# Sickle cell disease and hyperhemolysis: epidemiology, risk factors and new therapeutic approaches in France

**WORLD APHERESIS ASSOCIATION AND FRENCH SOCIETY FOR HEMAPHERESIS**
**April 27-29, 2016, PARIS-FRANCE**

**France Pirenne, EFS, INSERM, Henri Mondor Hospital, Créteil, France**

EFS

Instituts thématiques Inserm Institut national de la santé et de la recherche médicale

IMRB INSTITUT MONDOR DE RECHERCHE BIOMÉDICALE

GREX

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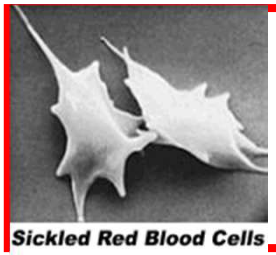
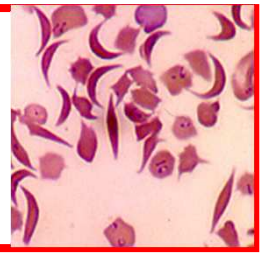
ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

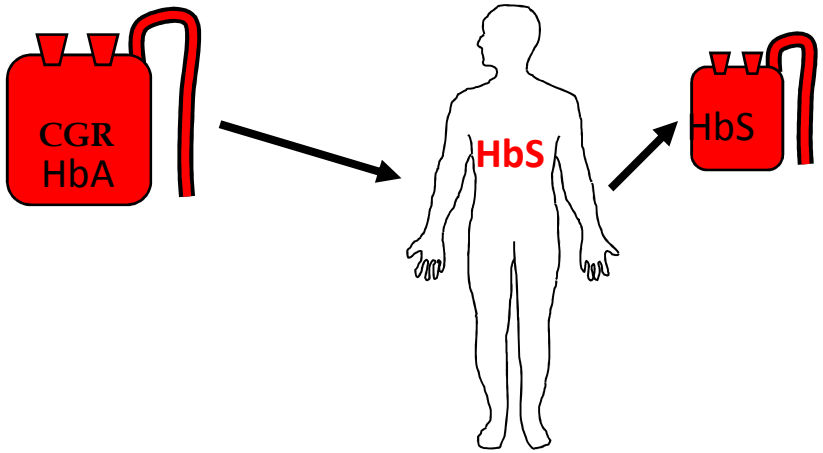



# Sickle Cell Disease



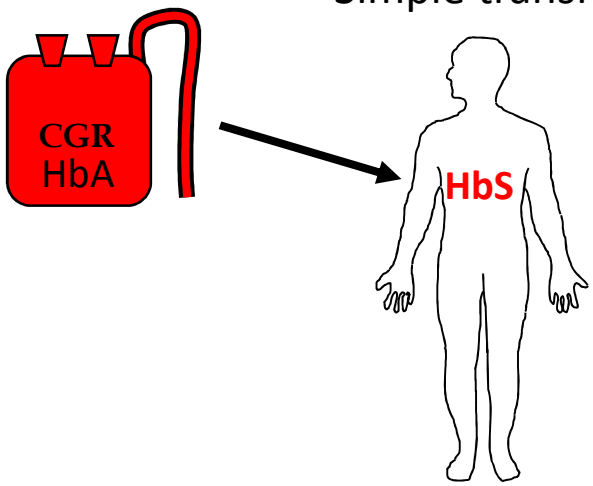
- Sickle Cell Disease (SCD) : most frequent inherited disordrer in France (20.000 patients): mutation of the globin β gene inducing an abnormal Hb, the HbS
- Polymerisation of HbS :
  - Fragility of the membrane : chronic hemolysis
  - Adhesion and low deformability of RBCs : vaso occlusion crisis (VOC)
  - Infections because of asplenia
- Transfusion remains the main treatment of the disease
  - For treatment : VOC, Acute Chest Syndrom, priapism…
  - For prevention
    - Pregnancy, surgery, high symptomatology…
    - Vasculopathy in children +++

Red cell exchange




30 % ↑ of HbA

Simple transfusion



# Hemolytic Transfusion Reaction in SCD : the most harmful effect of transfusion and alloimmunization

- Frequently delayed : 5 to 10 days following transfusion
  - Delayed hemolytic transfusion reaction (DHTR)
- Destruction of both transfused and autologous RBCs
  - Hyperhemolysis
- Profound reticulopenia : worsen the anemia
- Clinical presentation resembling a vaso occlusive crisis
  - Explains why DHTR is under recognized
- Additional transfusions exacerbate the anemia
- Specific Immuno hematological characteristics
  - Main cause : allo immunization
  - In some cases : no detectable antibodies…

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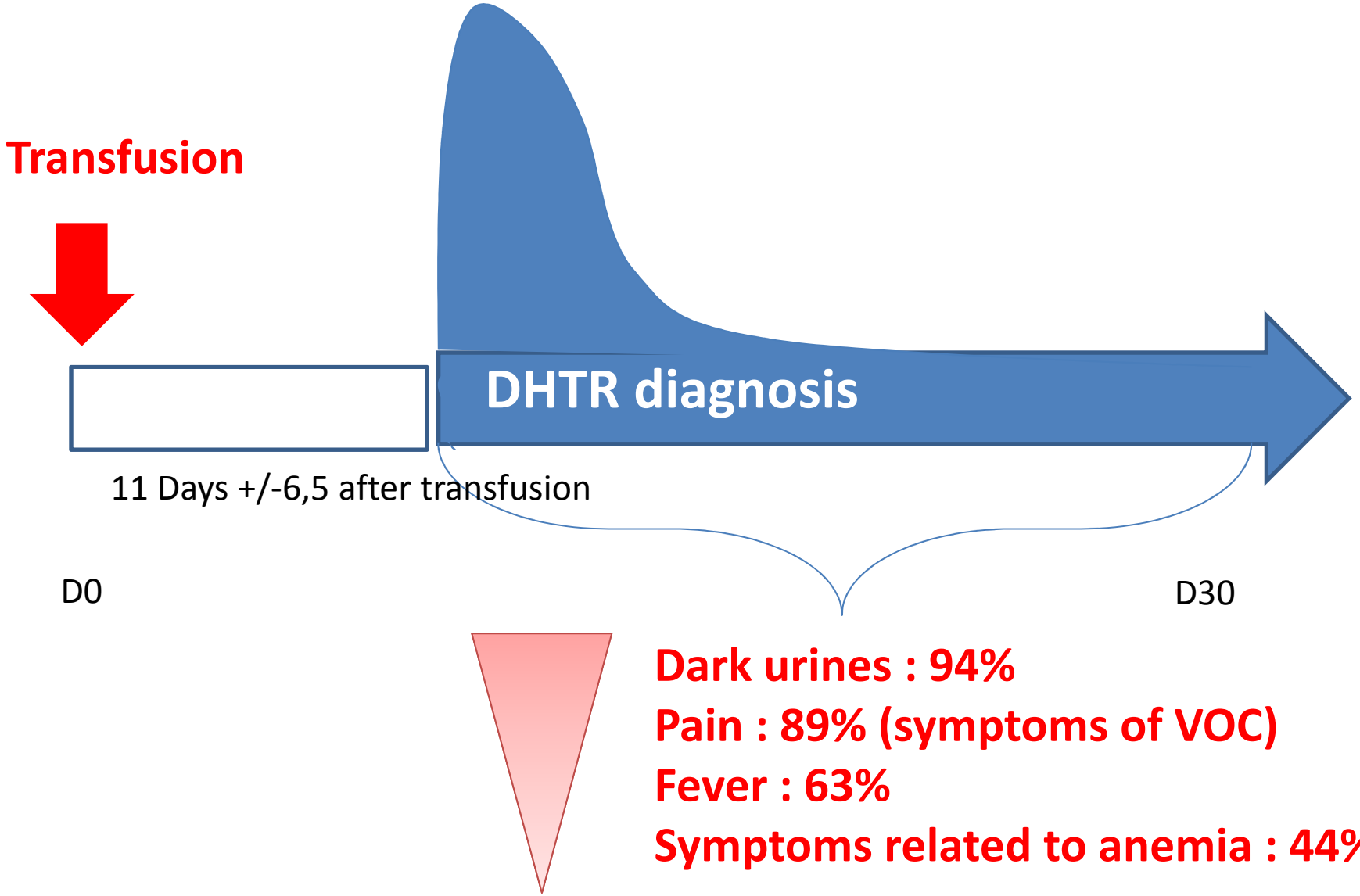
## Diagnosis of DHTR

- Retrospective analysis of cases at the Reference Center of Sickle Cell Disease in the Henri Mondor Hospital, during 11 years

  – 99 cases in 69 patients

  – 6 deaths

*Habibi, Pirenne, in preparation*


Transfusion
DHTR diagnosis
11 Days +/-6,5 after transfusion
D0
D30
Dark urines : 94%
Pain : 89% (symptoms of VOC)
Fever : 63%
Symptoms related to anemia : 44%

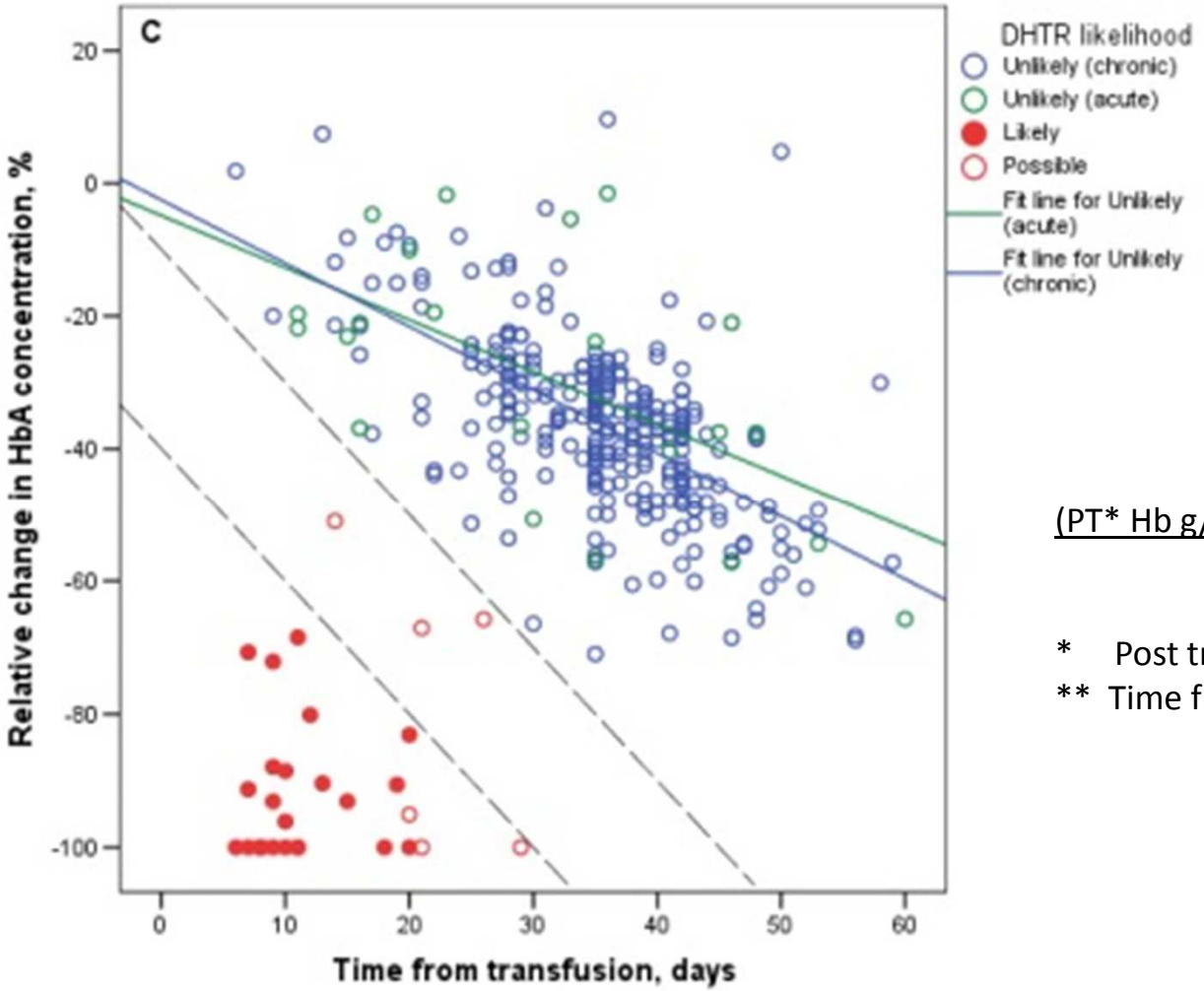
# DHTR : Biological parameters

| | Median | Missing data | |
|---|---|---|---|
| Post TF Hb** , g/dl | 9.5 [8.6-14] | 23 | |
| Biology at Emergency room (n=66) | | | |
| Delay between TF and readmission, days | 12± 6 | 0 | |
| Hb, g/dl | 7.8[6.9-9.3] | 5 | |
| LDH***, UI/l | 758[554-958] | 16 | |
| Biology at admission in ICU (n=41) | | | |
| Day of admission in ICU | 10[7-14] | 0 | |
| Hb, g/dl | 6[5-7.5] | 4 | |
| LDH***, UI/l | 1364[865-2350] | 6 | |
| Biology and delay (n=99) | | | |
| Lowest Hb, g/dl | 5.5[4.5-6.3] | 5 | |
| Day of lowest Hb | 12[8-14] | 6 | |
| delta Hb##, g/dl | 4.6[3.1-5.3] | 26 | |
| LDH***max , UI/l | 1335[798-2086] | 7 | |
| Day of LDH max | 10[7-13] | 13 | |
| lowest reticulocyt count, /mm³ | 180[121-240] | 14 | |
| Highest leucocytes | 18[15-24] | 15 | |

# Characteristics of dead patients

| Patient | TF indication | Previous DHTR | DHTR Antibody | Hb post TF | ∆Hb | Nadir Hb | Day of nadir Hb | LDH max | Day of LDH max |
|---|---|---|---|---|---|---|---|---|---|
| N 1 | VOC | No | none | 11.1 | 8.1 | 3 | 8 | 5453 | 8 |
| N 2 | Pregnancy ACS | Yes | antiHI | 8.2 | 3.2 | 5 | 7 | 5436 | 7 |
| N 3 | Delivery | Yes | none | 8,2 | 2,2 | 6 | 15 | 882 | 13 |
| N 4 | Leg ulcer | Yes | S, Lea | 7,6 | 3,7 | 3,9 | 14 | 4800 | 14 |
| N 5 | Surgery | Yes | none | 11,5 | 6,7 | 4,8 | 11 | 2898 | 10 |
| N 6 | School examen | No | S, non specific | 10,9 | 6,7 | 4,2 | 8 | 6181 | 8 |
| **Mean ± SD** | | | | **9.98 ± 1.64** | **5.1 ± 2.3** | **4.4 ± 1** | **10.5 ± 3.3** | **4275 ± 2002** | **10 ± 2** |
| median | | | | 10.9 | 5.2 | 4.5 | 9.5 | 5118 | 9 |

# Diagnostic nomogram of DHTR




$$\frac{\text{(PT* Hb g/dl x \%HbA) – (TFT** Hb g/dl x \%HbA)}}{\text{(TFT Hb g/dl x \%HbA)}}$$

* Post transfusion : Between 0 to 48H

** Time from Transfusion :
any time when DHTR is suspected

*Mekontso Dessap et al, Medicine, 2016, in revision*

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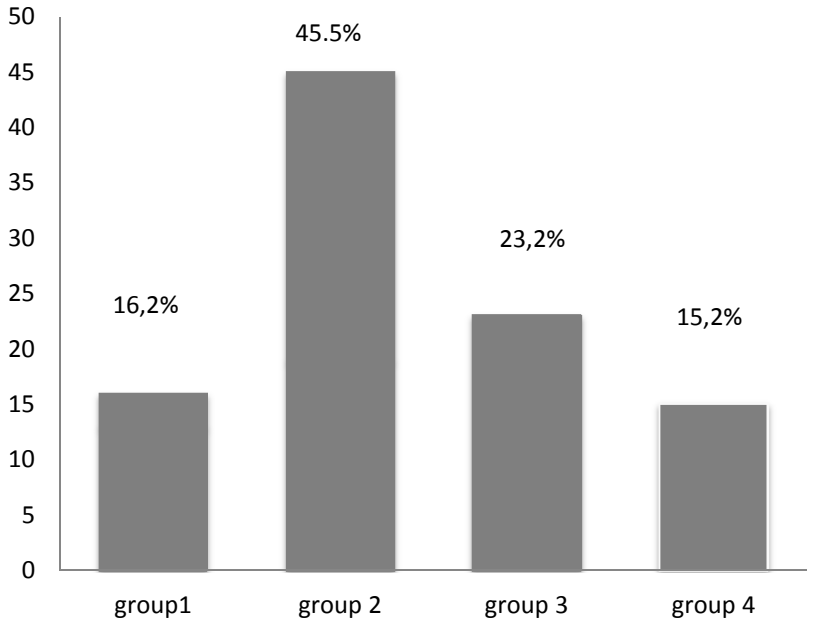
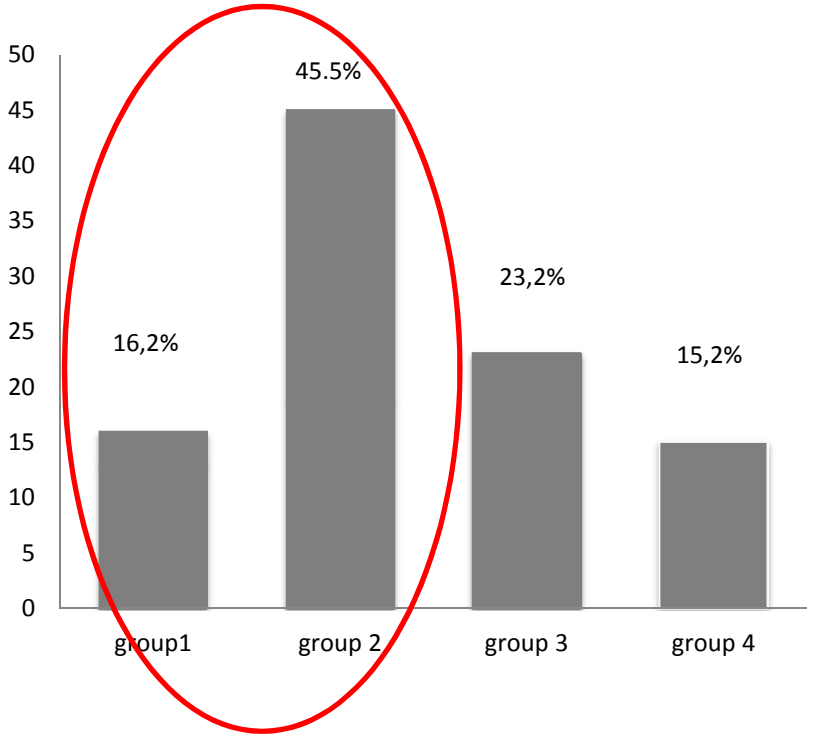
# Immuno-hematological characteristics of the 99 cases




group 1**:** DHTR in non-immunized patients who developed antibodies;
group 2: DHTR in previously immunized patients who developed newly formed antibodies;
group 3: DHTR in non-previously immunized patients who did not developed antibodies, and
group 4: DHTR in previously immunized patients who did not developed newly formed antibodies.

# Immuno-hematological characteristics of the 99 cases




Antibodies appeared in 62% of the cases

group 1: DHTR in non-immunized patients who developed antibodies;
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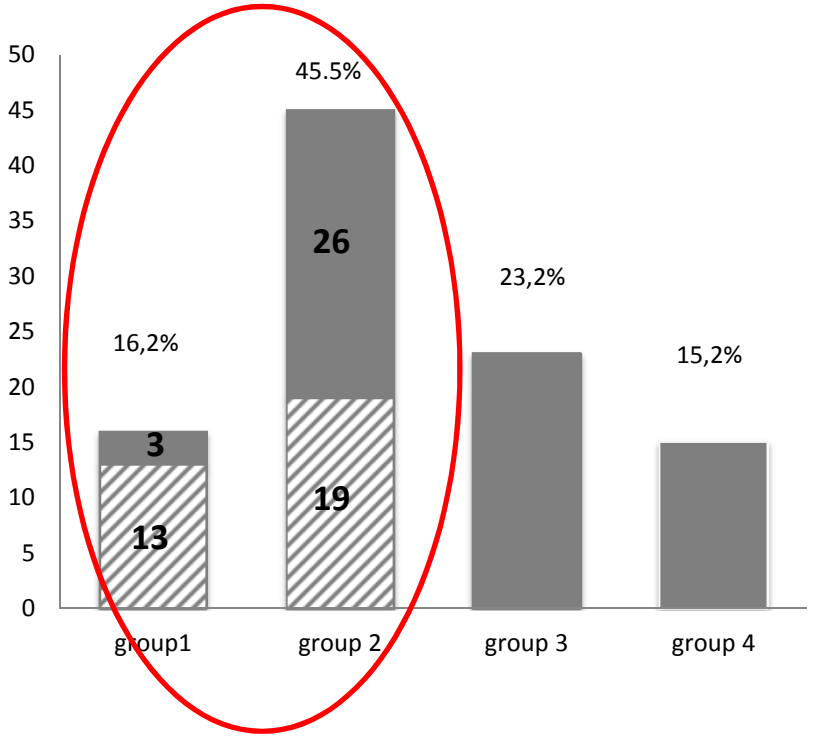
# Immuno-hematological characteristics of the 99 cases




Antibodies appeared in 62% of the cases

group 1**:** DHTR in non-immunized patients who developed antibodies;
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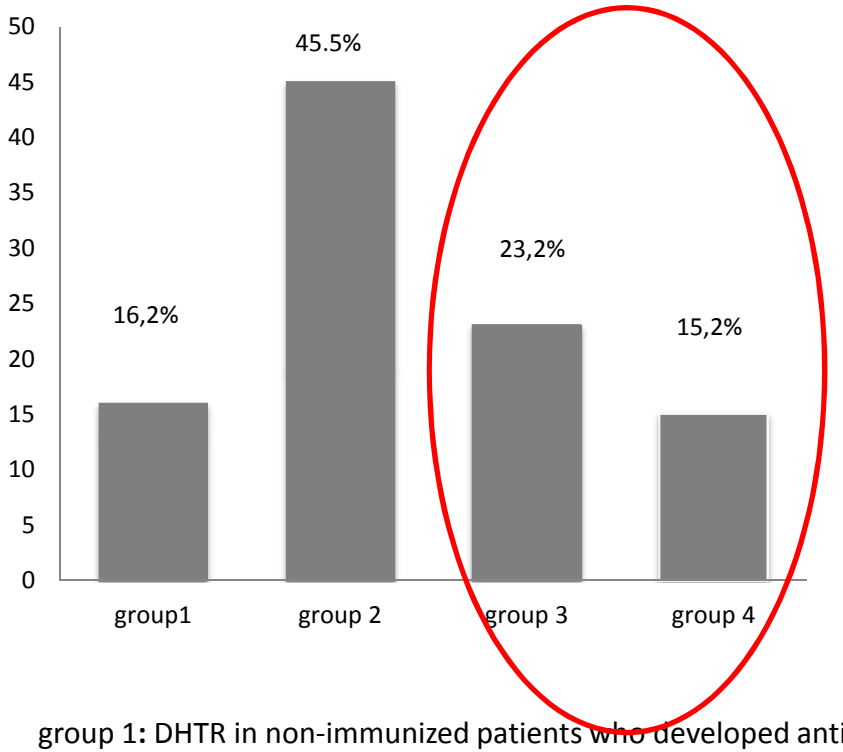
group 4: DHTR in previously immunized patients who did not developed newly formed antibodies.

antibodies known to be pathogenic (RH, FY, JK, MNS, high frequency..)

antibodies not known as pathogenic (AUS, auto)

# Immuno-hematological characteristics of the 99 cases




In 37% of the cases, newly formed antibodies were not detected

group 1: DHTR in non-immunized patients who developed antibodies;
group 2: DHTR in previously immunized patients who developed newly formed antibodies;
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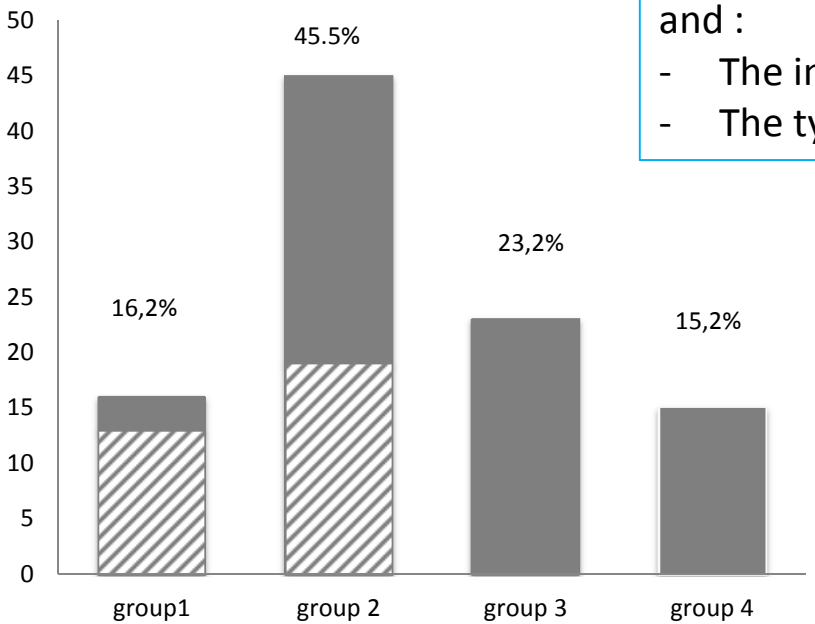
group 4: DHTR in previously immunized patients who did not developed newly formed antibodies.

# Immuno-hematological characteristics of the 99 cases




A severity score was calculated based on the number of severe complications

There is no statistical correlation between the severity score and :

- The immuno-hematological profile
- The type of antibodies that appear in groups 1 and 2

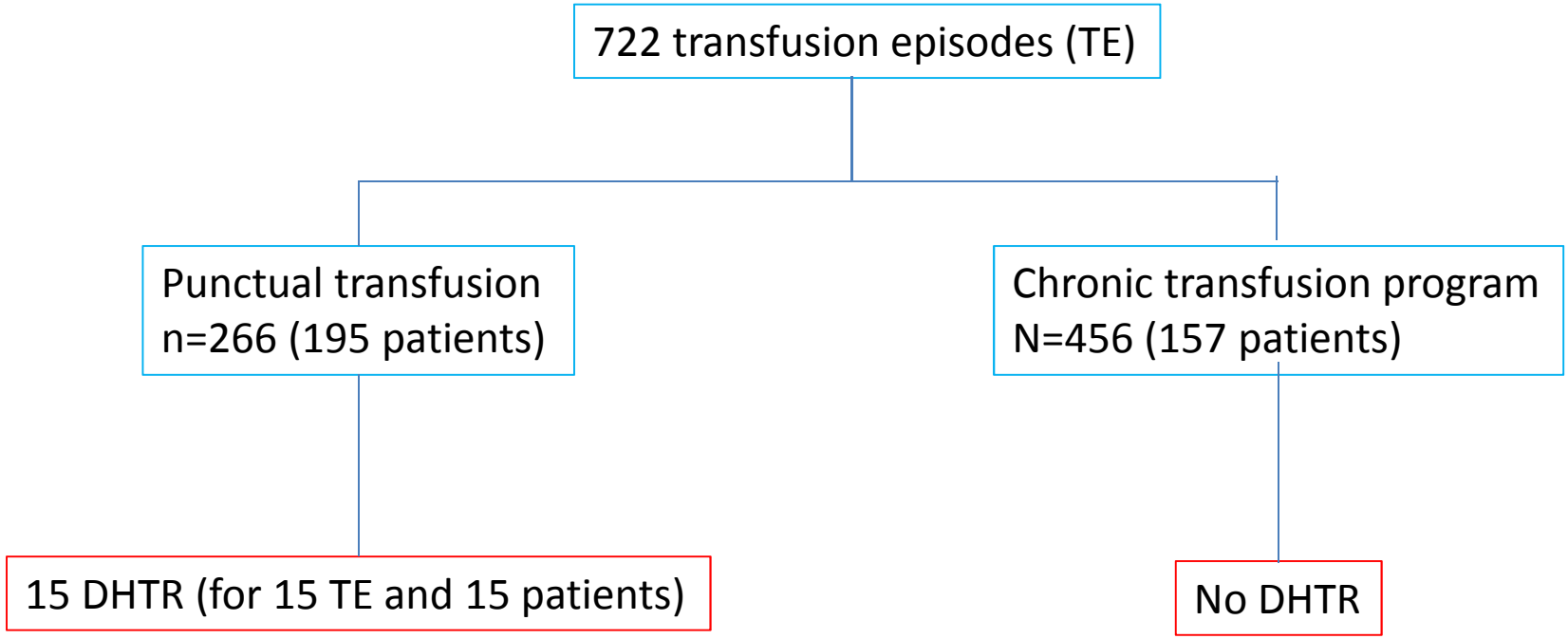
# Incidence of DHTR

- Prospective monocenter observational study : between november 2011 and june 2014 in the Henri Mondor hospital, Creteil

- Patients included :
  - age 18 and over with an intention to transfuse followed by effective transfusion in the hospital
  - DHTR confirmed by : rapid disappearance of HbA associated with 2 to 3 of the following criteria a few days to 3 weeks after transfusion
    - Symptoms of VOC
    - Dark urines
    - Worsening of anemia
    - Increase in LDH level

*Narbey, Habibi, Pirenne, in preparation*

# Incidence of DHTR



# Incidence and risks

- There is a significant higher risk to develop DHTR when patients are transfused punctually as compared to patients transfused on a transfusion program ($p<0,001$)

- Incidence of DHTR when patients are transfused punctually **:**
  - DHTR developed after 5.6% of the TE
  - 7.6% of the patients developed DHTR during the studied period

- Risk factors
  - punctual transfusion

  AND
  - history of DHTR, history of immunization, patients with few transfusions, pregnancy

## Prevention and treatment of DHTR in SCD

- In order to be efficient : action at 3 levels

1- The trigger of the reaction

2- The mechanism of destruction

3- The consequences of the hemolysis

# Prevention and treatment of DHTR in SCD

- In order to be efficient : action at 3 levels

## 1- The trigger of the reaction

- Allo immunization

Prevention

  - RH/K matched RBCs in non immunized patients
  - Extended matched RBCs in immunized patients

- But not only …
  - many cases with poorly significant antibodies (auto, AUS) and 30% of cases without detectable antibodies
  - Trigger unknown ….

# Prevention of immunization

VoxSanguinis
The International Journal of Transfusion Medicine
ISBT International Society of Blood Transfusion
*Vox Sanguinis* (2015) **108**, 262–267

DOI: 10.1111/vox.12217

**ORIGINAL PAPER**

## The use of rituximab to prevent severe delayed haemolytic transfusion reaction in immunized patients with sickle cell disease

F. Noizat-Pirenne,[1,2] A. Habibi,[2,3] A. Mekontso-Dessap,[4] K. Razazi,[4] P. Chadebech,[1,2] M. Mahevas,[2,3,5] B. Vingert,[1,2] P. Bierling,[1,2] F. Galactéros,[2,3] P. Bartolucci[2,3] and M. Michel[5]

8 patients highly immunized with history of DHTR were preventively treated with RITUXIMAB before a new transfusion

For all patients : No appearance of new formed antibodies,
**5 patients : non eventful clinical course**
**3 patients : mild DHTR**

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# Rituximab

- Rituximab can at least prevent recurrence of newly formed antibodies in high responders patients and potentially minimizes the risk of severe DHTR

- Confirmation that DHTR is complex in SCD and does not only rely on antibody mediated hemolysis

- Rituximab should be considered when a new transfusion seems inevitable in SCD patients with a previous history of severe DHTR linked to immunization

- **Caution is absolutely necessary in SCD because of the higher risk of infectious diseases**

## Prevention and treatment of DHTR in SCD

- In order to be efficient : action at 3 levels

  **2- The mechanism of destruction**

  **Antibody mediated hemolysis**

  action on effector cells (macrophages, NK) and complement

  Complement and effector cells involved without detectable antibodies ?

# Steroids

| + | - |
| --- | --- |
| • Frequently used<br>• Synergestic with IgIV<br>• Low cost | • VOC and ACS recurrence |

# Immunoglobulins

| + | - |
|---|---|
| • Frequently used | • Viscosity (but low Hb) |
| • Good benefit/risk ratio | • Kidney |
| • Animal models | |
| • Not immunsuppressing | |

**Delayed hemolytic transfusion reaction in children with sickle cell disease**

Mariane de Montalembert,[1] Marie-Dominique Dumont,[2] Claire Heilbronner,[3] Valentine Brousse,[3] Oussama Charrara,[4] Béatrice Pellegrino,[5] Christophe Piguet,[6] Valérie Soussan,[7] and France Noizat-Pirenne[8]

*Haematologica 2011*

# Other treatments to act on destruction

- Action of RITUXIMAB on destruction ?
  - Bachmeyer, 2009; Delmonte, 2013 : Rituximab was given to treat DHTR associated with other treatments

- Eculizumab
  - To stop complement activation and the cascade of events produced by the release of RBC content

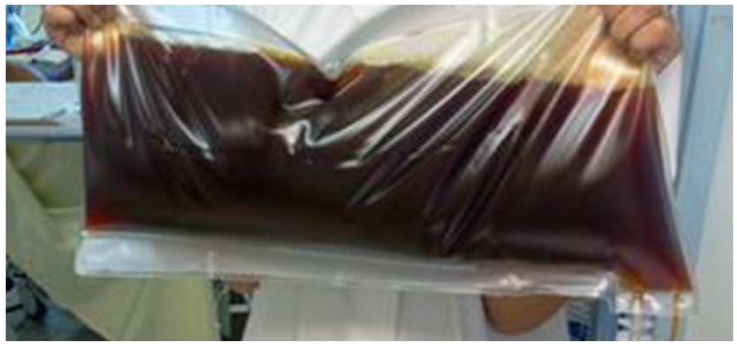
## Prevention and treatment of DHTR in SCD

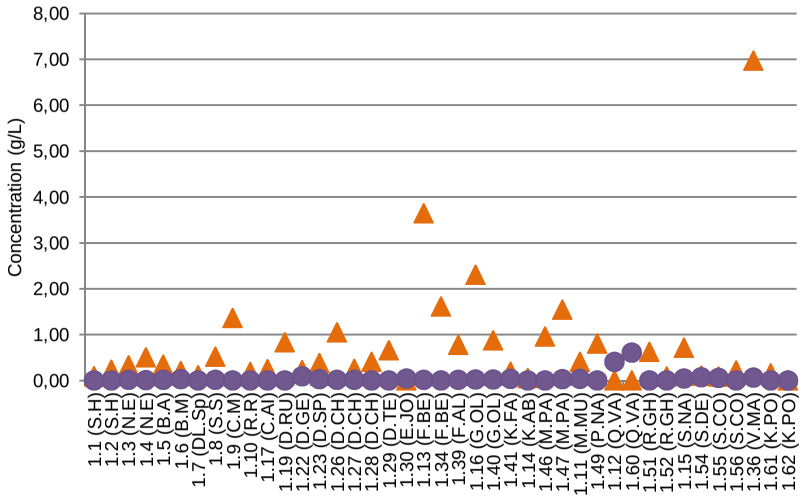
- In order to be efficient : action at 3 levels

### 3- The consequences of the hemolysis

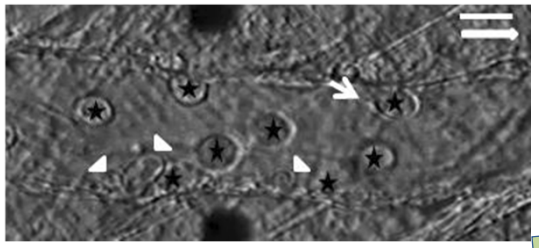
# Cases of severe DHTR

- In some cases, the evolution of DHTR can be disastrous :
  - Within a few hours : multi organ failure
  - Frequently linked with intra vascular hemolysis

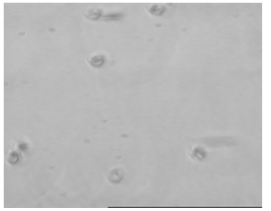


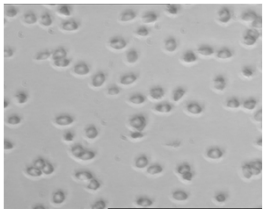


Leucocytes adhesion



RBC adhesion

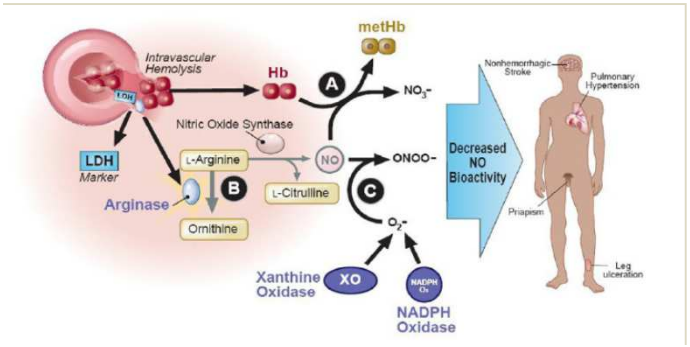




Endothelial cells

Free hemoglobin
Free heme

↘ NO Biodisponibility




Thrombosis

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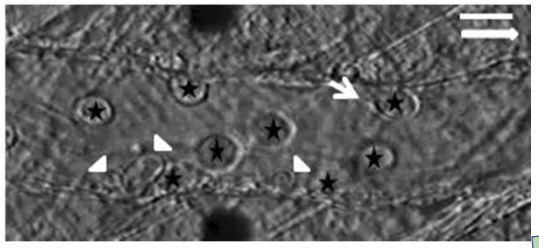
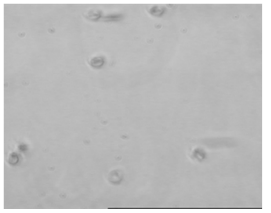
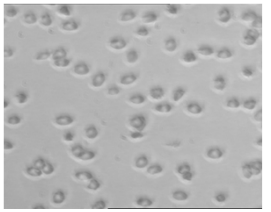
Leucocytes adhesion



RBC adhesion





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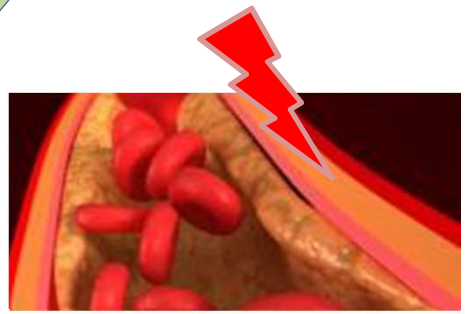


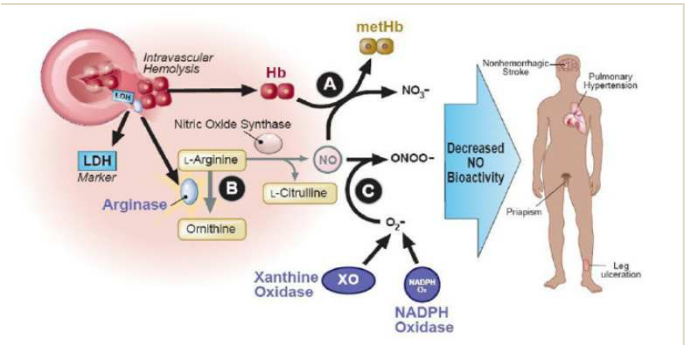
Free hemoglobin
Free heme

Extracellular hemin crisis triggers acute chest syndrome in sickle mice

JCI 2014

Samit Ghosh, Olufolake Adetoro Adisa, Prasanthi Chappa, Fang Tan, Kesmic Ann Jackson, David Robert Archer, and Solomon Fiifi Ofori-Acquah

↘ NO Biodisponibility




Thrombosis

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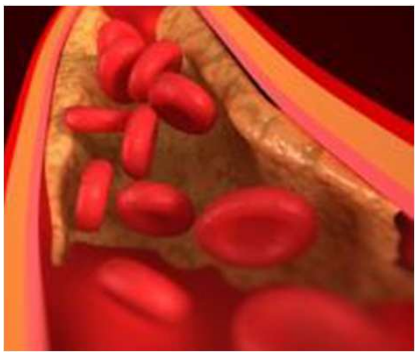
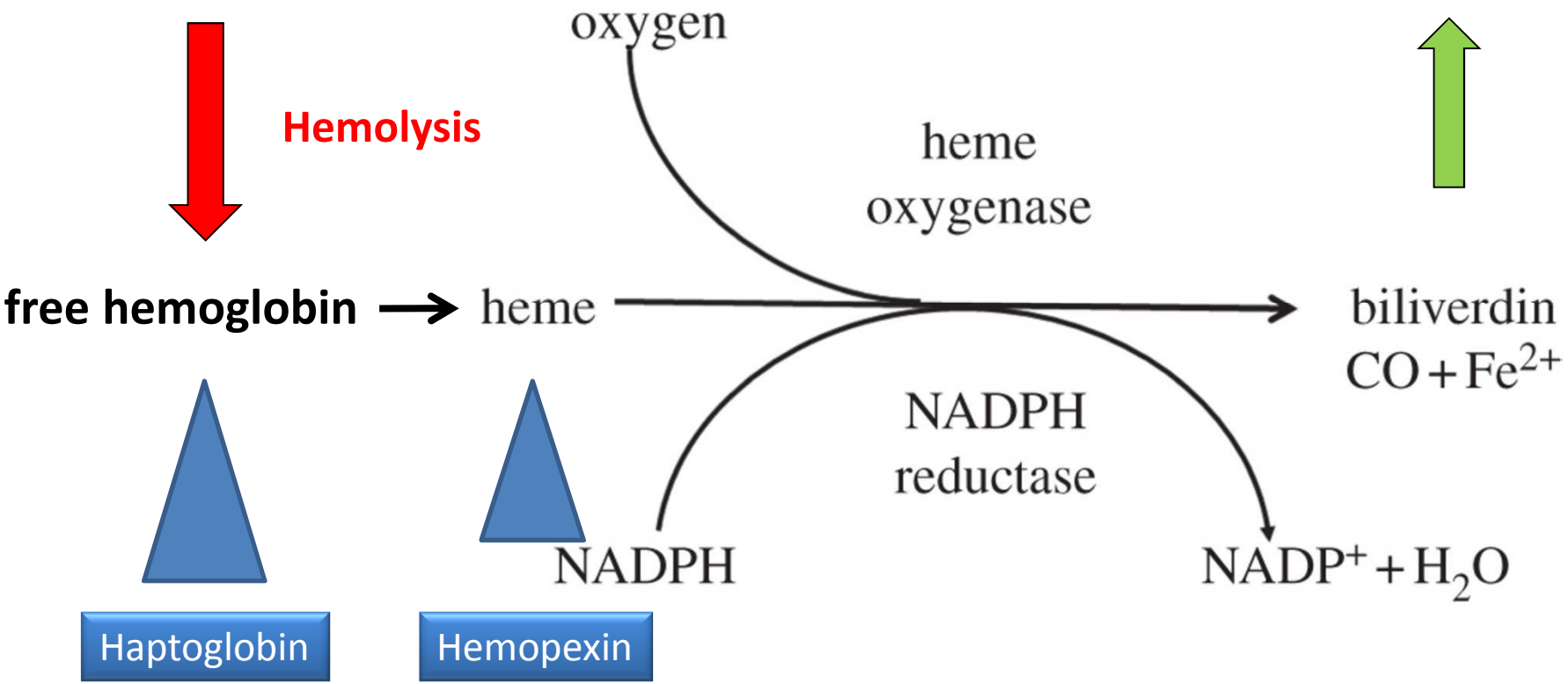
# Intra vascular hemolysis



Anti inflammatory
Anti oxidant

Hemolysis



# Intra vascular hemolysis

Anti inflammatory
Anti oxidant

**Hemolysis**

**free hemoglobin → heme**

oxygen

heme oxygenase

biliverdin
$CO + Fe^{2+}$

NADPH reductase

NADPH

$NADP^+ + H_2O$

Haptoglobin

Hemopexin

# Plasma Exchange

| + | - |
| --- | --- |
| ↘↘ free-hemoglobin | Not easy to perform RBC<br>– Low Hb level<br>– Faisability |

**Successful treatment of recurrent hyperhemolysis syndrome with immunosuppression and plasma-to-red blood cell exchange transfusion**

*Transfusion 2014*

*Erik J. Uhlmann, Shalini Shenoy, and Lawrence T. Goodnough*

## Other potential treatments to inhibit toxicity of RBC content

- Haptoglobin
- Hemopexin

# Rationale to use Eculizumab

**In the most severe cases of DHTR , there is a need of a treatment that stop hemolysis and the disastrous cascade of events that are induced by free heme and free hemoglobin as in SCD patients, haptoglobin and hemopexin are frequently overwelhmed because of chronic hemolysis**

# Rationale to use Eculizumab

**Complement activation likely involved in post-transfusion hyperhemolysis in SCD**

**First hit : yes when antibodies are produced**
**First hit : ?? low titer of antibodies against RBCs**

**Second hit : hypothesis : free heme**

blood 2013 122: 282-292
doi:10.1182/blood-2013-03-489245 originally published online May 21, 2013

**Complement activation by heme as a secondary hit for atypical hemolytic uremic syndrome**

Marie Frimat, Fanny Tabarin, Jordan D. Dimitrov, Caroline Poitou, Lise Halbwachs-Mecarelli, Veronique Fremeaux-Bacchi and Lubka T. Roumenina

# The use of Eculizumab in severe DHTR

**Life-threatening delayed hyperhemolytic transfusion reaction in a patient with sickle cell disease: effective treatment with eculizumab followed by rituximab**

BOONYASAMPANT ET AL. Volume 00, April 2015 TRANSFUSION

**Eculizumab salvage therapy for delayed hemolysis transfusion reaction in sickle cell disease patients**

Guillaume Dumas,[1] Anoosha Habibi,[1-3] Thierry Onimus,[4] Jean-Claude Merle,[5] Keyvan Razazi,[6] Armand Mekontso Dessap,[6] Frederic Galactéros,[2] Marc Michel,[1] Veronique Frémeaux Bacchi,[7] France Noizat Pirenne,[3,8] and Pablo Bartolucci[1-3]

BLOOD, 25 FEBRUARY 2016 • VOLUME 127, NUMBER 8

EFS

# TREATMENT Of DHTR

***Primum non nocere***

## AVOID

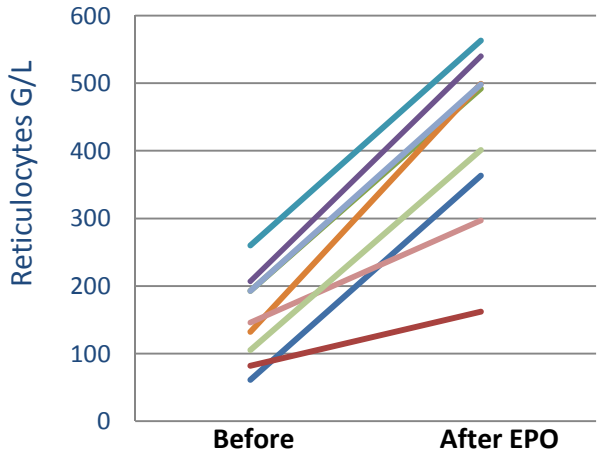
## Retransfusion

## only life-threatening complications should authorize a new transfusion

All known antibodies in every patient's history must be considered

transfusion matching should be extended to the main immunogenic blood groups: FY, JK and MNS

**Erythropoietin**

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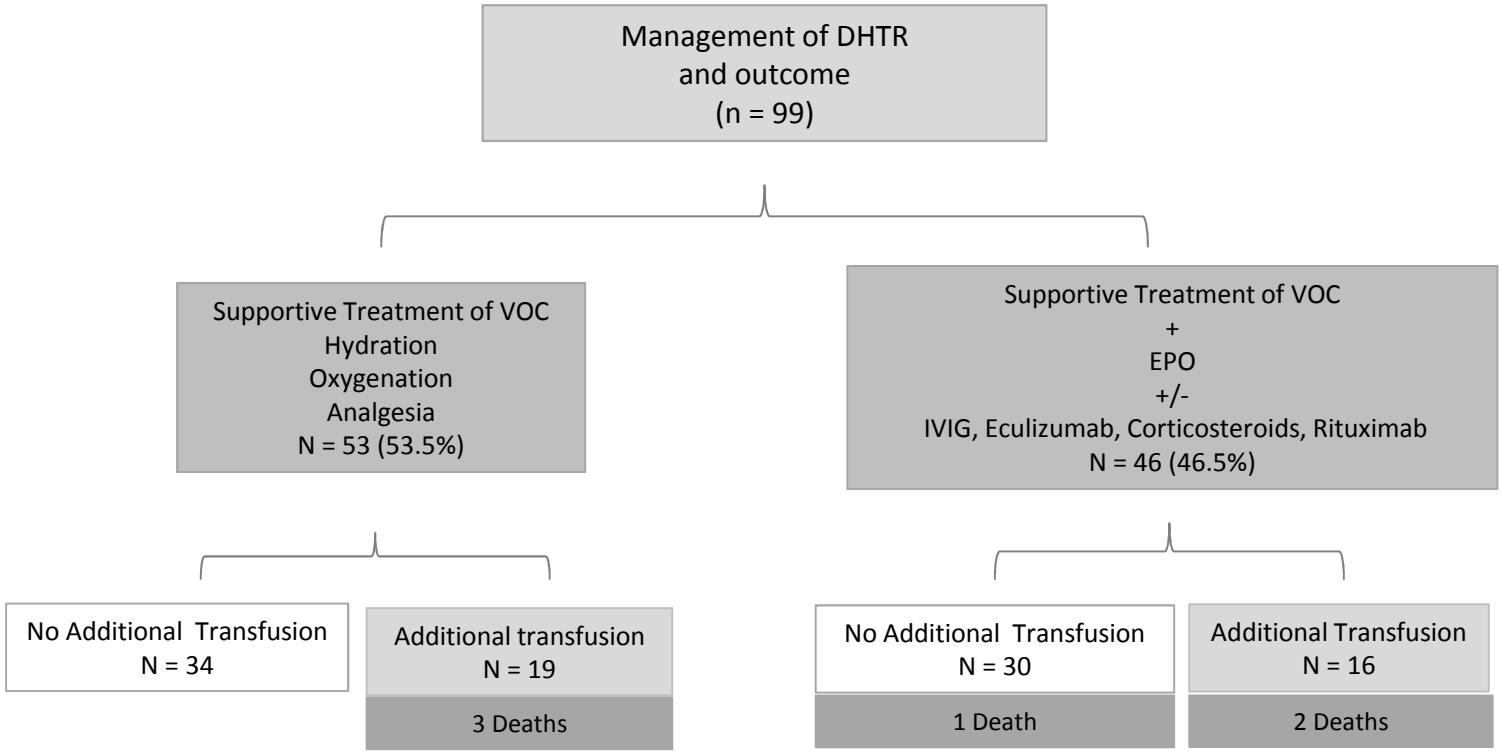
Management of DHTR
and outcome
(n = 99)
Supportive Treatment of VOC
Hydration
Oxygenation
Analgesia
N = 53 (53.5%)
Supportive Treatment of VOC
+
EPO
+/-
IVIG, Eculizumab, Corticosteroids, Rituximab
N = 46 (46.5%)
No Additional Transfusion
N = 34
Additional transfusion
N = 19
3 Deaths
No Additional Transfusion
N = 30
1 Death
Additional Transfusion
N = 16
2 Deaths

# Conclusions

- DHTR can be life threatening and under recognized
- Incidence is high when patients are punctually transfused
- Mechanism remains enigmatic in some cases
- New transfusion should be avoided
- Treatment has to be decided early in case of severe hyperhemolysis, but DHTR can resolve without specific treatment
- Prevention of immunization is necessary but not sufficient

**EFS-INSERM U955**
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Sadaf Pakdaman
Philippe Chadebech
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