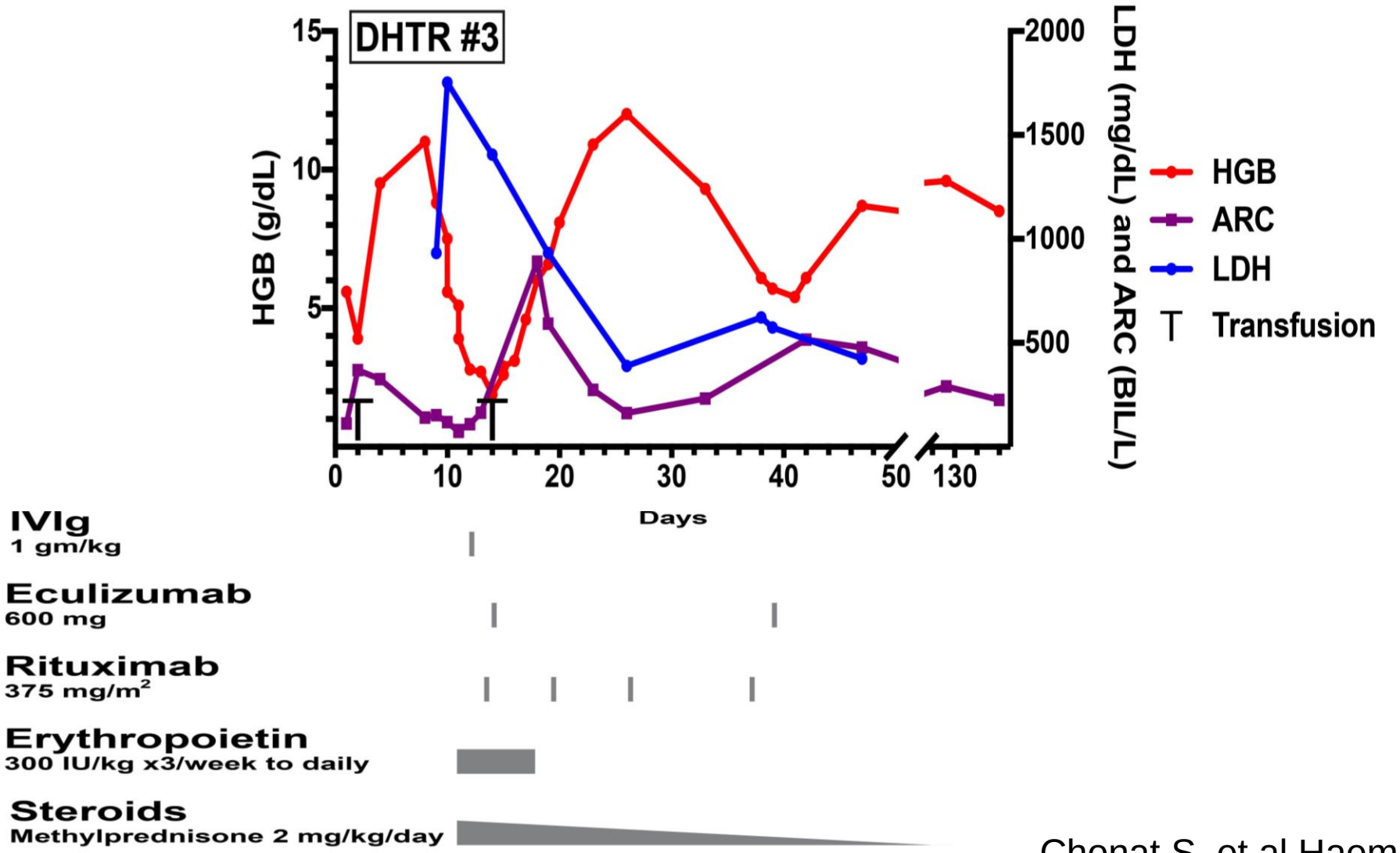
Introductory Case

- 14 year old female with HbSS with the following PMH:
  - DHTRs x 2
  - RBC Abs: anti-S, anti-Di^a , anti-Sd^a, cold agglutinin
- presented with significant VOC pain and Hb 5.6 g/dL- dropped to 3.9 g/dL within 24 hours of admission (no preceding RBC transfusion).
- Received 3 units of RBC units (extended matched, S-negative, Di^a-negative, crossmatch compatible)
- Discharged home with Hb of 9.5 g/dL.

Introductory Case

- Presented 8 days later with generalized pain, fever, hemoglobinuria, and bilirubin 7.3 mg/dL. HGB declined sharply with worsening of intravascular hemolysis (elevated free plasma hemoglobin 100 mg/dL, worsening hemoglobinuria, and LDH peak of 1753 U/L) along with elevated transaminases).
- DAT and antibody testing were again negative.
- On day 14, Hb =1.9 g/dL, creatinine doubled, with worsening pain, altered mental status, and development of new diffuse pulmonary edema requiring positive airway pressure support.

Introductory Case



Chonat S, et al Haematologica: 2018

RBC Alloimmunization Rates

Population	RBC alloimmunization
SCD	18-47 %
Thalassemia	5-19%
General	0.2% - 2.8%

Complications of Alloimmunization

Inventory / Cost

- Difficulty or impossibility of finding compatible RBC units
- Increased cost and risk to patient

Future BMT Implications

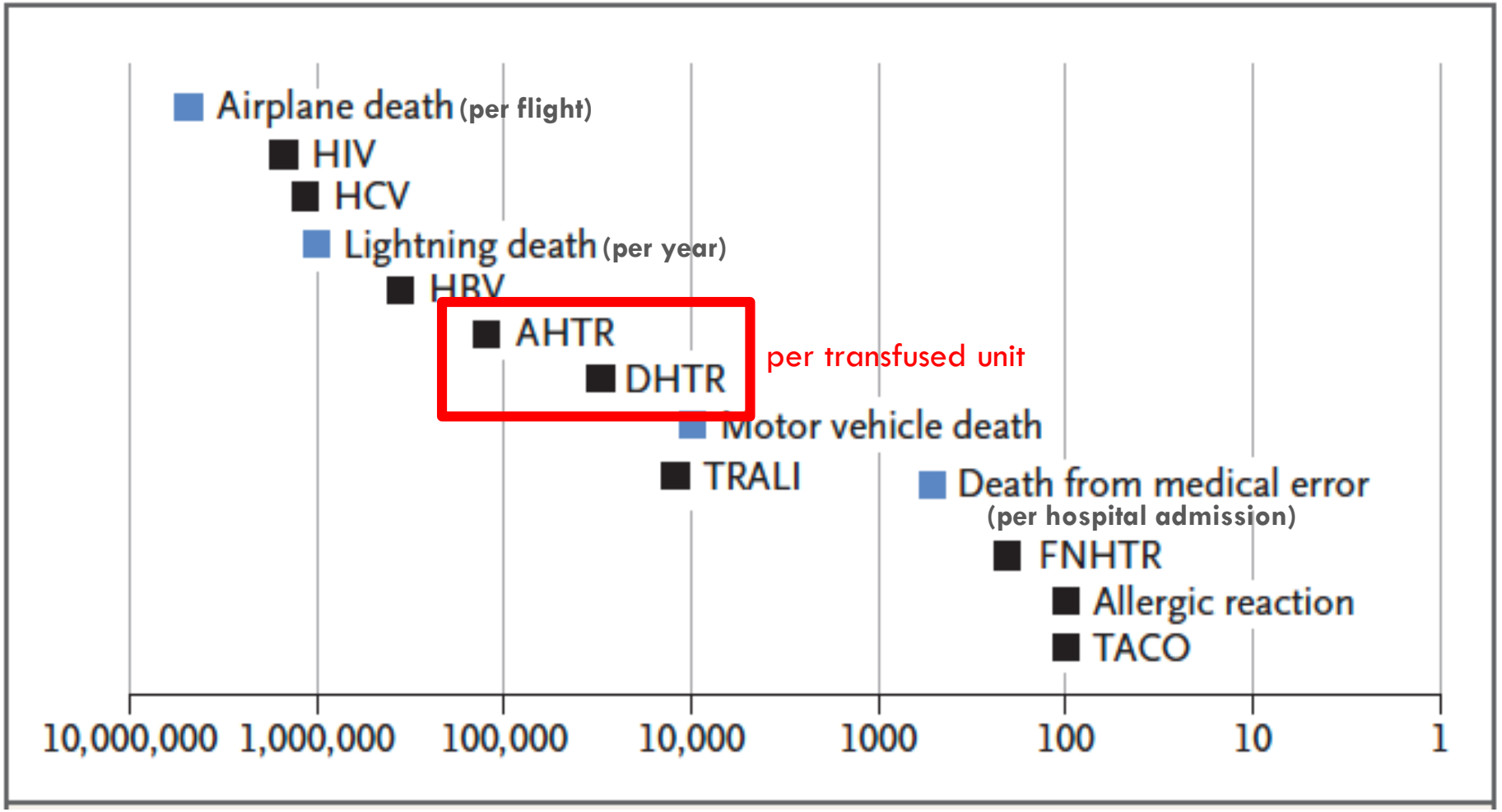
- Associations found in SCD patients between RBC Abs and HLA Abs
- Predisposition to graft rejection in SCD patients undergoing BMT (?)

Delayed Hemolytic Transfusion Reaction (DHTR)

- The most feared transfusion complication in SCD patients
- **Hyperhemolysis** (bystander hemolysis) unique to SCD
- **Ab-Negative DHTR**: Process attributed to antibody-independent macrophage activation.
- **Autoantibodies**: Further complicate clinical picture, and potentially contribute to hyperhemolysis
- **DHTR Treatment:** Remains controversial because the exact mechanisms remain unclear
  - EPO / IVIG / Steroids / Rituxan / Eculizumab / Bortezomib
  - Avoidance of RBC transfusion

Incidence of DHTRs

Infectious and Noninfectious Adverse Effects of RBC Transfusions

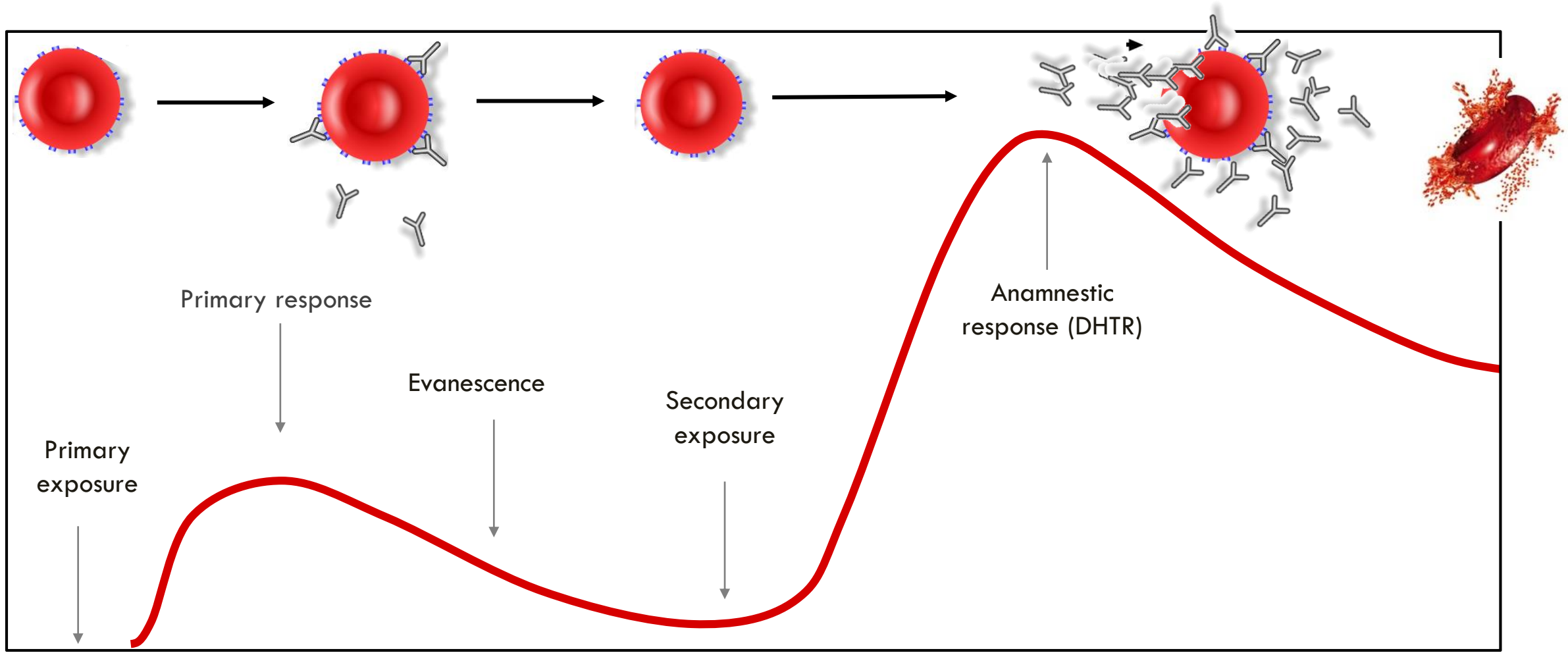


Carson JL, et al. N Engl J Med. 2017

DHTR incidence in SCD

Report	# of patients	# of transfusions	DHTR incidence (% of patients)	DHTR rate (per transfusion)
Vidler, et al. 2015 (PMID: 25753472)	220	2158	7.7%	1.1%
		591 (acute)		3.5%
		1567 (chronic)		0.1%
Narbey, et al. 2017 (PMID: 28924974)	311	694	4.8%	2.1%
		360 (acute)		4.2%
		334 (chronic)		0%

Pathophysiology of DHTR



DHTRs in Adult SCD: Presentations

Sign/Symptom (N=99)	Result (median, IQR)
Laboratory findings	
• Days after transfusion to DHTR Dx	10 days (8 to 14)
• ΔHb (post-trxn to DHTR)	4.6 g/dL (3.1 to 5.3)
• Hb nadir	5.5 g/dL (4.6 to 6.3)
• LDH max	1335 IU/mL (798 to 2086)
• Lowest retic count	180 k/µL (121 to 240)
Clinical findings at DHTR presentation	
• Hemoglobinuria	96%
• Pain	89%
• Fever	64%
• Symptoms of anemia	44%
Complications during DHTR	
• ACS	50%
• Hepatic impairment	35%
• Renal failure	10%
• Death	6%

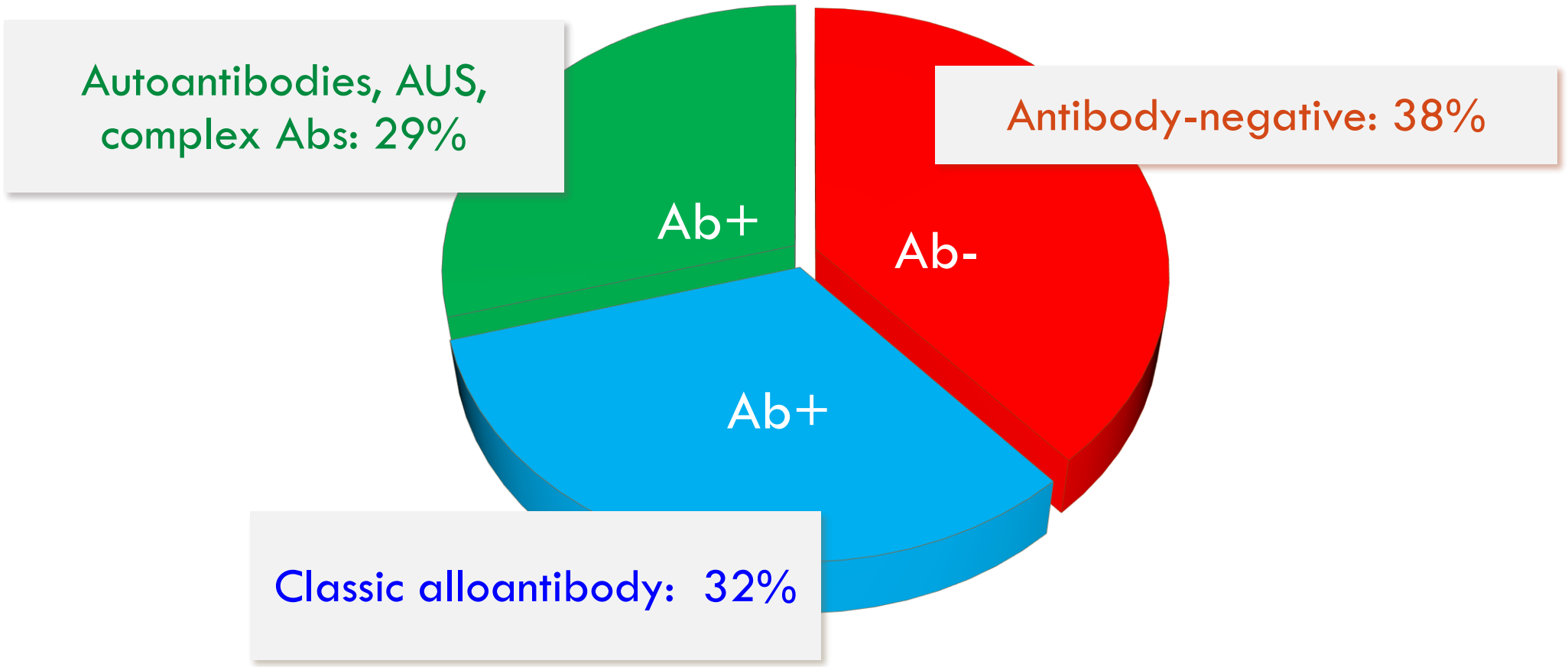
Habibi A, et al. Am J Hematol. 2016

DHTRs in adult SCD: Treatment and Outcomes

Treatment (n: 99)	Result (%)
Non-specific supportive care measures	54%
EPO (10,000–60,000 IU total)	45%
+ Corticosteroids	3%
+ IVIG	4%
+ Eculizumab	2%
+ Rituximab	2%
Transfusion	35%
• Determined ineffective	• 69% (24/35)
• HbA = 0% within 2 wks of trxn	• 51% (18/35)
• Died of exacerbated hemolysis and MOF	• 14% (5/35)

Habibi A, et al. Am J Hematol. 2016

DHTRs in SCD: Immunohematological Characteristics



Habibi A, et al. Am J Hematol. 2016

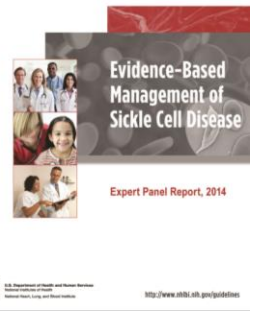
Prevention of DHTRs

1. **Reduce risk of RBC alloimmunization**
   - Prophylactic matching (for Rh (C/c, E/e, K)
   - Judicious use of RBC transfusions (i.e. avoid transfusion for simple VOC)
   - **Identify Antibody "Responders" from "Non-responders" (in the future)**

2. **Reduce the risk of missing the detection of a "transient" alloantibody**
   - Follow-up antibody screens at set intervals (4-12 weeks) after every episodic transfusion
   - Avoid multi-site transfusion
   - Thorough transfusion history
   - Reliable inter-institutional blood bank communication

3. **Reduce the risk of re-exposure to an "evanesced" alloantibody**
   - Avoid multi-site transfusion
   - Thorough transfusion history
   - Reliable inter-institutional blood bank communication

RBC Antigen Matching



Study	N	Matching	Patient % w/ AlloAbs	Rate (AlloAb/100 units)
Rosse et al. 1990	1044	ABO, D	27%	n/a
Vichinsky et al. 1990	107	ABO, D	30%	n/a
Aygun et al. 2002	140	ABO, D	37%	2.8
Castro et al. 2002	351	ABO, D	29%	3.8
Sakhalkar et al. 2005	387	ABO, D	31%	1.7
Vichinsky et al. 2001	61	Limited (C, E, K)	11%	0.5
Sakhalkar et al. 2005	113	Limited (C, E, K)	5%	0.26
Chou et al. 2013	182	Limited (C, E, K)*	44%	0.33
Debaun et al. 2014	90	Limited (C, E, K)	4.5%	0.28
Lasalle-Williams et al. 2011	99	Extended matching†	7%	0.1
Tahhan et al. 1994	40	Extended matching §	0%	n/a

* from African-American donors † C, E, K, Fy^a, Fy^b,Jk^a, Jk^b, § C, E, K, Fy^a, Fy^b,Jk^a, Jk^b, S

Judicious use of RBC transfusions is recommended during acute sickle cell events

Table V. Multivariate analysis of the effect of inflammatory events on alloimmunization.

N = 52 patients (3166 transfusions)	Odds ratio (OR)	95% Confidence interval
Events		
Any inflammatory event	8·9	5·9–13·5
ACS	13·2	8·4–20·8
VOC	8·7	3·5–21·5
Elective surgery	4·6	1·9–11·1
AFI	4·1	1·7–9·7
SS/AIS/Aplastic Crisis/Priapism	4·0*	1·6–10·1
Match level		
1–4 vs. 0	0·18†	0·12–0·28
Storage solution		
CPD(A) vs. AS	1·4†	0·92–2·12

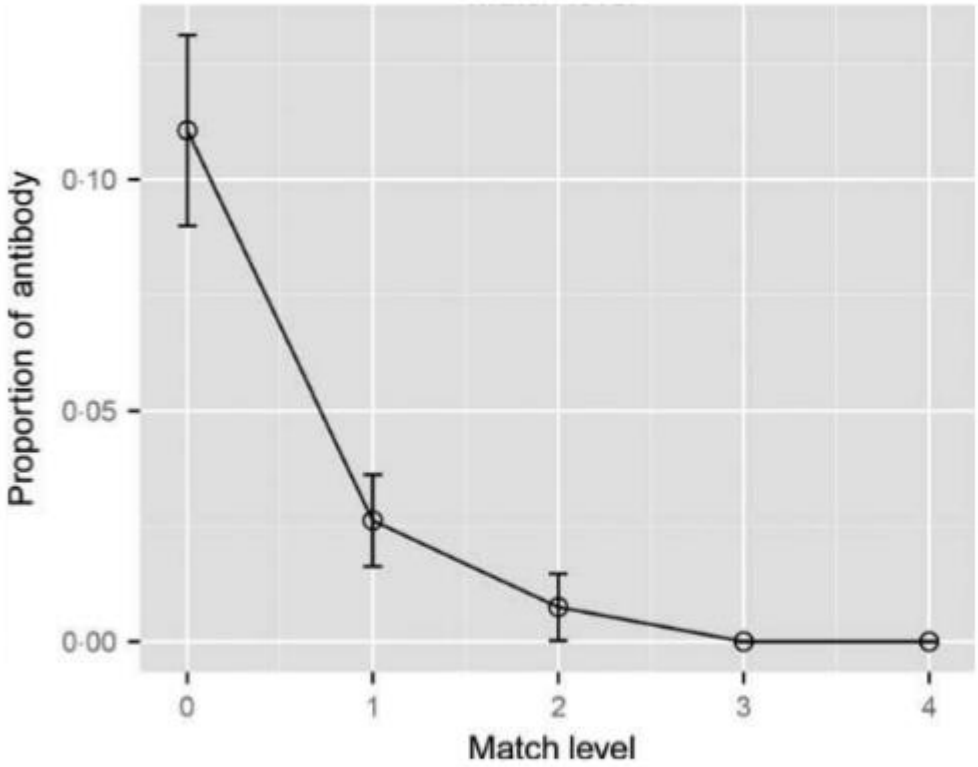
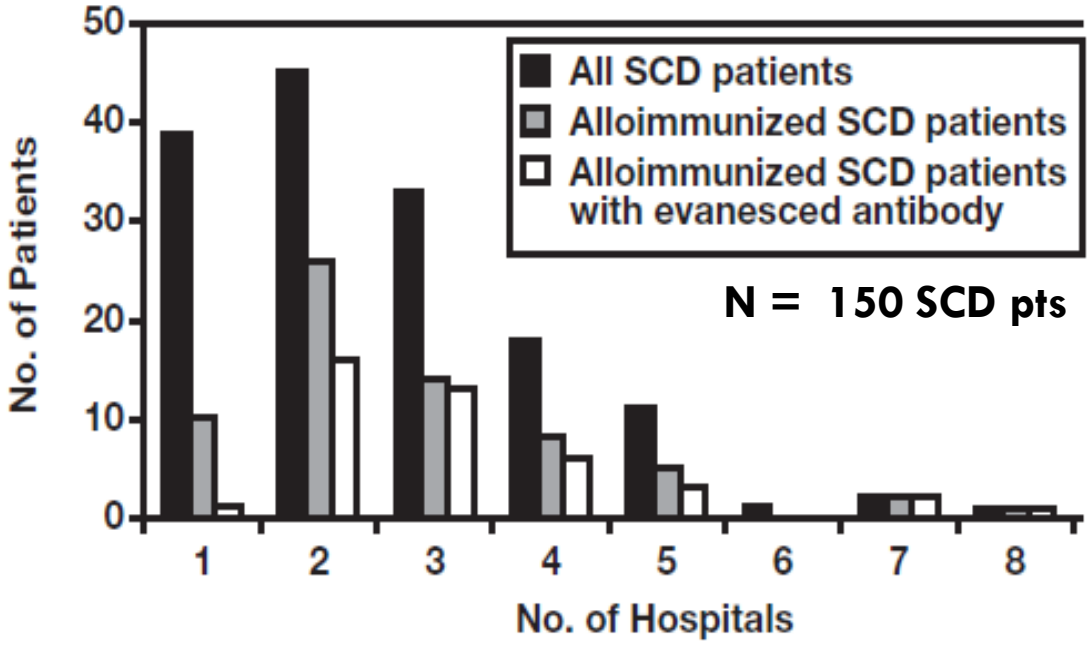


Figure 1. Effect of level of matching on alloimmunization. Graph represents effect of red blood cell (RBC) unit level of matching on probability of alloimmunization with error bars illustrating 95% confidence intervals (±2 standard deviations) ($P < 0·0001$ for trend).

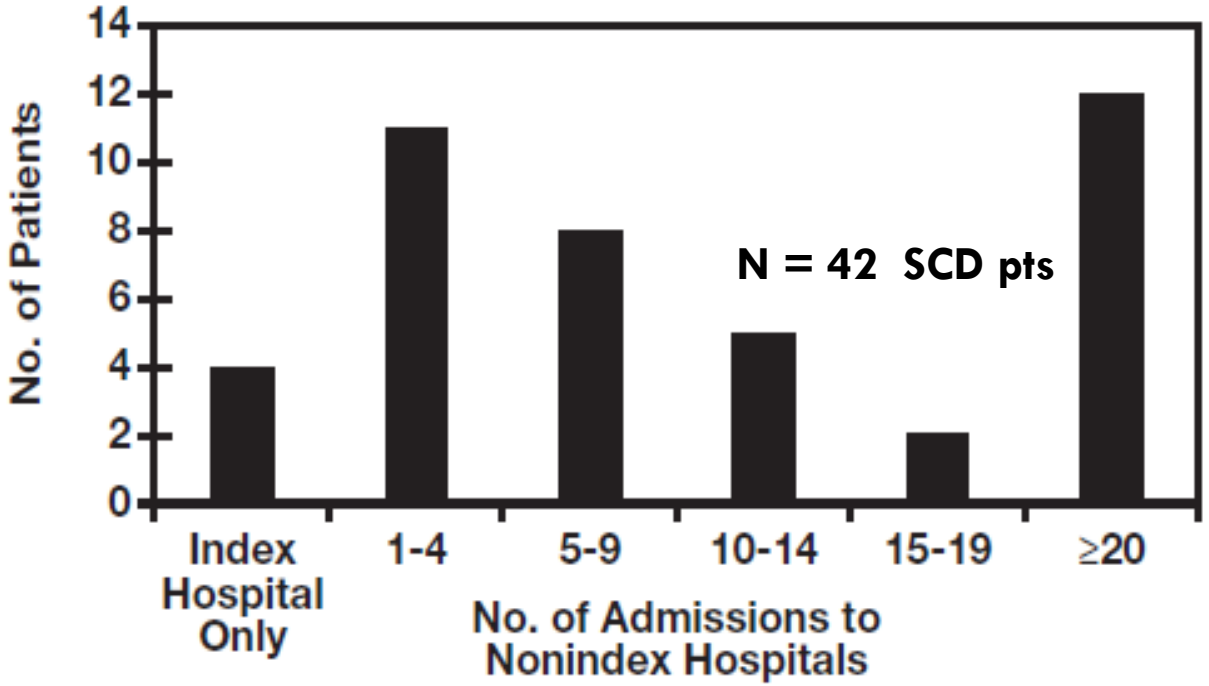
Fasano RM, et al. Br J Haematol. 2015

Multi-site transfusion in SCD patients

Number of hospitals in which SCD patients receive ≥1 RBC transfusion



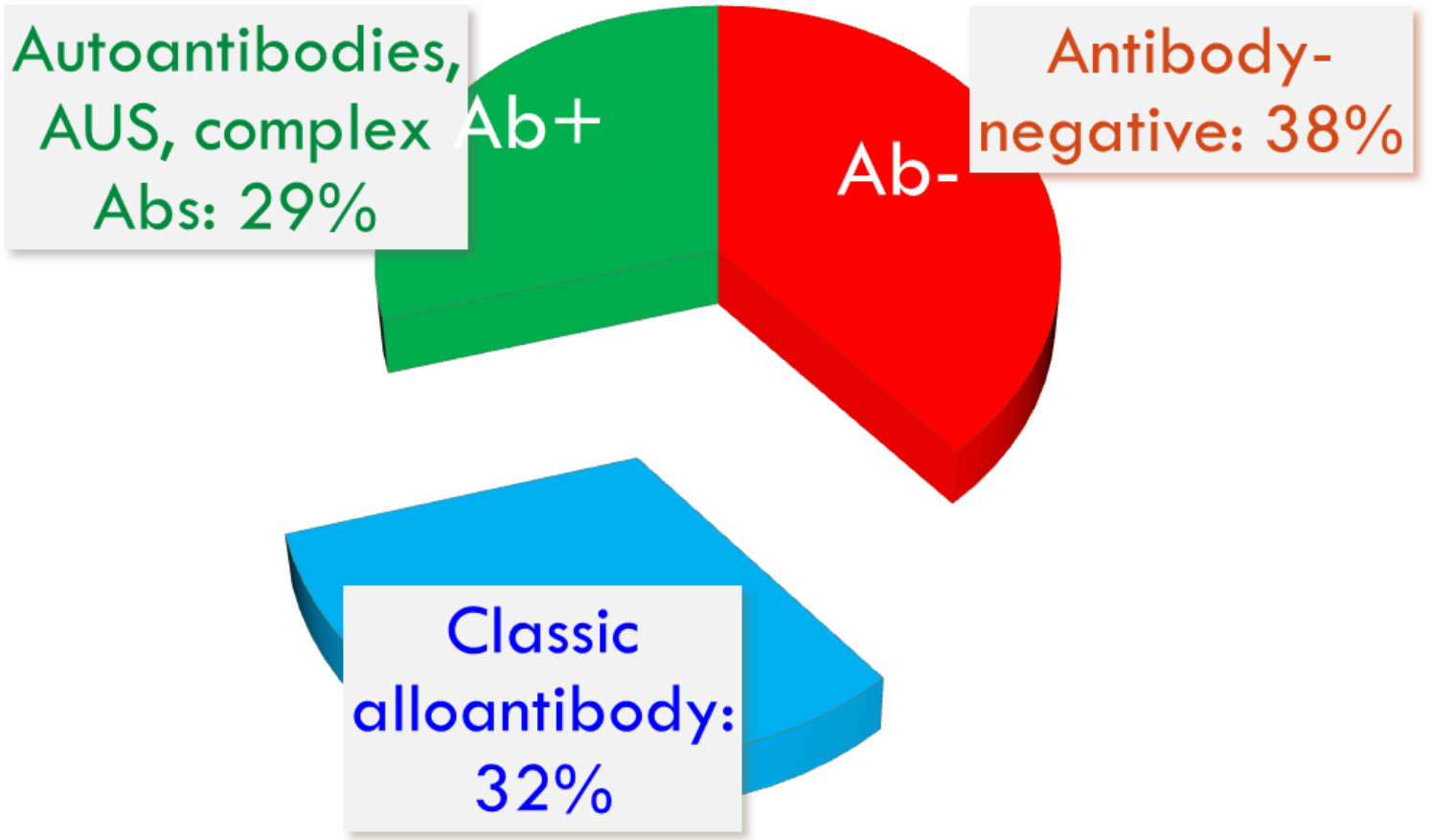
Number of hospital admissions for transfusion in SCD patients with evanesced antibodies



Harm SK, et al. Am J Clin Pathol 2014

Prevention of DHTRs

1.
2.
3.



ion

Clinical considerations?

1. What are the ways to prevent and treat recurrent DHTRs in SCD patients?
   - **Transfusions** (matching, transfusion triggers, complex evaluations by BB/IRL)?
   - **Medications** (EPO, IVIG, steroids, Rituxan, Eculizumab, Bortezomib, etc…)?

2. Are all DHTRs created equal?
   - **Ab+** vs. **concurrent alloantibodies with autoantibodies** vs. **Ab-**?

3. How to manage SCD patients when transfusion is unavoidable?
   - Eg. Life threatening DHTRs, Moya moya surgery, curative BMT, etc?

4. Should definitive therapies be delayed/avoided if transfusions are needed?

Conclusions

- Transfusions should be used judiciously in the management of acute sickle cell complications
- DHTRs in 5-7% of SCD patients which can be life-threatening (6% mortality).
- Clinicians and blood banks should attempt to reduce the risk alloimmunization by providing phenotypically matched RBCs.
- It is **imperative** to get a transfusion history from every patient in order to minimize risk of DHTRs.
- Clinicians and blood banks should recognize patients with Ab-negative DHTRs and avoid RBC transfusions in those patients.
- Ab-negative DHTRs are impossible to prevent with current Blood Bank best practices when there never has been an Ab ever identified
- More investigation is needed to understand the mechanisms involved in antibody-mediated and antibody-independent clearance of RBCs to prevent/treat DHTRs.

Thank you.




Reducing complications of therapeutic blood transfusion in sickle cell disease

- Introduction
- Use of blood transfusion during acute illness
- Delayed hemolytic transfusion reactions
- Management of chronic transfusion

CME & CNE available

ghpc.gsu.edu/cme

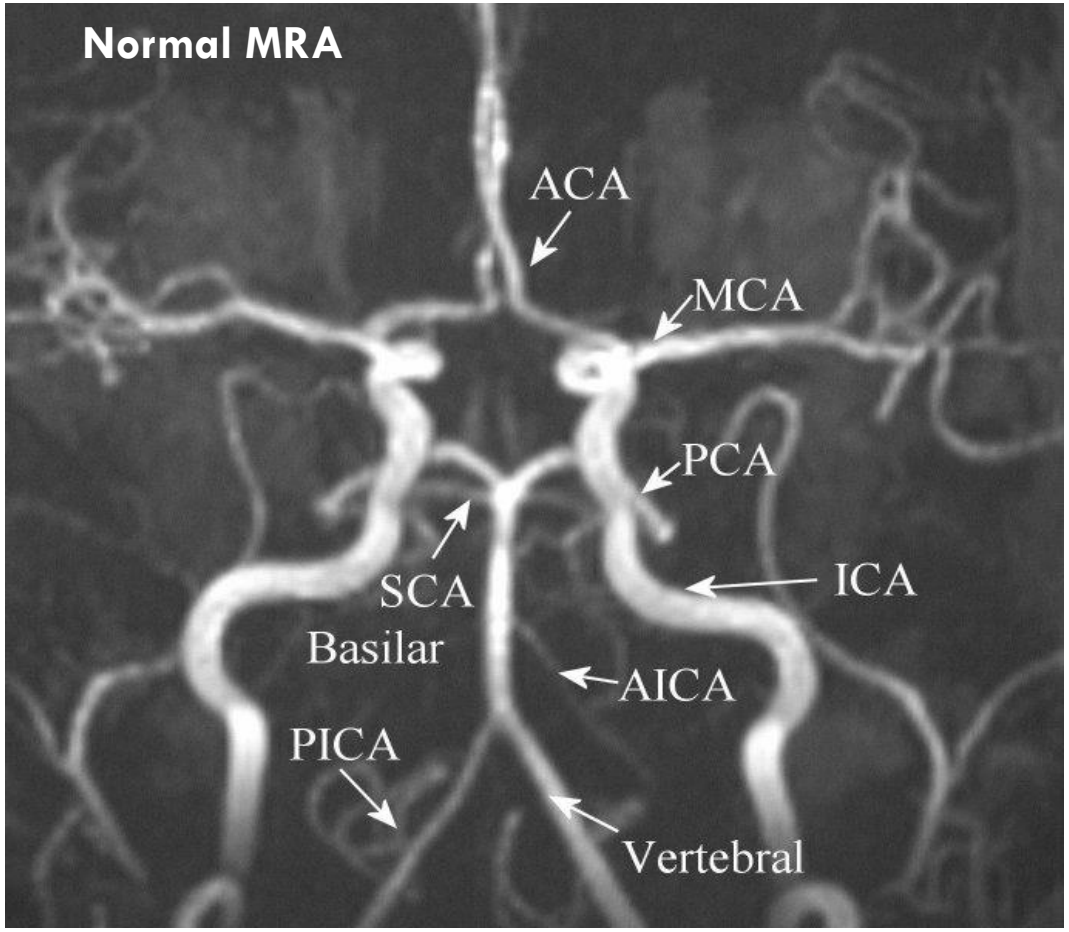
REdHHoTT
Improving transfusion practice through data sharing and education

Supplemental slides

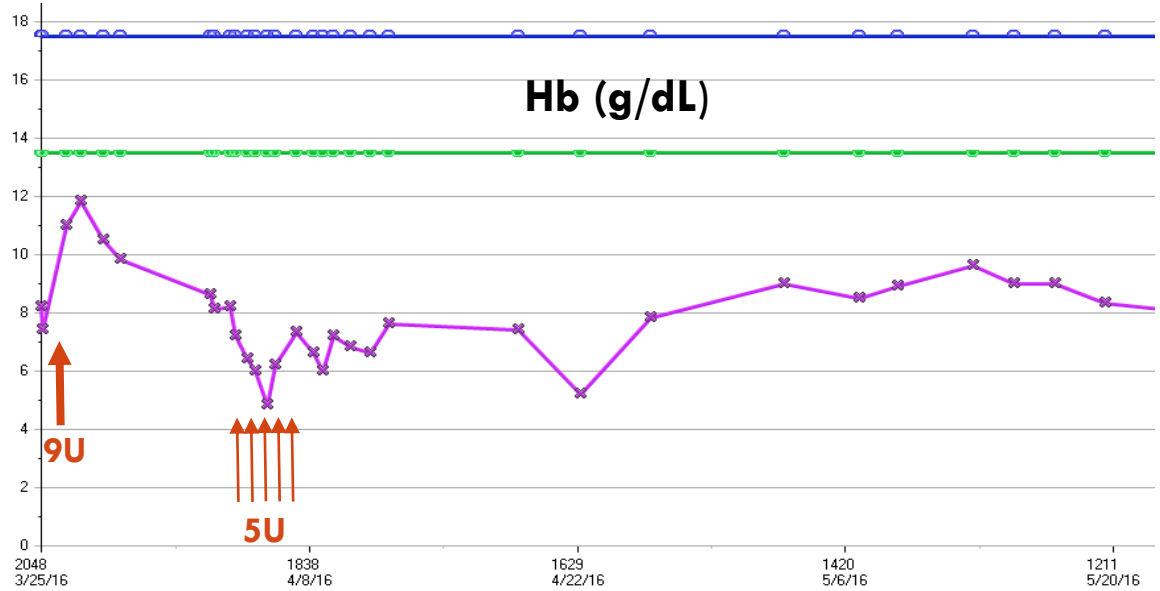
Case- presentation

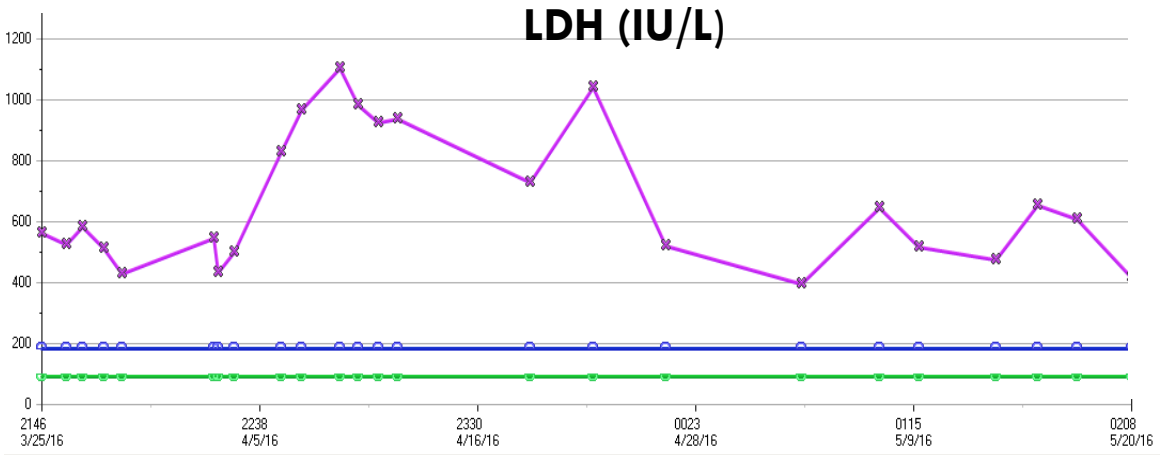
- Patient presents acute VOC, with word-finding difficulties -an expressive aphasia (previous strokes presented similarly).
- PE: NIHSS 5 for (mental status questions: could not say month), RUE drift, R facial weakness, and mild anomia.
- MRI upon admission: no acute stroke and chronic L ACA/MCA distribution encephalomalacia
- **Dx: Sickle Cell Crisis/w recrudescence of old stroke symptoms (AKA no acute stroke)**
- **Primary team requested an emergent red cell exchange.**

Case- baseline MRA

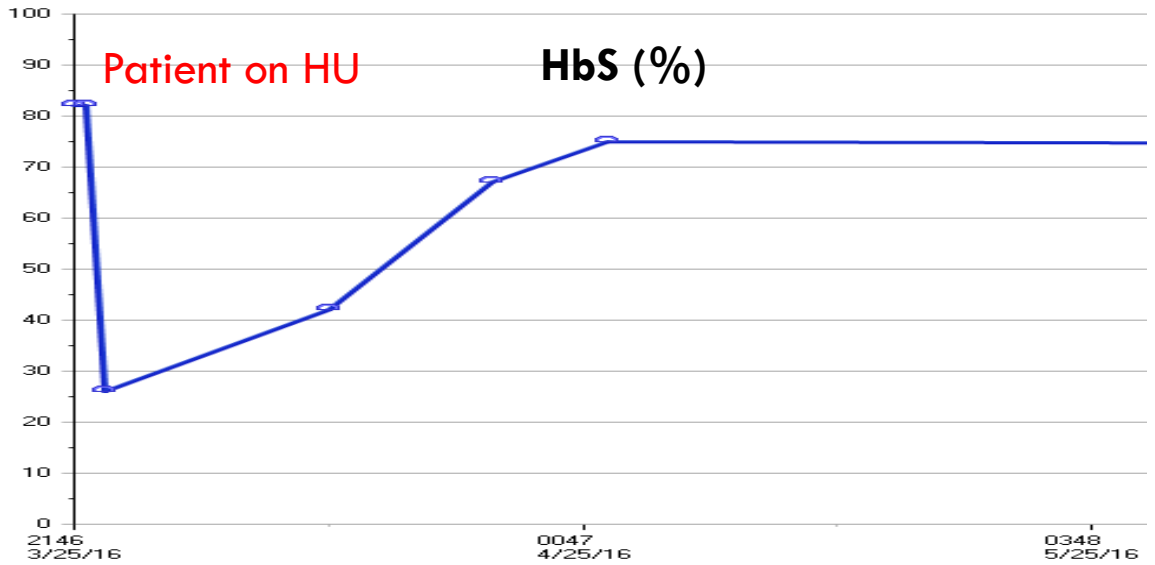


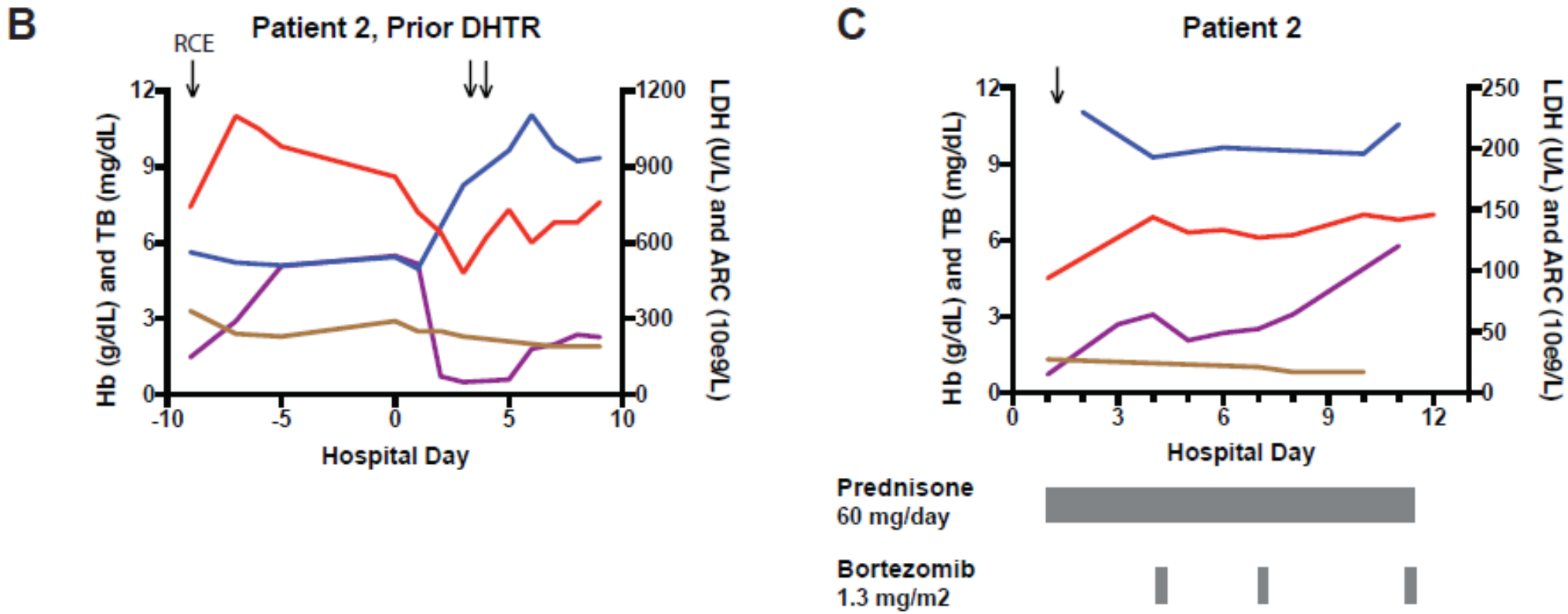
Case- DHTR (No antibody identified)





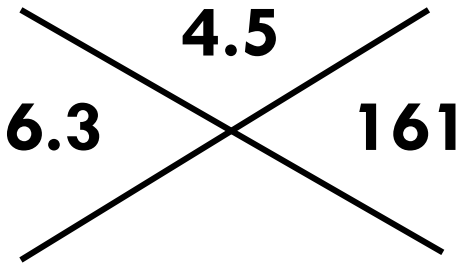
- Patient re-presented 7 days post RBCX with diffuse intense body pain in bilateral arms, legs and back
- Received 5 more units RBCs for dropping Hb.
- UA: + hemoglobinuria (D+7, D+28). Ab screens and DAT: negative



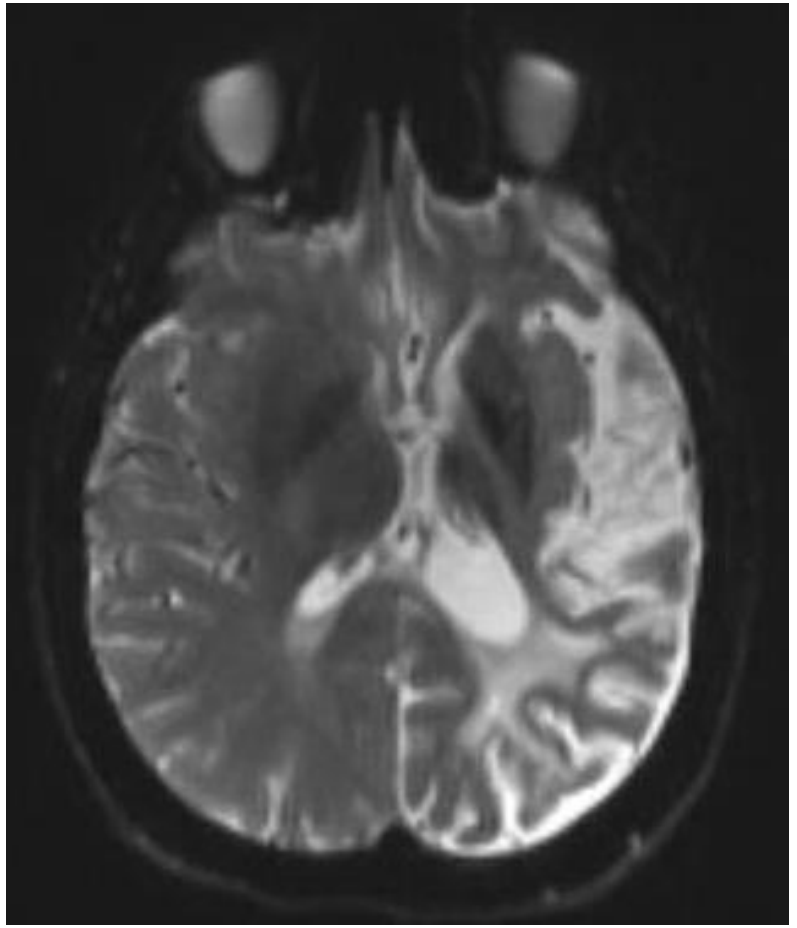

B
Patient 2, Prior DHTR
RCE
Hb (g/dL) and TB (mg/dL)
LDH (U/L) and ARC (10e9/L)
Hospital Day
C
Patient 2
Hb (g/dL) and TB (mg/dL)
LDH (U/L) and ARC (10e9/L)
Hospital Day
Prednisone
60 mg/day
Bortezomib
1.3 mg/m2

Case continued…

- Patient re-presented to clinic with pain and new worsened right hemiparesis and expressive aphasia.
- MRI showed a new left MCA ischemic stroke



ANC 2900 /µL
Retic: 1.7%
ARC: 14.8k /µL



- Dx: Aplastic crisis, new ischemic stroke

- **What to do**

Why Rituximab?

- Rituximab targets CD20, and induces B cell depletion
  - should inhibit primary or secondary immune response to blood group antigens
- Rituximab is effective in depleting B cells in NHL
- Rituximab has been effective in treating many autoimmune disorders that Ab-dependent (e.g. AIHA, ITP, TTP, SLE, etc…)

- Is Rituximab effective in Ab-negative DHTRs?

Prevention of DHTR with Rituximab

- Retrospective analysis of 8 SCD patients with multiple antibodies and with previous history of life threatening DHTR (1 to 4 episodes of DHTR)

- **Pre-Treatment**
  - 2 different Rituximab regimens depending on the patient condition
    - Ritux 1,000 mg x2, 2 weeks apart, (D-30, D-15) before the procedure
      - planned surgery requiring transfusion
    - Ritux 1,000 mg x1
      - acute conditions requiring urgent transfusion
    - In all cases, 10 mg of methylprednisolone (usual dose 100 mg)

- **Transfusion**
  - Extended matched RBCs (Rh/K/Fy/Jk/MNS) and negative for previous antibodies

Noizat-Pirenne, Vox Sanguinis, 2015

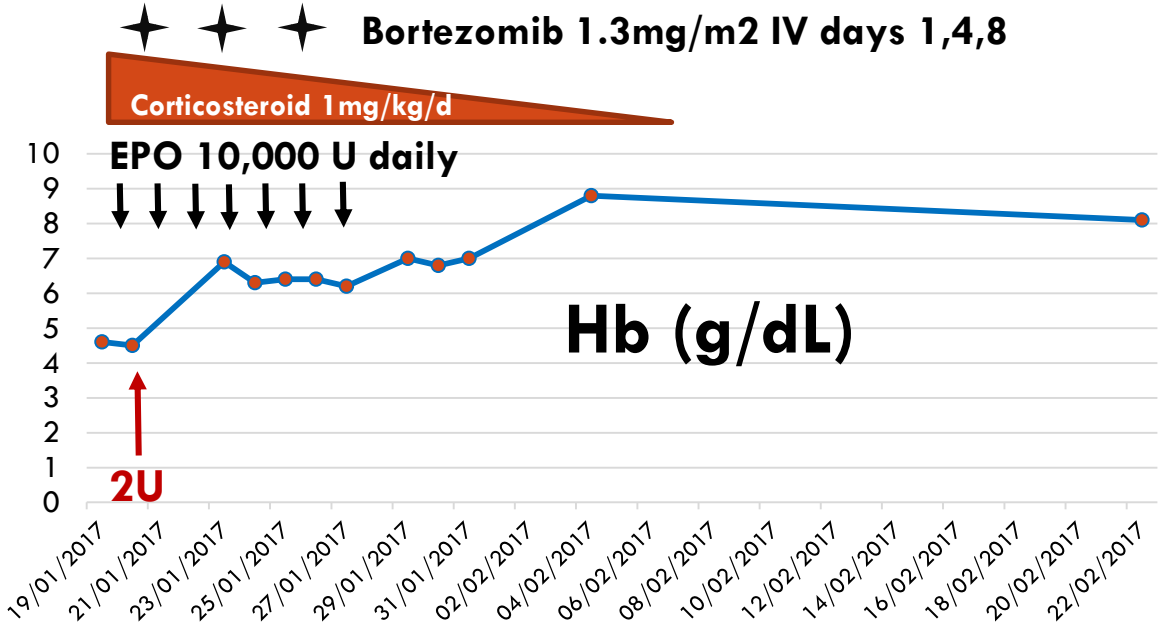
Prevention of DHTR with Rituximab

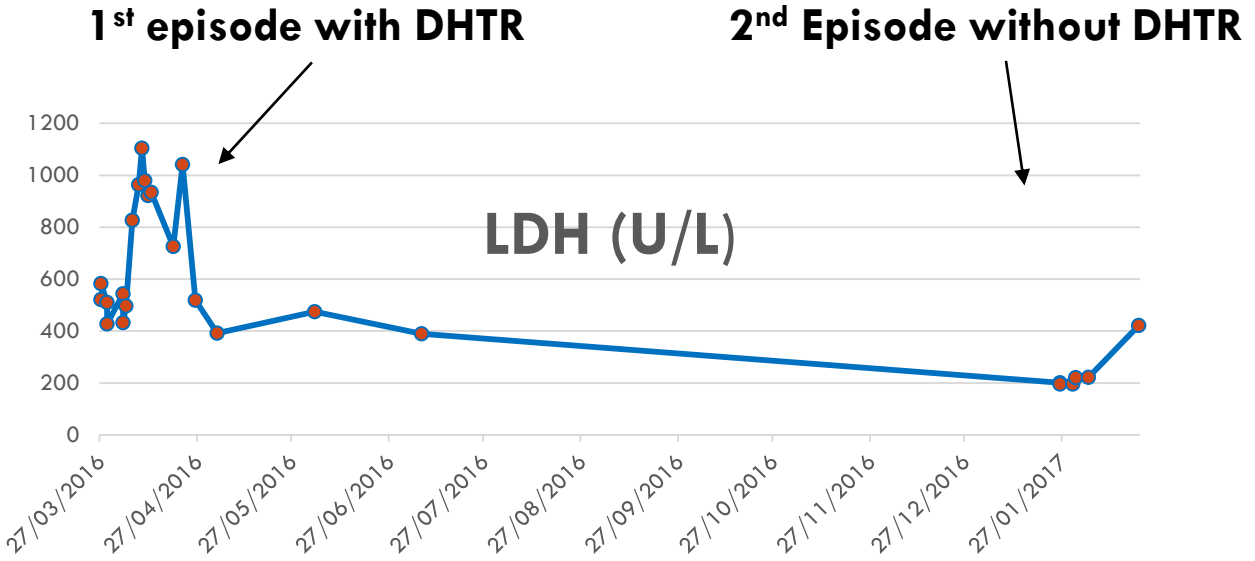
- **Clinical course (N=8)**
  - Median drop of Hb from post-trxn Hb: **1.3 g/dl** (range 0 to 3.8 g/dl)
  - Median LDH max: **461 IU/mL** (range: 271-1180)
  - 5 patients : no DHTR
  - 3 patients : mild DHTR
    - 2 patients had mild clinical symptoms of intravascular hemolysis and/or exacerbation of VOC

- **Post transfusion serologic testing**
  - In all patients : no new formed antibodies, DAT remains negative

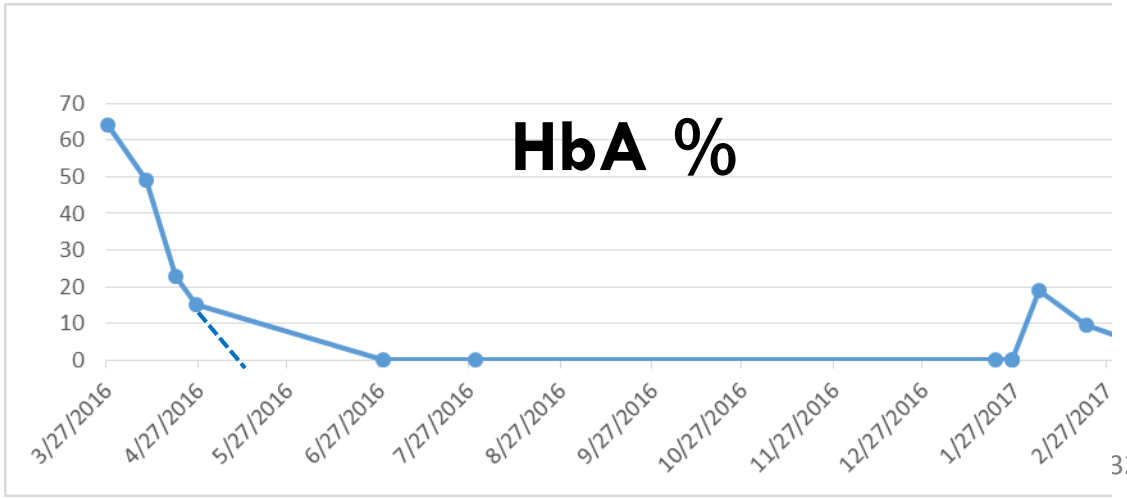
Noizat-Pirenne, Vox Sanguinis, 2015

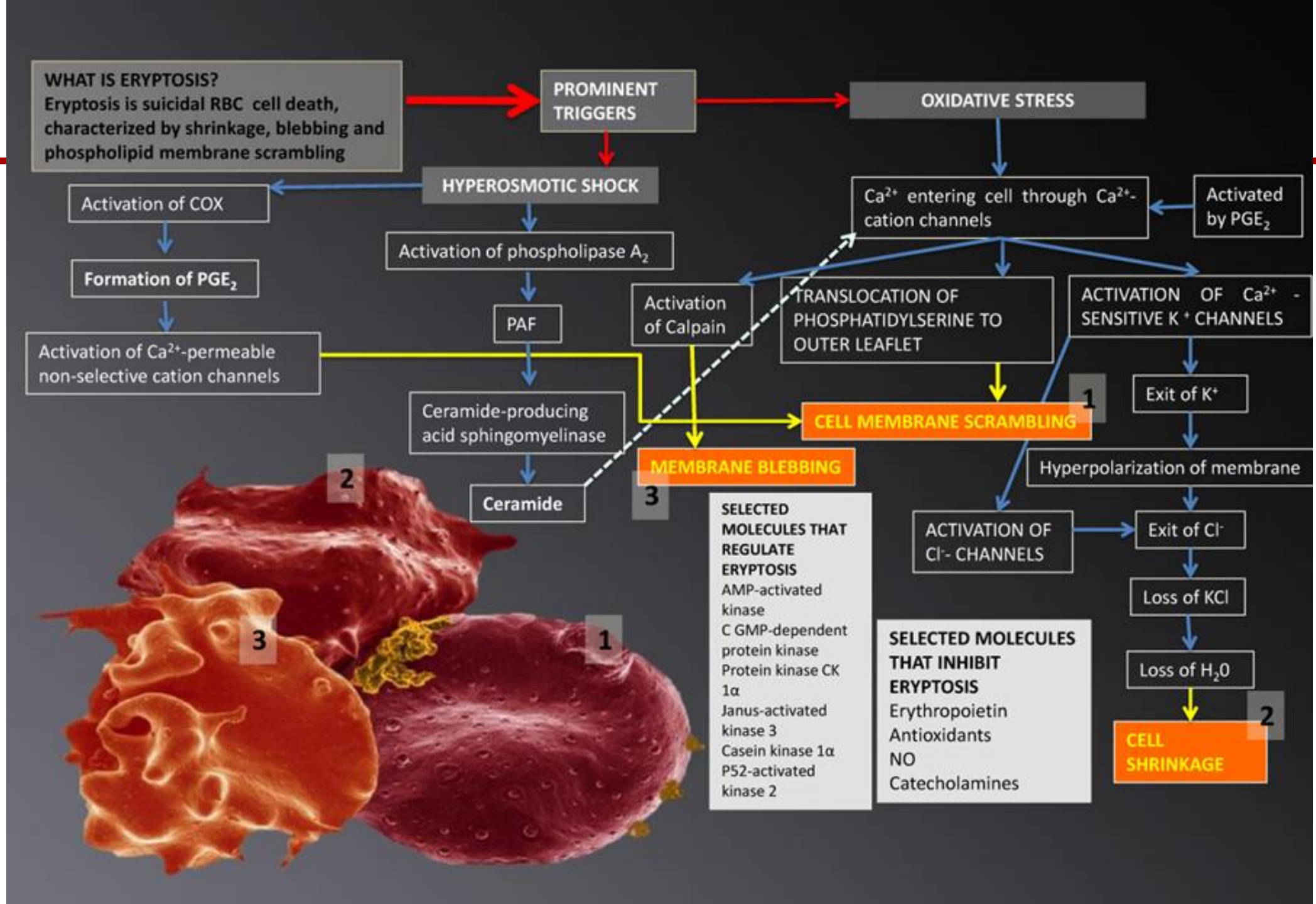
Case continued… No evidence of DHTR



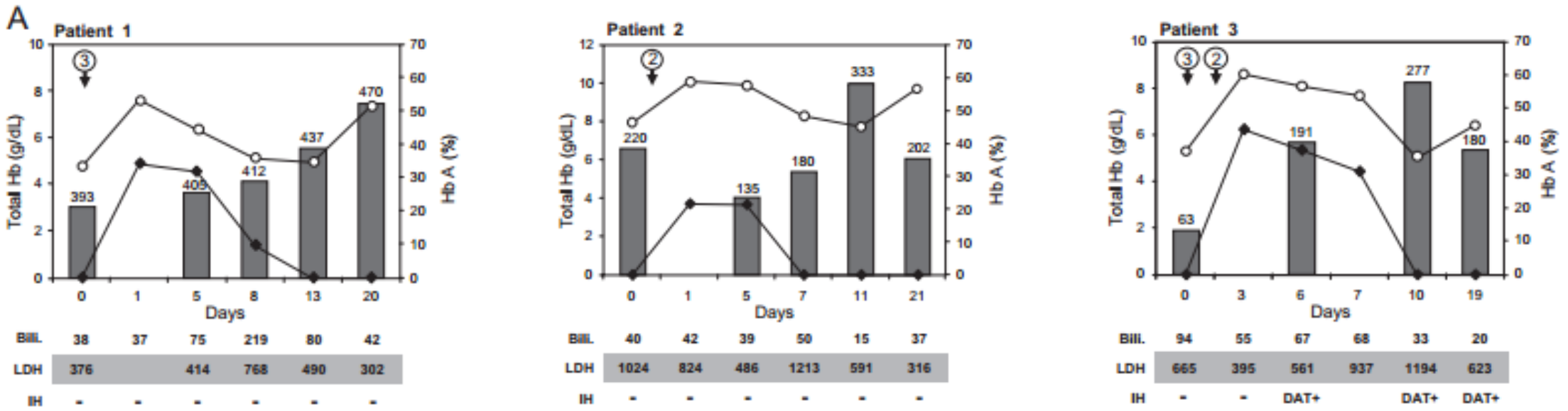


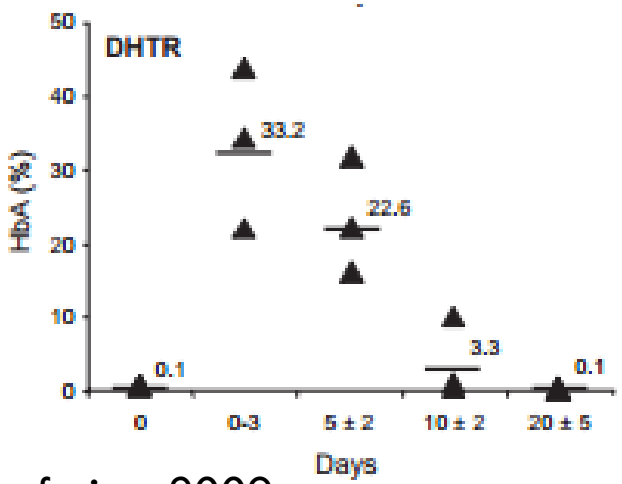
UA: all negative for hemoglobinuria Ab screens and
DAT: negative

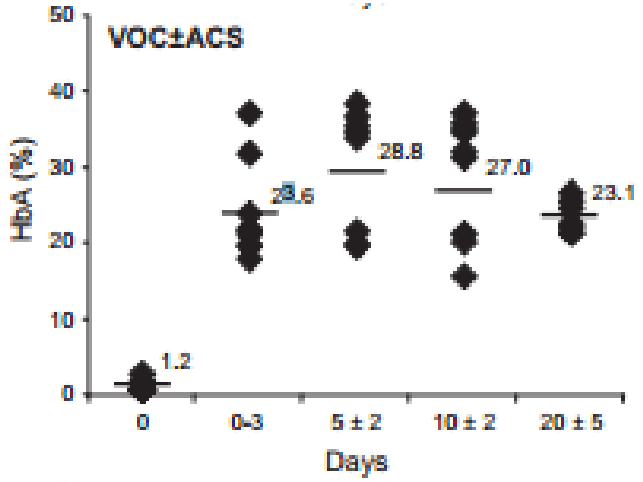



WHAT IS ERYPTOSIS?
Eryptosis is suicidal RBC cell death, characterized by shrinkage, blebbing and phospholipid membrane scrambling
PROMINENT TRIGGERS
OXIDATIVE STRESS
HYPEROSMOTIC SHOCK
Activation of COX
Formation of PGE_2
Activation of phospholipase A_2
PAF
Activation of Ca^{2+}-permeable non-selective cation channels
Ceramide-producing acid sphingomyelinase
Ceramide
Ca^{2+} entering cell through Ca^{2+}-cation channels
Activated by PGE_2
Activation of Calpain
TRANSLOCATION OF PHOSPHATIDYLSERINE TO OUTER LEAFLET
ACTIVATION OF Ca^{2+} - SENSITIVE K^+ CHANNELS
CELL MEMBRANE SCRAMBLING
1
Exit of K^+
Hyperpolarization of membrane
MEMBRANE BLEBBING
3
2
ACTIVATION OF Cl^- - CHANNELS
Exit of Cl^-
Loss of KCl
Loss of H_2O
CELL SHRINKAGE
2
SELECTED MOLECULES THAT REGULATE ERYPTOSIS
AMP-activated kinase
C GMP-dependent protein kinase
Protein kinase CK 1α
Janus-activated kinase 3
Casein kinase 1α
P52-activated kinase 2
SELECTED MOLECULES THAT INHIBIT ERYPTOSIS
Erythropoietin
Antioxidants
NO
Catecholamines
3
1

Published Cases of Ab-negative DHTR





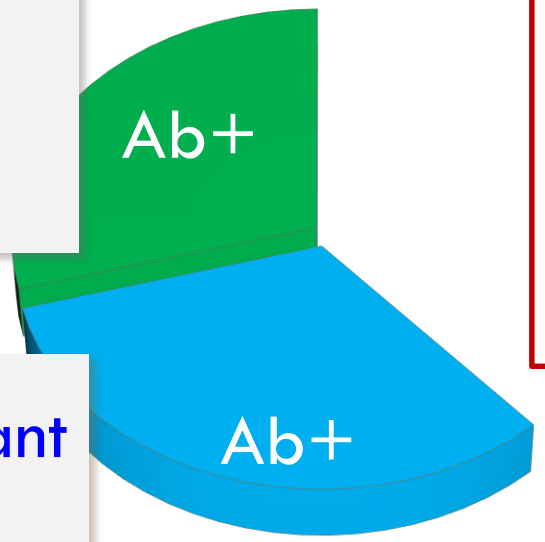


Chadebech P, et al. Transfusion. 2009

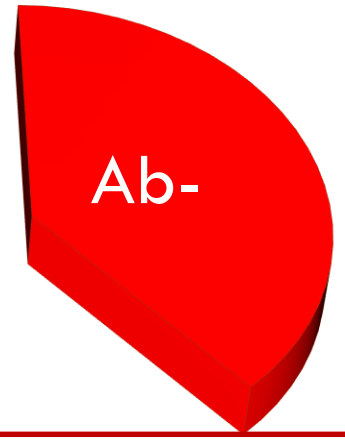
DHTRs in SCD: Immunohematological Characteristics

Autoantibodies, AUS, complex Abs: 29%

New classically significant alloAb: 32%



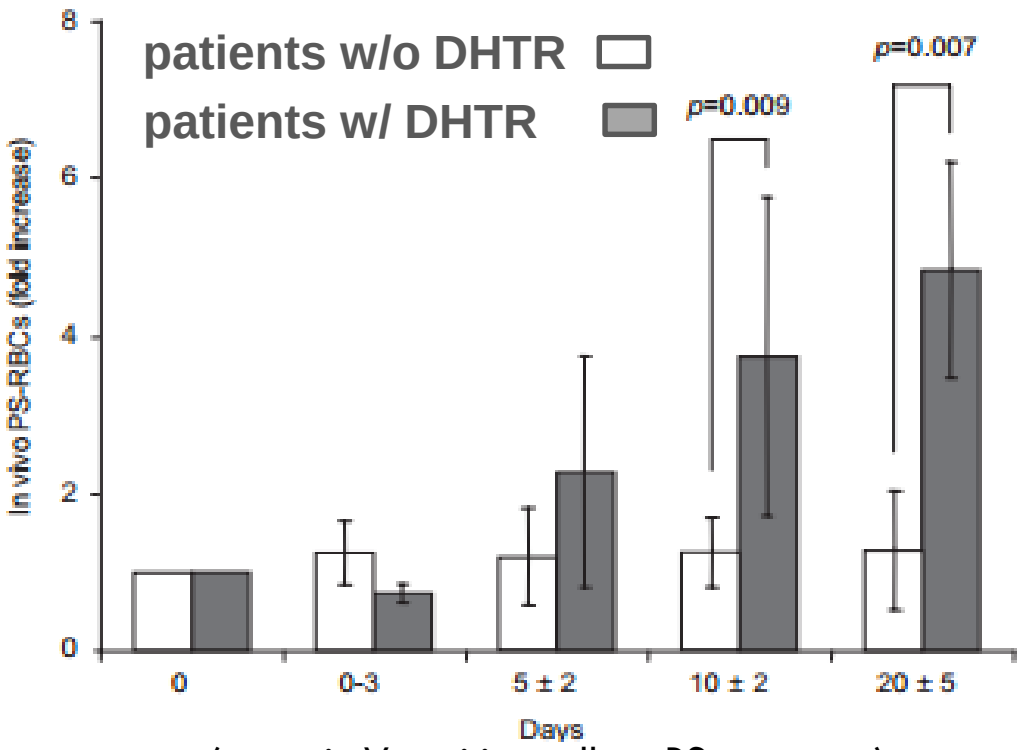
Antibody-negative: 38%



Habibi A, et al. Am J Hematol. 2016

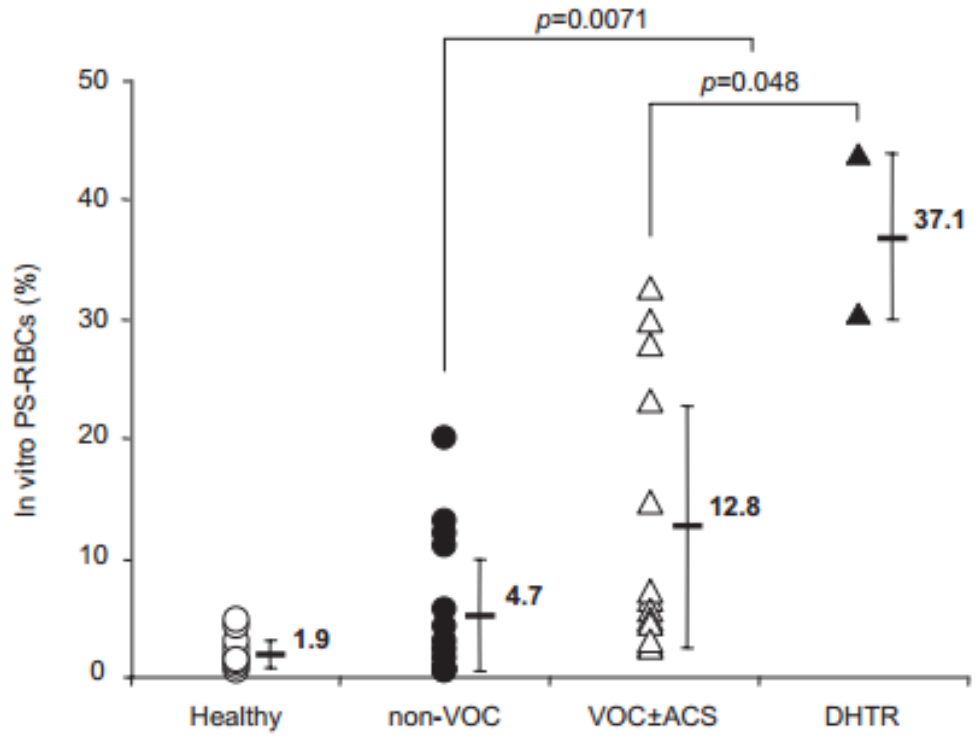
Potential mechanism for Ab-negative DHTRs:
Suicidal RBC death from PS exposure

In vivo PS-RBC % increase from pre-transfusion



(annexin V-positive cells = PS exposure)

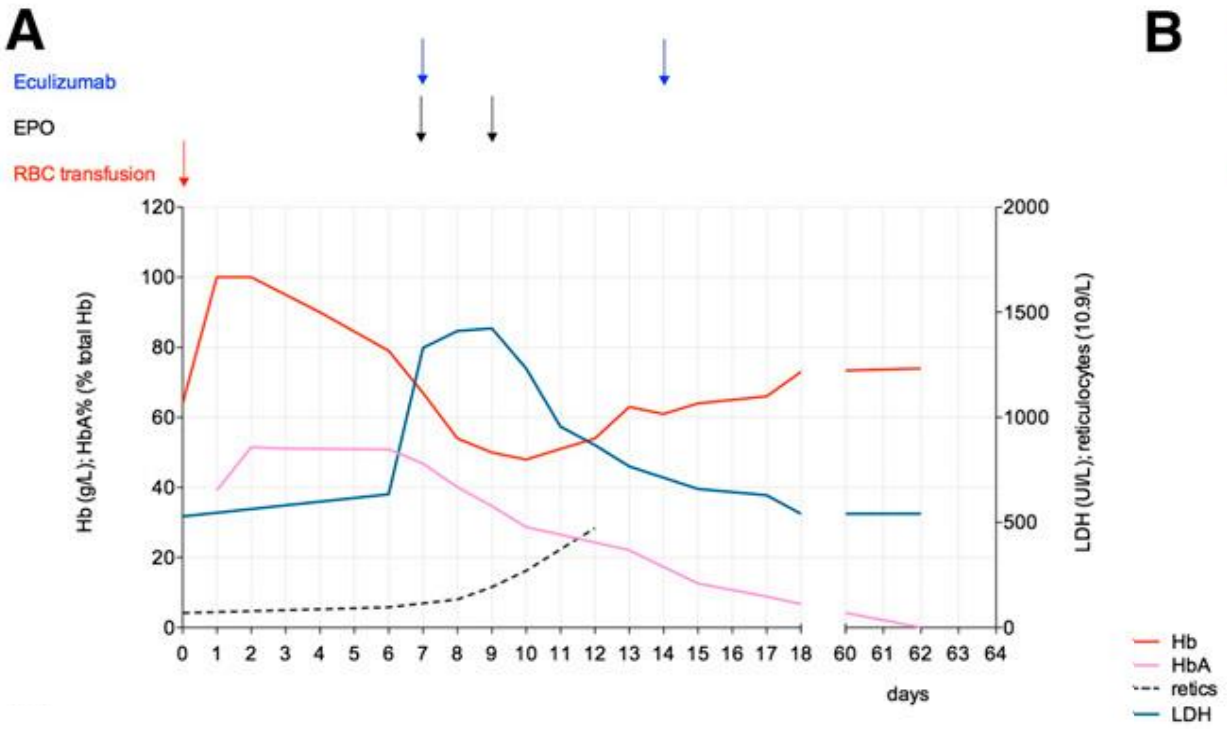
In vitro: patient plasma with donor RBCs

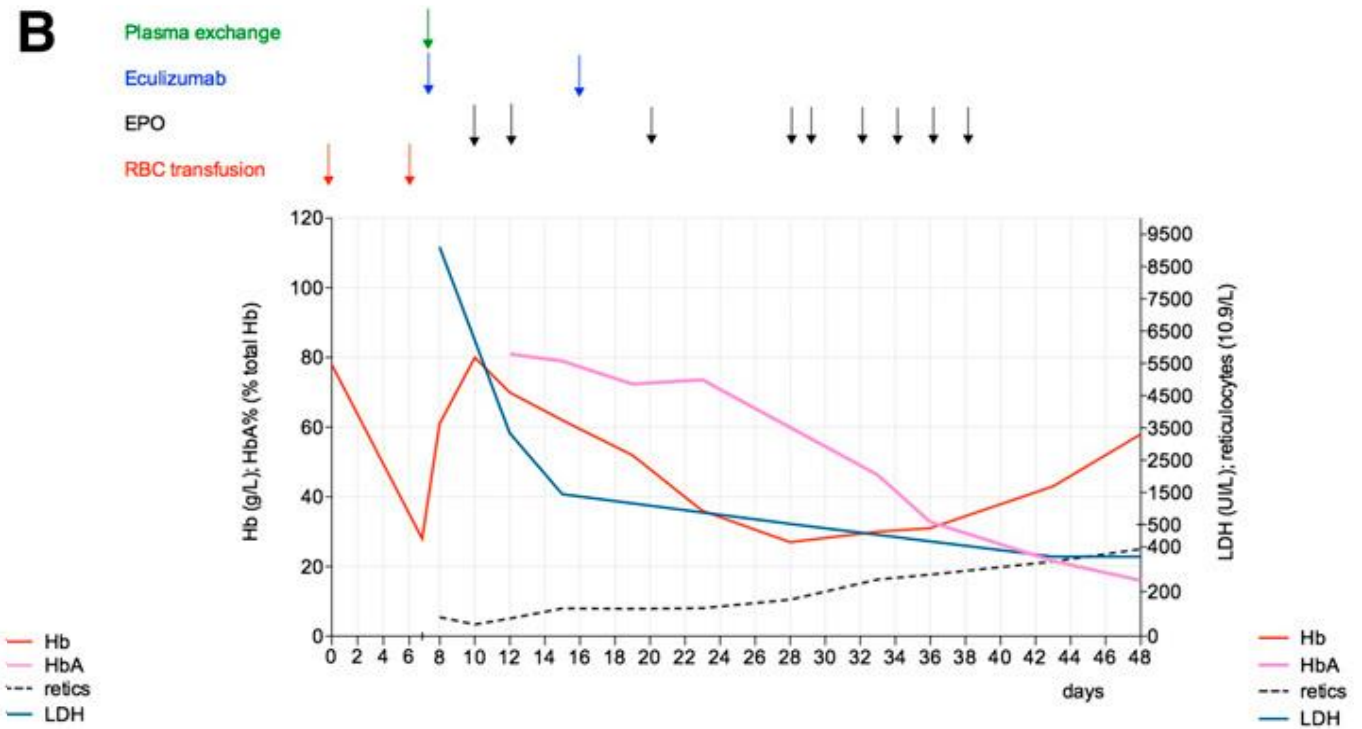


- PS exposure is a signal for eryptosis—suicidal RBC death—involving membrane shedding and leading to the physiologic clearance of apoptotic cells by **macrophages**, via specific PS receptors

Chadebech P, et al. Transfusion. 2009

Eculizumab salvage therapy for Ab-negative DHTRs in SCD patients





20 yr male w/ HbSS- developed severe VOC/dark urine 6 days post 6 U RBCs for acute stroke

- Dx: DHTR with negative DAT and reticulocytopenia
- **EPO and Eculizumab** given with improvement of VOC and hemoglobinuria within 24 hrs of 1st dose of Eculizumab

- 17 yr male w/ HbSS- severe ACS and dark-colored urine 7 days post 2 U RBCs to treat VOC. MSOF developed after another RBC transfusion (2 U).
- Dx: DHTR with negative DAT and reticulocytopenia
- **EPO and TPE followed by Eculizumab** given with gradual improvement over subsesequent 40 days.

Dumas G. et al. Blood 2016

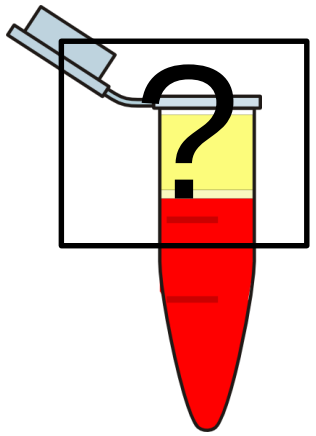
Why Bortezomib?

- Proteasome inhibitor which blocks NF-κB activation
  - Causes accumulation of misfolded proteins
  - Leads to cell apoptosis, particularly plasma cells.
- Bortezomib effective treatment of multiple myeloma and NHL.
- Bortezomib shown to ameliorate clinical manifestations of refractory SLE*
- Selective apoptosis also occurs in monocytes and monocyte-derived DCs**

*Alexander T, et al. Ann Rheum Dis. 2015

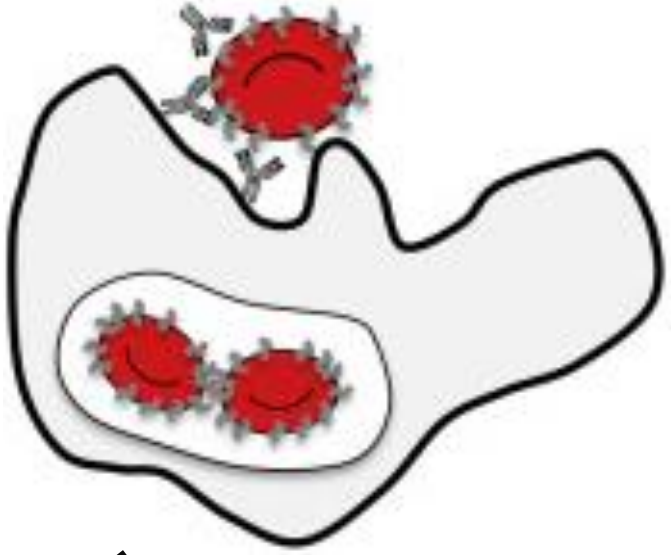
**Arpinati M, et al. BMT. 2009

Ab-negative DHTRs: Suicidal Red Cells
Proposed mechanism



PS exposure

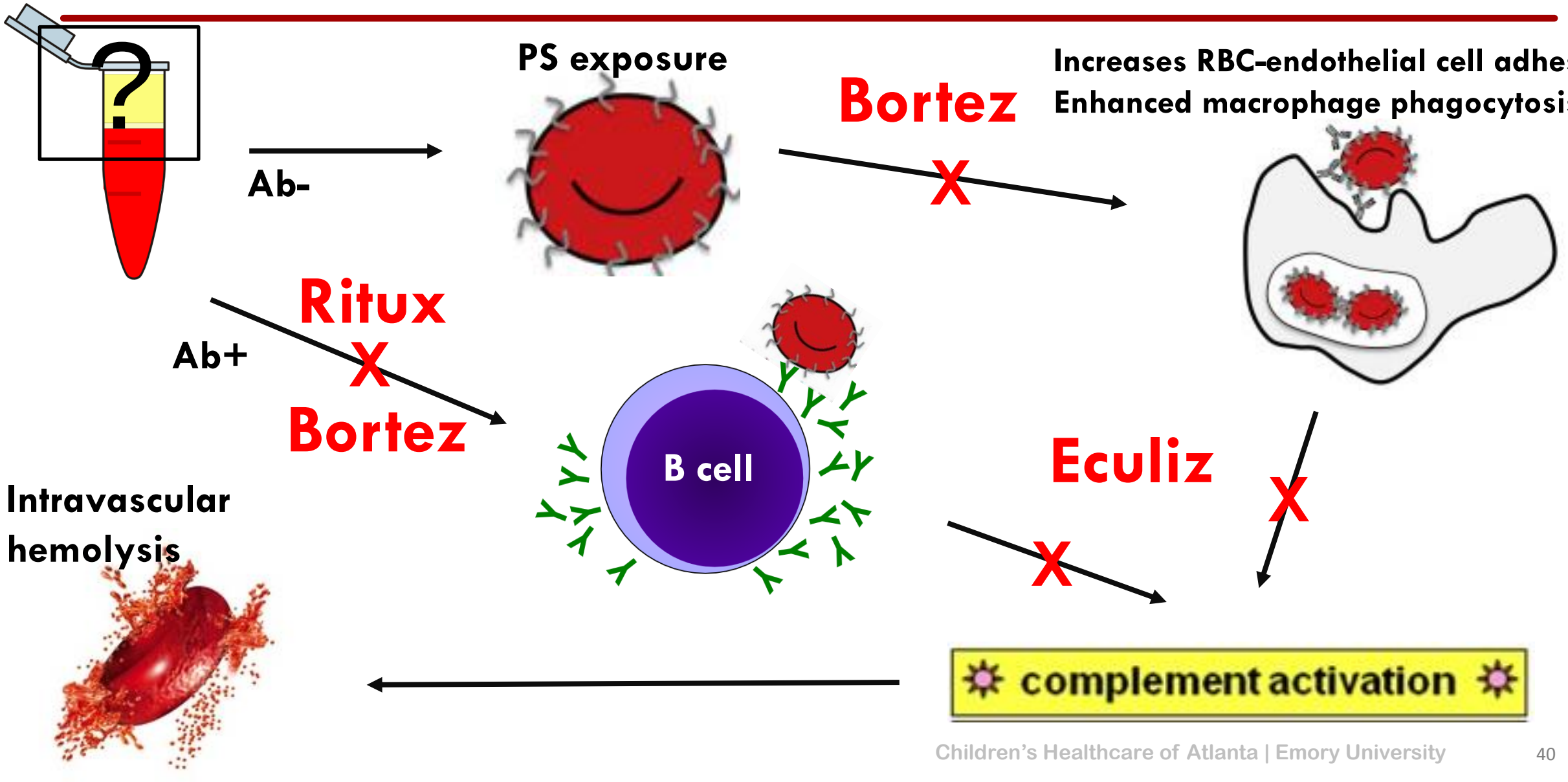
Increases RBC-endothelial cell adhesion
Enhanced macrophage phagocytosis



complement activation

Intravascular hemolysis

DHTRs: Proposed mechanism and potential treatments



Dilemna:

- **Patient needs a neurosurgical intervention (EDAS) for severe moya moya dz.**
  - **Proceed or not proceed?**