How we treat delayed haemolytic transfusion reactions in patients with sickle cell disease

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Summary

Transfusion therapy is effective in the prevention and treatment of many complications of sickle cell disease (SCD). However, its benefits must be balanced against its risks, including delayed haemolytic transfusion reactions (DHTR). Not only is the relative rate of alloimmunization higher in patients with SCD than in other patient populations, but attendant risks associated with DHTR are even greater in SCD. Clinicians’ awareness of DHTR events is poor because symptoms of DHTR mimic acute vaso-occlusive pain and immunohaematology findings are often negative. Transfusions delivered in the acute rather than elective setting appear to confer a higher risk of DHTR. Management of DHTR in SCD depends on the clinical severity, ranging from supportive care to immunosuppression, and optimization of erythropoiesis. DHTR must be considered in any recently transfused patient presenting with acute sickle cell pain. Meticulous documentation of transfusion and immunohaematology history is key. We anticipate an increase in DHTR events in SCD patients with the increasing use of red blood cell transfusion therapy.

Keywords: delayed haemolytic transfusion reactions, hyperhaemolysis, vaso-occlusive crisis, alloantibodies, sickle cell disease.

Blood transfusion is the most effective treatment for the prevention and management of many acute and chronic complications of sickle cell disease (SCD), including primary and secondary stroke prevention, acute management of stroke, acute chest syndrome (ACS), splenic sequestration and acute multi-organ failure (Adams et al, 1998; DeBaun et al, 2014; National Institutes of Health: National Heart Lung and Blood Institute 2014). The beneficial effects of transfusion therapy observed in recent clinical trials are expanding their indication and utilization, contributing to an increased use of blood (Drasar et al, 2011; Steinberg, 2014). Nonetheless, transfusion is not without risks; haemolytic transfusion reactions and iron overload are common side effects, while transmission of infections remains a significant issue in some countries. Alloimmunization to red blood cell (RBC) antigens is a major complication of transfusion and the underlying cause of the majority of delayed haemolytic transfusions reactions (DHTR). DHTR occurs from 24 h up to 21 d after a transfusion. It is a secondary immune phenomenon, typically arising after recrudescence of an alloantibody to which the patient had been immunized, but which later evanesced and became undetectable by the standard compatibility tests. However, particularly in the setting of SCD, new alloantibodies are not always detected in the plasma or eluate of the patients with an otherwise typical DHTR, invoking other pathological mechanisms. A key challenge is that DHTR in SCD is under-recognized, not only because it mimics the clinical events in acute vaso-occlusive crisis (VOC), but because serological markers of alloimmunization are often equivocal. Furthermore, DHTR itself often triggers VOC in SCD, which can lead to life-threatening complications. The first DHTR cases in SCD were probably documented by Diamond et al (1980), who described four patients with DHTRs presenting as sickle cell crises. Diamond emphasized then, that DHTR should be considered whenever a recently transfused patient with SCD presents with VOC-type symptoms.

There is a current lack of evidence in the area to inform best practice. Here, we present five real-life case scenarios that highlight the risks and challenges in the management of DHTRs.

Cases

Case 1

A 54-year-old multiparous British woman of African-Caribbean origin with sickle cell anaemia (HbSS) and a
previous history of ACS requiring ventilator support and RBC immunization (anti-Jk^k, -McC^a, -C and –E and a warm reacting anti-HI) had a historical DHTR treated with intravenous (IV) steroids. She repeatedly declined hydroxychloroquine therapy. She presented with acute pain typical of a VOC, chest and back pain. He had had a previous DHTR episode with a history of anti-Fya antibody. A chest radiograph demonstrated left basal consolidation and a small pleural effusion. He was initially treated with IV antibiotics, but because of increasing oxygen requirements he was given an exchange RBC transfusion (total 13 RBC units exchanged), and subsequently transferred to critical care for non-invasive ventilator support. Pain control was - unusually difficult to manage and required patient-controlled analgesia (PCA). After achieving some respiratory improvement, he deteriorated 5 d after his initial transfusion, with increasing oxygen requirements, fevers, haemoglobinuria and a recurrence in his pain. Blood samples were grossly haemolysed with severe anaemia (Hb 39 g/l, baseline 95 g/l), relative reticulocytopenia (102 × 10^9/l, baseline 111 × 10^9/l), and rise in LDH (3664 iu/l, baseline 422 iu/l). After receiving three units of RBCs, the patient developed haemoglobinuria with a further drop in the post-transfusion Hb level to 61 g/l after 5 d, this time with a reticulocytosis (789 × 10^9/l). A DHTR was suspected but the DAT was negative. She remained haemodynamically stable, reticulocytes continued to recover and a decision was made to withhold transfusion of RBC. Her Hb recovered without further supportive therapy. Subsequent DATs were negative until 17 d post-transfusion only; all RBC alloantibody screens, as well as RBC + DAT was negative with no new antibodies identified. He was transfused a further six units of RBCs under immunosuppressive cover, which included a total of 2 kg IVIg and one dose of IV methylprednisolone. A rapid improvement was seen. To date, he has not required subsequent blood transfusions (Fig 1B).

Case 3
A 52-year-old Jamaican woman with HbS/β^0 thalassaemia received a two unit pre-operative RBC transfusion 6 d prior to an elective hysterecomy. Surgery was uneventful with minimal blood loss and she was discharged after 48 h. On post-operative day 9 (15 d post-transfusion), she re-presented as an emergency with fever and abdominal pain. IV antibiotics were commenced and the patient was admitted for presumed post-operative sepsis. Laboratory results demonstrated a fall in Hb to 65 g/l (baseline 86 g/l), relative reticulocytopenia (210 × 10^9/l, baseline 447 × 10^9/l), and rise in LDH (3664 iu/l, baseline 422 iu/l). After receiving three units of RBCs, the patient developed haemoglobinuria with a further drop in the post-transfusion Hb level to 61 g/l after 5 d, this time with a reticulocytosis (789 × 10^9/l). A DHTR was suspected but the DAT was negative. She remained haemodynamically stable, reticulocytes continued to recover and a decision was made to withhold transfusion of RBC. Her Hb recovered without further supportive therapy. Subsequent DATs were negative until 17 d post-preoperative (initial) transfusion / 2 d post-second transfusion (IgG 1+ only); all RBC alloantibody screens, as well as RBC eluate analysis, were negative.

Case 4
A 56-year-old British woman of Jamaican origin with HbSC presented with left sided weakness, dysphagia and headache. Magnetic resonance angiography of the head demonstrated infarction in the territory of the right posterior inferior cerebellar artery. She was given a RBC exchange transfusion; Hb increased from 102 to 111 g/l post-exchange (baseline Hb 108 g/l), and total HbS+HbC from 93.3% to 11.8%. Neurological symptoms swiftly improved post-exchange transfusion to allow discharge after 10 d. She re-presented 8 d post-transfusion with a VOC (abdominal and back pain), plus haemoglobinuria and fever. Her Hb had dropped significantly to 77 g/l, LDH was elevated at 1563 iu/l (baseline 419 iu/l) and total bilirubin 42 μmol/l (baseline bilirubin 9 mol/l).
Fig 1. Sequence of events and intervention in the three case scenarios. (A) Case 1: 54-year-old African British woman with HbSS. As the patient continued to haemolyse, rituximab was added as third line immunosuppression. At the peak of haemolysis, the patient developed reticulocytopenia of $59.9 \times 10^9/l$ (from a baseline of $178.9 \times 10^9/l$); (B) Case 2: 18-year-old West African man with HbSS; (C) Case 3: 52-year-old Jamaican woman with HbS/b0 thalassaemia. Hb, haemoglobin; DAT, direct antiglobulin test; LDH, lactate dehydrogenase; H, total haemoglobin A; HbS%, haemoglobin S percentage of total haemoglobin; Retics, reticulocytes; RBS, red blood cell; EPO, erythropoietin; IV, intravenous; IVIg, intravenous immunoglobulin.
13 μmol/l). Immunohaematology results demonstrated a positive DAT (IgG 3+ and C3d 1+) with a new anti-S antibody. She was haemodynamically stable, and was given one dose of IV methylprednisolone 500 mg. Her reticulocyte count initially remained at baseline (but subsequently increased to 493 × 10⁹/l (from 160 × 10⁹/l)) with Hb improvement, allowing discharge after 1 week. A decision was taken to initiate a post-stroke exchange transfusion programme with S-negative blood. Subsequent blood transfusions have been uneventful, not only in the elective exchange setting, but also in acute settings, including an admission with a large subdural haematoma.

Case 5

A 14-year-old African-American female with HbSS, presented with acute vaso-occlusive pain localized to the chest and abdomen. PCA led to resolution of the chest pain, but no improvement in the abdominal pain. By day 5, the Hb level had decreased from a baseline of 90 g/l to 70 g/l; she was transfused RBC with an appropriate rise in Hb to 99 g/l. Six days after transfusion, she developed fever and worsening abdominal pain with a drop in Hb to below pre-transfusion levels (57 g/l). The indirect antibody test (IAT) and DAT were negative and she received a second RBC transfusion with only a modest increment in Hb to 70 g/l followed by a rapid decline to 45 g/l; LDH increased to 11,244 iu/l and Hb A was not detected by Hb electrophoresis, findings consistent with a DTHR. Methylprednisolone (2 mg/kg/d) and erythropoietin (750 u/kg/d) were initiated and she received one dose of IV Ig (1 g/kg). Her Hb continued to decline to a nadir of 30 g/l, serum LDH rose to 22,270 iu/l, and plasma Hb was 4.68 g/l. Repeat IAT remained negative, but polynegative DAT was weakly positive. However, no alloantibody was identified by elution. The patient’s clinical status rapidly deteriorated with development of hypertension, renal failure, overt pancreatitis and consumptive coagulopathy. She became unresponsive with a fixed dilated right pupil; a head CT scan revealed a large right temporo-parietal epidural haematoma with midline shift, requiring neurosurgical evacuation. An interval CT, 24 h later, showed a new right occipital epidural haematoma, requiring a second neurosurgical procedure. A total of 11 units of RBC were transfused in the perioperative period. Despite 2 weeks of treatment with daily methylprednisolone and erythropoietin, the patient remained anaemic. A trial of plasma exchange followed by RBC exchange resulted in a sustained rise in Hb levels. Methylprednisolone was tapered off and the patient discharged 5 weeks later, with remarkable recovery of her physical and cognitive function. The patient continues to receive monthly exchange RBC transfusions with no further complications. No alloantibodies were ever detected in this patient. In the elective setting she was able to receive RBC transfusion without complications.

Epidemiology and risk factors for DTHR

Alloimmunization and haemolytic transfusion reactions (HTRs) are seen in all transfused populations. In a prospective study of a non-SCD population, alloimmunization occurred in 2.6% of recipients with no previous history of alloimmunization, but 8.9% of those with previously detectable antibodies developed additional alloantibodies (Heddle et al, 1995). However, despite this high frequency of alloimmunization, only 0.05% had clinical evidence of DTHR. In SCD, rates of alloimmunization range from 7 to 49% (Vichinsky et al, 1990; Garratty, 1997; Aygun et al, 2002; Castro et al, 2002; Lasalle-Williams et al, 2011; Wahl et al, 2012; O’Suoi et al, 2013). Clinically-significant DTHRs have been reported in 4–11% of patients with SCD who receive transfusions (King et al, 1997; Talano et al, 2003; de Montalembert et al, 2011; Vidler et al, 2015). The antigens most frequently involved in alloimmunization in SCD belong to the Rh and Kell blood groups, followed by Kidd, Duffy, Lewis and MNS blood group systems (Rosse et al, 1990; Vidler et al, 2015). Despite Rh and Kell antigen matching of transfused units, immunization to these antigens remains high (Chou et al, 2013; Mijovic et al, 2013).

It has been suggested that one possible reason for the relatively high incidence of alloimmunization observed in the SCD population is the mismatch in the RBC antigens expressed in patients of African descent and donors of primarily Northern European descent. Alloimmunization rates are lower in patients who are transfused RBC units from ethnically similar donors [e.g., in Ugandan (Natukunda et al, 2010) and Jamaican (Olujohungbe et al, 2001) populations] but the lower alloimmunization rate in SCD populations in these countries may also be related to an overall lower exposure to RBC transfusion. In the US, using a strategy of purposefully sourcing black donors has reduced HTR rates significantly lower than expected, even after accounting for total number of RBC units transfused (Rosse et al, 1990). Second, females are also at increased risk, owing in part to increased RBC antigen exposure in pregnancy (Reisner et al, 1987; Schonewille et al, 2015 John Wiley & Sons Ltd, British Journal of Haematology.
Pathophysiology

The key cause of DHTR is a recipient’s production of alloantibodies against the donor’s RBC antigens after transfusion. These alloantibodies may be either new antibodies, or evanescent antibodies that were undetectable prior to transfusion, but where re-exposure to a RBC antigen triggered an anamnestic antibody response and activation of complement (Stowell et al., 2012). Antibody evanescence, a phenomenon crucial to the pathogenesis of DHTR in general, has been reported for 37–51% of antibodies detected in the series reported by Rosse et al. (1990) and Harm et al. (2014). The latter group reported that in 63.6% of alloimmunized patients, one or more alloantibodies evanesced; evanescing antibodies were found in all blood group systems.

We use DHTR as an umbrella term, including cases without serological confirmation of a new alloantibody, but with unequivocal evidence of marked haemolysis following a blood transfusion within the given timeframe. The terms ‘hyperhaemolytic’ transfusion reaction (HHTR) and “hyperhaemolysis” syndrome are often used to describe cases of more severe haemolysis, where Hb drops below pre-transfusion levels due to destruction of both autologous and transfused RBCs. The first cases of non-sickle HHTR were described over 50 years ago (Stewart & Mollison, 1959; Heidt et al., 1960; van der Hart et al., 1963; Davey et al., 1980) with subsequent cases in SCD (King et al., 1997; Petz et al., 1997). Petz and Garratty (2004a) found that DHTR in SCD frequently manifested with atypical symptoms, such as painful crisis or haemoglobinuria; notably, new allo-antibodies were never detected in 20% of cases, and in 7% they were detected 72 h or longer after haemolysis became evident. These authors felt that there were sufficient distinctive features of these haemolytic episodes to be considered as an entity, which they termed sickle cell haemolytic transfusion reaction syndrome, rather than the somewhat confusing ‘hyperhaemolysis’ (Petz & Garratty, 2004a).

The pathophysiology of RBC alloimmunization in SCD was comprehensively and recently reviewed by Yazdanbakhsh et al. (2012). Several mechanisms have been postulated to explain haemolysis in situations of either ‘hyperhaemolysis’ (where some destruction of autologous cells is implied) and/or haemolytic transfusions reactions without an identified causative RBC alloantibody. Putative mechanisms include:

1. Bystander haemolysis (King et al., 1997). Bystander haemolysis was first described by Petz and Garratty (2004b) as ‘immune haemolysis of cells that are negative for the antigen against which the antibody is directed’.
   - Complement membrane attack complex (‘MAC’) in sickled RBC
   - Sickled RBC expose cryptic antigens, high levels IgG
   - Complement-mediated antibody reactions to other transfused proteins (plasma proteins, HLA) (Garratty, 1997).

2. Auto-antibody formation (Zimring et al., 2007)
   - Alloimmunization promotes autoantibody formation.
   - Autoantibody formation is higher in SCD than non-SCD populations with a cumulative incidence 6–10% (Castellino et al., 1999; Aygun et al., 2002; Garratty, 2004; Young et al., 2004; Lasalle-Williams et al., 2011). Studies suggest that alloantibody binding to the RBCs induce conformational changes in the antigen epitope that signal production of autoantibodies. Also, there may be a subset of patients who are genetically predisposed to develop RBC autoantibodies that could reflect an overall dysfunction of the immune system (Castellino et al., 1999).

3. HLA antibody formation (Win et al., 2001): instead of antibodies against specific RBC antigens

4. Suppression of erythropoiesis (Petz et al., 1997; Petz, 2006)

5. Instigation of an acute pain episode, RBC sickling and consequential autologous haemolysis

6. Excessive eryptosis via exposure of phosphatidylserine (PS) on RBC membrane signals suicidal RBC death: ‘eryptosis’ (Chadebech et al., 2009). PS exposure increases complement binding and potentiates destruction by macrophages through PS receptors.

The concept of HHTR is particularly complicated in SCD because of the ongoing background of chronic haemolysis upon which an acute DHTR event occurs. Furthermore, as DHTR itself can trigger another VOC, patients with SCD tend to develop brisker haemolysis. In SCD the distinction between haemolysis of ‘self’ RBCs as against ‘transfused cells’ can be traced by Hb electrophoresis that differentiates ‘self’ RBCs (HbS) from ‘transfused’ RBCs (HbA). In a classical DHTR event, the lysis is mainly that of the transfused cells that have been transfused RBCs (HbA).
resulting in a sharp decrease in HbA% and a concomitant increase in HbS%.

**Defining and diagnosing DHTTR**

Definitions of DHTTR vary and are ambiguous for many reasons, as discussed above, but a key feature is an acute drop in Hb in the context of a recent blood transfusion. Temporal association with transfusion does not prove that the haemolysis is a genuine transfusion reaction given the ongoing baseline haemolysis in patients with SCD. While DHTTR mimics the events of a vaso-occlusive sickle crisis, it often triggers another acute VOC resulting in a brisker haemolysis, that may lead to other life-threatening complications such as multi-organ failure, as observed in Case 5. Furthermore, immune-haematology findings are often negative and serological findings do not necessarily correlate with clinical severity.

The literature acknowledges the difficulties in defining DHTTR in SCD. We suggest a definition of DHTTR in SCD as a significant drop in Hb (of more than 25%) occurring between 1 and 21 d post-transfusion. The drop in Hb is associated with immuno-haematology evidence (new RBC antibodies, positive DAT) with high performance liquid chromatography evidence of destruction of transfused cells over self RBCs (accelerated increase in HbS% and corresponding fall in HbA%), evidence of prominent haemolysis (striking rise in LDH, haemoglobinuria), and relative reticulocytopenia (compared to baseline). Alternative causes of Hb drop must be excluded, notably perioperative blood loss, transient RBC aplasia due to infection and glucose-6-phosphate dehydrogenase deficiency.

**Diagnosing DHTTR**

Delayed haemolytic transfusion reactions in SCD encompasses a spectrum of clinical severity, ranging from mild cases when patients can be monitored closely as outpatients, to fatal cases (El-Husseini & Sabry, 2010). Symptoms include fever and pain, typically described as an acute VOC, but crucially in the context of a recent RBC transfusion. On direct questioning, patients may also report ‘dark’ or ‘Coca-Cola

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Fig 2. Proposed pathway for diagnosis and management of DHTTR in SCD. SCD, sickle cell disease; VOC, vaso-occlusive crisis; Hb, haemoglobin; DHTTR, delayed haemolytic transfusion reaction; G6PD, glucose-6-phosphate dehydrogenase; DAT, direct antiglobulin test; LDH, lactate dehydrogenase; HbA, haemoglobin A; HbS, haemoglobin S; ESA, erythropoiesis-stimulating agent.

Note: There is no one clear diagnostic test for DHTTR in SCD.
Table I. Differentiating DHTR from VOC episodes in SCD.

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<thead>
<tr>
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<th>DHTR</th>
<th>VOC episodes</th>
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</thead>
<tbody>
<tr>
<td>Context</td>
<td>Recent RBC transfusion (within 2–21 d)</td>
<td>Any</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Pain, fever, haemoglobinuria</td>
<td>Pain, fever</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>Variable – relative reticulocytopenia or elevated</td>
<td>Frequently elevated from baseline unless transient RBC aplasia from acute infection e.g. Parvovirus</td>
</tr>
<tr>
<td>LDH</td>
<td>Highly elevated (several times patient’s baseline LDH)</td>
<td>Mildly elevated compared to patient’s baseline LDH</td>
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<tr>
<td>Hb quantitation</td>
<td>Decrease in Hb to below post- and sometimes, pre-transfusion level, rapid clearance of HbA% with concomitant increase in HbS%</td>
<td>Unchanged or mild decrease from baseline (or if transfused, appropriate increase in Hb)</td>
</tr>
<tr>
<td>Immunohaematology</td>
<td>DAT positive (~75%) or negative (~25%); new RBC alloantibody detected in some cases May require RBC eluates</td>
<td>DAT negative</td>
</tr>
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DHTR, delayed haemolytic transfusion reaction; VOC, veno-occlusive crisis; SCD, sickle cell disease; RBC, red blood cell; LDH, lactate dehydrogenase; DAT, direct antiglobulin test.

coloured’ urine indicative of haemoglobinuria. The timing of presentation is variable. Our cases occurred from 5 to 16 d following a transfusion, consistent with previous reports (Talano et al, 2003; de Montalembert et al, 2011). However, instances of very delayed HTR, occurring as late as 4 weeks post-transfusion have been reported. We suggest an algorithm and work-up for diagnosing DHTR (Fig 2) and features that differentiate DHTR from acute VOC in SCD (Table I).

It is important to obtain previous transfusion records of the patient and antibody testing from other centres. A suspected DHTR should be promptly investigated to facilitate future sourcing of blood should the patient require transfusion. Timing of testing is important; given that clinical manifestations typically occur 6–10 d post-transfusion at a time when serology may be negative, sequential testing at outpatient follow-up or deterioration after discharge may capture a positive antibody finding that was initially negative.

The full blood count in DHTR demonstrates a marked drop in Hb. At the severe end of the spectrum (‘hyperhaemolysis’), Hb falls below pre-transfusion levels, implying that both recipient and donor RBCs are lysed. Haemolytic markers, in particular LDH, become more abnormal than at baseline. An unexplained finding is the reticulocyte count in DHTR; not only do many patients lack a compensatory, appropriate reticulocytosis, but many also appear to exhibit frank reticulocytopenia in the most acute stages as demonstrated in Cases 1 and 3. While Case 1 demonstrated absolute reticulocytopenia, Case 3 had an inappropriately low reticulocyte count at peak haemolysis. As expected, reticulocytosis heralds Hb increase and recovery from DHTR. Indeed, the duration and severity of the DHTR may be related to the patient’s ability to mount a reticulocyte response – Case 1 demonstrates a drawn-out DHTR period with a slow erythropoietic recovery despite EPO therapy. Hb recovery is possibly dependent on age of the patient, with the cumulative effects of bone marrow infarction in older patients hindering erythropoietic recovery. Other factors that may influence erythropoietic recovery are iron deficiency (absolute or functional), inadequate erythropoietin response or suppression of erythropoiesis by pro-inflammatory cytokines (e.g. tumour necrosis factor α, γ-interferon).

Quantitation of HbA% and HbS% helps define whether haemolysis is mainly lysis of donor cells (brisk reduction in HbA% and swift increase in HbS%), in contrast with autohaemolysis. Sequential testing of HbA% and HbS% helps to capture the trajectory of the haemolysis.

Direct antiglobulin test is imperative for all immune-mediated haemolytic episodes. While, by definition, positive in non-sickle DHTR in general, it was found to be negative in 21–26% DHTR episodes (Petz & Garratty, 2004b; Vidler et al, 2015) and in 7 of the 9 DHTR episodes in the series reported by Talano et al (2003). Thus, while a positive DAT is diagnostic, a negative DAT does not rule out DHTR in SCD. If the DAT is positive, and no new alloantibody is detectable in the plasma, antibody elution should be performed in an attempt to identify the antibody. Alternatively, the presence of a newly detected alloantibody in the plasma confirms the diagnosis of DHTR. If testing is initially negative, it is important to retest to maximize the chance of ‘capturing’ an alloantibody at peak titres. However, in ours and others’ experience, new RBC alloantibodies are frequently not detected (Talano et al, 2003; de Montalembert et al, 2011; Vidler et al, 2015). Again, this contrasts with most non-sickle DHTRs, suggesting that mechanisms other than antibody-mediated RBC destruction are involved.

**Patient management**

Decision to treat should be guided by clinical status of the patient and not on laboratory values – neither positive DAT nor identification of a new alloantibody is a prerequisite for DHTR treatment. Current management of DHTR in SCD is empirical. There is no evidence base in SCD (other than case series and general consensus among practitioners) in the
management of DHTR, including no clinical trials on the use of immunosuppressive agents (steroids, IVIg and rituximab). Furthermore, these treatments may have particular issues for patients with SCD, e.g., rebound pain on steroid cessation (Darbari et al, 2008), IVIg and hyperviscosity risk (Vichinsky et al, 2001). We suggest a combination of four strategies: (i) supportive care (ii) optimization of erythropoiesis, (iii) consideration of immunosuppression and (iv) minimizing further RBC transfusion if possible. Throughout this process, and until a safe Hb is achieved and sustained, laboratory results (including Hb, reticulocyte count, LDH, and Hbs/HbA levels) must be monitored carefully. The initial aim is to get the Hb to a clinically feasible minimum, which may well be below the patient’s steady state Hb. A suggested pathway for diagnosis and management of DHTR is shown in Fig 2.

Supportive care

Patients are at risk of developing a Hb-induced tubulopathy and associated acute kidney injury, hence maintaining fluid balance, renal function and a good urine output, is of prime importance. Clinicians must be vigilant for the development of other acute sickle phenomena such as ACS, stroke and multi-organ failure.

Directed treatments

In severe cases of HTR, where intervention is warranted, a dual pronged treatment approach is suggested. First, some form(s) of immunosuppression must be instituted, and secondly, erythropoiesis must be optimized.

Immunosuppression. Corticosteroids, IVIg, and rituximab have been utilized in the treatment of DHTR/HHTR in SCD.

Corticosteroids: Corticosteroid therapy may be beneficial in DHTR; however, the potential risks of steroids need to be balanced against their potential life-saving effect and should not be withheld in life-threatening cases. Systemic corticosteroids decrease the severity of ACS and vaso-occlusive events, but when stopped abruptly, are associated with a rebound effect. Using a tapering regimen may ameliorate the rebound effect (as in Case 5). Neurological events have been noted in some patients receiving steroids; however, its causal relationship is unclear. These patients also had several co-factors including hypertension, hyperviscosity and high Hb levels (Elenga et al, 2008). The potential risks of steroids need to be balanced against their potential life-saving effect and should not be withheld in life-threatening cases. In children, we recommend 2 mg/kg/d of prednisolone with a maximum dose of 60 mg/d, followed by a very slow tapering (weeks) of steroids to prevent rebound vaso-occlusive pain events. In adults, we have used higher steroid doses (methylprednisolone 0.5 g daily) over a shorter period (up to 5 d) without undue toxicity.

IVIg and Immunomodulation: IVIg alone has been used successfully in DHTR (Cullis et al, 1995), but more commonly, it is used in combination with steroids (Win et al, 2001, 2010; Scheunemann & Ataga, 2010). Severe DHTR has been successfully managed using a combination of IVIg and steroids, and withholding transfusion (Win et al, 2010). We have used IVIg 1 g/kg/d for 1–2 d in both children and adults.

Rituximab: More recently, rituximab has emerged as a third line agent in refractory episodes of DHTR (Delmonte et al, 2013; Noizat-Pirene et al, 2015) or prophylactically in polyimmunized patients with previous DHTR (Noizat-Pirene et al, 2007; Bachmeyer et al, 2010). Rituximab dosing schedules varied in the literature; we have used both low-dose (100 mg IV once per week for 4 weeks) and standard-dose (375 mg/m2 weekly ×4). As rituximab causes significant depletion of B lymphocytes, its use must be used judiciously clinicians must be alert to the potential increased risk of infection in patients who are already immunocompromised.

Optimizing erythropoiesis. Innate erythropoiesis can be maximized with a combination of an erythropoiesis-stimulating agent (ESA) and IV iron. ESA can have particular benefit in the DHTR setting (de Montalembert et al, 2011), even when endogenous erythropoietin levels are elevated, because the erythropoietin level is inappropriately low for the degree of anaemia (Sherwood et al, 1986). In patients who develop renal failure, higher doses of ESA, ranging from 150 to 250 u/kg/d, may be necessary and appear to be well tolerated (Roger et al, 1991; Steinberg, 1991; Tomson et al, 1992). In a randomized trial utilizing ESA doses up to 800 u/kg/d (Nagel et al, 1993) in patients with renal failure, no side effects were observed. Similarly high doses have been used effectively in SCD cases with life-threatening DHTR (Little et al, 2006).

We suggest high-dose erythropoietin (250–800 u/kg/dose) three times weekly, with close monitoring for hypertension, thrombosis and bone pain. Intra-venous iron supplementation is indicated if transferrin iron saturation is <20%, due to the inability to mobilize stored iron adequately (Nagel et al, 1993).

Blood transfusion. Transfusion will be unavoidable in a subset of cases. If the patient has life-threatening anaemia with co-existent organ complications, e.g. ACS and respiratory failure, further transfusion to improve oxygen delivery can prove essential. Needless to say, blood units should be selected as stringently as possible, although this will often require additional donation testing or provision of blood from frozen stocks.

Other measures. Two case reports have highlighted the usefulness of apheresis procedures in the management of severe DHTHR in patients with SCD. Uhlmann et al (2014) described a modified plasma exchange procedure with return of donor RBCs in a patient with heart failure (Uhlmann et al, 2014);
Kalyanaraman et al (1999) described a case with acute renal failure following DHTR where a whole blood exchange significantly contributed to patient recovery. Apheresis procedures remain a somewhat heroic measure that should be reserved for selected cases with DHTR in SCD.

Future patient management

Once a patient has become alloimmunized, they are at increased risk of producing additional antibodies (Silvy et al, 2014), and thus at high risk for another DHTR. Future transfusion needs must be individually determined based on the particular risk benefit analysis for that patient.

More widely, DHTR events may be considered as a prompt to initiate hydroxy carbamide in the patients considered ‘untransfusable’ to maximize Hb levels and hopefully minimize transfusion needs in the longer term. This strategy is logical but needs an evidence base to transfer the idea of severe DHTR being an indication for hydroxycarbamide. Going one step further, such ‘untransfusable’ patients, who in our experience constitute 10% of alloimmunized SCD patients (Mijovic et al, 2013), could be candidates for allogeneic haemopoietic stem cell transplantation. However, it should be pointed out that peri-transplant procedures may well require blood transfusion until donor cells engraftment.

Strategies for prevention of DHTR in SCD

A simple, but possibly most effective, preventative strategy is to maintain good documentation of antibody history. Some countries in Europe, including National Health Service Blood and Transplant in the UK, operate national centralized electronic database systems; in the United States, regional centralized networks have been set up to prevent incompatible RBC transfusion to patients with evanesced alloantibodies, who often attend more than one hospital to receive transfusions (Harm et al, 2014). Where centralized databases are not available, laboratory information management systems must be carefully updated and additional measures, such as patient cards/letters or bracelets containing information about detected antibodies, should be implemented.

Strategies to prevent alloimmunization in SCD include (i) transfusion from ethnically-matched donors and (ii) extended RBC antigen matching. Notably, using ethnically-matched blood may reduce rather than eliminate DHTR given the significant RBC antigen heterogeneity in the black population (Chou et al, 2013). This is complicated by the high incidence of sickle cell trait in black donors; blood from donors with sickle cell trait causes blockages of the filters during leukodepletion (Beard et al, 2004); in addition, the use of AS donors complicates tracking of HbS versus HbA levels.

Prophylactic antigen matching for highly immunogenic Rh (D, C, E, c, e) and Kell antigens has been shown to dramatically decrease alloimmunization rates in SCD and is recommended in most developed countries. Further extension of antigen matching, to include Kidd, Fya and, whenever possible, Lewis and MNS system antigens, has the potential to reduce alloimmunization even further (Lasalle-Williams et al, 2011). However, the cost-benefit and reproducibility of such an approach needs to be determined. UK guidelines recommend performing an extended RBC phenotype of ABO, Rh, Kell, Kidd, Duffy and S/s antigens on SCD patients prior to receiving any transfusions (Milkins et al, 2013). With regard to antigen matching, all UK centres routinely prophylactically match RBC units for SCD patients for ABO, RhD, -C, -c, -E, -e and K antigens.

Molecular testing has revealed high rates of variant RHD/RHCE alleles and consequent partial Rh antigens giving rise to alloantibodies in patients deemed to be antigen-positive serologically (and the antibodies are sometimes consequently deemed to be autoantibodies as a result) (Chou et al, 2013). These data suggest a role for genotyping, at least for those patients with previous DHTR events, being rolled out into clinical practice.

Special case: peri-operative transfusion

Perioperative transfusions and DHTR represents a special case. In SCD, blood transfusion in the preoperative, elective setting is on the increase, guided by the recent TAPS (Transfusion Alternatives Preoperatively in SCD) study (Howard et al, 2013), which found increased complications in non-transfused patients. We note that in the TAPS trial, there was only one incidence of alloimmunization at 3 months follow-up from a transfusion group of 34. Lower rates of alloimmunization event in the TAPS trial compared to the 1995 (Vichinsky et al, 1995) study might relate to stricter RBC matching.

Case 3 in this article, and other case reports (McGlennan & Grundy, 2005) highlight some of the particular diagnostic dilemmas in assessing for DHTR peri-operatively. Interpretation of blood results can be particularly confusing in those who have had pre-operative transfusion with significant surgical blood loss. Furthermore, post-operatively, the patient is at risk for development of VOC and ACS in SCD, and this leads many practitioners to have a low threshold for transfusion. The decision to transfuse must therefore be made in the context of consideration of DHTR as a possible differential diagnosis in the post-operative transfusion setting.

Conclusion

Delayed haemolytic transfusion reactions in SCD continues to challenge clinicians from diagnostic, therapeutic and prophylactic perspectives. Diagnosis is best made by simply being aware of DHTR when patients present with acute pain following a recent transfusion; diagnosis cannot simply be put down to uncomplicated VOC without further investiga-
tion. Recognition of DHTR is vital; not only is DHTR itself potentially life-threatening, but giving a further transfusion in this setting can prove fatal. It is notable that most trigger transfusions for DHTR events are delivered in the acute rather than elective setting; thus, a patient presenting acutely again (after receiving transfusion for their first VOC) should provide a prompt to consider DHTR. Management of DHTR is dictated by the clinical severity. Many patients can be managed conservatively, with close clinical and laboratory monitoring until reticulocytes and Hb recover. If patients do need an intervention, further blood transfusion should be kept at the clinically feasible minimum with immunosuppression and optimization of erythropoiesis. While data are limited, plasma and/or RBC exchange transfusion may also be considered in selected cases, especially in patients who develop additional complications that require repeated transfusion.

Prevention is the key to reducing the overall burden of DHTR in the SCD population; extended RBC phenotyping and antigen matching of transfused units have significantly reduced alloimmunization rates. There is growing evidence for genetic testing of patients with SCD, particularly for RHD and RHCE loci, which warrants further evaluation from both clinical and cost-effectiveness standpoints. The future may see molecular testing of both patients and donors, as high throughput sequencing becomes less costly. This would enable an even more individualized approach to transfusion medicine.

Acknowledgements
We thank Dr Elliott Vichinsky (Children’s Hospital and Research Center Oakland, Division of Hematology/Oncology, Oakland, CA 94609, USA, for his incise and helpful comments. We also thank Claire Steward and Robin Swabey for help in preparation of the manuscript.

Author contributions
All authors reviewed the literature, wrote and contributed to the manuscript.

Conflict of interest
The authors declare no competing financial interest.

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