# Hyperhaemolysis
# Macrophage the culprit!

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# Disclaimer

- There are no conflicts of interest

## Hyperhaemolysis Syndrome (HS)
## Post-Transfusion Hyperhaemolysis Syndrome: (PTHS)

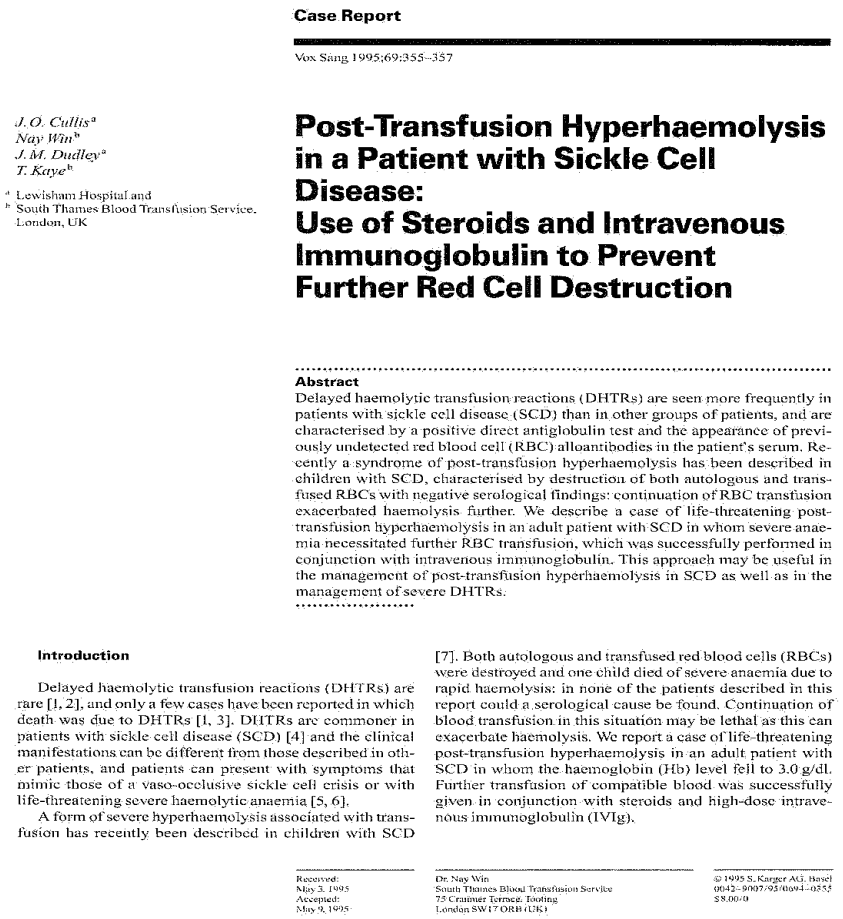
HS is a rare complication of blood transfusion which can be fatal.
Reported in both SCD patients and Non SCD (Thalassaemia and others )

Cullis J O, Win N, Dudley J M, Kaye T.

Post-Transfusion Hyperhaemolysis in a patient with Sickle Cell Disease: Use of Steroids and Intravenous Immunoglobulin to Prevent Further Red Cell Destruction

Correspondence: N Win

Published: *Vox Sang* **1995**; 69: 355-357
Doi. 10.1111/j.1423-0410.1995.tb00373.x



Case Report

Vox Sang 1995;69:355–357

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# Post-Transfusion Hyperhaemolysis in a Patient with Sickle Cell Disease: Use of Steroids and Intravenous Immunoglobulin to Prevent Further Red Cell Destruction

**Abstract**

Delayed haemolytic transfusion reactions (DHTRs) are seen more frequently in patients with sickle cell disease (SCD) than in other groups of patients, and are characterised by a positive direct antiglobulin test and the appearance of previously undetected red blood cell (RBC) alloantibodies in the patient's serum. Recently a syndrome of post-transfusion hyperhaemolysis has been described in children with SCD, characterised by destruction of both autologous and transfused RBCs with negative serological findings: continuation of RBC transfusion exacerbated haemolysis further. We describe a case of life-threatening post-transfusion hyperhaemolysis in an adult patient with SCD in whom severe anaemia necessitated further RBC transfusion, which was successfully performed in conjunction with intravenous immunoglobulin. This approach may be useful in the management of post-transfusion hyperhaemolysis in SCD as well as in the management of severe DHTRs.

## Introduction

Delayed haemolytic transfusion reactions (DHTRs) are rare [1, 2], and only a few cases have been reported in which death was due to DHTRs [1, 3]. DHTRs are commoner in patients with sickle cell disease (SCD) [4] and the clinical manifestations can be different from those described in other patients, and patients can present with symptoms that mimic those of a vaso-occlusive sickle cell crisis or with life-threatening severe haemolytic anaemia [5, 6].

A form of severe hyperhaemolysis associated with transfusion has recently been described in children with SCD [7]. Both autologous and transfused red blood cells (RBCs) were destroyed and one child died of severe anaemia due to rapid haemolysis; in none of the patients described in this report could a serological cause be found. Continuation of blood transfusion in this situation may be lethal as this can exacerbate haemolysis. We report a case of life-threatening post-transfusion hyperhaemolysis in an adult patient with SCD in whom the haemoglobin (Hb) level fell to 3.0 g/dl. Further transfusion of compatible blood was successfully given in conjunction with steroids and high-dose intravenous immunoglobulin (IVIg).

Received:
May 3, 1995
Accepted:
May 9, 1995

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0042–9007/95/0694–0355
$8.00/0

# First case treated with IVIG/steroids 1995

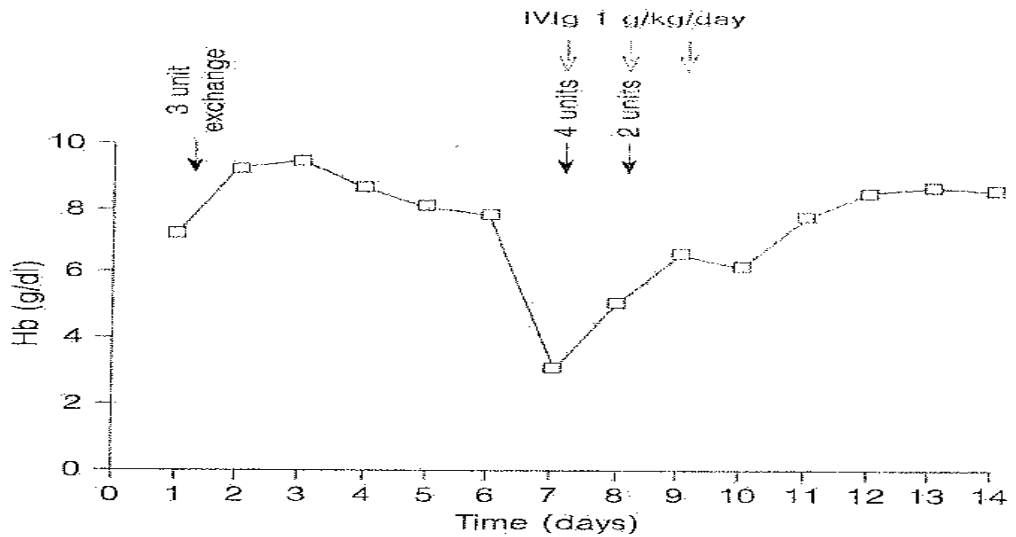



**Fig. 1.** Serial haemoglobin levels in relation to RBC transfusion in case described.

## The Term Syndrome was coined by Prof Petz

i) patient developed severe anaemia after transfusion suggested not only the transfused cells were haemolysed, but destruction of patient's own RBC may play a role resulting in significant decrease in Hb level.

ii) it may manifest as acute or delayed haemolytic transfusion reactions (HTR), serological studies may not provide an explanation for the HTR. Some DAT pos. Some new alloantibodies from others not ongoing haemolysis with subsequent transfusion despite providing antigen matched RBC.

Ref: Petz, L.D;Calhoun, L;Shulman,I; Johnson, C; Herron, R. (1997)
The sickle cell haemolytic transfusion reaction syndrome. *Transfusion*, 37,382-392.

iii) presenting features: relative reticulocytopenia and Petz et al[1] have initially suggested that the apparent increase of the rate of haemolysis of autologous RBC was due to transfusion "suppression of erythropoiesis.
**Petz et al** described 4 Delayed and 1 acute form of HHS. Each patient received additional transfusion (mean 13 units) and were discharged on days 2,24,29,36 and 52 after the admission

iv) if possible stop transfusion and administration of corticosteroids appears to be an important therapeutic measure.

1) **Development of severe anemia:**
**Destruction of both autologous and transfused RBCs**

Serial measurement of high performance liquid chromatography (HPLC) analysis of the urine during haemolysis confirming both HbA and HbS.
Intravascular haemolysis (contact lysis/Haemoglobinuria)
Extravascular haemolysis (erythrophagocytosis/high bilirubin)

Ref: Win, N, Doughty, H; Telfer, P; Wild, B; Pearson, T. (2001).
Hyper-haemolytic transfusion reaction in SCD Transfusion, 41, 323-328.

## 2) Acute and delayed forms / classification

Acute form < 7 days after receiving transfusion.
DAT is negative.
With no detectable new red cell allo-antibodies in the post-transfusion samples.

The delayed form > 7 days. The DAT is positive.
New allo-antibodies are often identified.
Haemolysis occurs despite providing compatible antigen matched units.

7 different publications
28 cases
15 acute from
13 delayed from

Ref: Win et al (2008) Hyperhemolysis syndrome in SCD case report and literature review. *Transfusion*; 48:1231-1238.

# Hyperhaemolysis Syndrome (HS)

Majority used the term “hyperhaemolysis syndrome” (HS) followed by “hyperhaemolysis” and “Delayed Haemolytic Transfusion Reactions”. (DHTR)

It is crucial to distinguish( HS) and classical Delayed Haemolytic Transfusion Reactions. (DHTR)

Two different distinct complications of transfusion with the differences in their clinical course, management and outcome.

## Difference between (**DHTR**) and **HS**

| | DHTR | HS* |
|---|---|---|
| **Symptoms** | May or may not be present | Fever, painful crisis generally present |
| **Time of onset** | >7 days | Acute HS: <7 days **<br>Delayed HS: >7-15 days |
| **Haemolysis** | Extravascular | Intravascular / Extravascular |
| **Hb outcome** | ≥Pretransfusion Hb level | <Pretransfusion Hb level |
| **Reticulocyte count** | ↑ | ↓ **(from base line)** |
| **Serological study** | DAT (+) : Detection of responsible Allo Ab | Acute form DAT (-) :No new Allo Ab**<br>Delayed from DAT (+) : New allo Ab formed |
| **Transfusion** | ➢ Transfused if indicated<br>➢ No haemolysis if avoid Antigen negative unit<br>➢ Expect Hb increment<br>➢ Anemia corrected | ➢ Avoid transfusion<br>➢ Transfusion even with Antigen Negative phenotype matched units may further exacerbate haemolysis worsen anemia / even death.<br>➢ **HS" may recur "** |
| **Treatment** | | IVIg/steroids (first line therapy) |

**Petz, et al (1997) *The sickle cell haemolytic transfusion reaction syndrome. *Transfusion*, 37,382-392**

**Win et al (2008)** Hyperhemolysis syndrome in SCD case report and literature review. *Transfusion,* 48:1231-1238.**

# Proposed mechanism

1) Suppression of erythropoiesis (Petz et al 1997)

2) Bystander haemolysis (King's et al 1997) (Garraty 1996)

3) Red cell destruction by activated macrophages (Win et al 2001)

## Red Cell destruction by activated Macrophages
## Acute form: No evidence of antibody mediated red cell destruction

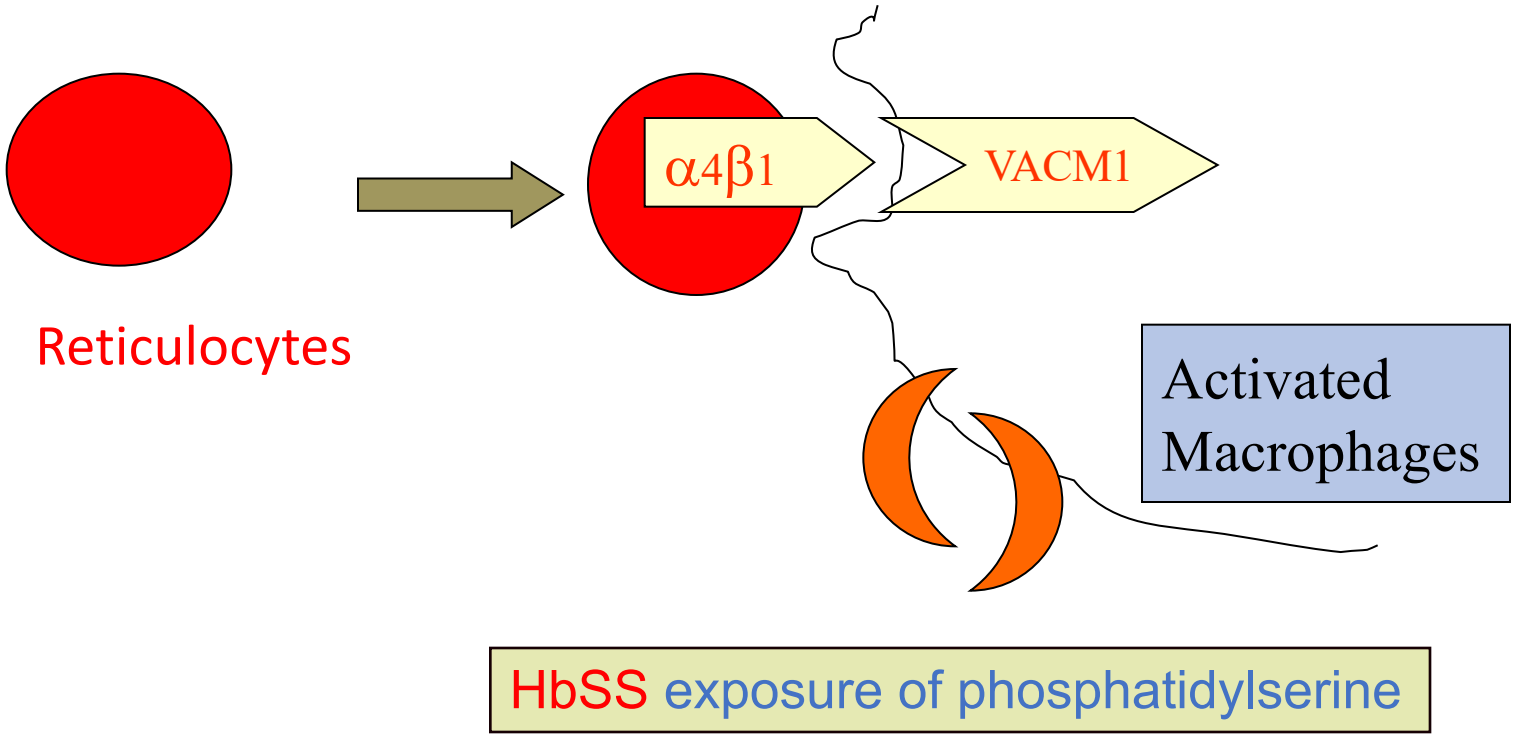
1) Mechanism of destruction of Autologous Cells in SCD patients
Both HbSS and sickle reticulocytes are destroyed by contact lysis via hyperactive macrophages.

Ref: Win, N; Doughty, H; Telfer, P; Wild, B; Pearson, T. (2001) Hyper-haemolytic transfusion reaction in SCD. *Transfusion*, 41, 323-328.

2) Mechanism of destruction of HbAA (Transfused RBC or Patient's RBC in Non SCD patients)

Ref: Win, N. (2009) Hyperhemolysis syndrome in SCD. *Expert Rev Hematology*,2(2), 111-115.

# Destruction of Reticulocytes / sickle cells by activated macrophages




Win et al 2001. *Transfusion*: 41,323-328

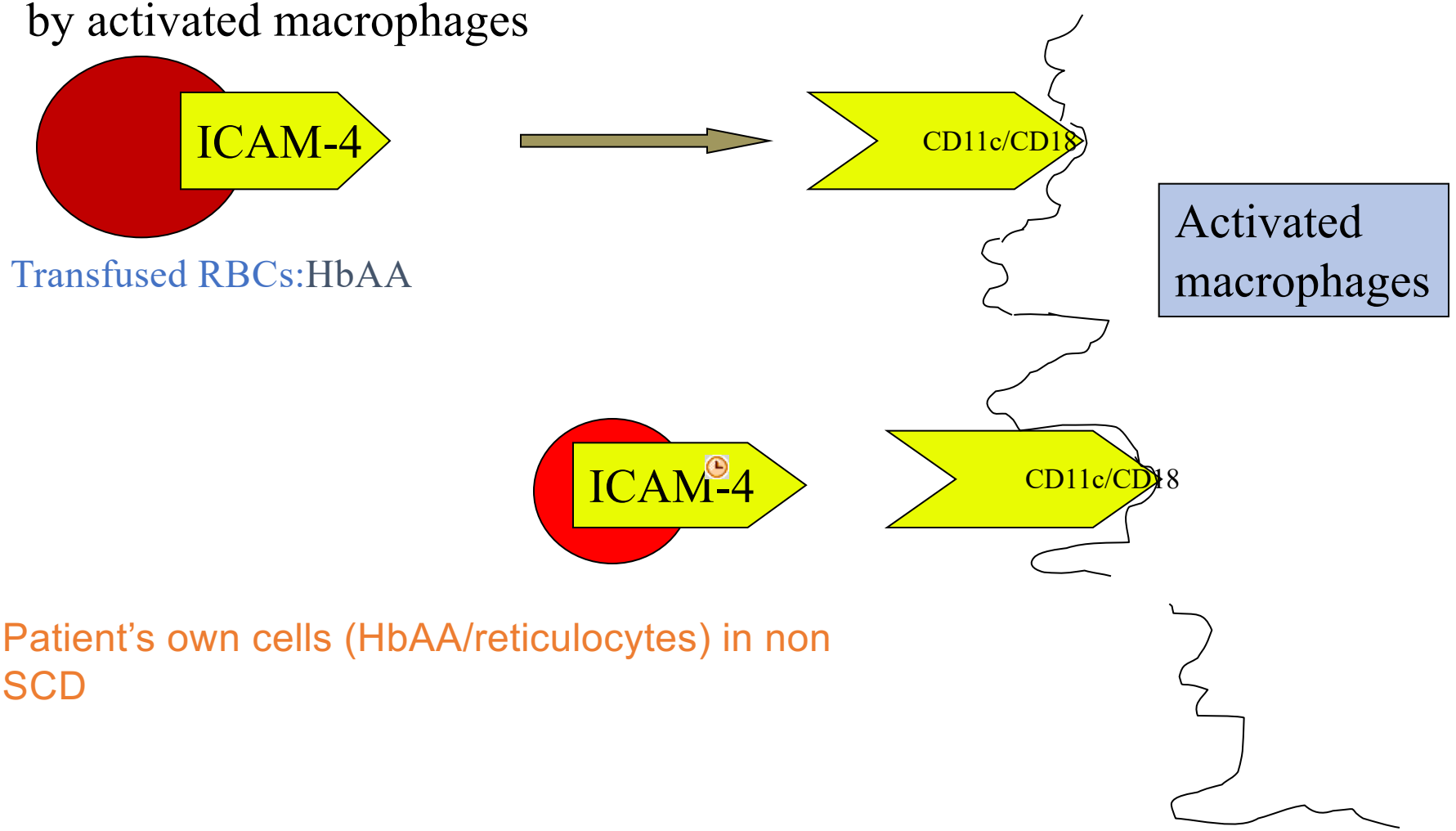
# Interaction between HbAA RBCs / Macrophages

Studies have shown that Intercellular Adhesion Molecule-4 (ICAM-4), a glycoprotein expressed on red blood cells and erythroid precursor cells interacts with macrophages via integrin receptors CD11c/CD18.

Inhibition of eryhrophagocytosis by anti-ICAM-4 and anti-integrin antibodies support the role of contact lysis of red cells by macrophages.

Ref: Ihanus et al: Red cell ICAM4 is a ligand for macrophage integrin CD11c/CD18 *Blood*,**109**,802-810(2007).

Destruction of transfused cells HbAA / reticulocytes by activated macrophages




Transfused RBCs:HbAA

Patient's own cells (HbAA/reticulocytes) in non SCD

Ref: Win, N. (2009) Hyperhemolysis syndrome in SCD. Expert Rev Hematology, 2(2),111-115.

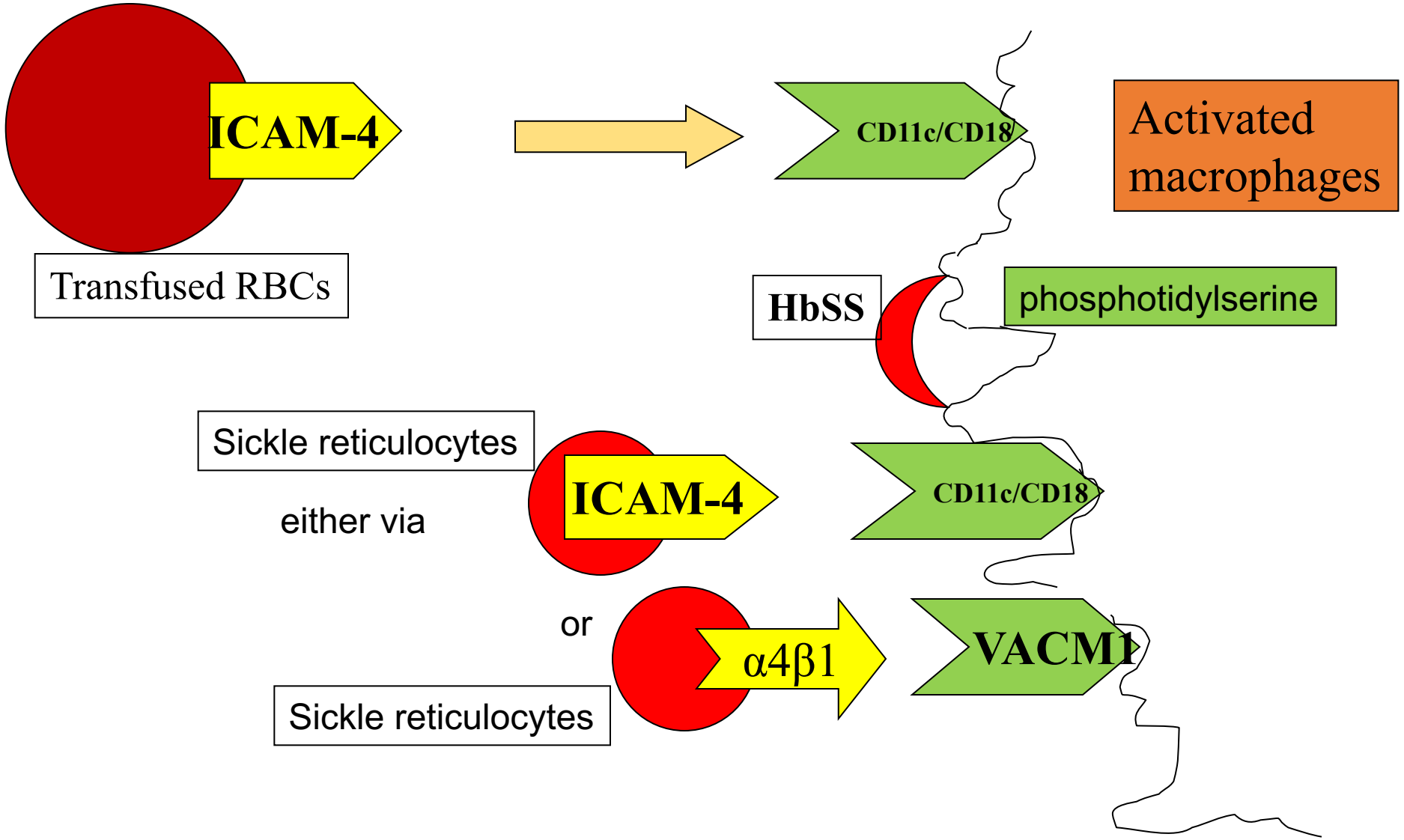
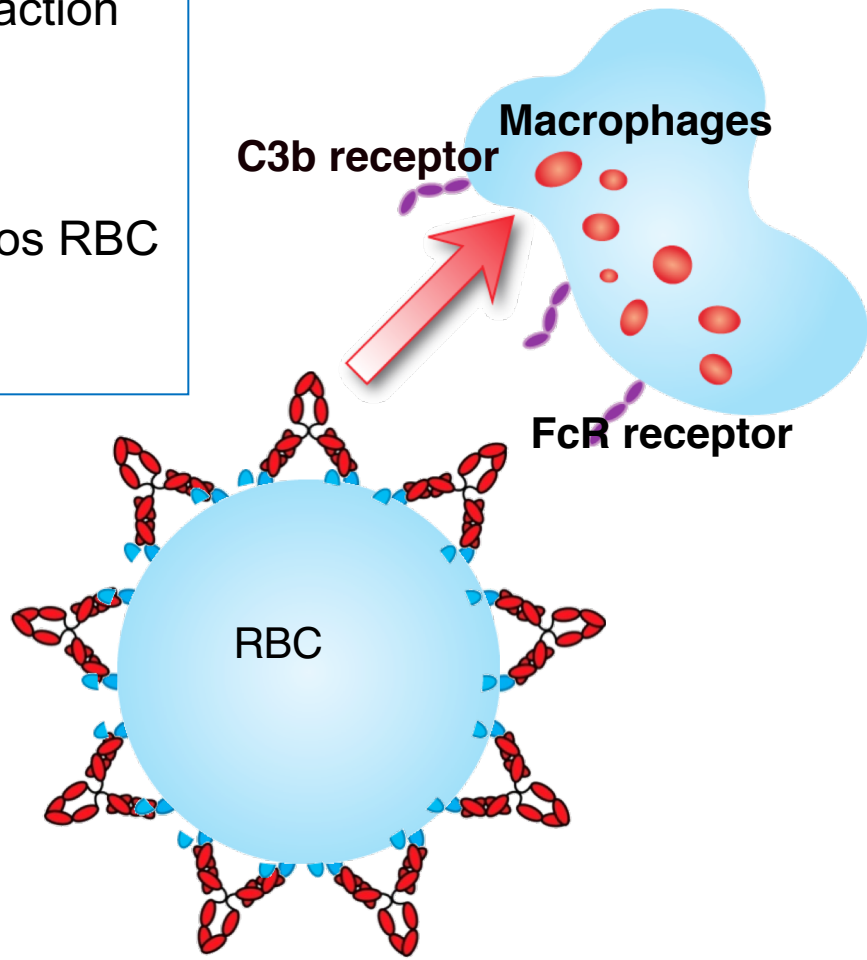



**Fig 2: Interaction and destruction of transfused cells and patient's own cells (HbSS/ reticulocytes) by activated macrophages**

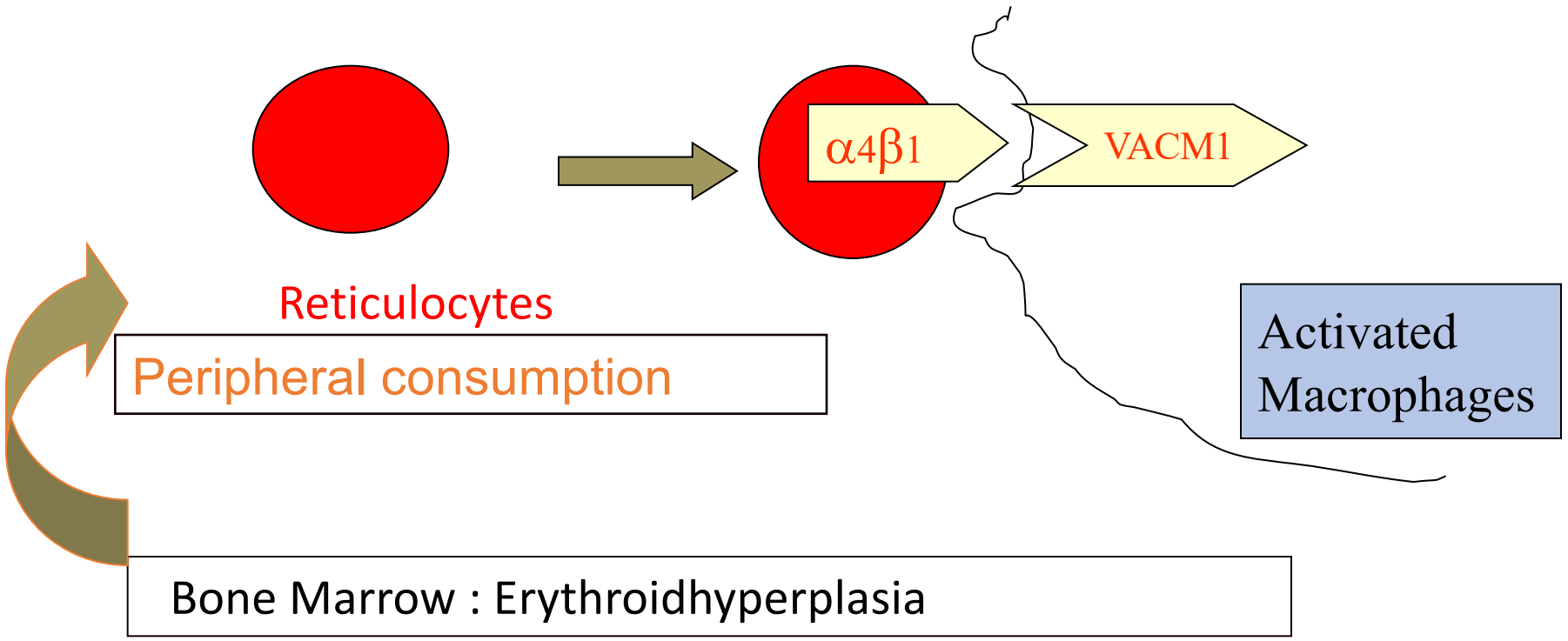
Delayed haemolytic Transfusion Reaction

DAT pos
Amnestic antibody response
Antibody coated on transfused Ag pos RBC
Destroyed by Macrophages
Mainly Extravascular haemolysis



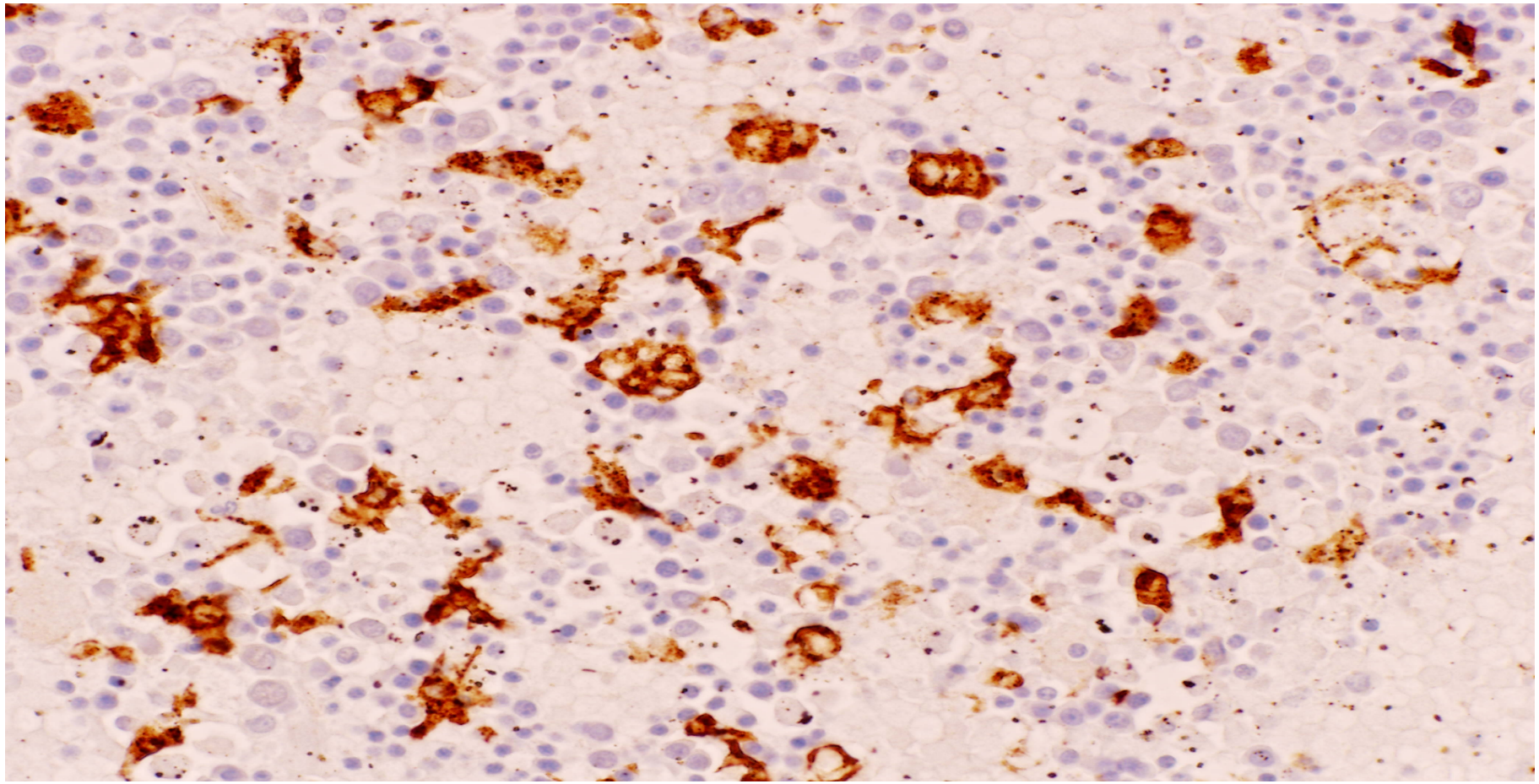

Delayed form of HS: If Macrophages are hyperactive it may lead to hyperhaemolysis via adhesion mechanism

## 3) Destruction of Reticulocytes by peripheral consumption
## Morphological evidence: (2001/2014/2019)




Ref: Win et al 2001. Transfusion: 41,323-328
Danaee et al 2014 Trans Med 4:244
Win et al 2019. BJH: doi: 10.1111/bjh.15925.

Bone Marrow (CD68)



**PM** findings: Widespread macrophage activation / erythroid hyperplasia

Ref: Histopathological evidence for macrophage activation PTHS. Win N, Lucas S, Hebbali S, McKernan A, Hamilton R, Robinson I, Chen F. 2019, **doi: 10.1111/bjh.15925**.

# Further evidences of Macrophage activation

1) Ferritin: Non specific marker for macrophages (2012/2014/2018/2019)

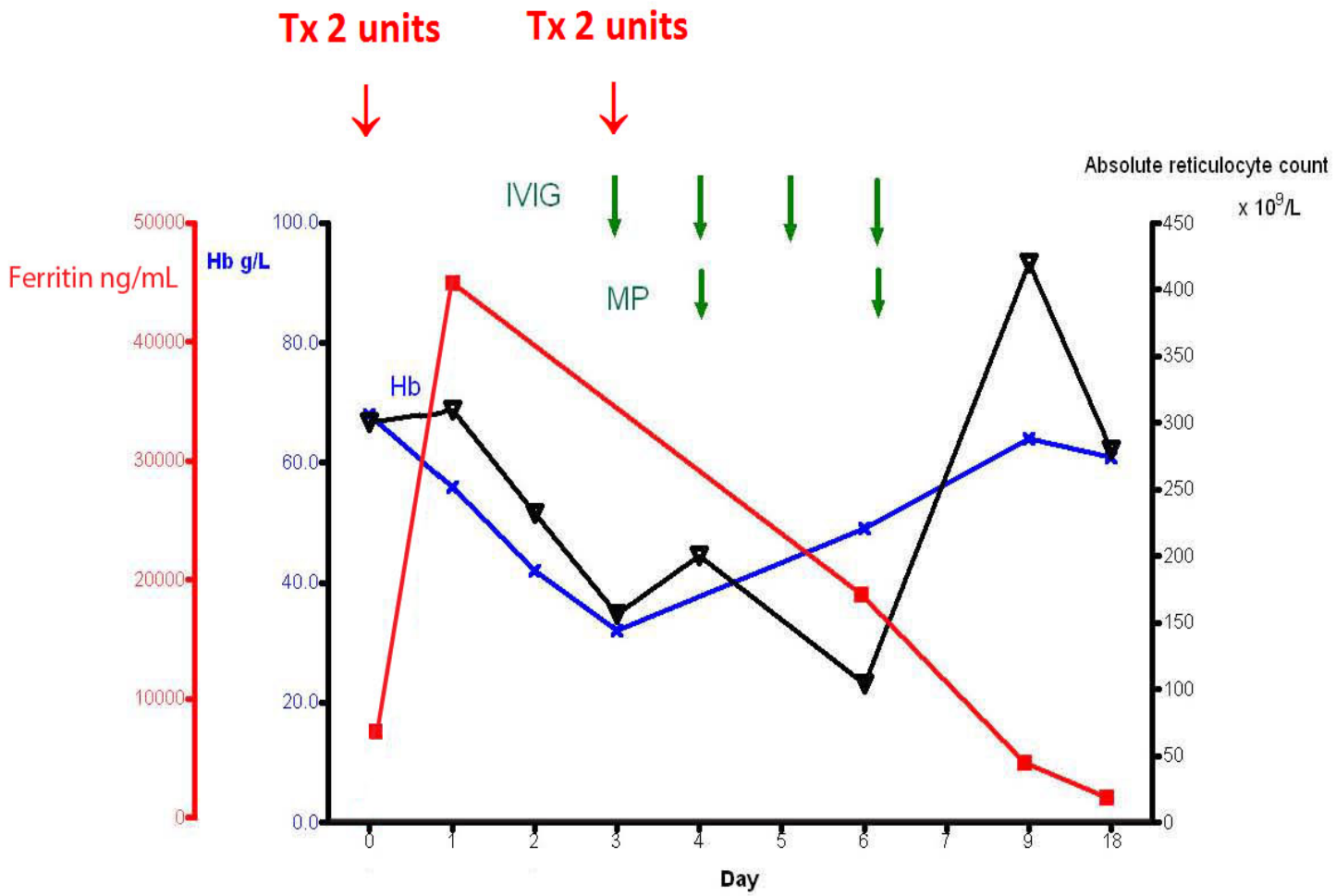
2) Histopathological findings supporting Macrophage activation (2019)

## Indirect evidence of Macrophage activation (2012 / 2014 / 2018 / 2019)

Ferritin: non specific marker for macrophages
correlates with disease activity / clinical response.

Ref: Win, N; Lee, E; Needs, M; Chia, L.W; Stasi, R. (2012) Measurement of macrophage marker in hyperhaemolytic transfusion reaction: a case report. *Transfusion Medicine*, 22, 137-141

Rogers, M; Smith, G. (2014) Letter to Editor: Hyperhaemolysis in a patient with CLL *Transfusion Medicine* **[doi: 10.1111/tme.12104].**




**Evidence of Macrophage activation**

Changes of absolute retic, **ferritin**, transfusion & treatment with IVIG/IVMethylprednisolone.

Ref: Win et al. *Transfusion Medicine*: Measurement of macrophage marker 2012,22,137-141.

# Histopathological evidence of Macrophage activation (2019)

**Fatal PTHS:**

26 yr old (HbSS SCD) died 4 hrs after receiving blood transfusion.

- DAT neg: No RBC antibodies detected
- Acute form of Hyperhaemolysis
- Histopathology: showed enlarged macrophages phagocytosis of RBC (Liver / Spleen / Bone Marrow)
- Immunostain (CD68) was also used.

Thal intermedia pregnant patient: not responding to 2 courses of IVIG/steroids/Rituximab ends up with splenectomy. Spleen tissue histopathology showed as above.

Ref: Win N**,** Lucas S, Hebbali S, McKernan A, Hamilton R, Robinson I, Chen F. Histopathological evidence for macrophage activation driving post-transfusion hyperhaemolysis syndrome. *British Journal of Haematology*, 2019, **doi: 10.1111/bjh.15925**.

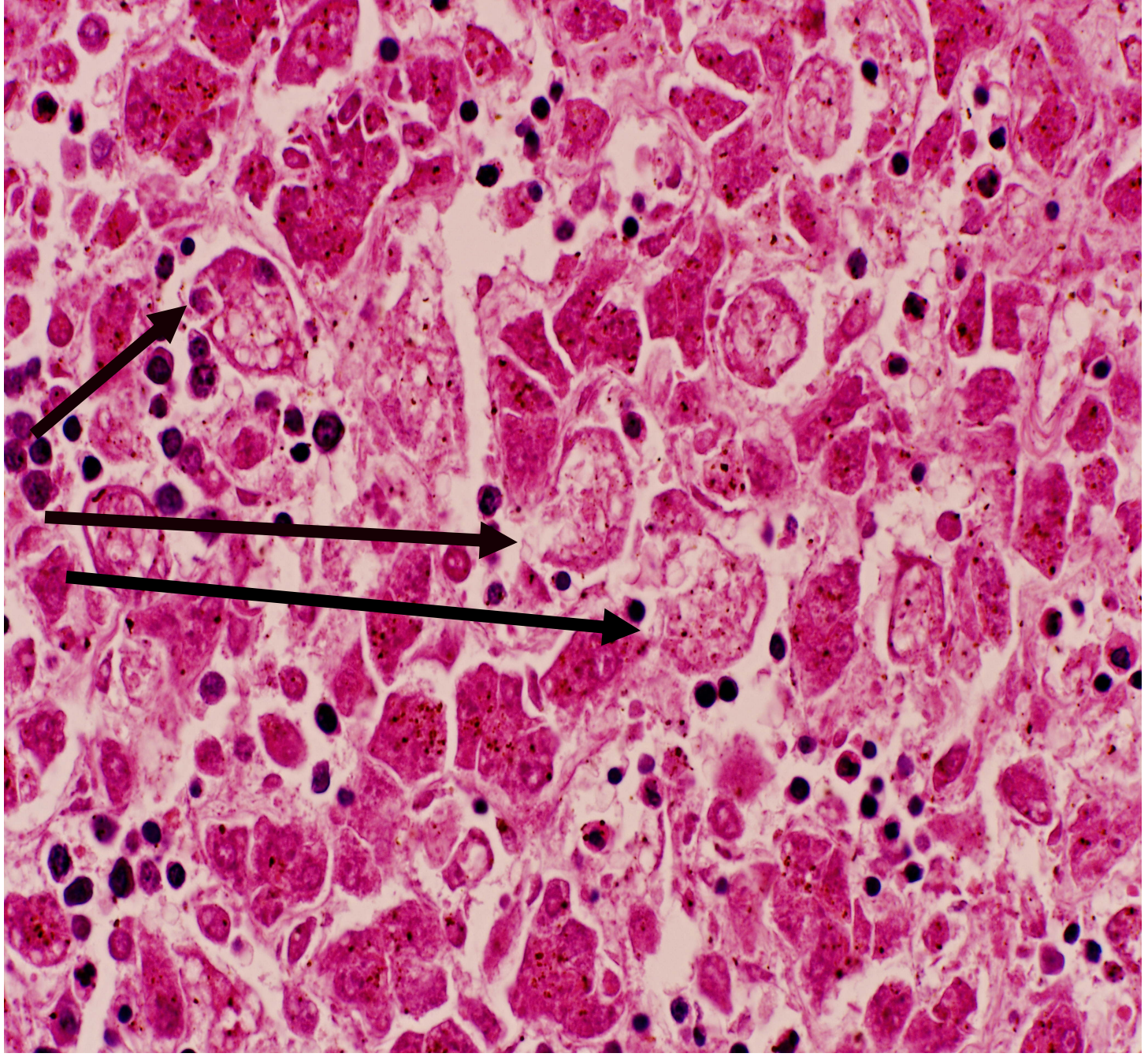


**Fig 3: High power H&E histology of liver kuffer cells shows phagocytosed red cells seen as Ghosts cells**

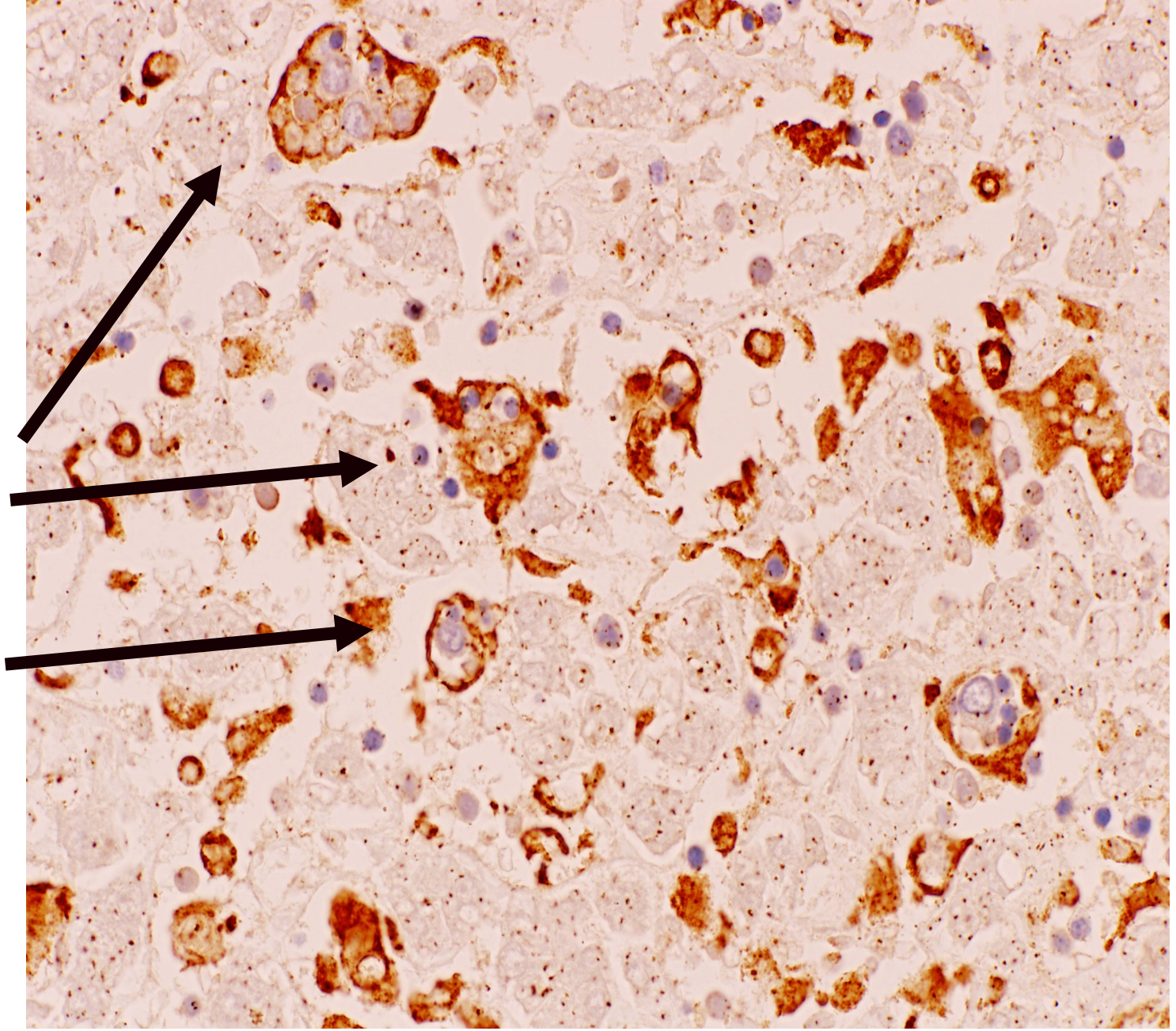


**Fig 4: High power (CD68 Immunostain) histology of bone marrow shows enlarged macrophages with phagocytosed red cells and other haemopoietic cells**

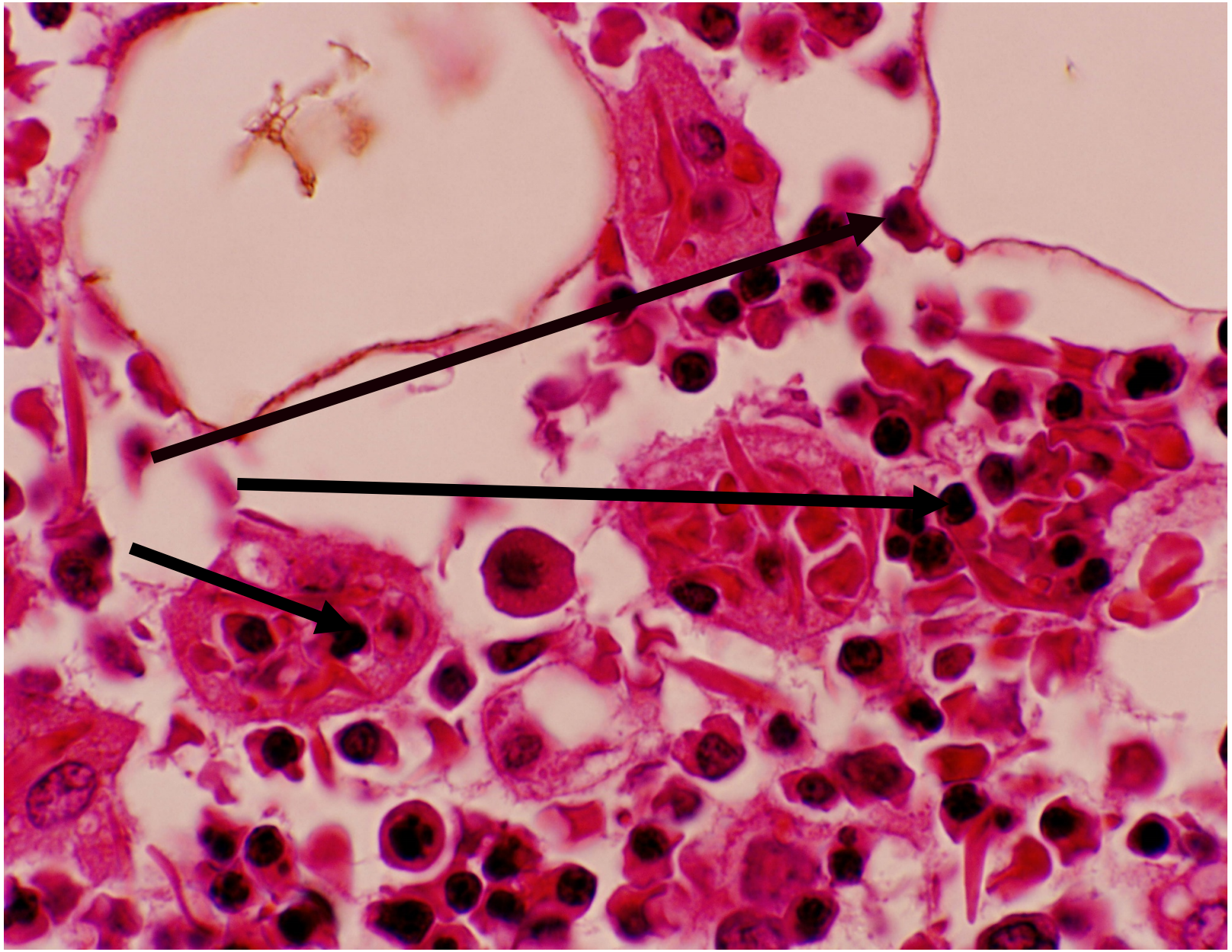


**Fig 2: High power H&E histology of bone marrow shows enlarged macrophages, phagocytosis of sickled and non-sickled red cells**

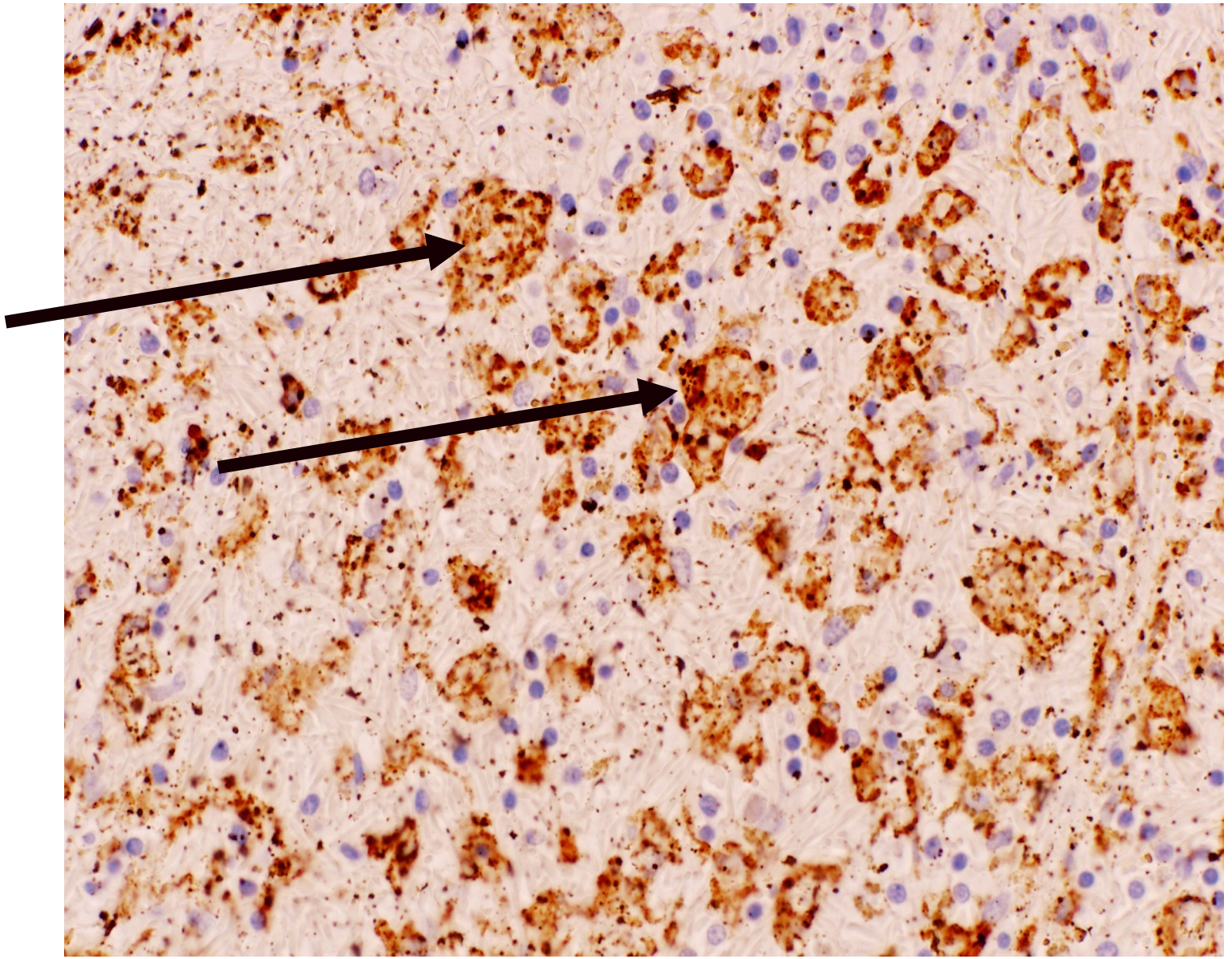


**Fig 5: High power (CD68 Immunostain) histology of splenic sinusoids shows enlarged macrophages with phagocytosed ghosts cells**

## Treatment with IVIG/steroids: therapeutic response

Response manifested by

i) a rise in Hb and

ii) a rise in reticulocyte count

iii) drop in Ferritin

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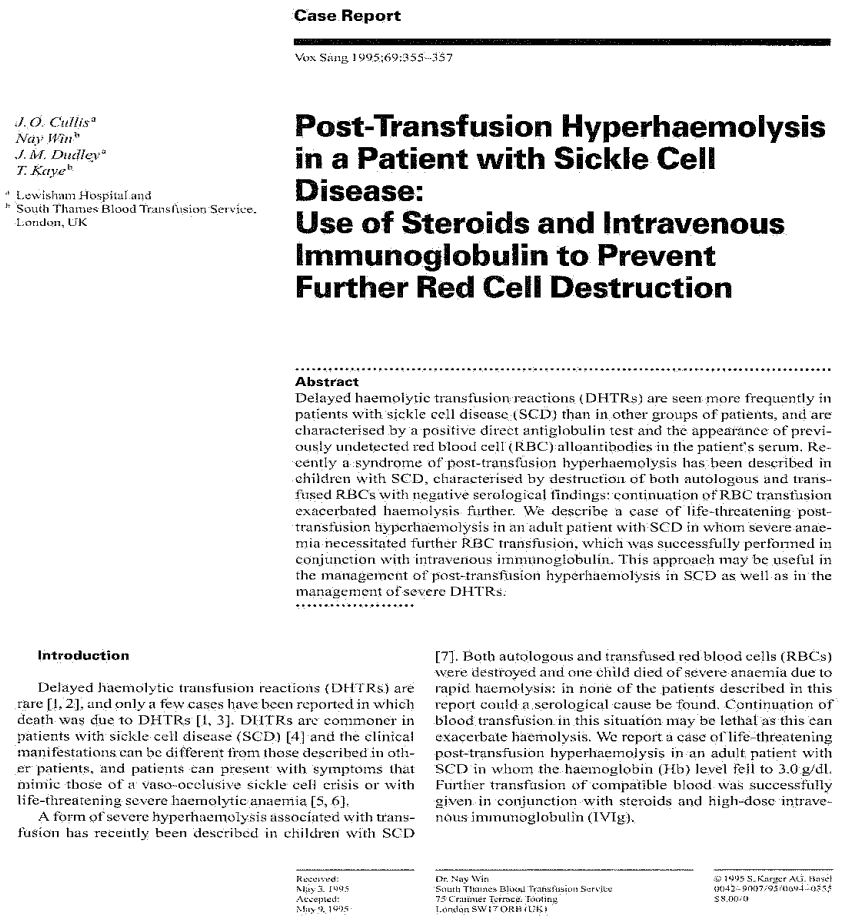

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**Post-Transfusion Hyperhaemolysis in a Patient with Sickle Cell Disease: Use of Steroids and Intravenous Immunoglobulin to Prevent Further Red Cell Destruction**

**Abstract**

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**Introduction**

Delayed haemolytic transfusion reactions (DHTRs) are rare [1, 2], and only a few cases have been reported in which death was due to DHTRs [1, 3]. DHTRs are commoner in patients with sickle cell disease (SCD) [4] and the clinical manifestations can be different from those described in other patients, and patients can present with symptoms that mimic those of a vaso-occlusive sickle cell crisis or with life-threatening severe haemolytic anaemia [5, 6].

A form of severe hyperhaemolysis associated with transfusion has recently been described in children with SCD [7]. Both autologous and transfused red blood cells (RBCs) were destroyed and one child died of severe anaemia due to rapid haemolysis; in none of the patients described in this report could a serological cause be found. Continuation of blood transfusion in this situation may be lethal as this can exacerbate haemolysis. We report a case of life-threatening post-transfusion hyperhaemolysis in an adult patient with SCD in whom the haemoglobin (Hb) level fell to 3.0 g/dl. Further transfusion of compatible blood was successfully given in conjunction with steroids and high-dose intravenous immunoglobulin (IVIg).

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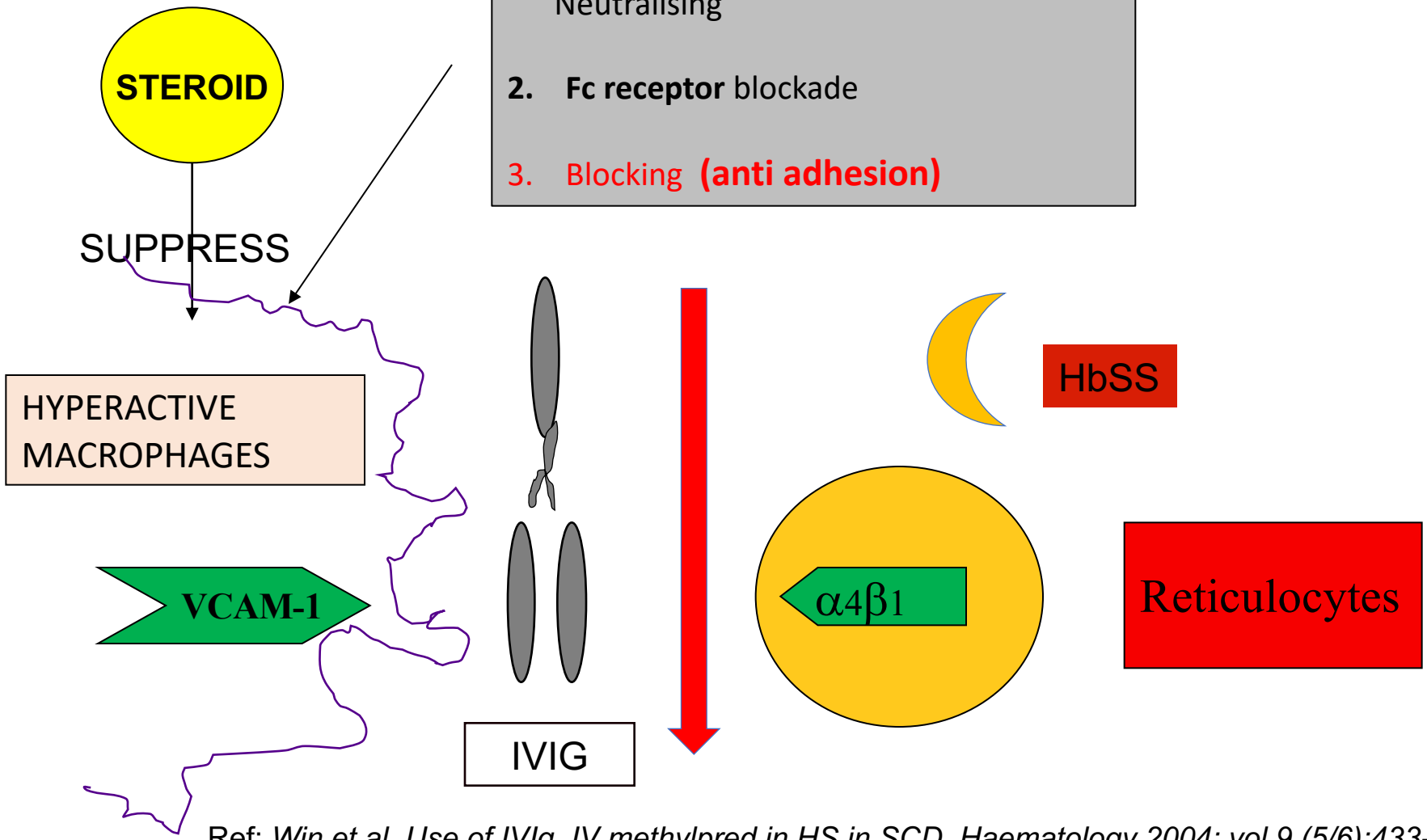
(Possible mechanism ) Use of IV Methylpred and IVIG in HS in SCD (Win et al 2004). *Haematology* 9,433-436.

# Possible Mechanism of Steroid / IVIG Therapy

1. IVIG Immunomodulation
   Produce IL-1 receptor antagonist
   Neutralising

2. **Fc receptor** blockade

3. Blocking **(anti adhesion)**




Ref: *Win et al. Use of IVIg, IV methylpred in HS in SCD. Haematology 2004; vol 9 (5/6):433-436.*

# IVIG Guidelines/Recommendations (2007 / 2011 / 2017)

1) IVIG may be considered among the options for treatment of serious, life threatening *HTRs*
*Ref: Guidelines on use of IVIG for Hematologic Conditions: Anderson et al (Trans Med Review vol 21, No 2, Suppl1 (April) 2007 ppS9-S56) IVIG Haematology and Neurology Expert Panels, Canada.*

2) IVIH has been used successfully in combination with corticosteroids (Post-transfusion Hyperhaemolysis).
*Ref: UK Guidance Clinical guidelines for immunoglobulin use (*https://www/gov.uk/government/publications/clinical-guidelines-for-immunoglobulin-use-second-edition-update) *(2nd edition: 15 Nov 2011)*

3) British Society of Haematology: Davis B.A., et al. (2017) Guidelines on red cell transfusion in sickle cell disease. Part 1: principles and laboratory aspects. *British Journal of Hematology*, **176**, 179-191.

# Management

Avoid further transfusion: even transfusion with crossmatched compatible antigen neg units may further exacerbate haemolysis: prolong the course: even death.

Rapid severe haemolysis patient might require additional transfusion.

Combined IVIG/steroids therapy

a) IVIG and steroid cover should be given the same time.
   IVIG 0.4g/kg/day for 5 days or
   1g/kg/day x 2 days
b) IV methyprednisolone 0.5 to 1G/day x 2 days
   4mg/kg (paediatric patients) x 2 days.
   Response to therapy takes about 4 to 5 days.

Ref: British Society of Haematology: Guideline 2017

1) Cullis, J O., Win N, Dudley J M, Kaye T. (1995) Post-Transfusion Hyperhaemolysis in a patient with Sickle Cell Disease: Use of Steroids and IVIg to Prevent Further Red Cell Destruction. *Vox Sang,* 69:355-357.
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3) Win, N., Tullie, Y., Needs, M., Chen, F.E., Okpala, I. (2004) Use of IVIg and IV methylprednisolone in hyperhaemolysis syndrome in sickle cell disease. *Haematology,* **9**, 433-436.
4) Win, N., New, H., Lee, E., De La Funete, J. (2008) Hyperhemolysis syndrome in sickle cell disease: case report (recurrent episode) and literature review. *Transfusion*, **48**, 1231-1238.
5) Win, N., Sinha, S., Lee, E., Mills, W. (2010) Treatment with IVIg/steroids may correct severe anemia in hyperhemolytic transfusion reactions: case report and literature review. *Transfusion Medicine Reviews*, **24,** 64-67.
6) Win, N., Lee, E., Needs, M., Chia, L.W., Stasi, R. (2012) Measurement of macrophage marker in hyperhaemolytic transfusion reaction: a case report. *Transfusion Medicine*, **22**, 137-141.
7) Win, N., Lee, E., Needs, M., Homedia, S., Stasi, R. (2014) Profound sustained reticulocytopenia and anaemia in an adult patient with sickle cell disease. *Transfusion Medicine*, **24**, 418-420.

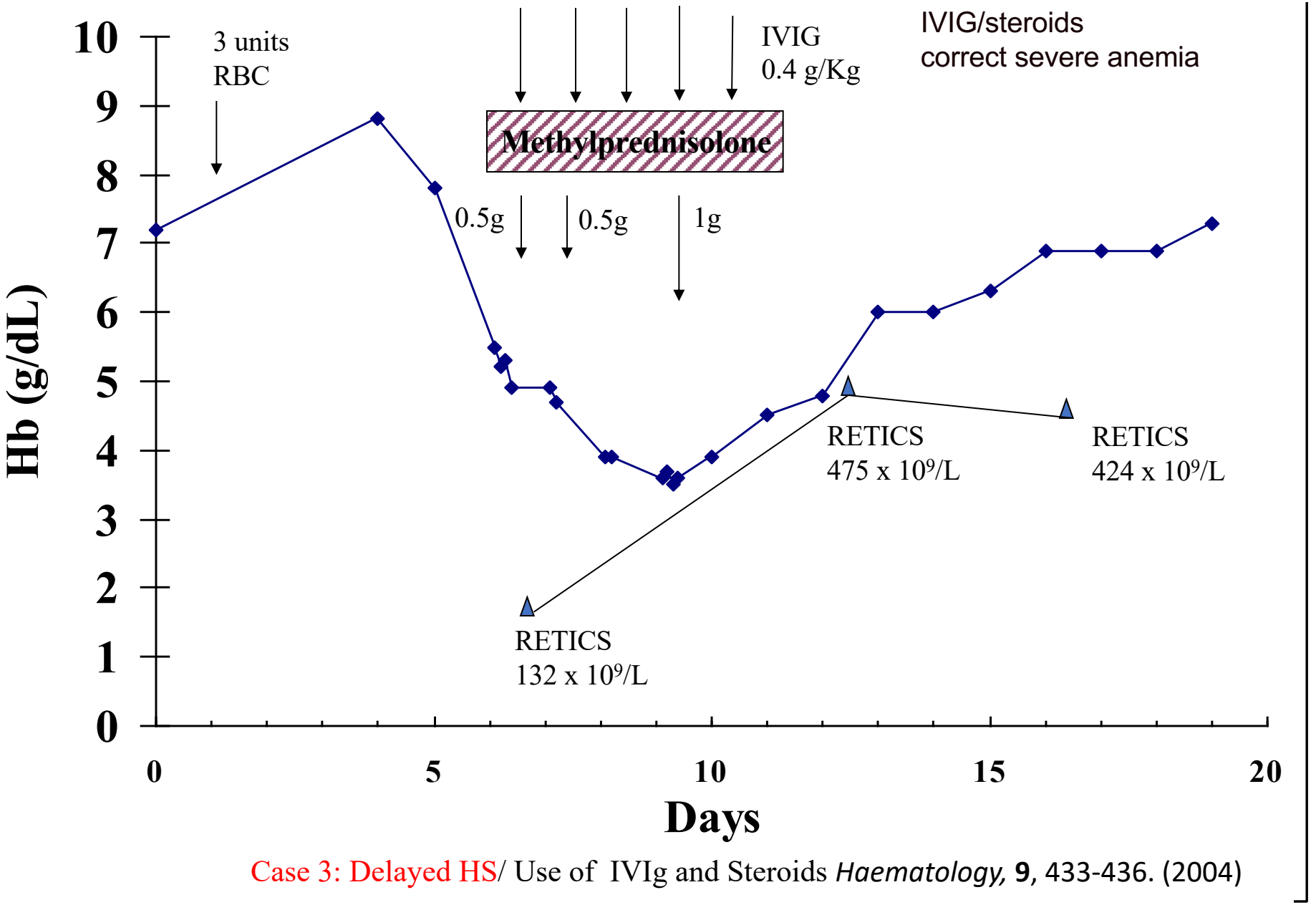
# Hb and reticulocyte Response to IVIG

From our institution we have previously reported 9 patients[1-7]
(six acute and three delayed form) in seven different publications.

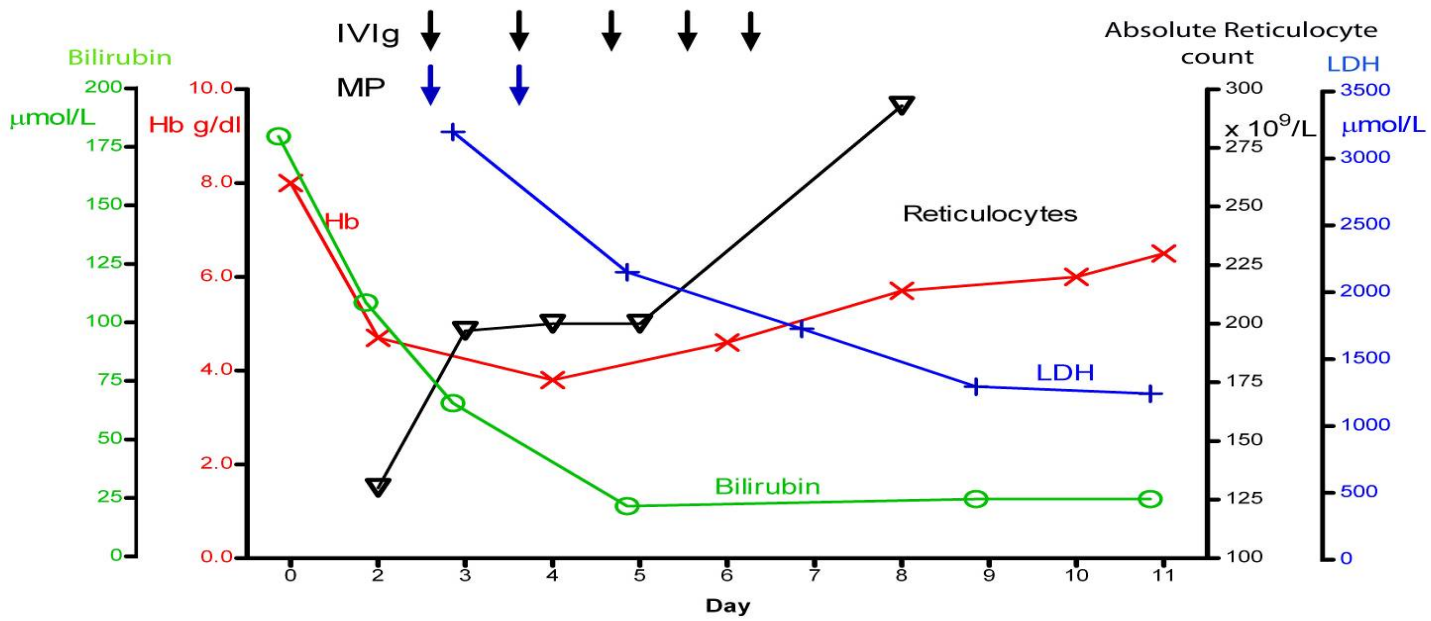
In 7 patients, response was achieved within 4/5 days of therapy
(increase in reticulocyte count together with a rise in Hb level).
No 8: (Paediatric patient) presented with recurrent PTHS[4] and additional course of IVIG/steroids was given; response after day four of therapy.
No 9: Presented with parvo induced red cell aplasia followed by HS after Transfusion.[7]

Transfusion support:
7 patients required additional transfusion with IVIG/steroids cover and in 2 delayed form, transfusion was avoided.[3,5]

Erythropoietin (Epo) Not standard therapy
Only prescribed in two patients with indication.
One already on Epo for chronic renal failure.[2]
Another due to parvo induced red cell aplasia.[7]




Case 3: Delayed HS/ Use of IVIg and Steroids *Haematology,* **9**, 433-436. (2004)




Changes of **Hb level**, absolute retic, **bilirubin** and **LDH**: treatment with IVIG/IVMP.

Case 5: Delayed form HS Win et al: Treatment with IVIG/steroids may correct severe anemia in HTR *Trans Med Rev, V*ol 24, No1, 2010:64-67.

# Response to IVIG/Steroids

Danee et al[17] reported 8 cases of PTHS with SCD from their institution (6 acute form and 2 delayed HHS).
Prescribed a high dose (1g/kg/day for 2 days) + steroids (a total dose of 2g/kg).
There were no adverse events and concluded that
”the combination of IVIG and steroids has always been successful in halting haemolysis thus far, only two patients require additional transfusion, no need to use additional immunosuppressive medication.”

Ref: Danaee, A., Inusa, B., Howard, J., Robinson, S. (2015) Hyperhemolysis in patients with hemoblobinopathies: a single-center experience and review of the literature. *Transfusion Medicine Review*, **29**, 220-230.

## Classical DHTR may transformed into HS if Macrophages are activated

DHTR can be prevented by IVIg / steroids after transfusion of incompatible RBC units

Ref: Transfusions of least-incompatible blood with intravenous immunoglobulin plus steroids cover in two patients with rare antibody. **Win N,** Needs M, Thornton N, Webster R, Chang C.
*Transfusion*, 2018, http://dx.doi.org/10.1111/trf.14648.

Prevention of haemolytic transfusion reactions with intravenous immunoglobulin prophylaxis in U- patients with anti-U. **Win N,** Almusawy M, Fitzgerald L, Hannah G, Bullock T.
*Transfusion*, 2019; Vol 59, **6**: 1916-1920.

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- Immunostain (CD68) was also used.

Thal intermedia pregnant patient: not responding to 2 courses of IVIG/steroids/Rituximab ends up with splenectomy. Spleen tissue histopathology showed as above.

Ref: Win N**,** Lucas S, Hebbali S, McKernan A, Hamilton R, Robinson I, Chen F. Histopathological evidence for macrophage activation driving post-transfusion hyperhaemolysis syndrome. *British Journal of Haematology*, 2019, **doi: 10.1111/bjh.15925**.

If not responding to first line therapy (IVIg/Steroids) what next?

# SHOT report in UK HHS

Since HHS has been well established as a separate syndrome in UK, Serious Hazard Of Transfusion (SHOT, UK), an independent haemovigilance scheme, has been collecting data on PTHS.[58]

Between 2010-2017, 30 cases reported, one patient had a recurrent PTHS.

The first fatal case of PTHS in the UK (a 10-year-old child with SCD) was reported in the 2010 SHOT report.[3]

There were 6 cases reported in 2017, 5/6 symptoms improved with IVIG/methylprednisolone therapy but one patient died of acute chest syndrome.

Although majority response to IVIG/steroids therapy patient may die of severe rapid acute haemolysis as described in SHOT report.

If not responding to first line therapy (IVIg/Steroids) what next?

# It’s a New Day: Collaborative medicine saves sickle cell patient

Pictured: Air Force Capt. (Dr) Lauren Lee, hematology/oncology fellow at Brooke Army Medical Center, reflects on the care BAMC staff members provided Alexis Piper, a patient with sickle cell anemia, who nearly died from a rare condition called hyperhemolysis. Hyperhemolysis syndrome is a potentially fatal transfusion complication. Many BAMC staff members collaborated to find a treatment for the life-threatening condition.

https://www.airforcemedicine.af.mil/News/Display/Article/1789917/its-a-new-day-collaborative-medicine-saves-sickle-cell-patient/

Brooke Army Medical Centre Public Affairs/Published March 20, 2019

# It’s a New Day: Collaborative medicine saves sickle cell patient

Air Force Master Sgt. Antwan Piper looks at his wife, Alexis, during a video interview about her disease and her recovery at Brooke Army Medical Center. Alexis Piper has had sickle cell anemia since she was 7 years old. She nearly died in 2018 after developing a rare condition called hyperhemolysis. Hyperhemolysis syndrome is a potentially fatal transfusion complication.


https://www.airforcemedicine.af.mil/News/Display/Article/1789917/its-a-new-day-collaborative-medicine-saves-sickle-cell-patient/

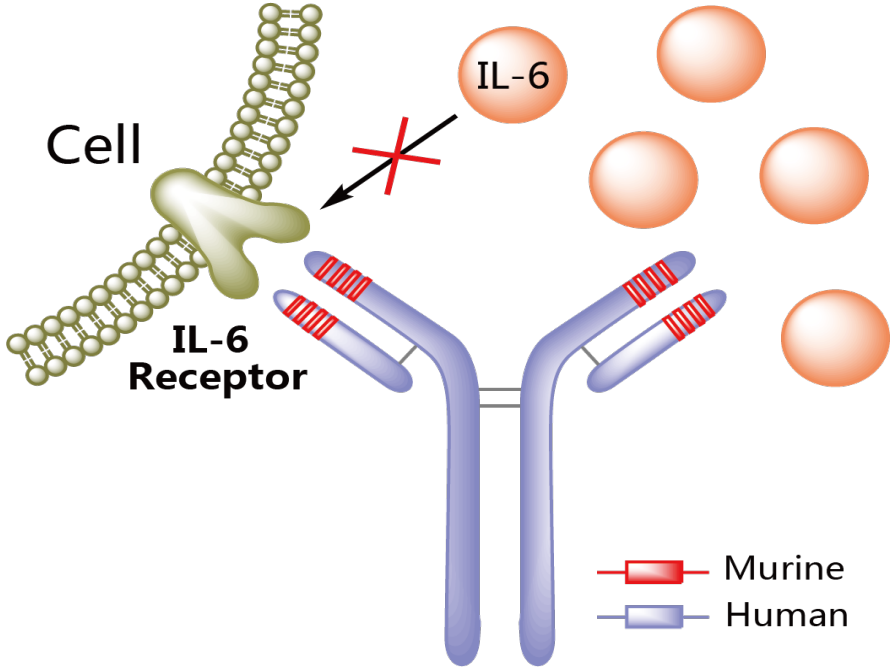
## It’s a New Day: Collaborative medicine saves sickle cell patient

“Lee began researching other treatment options. She contacted an expert in England, Dr Win Nay, who had published extensive research about sickle cell patients with hyperhemolysis……”

## Case Summary

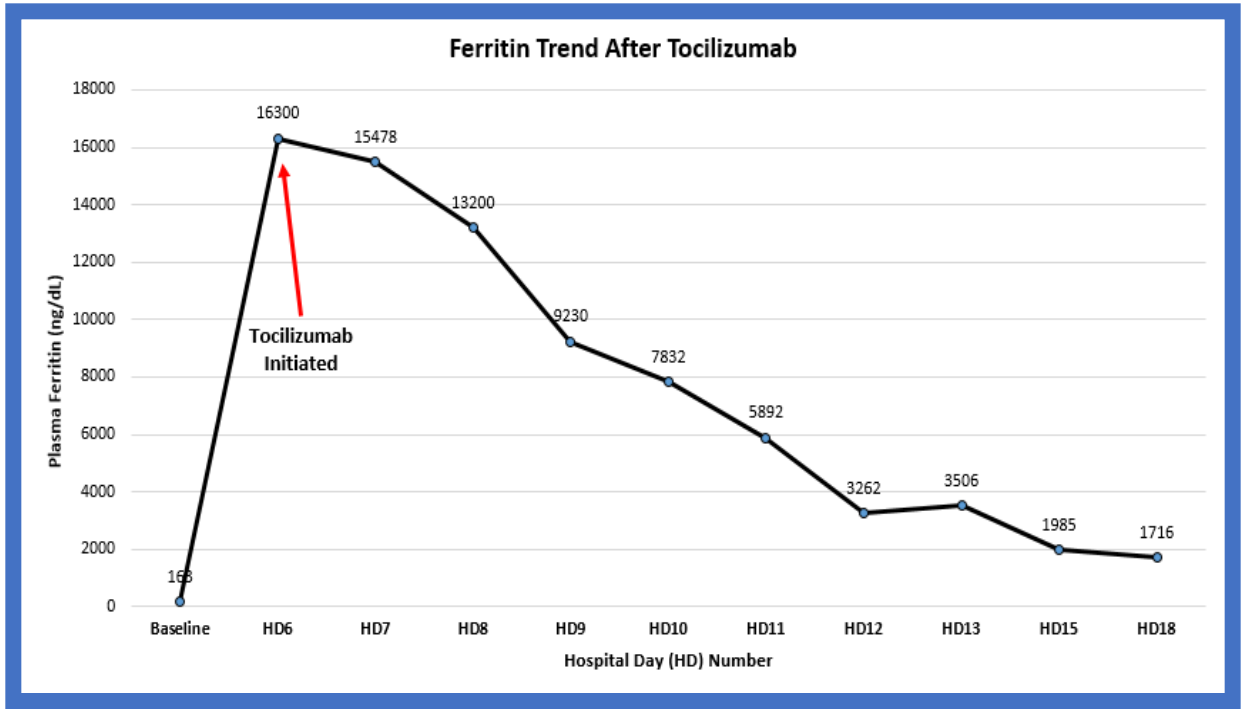
- **Day 1** 36 yr old HbSS SCD Hb 55g/L with (anti-e/anti-Fya/anti-C and anti-K) 2 Units transfused
- **Ferritin 157 ng/ml (range 13-150)** Post Transfusion Hb level 88g/L
- **Day 6** admitted with fever, joint pain
- **Day 8** Hb dropped to 51g/L, reticulocytopenia recorded
- PTHS suspected and commenced (IVIG)x 5days and steroids Methyl Pred 1 g/day
- **Day 10** Hb 33g/L and **Day 11** Hb 21 g/L
- On Day 10 received e-mail from LL. Advice: to check Ferritin (Ferritin 100 fold increased 16,300 ng/ml) Macrophage activation is associated with hyperferritinemia.
- **Tocilizumab** humanized **monoclonal antibody**, against **IL-6 receptor**; bindings of Toxilixumab to receptor prevents cytokine IL6 from exerting pro-inflammatory.
- Tocilizumab has been successfully used in Macrophage Activation Syndrome.
- Hemoglobin-based oxygen carriers, such as **HBOC-201**, a **purified polymerized bovine hemoglobin** (Hb) was also prescribed in this case.

# Tocilizumab



Lee, L.E., Beeler, B.W., Henderson, A.T., Graham, B.C., Osswald, M.B., Win, N., Cap, A.P. (2018) Targeting Macrophage Activation in Hyperhemolysis Syndrome with Novel Use of Tocilizumab. *Blood.*
doi: https://doi.org/10.1182/blood-2018-99-110529

## RESULTS



# Another / second case treated with Tocilizumab

- **Day 1**: 33 yr old HbSS SCD Hb 63g/L (DAT neg / No antibody)12 Units automated exchange transfusion Post Hb 12g/L. In view of the past history of recurrent HS (twice) she was prescribed IVIG 0.5g/kg/day for 5 days and IV methylpred 500mg x 3days from **day one.**
- **Ferritin 340 ng/ml (range 13-150) Day 5 and Day 8 Hb** dropped to 97g/L and 47g/L.
- **Day 12** Hb dropped to 32 g/L, reticulocytopenia recorded.
- **Tocilizumab started 8mg/kg x 2 days/monitored.**
- **On Day 14 (2 days post treatment)**
- Ferritin (Ferritin dropped from 18,342 to 9923 ng/ml); reticulocyte count rose from 128 to 414x $10^9$ /L.
- Optimal dose not known (for Cytokine Storm/macrophage activation) the recommended dose is 4 days. Decided to withhold further dose.

Article in print: **Treatment of Post Transfusion Hyperhaemolysis syndrome in Sickle Cell Disease with the anti-IL6R humanised monoclonal antibody Tocilizumab**

Authors: Sivapalaratnam, S; Linpower, L; Agapidou, A; Jain, S; **Win N**, Tsitsikas D A.

*British Journal of Haematology*

Doi.10.1111/bjh.16103

bjh correspondence

## Treatment of post-transfusion hyperhaemolysis syndrome in Sickle Cell Disease with the anti-IL6R humanised monoclonal antibody Tocilizumab

Post-transfusion hyperhaemolysis syndrome (PTHS) is a rare post-transfusion reaction seen primarily in patients with sickle cell disease (SCD). It is characterised by brisk haemolysis of both transfused and recipient red cells, also known as 'By-stander' haemolysis, with a haemoglobin (Hb) drop below pre-transfusion levels, marked increase in serum ferritin (SF) and a fall in reticulocyte count. This is a potentially life-threatening complication as it can lead to severe anaemia while further transfusion may further aggravate haemolysis and should only be used as a last resort (Win 2019). The pathophysiology of PTHS is still poorly understood. 'By-stander' haemolysis, which is probably complement mediated, and suppression of erythropoiesis have been implicated while there is increasing evidence that macrophage activation and direct haemophagocytosis plays a key role (Win *et al*, 2019). Standard treatment in severe cases is with intravenous immunoglobulin (IVIG) 0·4 g/day for five days and high dose steroids (intravenous methylprednisolone 0·5 g/day for two days) (Win *et al*, 2008).

Tociluzimab is a humanised monoclonal antibody against soluble and membrane bound interleukin 6receptor (IL6R). The binding of tociluzimab to IL6R prevents the cytokine IL6 from exerting its pro-inflammatory effect. It is currently approved for the treatment of various autoimmune conditions, such as rheumatoid arthritis, systemic juvenile idiopathic arthritis and giant cell arteritis (https://www.nice.org.uk/guidance/ta247, https://www.nice.org.uk/guidance/ta238, https://www.nice.org.uk/guidance/TA518) and has been successfully used in the management of macrophage activation syndrome (Watanabe *et al*, 2016). In PTHS the hypothesis is that macrophage activation results in phagocytosis of patient and donor reticulocytes and mature red blood cells. There is some aneclotal evidence that tociluzimab may have a potential role in the management of PTHS, especially when resistant to standard measures, none of which has been published as a peer reviewed paper (Lee *et al*, 2018).

We report a case of PTHS treated with tociluzimab in a 33-year-old man with homozygous sickle cell disease (HbSS). The patient had a history of recurrent episodes of the acute chest syndrome (ACS) and also two previous episodes of PTHS following exchange transfusion for treatment of ACS. He was on treatment with hydroxycarbamide, varying from 1·5 to 2·0 g/day, for ACS prevention, but compliance was poor.

On this occasion, he was admitted to hospital with a painful crisis accompanied by type I respiratory failure and diffuse bilateral chest x-ray infiltrates, diagnostic of a new episode of ACS. He received an emergency automated red cell exchange transfusion (ARCET) with 12 units of red cells. His haemoglobin (Hb) and HbS percentage pre- and post-ARCET were 63 g/l and 93% and 120 g/l and 26% respectively.

Because of the previous two episodes of PTHS he was preemptively treated with IVIG 0·4 g/kg/day for five days and methyprednisolone 500 mg for 3 days, both commencing on the day of the exchange transfusion. Despite this, his Hb dropped sharply, from 97 g/l on Day 5 post-ARCET to 47 g/l on Day 8, and his haemolytic markers, such as bilirubin and lactate dehydrogenase (LDH), started increasing as did his serum ferritin. He had no detectable allo-antibodies and his direct antiglobulin test was negative. His Hb continued to drop (nadir 32 g/l on Day 12 post-ARCET). As he had been nursed in the intensive care unit from the beginning of his admission, ensuring very close monitoring, further transfusion was withheld with a view to be undertaken immediately as soon as he displayed any signs of haemodynamic compromise. Instead, he was treated with tocilizumab 8 mg/kg intravenously once daily for two days, which was not associated with any adverse reactions.

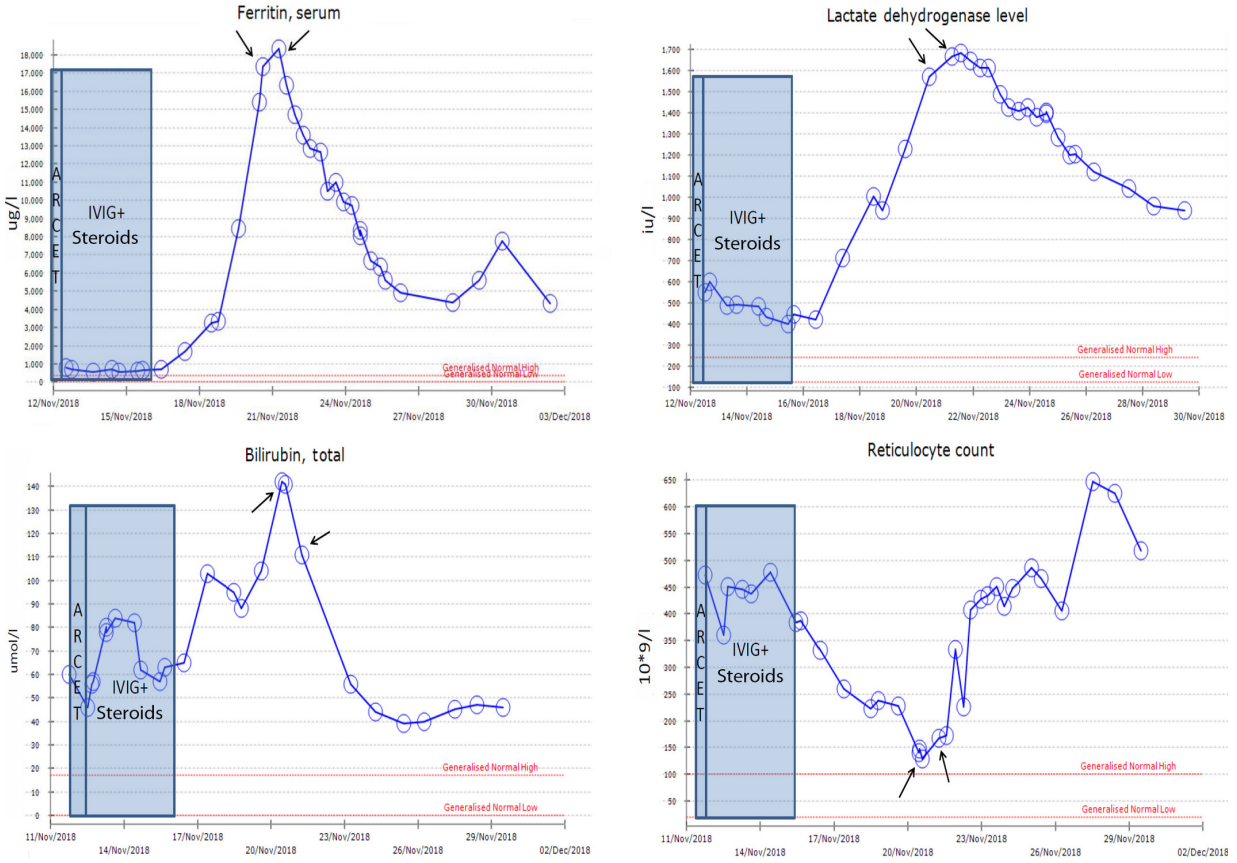
The patient's urine, previously deeply discoloured, appeared clear the morning after the first dose of tociluzimab. On day 2 after starting treatment with tocilizumab, his reticulocytes had increased, from 128 × $10^9$/l to 414 × $10^9$/l, SF had decreased, from 18 342 to 9923 μg/l, and his haemolytic markers immediately started to improve (Fig 1). There was no further drop in his Hb. His serum IL6 level measured before treatment was 140 pg/ml (reference range <7 pg/ml), which rose to 1400 pg/ml after the first dose and 3870 pg/ml after the second dose. We assume these results reflect effective blockade of the IL6R with subsequent increase in unbound circulating IL6.

The time for Hb recovery from its nadir value to the patient's baseline was not shorter than the previous two episodes of PTHS suffered by this patient. However, it should be noted that haematological and biochemical parameters



doi: 10.1111/bjh.16103

Response of serum ferritin, lactate dehydrogenase, bilirubin and reticulocyte count after two doses of tocilizumab (**arrows**). ARCET, automated red cell exchange transfusion; IVIG, intravenous immunoglobulin



# What next?

First line of therapy should be IVIG/steroids which is effective in both the acute and delayed form for HS.
No need of Epo therapy.

Preliminary reports demonstrated Tocilizumab was well tolerated with no adverse events.

PTHS is a potentially life threatening complication and Tocilizumab may be an important adjunct to its management.

Need further clinical trial to further assess safety, efficacy and to determine the optimal dose.

**Acknowledgement to**

all of the colleagues who referred cases to me

and Dr Fred Chen (Royal London Hospital/ Barts Health NHS Trust)

# Thank you

Questions?