Anti-C5 antibody treatment for delayed hemolytic transfusion reactions in sickle cell disease

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Running title: Anti-C5 antibody treatment for DHTTR

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Delayed hemolytic transfusion reaction (DHTR) is an unpredictable severe complication of transfusion in patients with sickle cell disease (SCD). It presents clinically as a vaso-occlusive crisis (VOC), often associated with the failure of one or more organs, after the transfusion of packed red blood cells (pRBC).\textsuperscript{1,2} Hyperhemolysis is encountered in the most severe forms. Both transfused and autologous RBC are lysed.

The mechanisms underlying DHTR remain unclear. Alloantibodies against RBC antigens were initially thought to underlie the pathophysiology, but no such antibodies are detected in about a third of cases.\textsuperscript{3}

RBC degradation products, such as hemoglobin and heme, are released into the bloodstream during intravascular hemolysis. These elements and heme-loaded membrane microvesicles have recently been implicated in inflammation and organ injury in DHTR.\textsuperscript{4} Complement is activated via the classical pathway, by alloantibodies, and/or via the alternative pathway, by free heme.\textsuperscript{5} Heme-dependent complement deposits on the endothelium contribute to organ damage.\textsuperscript{6} Due to these vascular lesions, hyperhemolysis often progresses to multiple organ failure and, in some cases, death.

These pathophysiological findings suggest that inhibitors of complement activation may be useful for treating DHTR with hyperhemolysis. Eculizumab is a monoclonal anti-C5 antibody that inhibits the cleavage of C5 into C5a by the C5 convertase, thereby preventing the late stages of the complement cascade. Anti-C5 therapy has been offered to several SCD patients for DHTR treatment at our SCD referral center in France since 2013.\textsuperscript{1,7} Other teams have also treated DHTR in patients with and without detectable allo- or autoantibody formation with Eculizumab, with promising results.\textsuperscript{8–11} The ASH guidelines include a conditional recommendation for the use of anti-C5 antibodies in patients with SCD presenting DHTR and ongoing hyperhemolysis, based on currently very low levels of certainty.\textsuperscript{12}

This retrospective study focuses on the biological and clinical findings and the effects of anti-C5 therapy on DHTR, for patients treated between 2013 and 2019 who experienced particularly severe DHTR.

DHTR was diagnosed\textsuperscript{1,2} on the basis of VOC signs occurring five to 20 days after pRBC transfusion, with no other identifiable cause of intravascular hemolysis, in association with at least one of the following signs:
- Rapid decrease in, or unexpectedly low, hemoglobin A (HbA) concentration (the diagnostic nomogram for DHTR diagnosis was used),

- Hemoglobinuria, as revealed by dark urine,

- Positive direct antiglobulin test (DAT) results or new antibody formation.

The criteria for the use of anti-C5 therapy was based either on the existence, at the time anti-C5 infusion was decided, of one or more organs with dysfunction and/or very low total Hb concentration (< 50 g/L), and/or a rapidly worsening clinical state.

Data were collected retrospectively from patient records. The clinical and biological findings available at transfusion, at the time of DHTR diagnosis and during follow-up were collected. We also noted patient sex, age, history of DHTR, pRBC transfusion, and antibody screens. We recorded the number of pRBC units and the indication of the transfusion(s) occurring within a timeframe compatible with DHTR (some patients had received pRBC on several occasions during the five to 20 days preceding DHTR). Clinical (hemoglobinuria, pain and VOC signs, organ failure) and biological (hemoglobin concentration, reticulocyte count, LDH, total bilirubin) findings at DHTR diagnosis were collected. The first clinical signs compatible with DHTR were noted, particularly pain indicative of VOC recurrence, and hemoglobinuria indicative of intravascular hemolysis. We collected follow-up data for biological tests, intensive care unit admission and discharge, organ failure and treatments. This study was performed in accordance with the Declaration of Helsinki.

We used R3.6.1 and lme4 for a linear mixed-effects model analysis of the relationship between Hb and LDH levels and anti-C5 treatment. Hb and LDH levels were modeled before and after treatment. An initial blind statistical analysis was performed, and several models were then proposed, with days and pRBC transfusions as fixed effects, and different combinations of random effects for subjects, days and pRBC transfusions. We obtained p-values for likelihood ratio tests of the full model with random effects against the model without additional terms, which we used to select the best model.

Eighteen SCD patients received anti-C5 treatment for DHTR with hyperhemolysis. All patients had signs of VOC five to 20 days after pRBC transfusion, with low HbA concentrations (<10 g/L) in 5 patients, a rapid decrease in HbA concentration in 10 patients (estimated by the nomogram as a high (N=4) or intermediate (N=6) risk of DHTR), hemoglobinuria in 11 patients, and positive DAT results or antibody formation in 12
patients (anti-MNS3, anti-KEL6, anti-RH10 + anti-RH20, anti-MNS5, anti-FY5 in one patient each, 1 developed multiple antibodies including anti-MNS3, anti-RH20 and auto-Ab, 2 developed auto-antibodies and 2 allo-antibodies for which the specificity could not be determined and 2 had positive DAT but no new Ab was subsequently identified). A 19th patient received anti-C5 antibody for hyperhemolysis but it was not possible to determine whether this patient had DHTTR due to hemolysis under extracorporeal membrane oxygenation$^{14}$, so this patient was excluded from the analysis.

The main characteristics of the patients are presented in Table 1. Sixteen patients (89%) had risk factors for DHTTR: a history of previous DHTTR ($N=2$), a history of RBC antibodies ($N=11$), or the administration of fewer than 12 pRBC units before the episode leading to DHTTR ($N=11$). Three patients had a history of ineffective pRBC transfusions, possibly due to previous undetected episodes of DHTTR. None of the patients were enrolled in chronic transfusion programs. Five patients underwent repeat transfusions before the diagnosis of DHTTR, which may have worsened their clinical presentation at diagnosis.

The findings at diagnosis and during follow-up, compared with those of a historical cohort$^1$ are presented in Table 1 (see Supplementary data for individual timelines). At diagnosis, the patients had particularly severe DHTTR, with parameters highly indicative of hemolysis (low Hb, high LDH concentrations), and the failure of at least one organ in 50% of cases ($N=9$): kidney failure ($N=7$), liver failure ($N=4$, including two with indications for liver transplantation), respiratory failure ($N=5$). Five patients had hemodynamic failure requiring treatment with vasoactive agents.

One to three anti-C5 doses were administered at one-week intervals (1 dose $N=6$, 2 doses $N=9$, 3 doses $N=1$), in association with other treatments (Table 1). Unfortunately, complement activation measurements were not performed for most patients. The number of pRBC units transfused was restricted as much as possible, to limit exacerbations of hyperhemolysis.

Remarkably, a worsening of clinical condition during follow-up occurred only in the hours immediately following anti-C5 infusion (i.e. due to the progression of preexisting organ damage due to DHTTR; $N=2$), or as a result of sepsis due to additional infectious complications ($N=2$). One patient suffered hemodynamic failure
within a few hours of anti-C5 infusion. One patient (16P) with kidney failure, hemodynamic failure and a severe hepatic alteration before anti-C5 infusion rapidly progressed to hepatic failure a few hours after the first infusion.

Outcome was favorable in 15 patients (83%), with a complete recovery of all failing organs. Three patients died (17%). All three had acute liver failure requiring emergency transplantation (already present at DHTR diagnosis in two of these patients). Two patients improved after one and two anti-C5 infusions and were able to undergo transplantation. However, both died from infectious complications due to encapsulated bacteria unrelated to anti-C5 treatment but promoted by the immunosuppressive regimen: ventilator-associated pneumonia 11 days after transplantation in patient 8H, and digestive and urinary infection 47 days after transplantation in patient 16P. No compatible organ could be found for patient 3C, who died one day after anti-C5 antibody infusion.

Despite the heterogeneity of the data, linear mixed-effect model analysis with adjustment to produce the best model ($p<0.05$) highlighted an influence of the anti-C5 antibody treatment on total Hb and LDH levels (Figure 1). The inversion of the slope for total Hb and LDH levels before and after anti-C5 treatment indicated that hyperhemolysis was stopped, or at least greatly decreased, by treatment. The gradual increase in Hb levels may also be due to the other treatments received by the patients, especially EPO (Table 1). The stimulation of erythropoiesis improves the reticulocyte count, and proportionally increases HbS. In several patients who received secondary RBC transfusion, HbA concentration was maintained post transfusion (e.g. patients 2B, 15O, 16P, 17Q).

In conclusion, this is the largest series to date of cases of severe DHTR with hyperhemolysis in SCD patients, treated with anti-C5 antibody. It demonstrates the effect of anti-C5 therapy against hyperhemolysis in DHTR, with remarkable beneficial effects on pre-existing organ failure and additional organ failure once the effects of the treatment are established. These findings consolidate the recommendation in the ASH guidelines to use anti-C5 antibody in patients with SCD and ongoing hyperhemolysis. Other anti-complement drugs may also be useful for treatment in this context. A prospective clinical trial would be required to determine whether all DHTR patients would benefit from anti-C5 therapy or whether such treatment is beneficial only for the most severe clinical presentations.
Acknowledgments

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References


<table>
<thead>
<tr>
<th>Table</th>
<th>This series</th>
<th>Habibi et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients ; DHTR episodes</td>
<td>18 ; 18</td>
<td>69 ; 99</td>
</tr>
<tr>
<td>Hb βS</td>
<td>18 (100%)</td>
<td>65 (94.2%)</td>
</tr>
<tr>
<td>Sex F/M</td>
<td>11 / 7</td>
<td>48 / 21</td>
</tr>
<tr>
<td>Age, years</td>
<td>24.6 ± 12.6</td>
<td>30 ± 9</td>
</tr>
<tr>
<td>Number of pRBC units in transfusion episode</td>
<td>2 ± 1.9</td>
<td>2 ± 3</td>
</tr>
<tr>
<td><strong>Transfusion indications</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preventive measure</td>
<td>5 (27.8%)</td>
<td>51 (51.5%)</td>
</tr>
<tr>
<td>Vaso-occlusive complications</td>
<td>11 (61.1%)</td>
<td>48 (48.5%)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (11.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Timeline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days from transfusion to DHTR diagnosis</td>
<td>8 [7-12.8]</td>
<td>10 [8-14] (MD=19)</td>
</tr>
<tr>
<td>Days from transfusion to anti-C5 infusion</td>
<td>10.5 [9-15.5]</td>
<td></td>
</tr>
<tr>
<td><strong>Biological findings in the emergency room</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Hb level, g/dL</td>
<td>63.5 [53.3-77.8] (NA=6*)</td>
<td>78 [69-93] (MD=5) †</td>
</tr>
<tr>
<td>LDH level, IU/L</td>
<td>1612 [825-2702] (NA=6*)</td>
<td>758 [554-958] (MD=16)</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPO</td>
<td>17 (94.4%)</td>
<td>45%</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>1 (5.6%)</td>
<td>3%</td>
</tr>
<tr>
<td>Plasma or albumin exchange</td>
<td>4 (22.2%)</td>
<td></td>
</tr>
<tr>
<td>IV immunoglobulins</td>
<td>9 (50%)</td>
<td>4%</td>
</tr>
<tr>
<td>Anti-CD20 antibody</td>
<td>7 (38.9%)</td>
<td>2%</td>
</tr>
<tr>
<td>Anti-C5 antibody</td>
<td>16 (100%)</td>
<td>2%</td>
</tr>
<tr>
<td>Secondary pRBC transfusion</td>
<td>14 (77.8%)</td>
<td>35%</td>
</tr>
<tr>
<td><strong>Extreme biological findings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest total Hb level, g/dL</td>
<td>30.5 [25.5-42.8]</td>
<td>55 [45-63] (MD=5) †</td>
</tr>
<tr>
<td>Highest LDH level, IU/L</td>
<td>3337 [2573-7986]</td>
<td>1335 [798-2086] (MD=7)</td>
</tr>
<tr>
<td>Lowest reticulocyte count, 10&lt;sup&gt;9&lt;/sup&gt;/L</td>
<td>46.1 [35.8-84.8] (MD=2)</td>
<td>180 [121-240] (MD=14)</td>
</tr>
<tr>
<td>Delta Hb‡, g/dL</td>
<td>57.5 [45.8-67.5] (MD=4)</td>
<td>46 [31-53] (MD=26) †</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU admission</td>
<td>17 (94.4%)</td>
<td>41 (40%)</td>
</tr>
<tr>
<td>ICU-stay duration, days</td>
<td>17.7 ± 10.2</td>
<td>6.2 ± 4</td>
</tr>
<tr>
<td>Hospital-stay duration, days</td>
<td>35.6 ± 25.3</td>
<td>15.9 ± 10</td>
</tr>
<tr>
<td>Transfusion-to-death interval, days</td>
<td>51.7 ± 47.9</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Death</td>
<td>3 (16.7%)</td>
<td>6%</td>
</tr>
</tbody>
</table>

**Table 1.** Clinical and biological findings at diagnosis and during follow-up. Continuous variables are expressed as means ± one standard deviation (SD) or medians [interquartile range], depending on whether they are normally or asymmetrically distributed. Categorical variables are expressed as numbers (%). For comparison with the largest published DHTR series, the data in column 2 are reprinted from Habibi et al.¹ with permission. The patients of our series, who received anti-C5 antibody, had very severe DHTR with hyperhemolysis (*p*-values in column 3 compare our patients with those of the historical series). * Six patients had not even been discharged, due to the severity of their DHTR, ** All patients in both series also received supportive VOC treatment, hydration, oxygenation, and analgesia. † Values were converted to g/L (from g/dL in Habibi et al.). ‡ Delta Hb is the difference between the highest and lowest values available post-transfusion.
Figure legends

Figure 1. Best mixed-effects model for total Hb and LDH during DHTR, before and after anti-C5 antibody infusion. Hb levels are predicted for a “theoretical” patient receiving anti-C5 antibody infusions on days 0 and 7 and no pRBC transfusions. Before anti-C5 antibody infusion, basal total Hb concentration in this model was 51.0 g/L (the intercept of the model), with an increase of +3.8 g/L for each pRBC unit transfused, and ongoing hemolysis at a rate of -4.4 g/L for each passing day (the fixed effects of the model). However, after anti-C5 antibody infusion, Hb levels gradually increased, with a basal Hb concentration of 46.2 g/L: the effect of each transfusion was an increase of 1.58 g/L for each pRBC unit transfused and an increase in Hb levels of 0.94 g/L.
Supplemental Data Legend: Figures for individual patient timelines, with main events. The scales are the same for all patients, to facilitate comparison. The timelines are discontinuous for longer follow-up periods, to maintain the same scale throughout.
Patient 1A

Notable events:
Day -8: hemodynamic failure, acute cor-pulmonale
Day -7: kidney and liver failure

Complement analysis on day 7:
- C3: 1270 mg/L (N=[660-1250])
- C4: 257 mg/L (N=[93-380])
- Soluble C5B9: 1797 mg/L (N=<450)

- Total Hb (g/L)
- HbA (g/L)
- LDH (IU/L)
- Total bilirubin (µmol/L)
- Reticulocyte count (10⁶/L) (refer to the black axis)
Patient 2B

- Total Hb (g/L)
- HbA (g/L)
- LDH (IU/L)
- Total bilirubin (µmol/L)
- Reticulocyte count (10⁹/L) (refer to the black axis)

△ 1 pRBC transfusion
- Anti-C5 infusion
☆ Organ failure
| Hepatic transplantation
| Death

Time (days relative to anti-C5 Ab infusion)
Patient 3C

Notable events:
Day -1: hemodynamic, kidney and liver failure
Day 0: respiratory failure
Day 1: death

- Total Hb (g/L)
- HbA (g/L)
- LDH (IU/L)
- Total bilirubin (µmol/L)
- Reticulocyte count (10⁹/L) (refer to the black axis)

△ 1 pRBC transfusion
| Anti-C5 infusion
☆ Organ failure
| Hepatic transplantation
| Death

Time (days relative to anti-C5 Ab infusion)
Patient 4D

Notable events:
Day -1: kidney failure
Patient 5E

Notable events:
Day -1: respiratory failure, acute cor-pulmonale
Day -7: kidney and liver failure
Day 12: sepsis

- Total Hb (g/L)
- HbA (g/L)
- LDH (IU/L)
- Total bilirubin (µmol/L)
- Reticulocyte count (10⁹/L) (refer to the black axis)

1 pRBC transfusion
Anti-C5 infusion
Organ failure
Hepatic transplantation
Death

Time (days relative to anti-C5 Ab infusion)
Patient 6F

Complement analysis:

<table>
<thead>
<tr>
<th>Time</th>
<th>CH50 (%)</th>
<th>C3 (mg/L)</th>
<th>C4 (mg/L)</th>
<th>Soluble C5B9 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>124</td>
<td>1080</td>
<td>266</td>
<td>856</td>
</tr>
<tr>
<td>Day 2</td>
<td>23</td>
<td>1030</td>
<td>283</td>
<td>1223</td>
</tr>
<tr>
<td>Day 14</td>
<td>30</td>
<td>1050</td>
<td>248</td>
<td>1406</td>
</tr>
</tbody>
</table>

- **CH50 (%)**: N=70-130
- **C3 (mg/L)**: N=660-1250
- **C4 (mg/L)**: N=93-380
- **Soluble C5B9 (mg/L)**: N<450

Graphical representation of patient data with:
- **Total Hb (g/L)**
- **HbA (g/L)**
- **LDH (IU/L)**
- **Total bilirubin (µmol/L)**
- **Reticulocyte count (10⁹/L)**

Legend:
- △ 1 pRBC transfusion
- Anti-C5 infusion
- Organ failure
- Hepatic transplantation
- Death

Time (days relative to anti-C5 Ab infusion)
Patient 7G

Notable events:
Day -1: respiratory failure
Day 2: sepsis
Patient 8H

Notable events:
Day -1: liver failure
Day 0: kidney, respiratory, hemodynamic failure
Day 1: first liver transplantation
Day 3: liver failure
Day 4: second liver transplantation
Day 14: sepsis
Day 15: death

Complement analysis:

<table>
<thead>
<tr>
<th>Day</th>
<th>C3 (mg/L) N=[660-1250]</th>
<th>C4 (mg/L) N=[93-380]</th>
<th>Soluble C5B9 (mg/L) N&lt;450</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1070</td>
<td>220</td>
<td>1527</td>
</tr>
<tr>
<td>Day 4</td>
<td>313</td>
<td>104</td>
<td>296</td>
</tr>
<tr>
<td>Day 6</td>
<td>359</td>
<td>129</td>
<td>224</td>
</tr>
<tr>
<td>Day 9</td>
<td>928</td>
<td>526</td>
<td>355</td>
</tr>
<tr>
<td>Day 13</td>
<td>1210</td>
<td>290</td>
<td>509</td>
</tr>
</tbody>
</table>

"1 pRBC transfusion
Anti-C5 infusion
Organ failure
Hepatic transplantation
Death

Refer to the black axis
Patient 9I

Notable events:
Day 0 (before anti-C5): acute cor-pulmonale, liver, kidney, hemodynamic failure

- Total Hb (g/L)
- HbA (g/L)
- LDH (IU/L)
- Total bilirubin (µmol/L)
- Reticulocyte count (10⁹/L) (refer to the black axis)

△ 1 pRBC transfusion
- Anti-C5 infusion
★ Organ failure
- Hepatic transplantation
| Death

Time (days relative to anti-C5 Ab infusion)
Patient 10J

Complement activity CH50 <20% on day 1 and 4 (N=[70-130])
Patient 11K

- Total Hb (g/L)
- HbA (g/L)
- LDH (IU/L)
- Total bilirubin (μmol/L)
- Reticulocyte count (10⁹/L) (refer to the black axis)

- ▲ 1 pRBC transfusion
- ▶ Anti-C5 infusion
- ✱ Organ failure
- ▪ Hepatic transplantation
- ■ Death

Time (days relative to anti-C5 Ab infusion)
Patient 12L

- Total Hb (g/L)
- HbA (g/L)
- LDH (IU/L)
- Total bilirubin (µmol/L)
- Reticulocyte count (10⁹/L) (refer to the black axis)

Diagrams with markers:
- 1 pRBC transfusion
- Anti-C5 infusion
- Organ failure
- Hepatic transplantation
- Death

Time (days relative to anti-C5 Ab infusion)
Patient 13M

- Total Hb (g/L)
- HbA (g/L)
- LDH (IU/L)
- Total bilirubin (μmol/L)
- Reticulocyte count (10⁹/L) (refer to the black axis)

Other events:
- 1 pRBC transfusion
- Anti-C5 infusion
- Organ failure
- Hepatic transplantation
- Death

Time (days relative to anti-C5 Ab infusion)
Patient 14N

Notable events:
Day 0 (a few hours after anti-C5): hemodynamic failure, acute cor-pulmonale
Day 1: respiratory failure

- Total Hb (g/L)
- HbA (g/L)
- LDH (IU/L)
- Total bilirubin (µmol/L)
- Reticulocyte count (10⁹/L) (refer to the black axis)

Legend:
- ▲ 1 pRBC transfusion
- ▲ Anti-C5 infusion
- ★ Organ failure
- ★ Hepatic transplantation
- ★ Death
Patient 150

- Total Hb (g/L)
- HbA (g/L)
- LDH (IU/L)
- Total bilirubin (µmol/L)
- Reticulocyte count (10⁹/L) (refer to the black axis)

- 1 pRBC transfusion
- Anti-C5 infusion
- Organ failure
- Hepatic transplantation
- Death

Time (days relative to anti-C5 Ab infusion)
Patient 16P

Much of the timeline is truncated to keep the same scale for all graphs.

Notable events:
Day -10: liver failure
Day 20: sepsis
Day 42: liver transplantation
Day 86: graft infection
Day 89: death

Complement analysis:

<table>
<thead>
<tr>
<th>Day</th>
<th>C3 (g/L)</th>
<th>C4 (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.57</td>
<td>0.09</td>
</tr>
<tr>
<td>8</td>
<td>0.42</td>
<td>0.07</td>
</tr>
<tr>
<td>10</td>
<td>0.72</td>
<td>0.13</td>
</tr>
<tr>
<td>15</td>
<td>0.99</td>
<td>0.18</td>
</tr>
<tr>
<td>21</td>
<td>1.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*Total Hb (g/L)  *HbA (g/L)  *LDH (IU/L)  *Total bilirubin (µmol/L)  *Reticulocyte count (10⁹/L) *(refer to the black axis)
Patient 17Q

Notable events:
Day -1: kidney and respiratory failure
Patient 18R

- Total Hb (g/L)
- HbA (g/L)
- LDH (IU/L)
- Total bilirubin (µmol/L)
- Reticulocyte count (10⁹/L) (refer to the black axis)

- 1 pRBC transfusion
- Anti-C5 infusion
- Organ failure
- Hepatic transplantation
- Death

Time (days relative to anti-C5 Ab infusion)