Eculizumab for complement mediated thrombotic microangiopathy in sickle cell disease


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Title: Eculizumab for complement mediated thrombotic microangiopathy in sickle cell disease

Running head: CM-TMA in sickle cell disease

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Despite being the first genetic disease described, SCD continues to afflict patients with immense pain, significant co-morbidities and premature death. SCD has only recently benefited from new interventions with L-glutamine (2017), voxelotor (2019) and crizanlizumab (2019) representing the first FDA approved medications for SCD since hydroxyurea in 1997. These interventions have demonstrated some ability to reduce vaso-occlusive pain crisis episodes, improve hemoglobin (HGB), or reduce markers of hemolysis and have largely been used as preventative care measures. While these and additional approaches, such as hematopoietic stem cell transplant and gene therapy, can improve SCD care, many patients with SCD continue to suffer from severe acute SCD complications that can result in organ damage and early death.\(^{(1, 2)}\)

Unfortunately, in these situations, supportive care remains the primary approach to alleviate complications. The lack of more targeted approaches in part reflects an incomplete understanding of the pathophysiology and accompanying pharmacological targets that could specifically mitigate acute disease complications. We present a summary of three cases of children with SCD who developed significant acute complications that demonstrate underlying CM-TMA. These cases include a delayed hemolytic transfusion reaction (DHTR), vasoocclusive crisis (VOC) and drug-induced immune hemolytic anemia (DIIHA).

Patient #1 is a 14-year-old male with a history of two episodes of DHTRs. At age 12y, he received a transfusion pre-operatively for hip core-decompression. He presented 7 days later with severe diffuse body pain, hemoglobinuria, fever, and total hemoglobin (HGB) of 9.4 g/dL with HbA at 17% (Figure 1A). Further testing revealed a previously
detected anti-U alloantibody, negative direct antiglobulin test (DAT) and evidence for intravascular hemolysis. On the night of admission, he became hypertensive with headache and sluggish mentation. A brain magnetic resonance imaging scan was normal. Hemoglobin dropped to 5.6 g/dL within 30 hours of admission, and the patient rapidly deteriorated to multiorgan failure (MOF) with thrombocytopenia (Figure 1A). Due to strong suspicion for DHTR with hyperhemolysis and CM-TMA, he was treated with eculizumab 600mg intravenously (IV) and erythropoietin 150 IU/kg to augment erythropoiesis. Over the next 24 hours, he developed a new consolidation in the left lung consistent with acute chest syndrome (ACS). He received one unit of crossmatch compatible, U-negative red blood cells (RBCs) after a dose of 1g/kg intravenous immunoglobulin (IVIG) on day 9. Over the next few days, he made a gradual improvement in clinical and laboratory status. Eculizumab 600mg was continued weekly for a total of 4 doses. Subsequent analysis revealed significant alternative complement pathway (ACP) activation at the peak of hemolysis, as evidenced by increased Bb levels, anaphylatoxins (C3a and C5a) and terminal complement complex (C5b-9) (Table 1). Testing for complement regulatory genes (CFH, CFI, MCP (CD46), CFB, CFHR5, C3, THBD, DGKE, PLG, ADAMTS13 and MMACHC) revealed a homozygous deletion of complement factor H-related protein (CFHR) 3 and CFHR1, but criteria for DEAP-HUS (Deficiency of CFHR plasma proteins and Autoantibody Positive form of HUS) were not met due to absence of factor H autoantibodies.

Patient #2 is a 15-year-old female with a history of VOC episodes and DHTR, who was transferred to our care at age 9. Since transfer, she suffered from multiple episodes of
VOC, each accompanied with a drop in HGB. Five of these episodes are shown in the dotted lines (Figure 1B, top). During episode #3, with no recent RBC transfusion history, the patient presented with an HGB of 6.7 g/dL which decreased to 3.8 g/dL over five days, accompanied by lethargy, hypoxia and respiratory distress. Given her past history of DHTR and rapid decompensation, one dose of eculizumab 900mg IV was administered along with erythropoietin 210 IU/kg daily and IVIG at 1g/kg. The patient’s symptoms resolved with a rise in HGB and she was discharged home within four days.

Six months later, she presented again (#4) with VOC, worsening hypoxia, and HGB 7.9 g/dL (Figure 1B, bottom). On admission, she received erythropoietin and IVIG at the doses outlined above. The HGB dropped to a nadir of 3.6 g/dL over three days, associated with severe headaches. Due to inadequate response to the above measures, eculizumab 900mg was administered with rapid improvement in 48 hrs.

Complement analyses (Table 1) indicated clear evidence of ACP activation during episodes #1 and #5, as shown by elevation of Bb levels. During episodes #3 and #4, eculizumab was administered in anticipation of worsening organ function, which could explain why the complement activation markers were not significantly elevated (unlike in other episodes) and why her rapid HGB response and prompt reversal of organ dysfunction were observed. Complement gene evaluation revealed a heterozygous deletion of CFHR3/CFHR1.

Patient #3 is a 3-year-old male with a history of splenectomy at age two for recurrent acute splenic sequestration. He presented with fever, tachypnea, HGB of 7 g/dL, with a negative chest x-ray (CXR). He developed increased work of breathing within 3 hours of
receiving ceftriaxone. Repeat CXR revealed bilateral infiltrates requiring emergent intubation. HGB dropped to 4 g/dL, and he received extended phenotype-matched and crossmatch-compatible RBC transfusions. Additional laboratory work-up revealed intravascular hemolysis and MOF (Figure 1C). DAT was positive for complement component 3 (C3) only. The patient required escalation of respiratory support to high frequency oscillatory ventilation and nitric oxide. Oliguric acute kidney injury (AKI) and hypertension required the use of continuous renal replacement therapy and multiple anti-hypertensives. Multiple common and rare causes for his rapid multi-organ failure were entertained including sepsis (negative blood/respiratory cultures), cold agglutinin syndrome (negative testing for mycoplasma serology and Donath-Landsteiner antibody), hemophagocytic lymphohistiocytosis (normal soluble IL2 receptor, CD107a, perforin/granzyme B, Epstein Barr and cytomegaloviral load) and ceftriaxone-induced hemolysis. As his clinical course was consistent with CM-TMA, complement inhibition with eculizumab 600mg IV was initiated on day 5. Following eculizumab, he demonstrated a rapid response with weaning of his ventilator support and dialysis, along with the reduced need for blood products. Follow up testing was notable for strongly positive ceftriaxone-dependent antibodies consistent with DIIHA. Complement analyses confirmed significant activation of ACP (factor Bb elevation, see Table 1). Proximal and terminal complement pathway activation were likewise observed as indicated by increased C3 and C5 activation and C5b-9, respectively. Additionally, hypocomplementemia with reduced C3, C4, and CH50, seen in this patient, suggests worse disease. These markers improved following complement inhibition, which correlated with improved clinical status within eleven days of initial presentation. Eight
months after this episode, this patient’s renal function is gradually improving. He remains on eculizumab 300mg every two weeks pending full renal recovery. Complement genetic analysis was negative.

All patients and/or their guardians of cases described in this report provided written consent for the off-label use of eculizumab. These patients received meningococcal and pneumococcal vaccinations as part of routine SCD standard-of-care or given right before eculizumab and continued on a prophylactic antibiotic regimen while on treatment.

Complement-mediated TMA from an underlying mutation involving the complement regulatory genes is traditionally called ‘atypical HUS’. CM-TMA occurring secondary to complement amplifying disorders such as hematopoietic stem cell transplantation, malignancy, infections, or autoimmune diseases are termed ‘secondary HUS’.(3, 4) The above clinical vignettes suggest that some complications of SCD may also reflect complement activation-induced secondary HUS (Figure. 2). As SCD is a chronic hemolytic condition and plasma free HGB and heme can activate complement,(5, 6) additional complement activation during episodes of disease exacerbation may lead to increased hemolysis and a sustained positive feedback loop that leads to life-threatening anemia. As sickle erythrocytes are uniquely susceptible to complement-mediated damage,(7) this may also result in further exacerbation of the disease. Heme-dependent endothelial dysfunction seen in SCD can also be modulated by complement activation.(8) In addition, heme can regulate the coagulation system, which likely
reflects another important feature of SCD pathophysiology that contributes to inflammation and thrombosis. Increased C5a production has also been shown to cause acute lung injury and vasoocclusion in animal models. Prior reports suggested the role of ACP in the pathophysiology of SCD, and a protective effect of eculizumab in some settings. However, the present data suggest that some complications in SCD may not only reflect exuberant ACP activation, but actually represent an underlying CM-TMA. In these situations, chronic hemolysis and endothelial dysfunction may saturate scavenging and detoxifying mechanisms, reducing the capacity of patients with SCD to manage elevated plasma HGB and heme during periods of crises. In two of our patients, we detected variants in the complement genes. The *CFH* gene encodes soluble plasma factor H, which is a principal inhibitor of ACP. Further genetic studies focusing on complotype are needed to help understand its role in SCD. Figure 2 compares salient clinical features seen in aHUS and CM-TMA in SCD. In this way, the underlying pathophysiology of SCD may prime individuals for secondary HUS through ACP activation, with a subset of these patients reaching an inflection point during periods of crises (second-hit) that lead to additional hyperhemolysis and MOF. Case #3 is the first report of successful use of eculizumab to treat life-threatening DIIHA. Ceftriaxone-induced hemolysis often occurs secondary to IgM anti-ceftriaxone antibodies, which typically engage the classical complement pathway and induce hemolysis. However, the impact of eculizumab did not appear to reflect inhibition of IgM-induced hemolysis as marked activation of APC was evidenced by increased levels of factor Bb, which likewise responded to eculizumab, consistent with a positive feedback loop that drives additional APC activation in these patients. While rare, this event in
particular holds public health and preventative importance, as ceftriaxone is a widely used medication in SCD, and there is a high prevalence of ceftriaxone-induced RBC antibodies in these patients.(16)

This collection of cases therefore emphasizes a previously underappreciated role of TMA and complement across a broad range of SCD presentations, and may reflect novel insight into the pathophysiology of acute exacerbations of SCD that may be sensitive to complement inhibition to avoid severe hemolytic complications in SCD.

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Authorship Contributions

S.C. collected and analyzed the data and wrote the manuscript. S.G., J.G.N., M.O.Q., J.B., A.T., P.E.Z., M.R., C.D.J. collected and analyzed the data and reviewed the manuscript. H.H.S., C.B., C.H.J., R.M.F. and S.R.S. analyzed the data and provided critical revisions to the manuscript.

Disclosure of Conflicts of Interest
S.C. is a scientific advisor to Alexion and Agios pharmaceuticals. M.R.R. is a scientific advisor to Bluebird Bio. C.D.J. receive research funds from Terumo BCT, Octapharma and Medtronics. The remaining authors declare no competing financial interests.

References


Table 1. Evaluation of complement pathway during acute sickle cell crises

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<tr>
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<th>DHTR Patient #1</th>
<th>VOC Episodes Patient #2</th>
<th>DIIHA Patient #3</th>
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<td>C3 (71-150 mg/dL)</td>
<td>110</td>
<td>ND</td>
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<tr>
<td>C4 (15.7-47 mg/dL)</td>
<td>14.2</td>
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<tr>
<td>CH50 (101-300 units)</td>
<td>362</td>
<td>ND</td>
<td>ND</td>
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<td>Bb (0.49-1.42 mcg/mL)*</td>
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<td>1.41</td>
<td>1.9</td>
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<td>375.5</td>
<td>127.2</td>
<td>124</td>
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<tr>
<td>C5a (2.74-16.33 ng/ml)</td>
<td>&gt;37.3</td>
<td>31.26</td>
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<tr>
<td>SC5b-9 (≤ 244 ng/mL)</td>
<td>1208</td>
<td>307</td>
<td>97</td>
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<tr>
<td>Day of eculizumab administration</td>
<td>Days 8, 15, 22 and 29</td>
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DHTR - delayed hemolytic transfusion reaction, VOC – vasoocclusive crisis, DIIHA – drug induced immune hemolytic anemia, C3 - complement component 3, C4 - complement component 4, CH50 - screening test for total complement activity, Bb – complement component fragment Bb, C3a - complement component fragment 3a, C5a - complement component fragment 5a, C5b-9 - complement component fragment 5b-9.
component fragment 5a, sC5b-9 - soluble membrane attack complex, ND- not done. *8 week labs were performed few hours prior to eculizumab administration. The whole blood samples for all testing was collected in EDTA anticoagulant tubes and plasma was obtained within 2 hours of collection, and stored in -80C till they were ready for analysis with single thawing. This method of plasma collection in EDTA results in chelation of calcium and magnesium, thus preventing any in vitro complement activation. All testing was obtained in a CLIA certified hospital-based clinical laboratory. All normal values are in parentheses under each value except in patient #3, day 5* Bb normal ranges were 1.32 – 4.18 mcg/mL due to variability seen with different ELISA kits. Patient #1, day 8 and day 22 signifies the complement evaluation a few hours prior to respective dosing of eculizumab. Patient#2 presented with 5 episodes of VOC (each column represents episodes 1 through 5) with complement function testing coinciding with a drop in HGB of greater than 2 g/dL from baseline. Eculizumab were dosed a few hours after samples were collected during episodes #3 and #5. Patient #3, days 5, 22 and 8 weeks reflect the complement evaluation. Complement proteins C3 and C4 signify the quantitative serum levels. Fragment Bb is a serine protease that in combination with hydrolyzed C3 (C3H2O) generates C3 convertase (C3bBb), which augments the cleavage of C3 to generate C3a and C3b. Anaphylatoxins, C3a and C5a are involved in local inflammation and tissue damage, while C3b results in RBC opsonization and deposition on endothelium. Terminal complex, C5b-9 contributes to intravascular hemolysis.
1A) DHTR. The patient received a transfusion (black arrows) on day one and presented with DHTR on day 7 with a HGB of 9.4 g/dL, which dropped to a nadir of 5.6 g/dL by day 8 along with absolute reticulocytopenia, as expected in DHTR. Thrombocytopenia coincided with this severe anemia and elevations in LDH, total bilirubin, and AST. Other evidence for intravascular hemolysis included reduced haptoglobin (<14; reference 30-120 mg/dL) and elevated plasma free HGB (120; reference < 30 mg/dL) not shown. Eculizumab (E) 600mg was initiated on day 8 and given weekly for a total of four doses. The patient developed ACS and received one unit of crossmatch compatible, U-negative RBCs after a dose of intravenous immunoglobulin (IVIG) on day 9. The resolution of hemolysis was evidenced by improvement in LDH and HGB following the first dose of eculizumab and maintained throughout the hospital stay. Thrombocytopenia recovered within a week of complement inhibition. The patient required an additional transfusion on day 19 from rebound anemia likely secondary to frequent blood draws, since markers of hemolysis and complement activation on day 22 did not worsen. Immunohematology work-up during this DHTR episode: DAT negative, historical anti-U detected. Note: The initial three LDH values were greater than 4000 U/L (upper limit of our clinical lab detection). Eculizumab was dosed based on the weight of the patient, as per the guidelines for loading dose for children with aHUS.

1B) VOC. Grey colored dotted bars depict the time points when the patient presented with VOC and also corresponds to complement testing (numbered #1 through #5). Eculizumab was administered for episodes #3 and #4 as described. Lower graph
represents episode #4 of VOC and shows an initial drop in HGB and platelets by day 4, when erythropoietin (P) 210 IU/kg was commenced along with a dose of IVIG (I) 1g/kg on days 4 and 5, respectively. Given the continued deterioration in HGB to a nadir of 3.6 g/dL along with severe headache and worsening hypoxia, eculizumab (E) 900mg was administered on day 7 after the blood for complement function analyses was collected. Rapid improvement in hemolysis and clinical status was observed within 48 hours of a single eculizumab dose. Eculizumab was dosed based on the weight of the patient, as per the guidelines for loading dose for children with aHUS.

1C) DIIHA. The patient presented on day 0 to the hospital with fever and received ceftriaxone; HGB dropped within 3 hours to 4 g/dL. Black arrows on the graph denote blood transfusions. Additional laboratory workup showed evidence of intravascular hemolysis, confirmed by elevated LDH > 4000 U/L, presence of schistocytes on blood smear and elevated plasma free HGB (not shown). Multi-organ failure was evident with a peak of creatinine at 2.36 mg/dL (baseline 0.3 mg/dL), and aspartate transaminase/alanine transaminase at 1002/70 U/L along with rise in total bilirubin. Black colored bars at the bottom of the graph depict the time points when various supportive care measures and eculizumab were administered. Shortly after the initiation of eculizumab, hemolysis decreased, as shown by the rapid drop in LDH, and the patient required less transfusion support. Thrombocytopenia improved. He had initial improvement in creatinine on CRRT, with a brief increase when CRRT was weaned. This rise was not sustained, and creatinine levels decreased promptly without any additional intervention except continued eculizumab therapy. By day 25, blood counts and chemistry were
within normal limits. The rebound thrombocytosis persisted for few weeks before trending back to the patient’s baseline. Note: The initial three LDH values were greater than 4000 U/L (upper limit of our clinical lab detection). Eculizumab was dosed based on the weight of the patient, as per the guidelines for loading dose for children with aHUS. Blood smears from days 4 and 5 showing the presence of schistocytes and helmet cells (black arrows), along with paucity of platelets. DHTR- delayed hemolytic transfusion reaction, VOC- vasoocclusive crisis, DIIHA- drug-induced immune hemolytic anemia, HGB – hemoglobin, ARC – absolute reticulocyte count, E- eculizumab, LDH – lactate dehydrogenase, T.Bili- total bilirubin, AST- aspartate transaminase, ACS- acute chest syndrome, IVIG – intravenous immunoglobulin, CRRT- continuous renal replacement therapy

Figure 2. A model for CM-TMA in SCD

Increased understanding of complement-mediated conditions such as aHUS and paroxysmal nocturnal hemoglobinuria has renewed interest in understanding the specific role of complement in hyperhemolysis and innate immunity. Sickle cell disease is a prototypical disease of chronic hemolysis, where increased levels of free plasma hemoglobin and heme with an insufficient, and thus ineffective scavenging mechanism by haptoglobin and hemopexin, leads to a state that is primed for complement activation. In addition, sickle erythrocytes themselves appear to be uniquely susceptible to complement-induced hemolysis, thereby further amplifying complement activation and additional hemolysis. Like in aHUS, the already inflamed endothelium in patients with SCD can be further modulated from increased hemolysis, complement activation,
coagulation dysfunction, and other plasma proteins. Further genetic studies focusing on complement type are needed to help understand its role in SCD, as they can modulate the homeostatic balance of complement activity. Triggers such as pain crisis, acute chest syndrome, infection, etc. can drive this vulnerable state very quickly into a complement activated state, which, if unregulated, can result in common and terminal complement pathway activation that can lead to catastrophic damage in end organs and even death. Irrespective of the instigating cause, once complement-mediated hyperhemolysis is set off, it could result in a positive feedback loop causing further complement activation and a precipitous drop in hemoglobin and risk of sudden death. The table on the right parallels the resting and enabling state seen in SCD to patients with aHUS.

aHUS- atypical hemolytic uremic syndrome, CM-TMA: complement-mediated thrombotic microangiopathy, SCD- sickle cell disease, MAHA- microangiopathic hemolytic anemia, RBC- red blood cell
**Figure #2**

**Susceptible state**
- Ongoing hemolysis
- Ineffective scavenging mechanism
- Sickle erythrocyte
- Inflamed endothelium
- Plasma factors/pro-coagulant state
- Genetics

**Second Hit (triggers)**
- Hemolytic transfusion reaction
- Vasoocclusive crisis
- Acute chest syndrome
- Drugs
- Infection
- Chronic inflammation, others

**Complement**
- Activation
- Inadequate regulation
- Ineffective clearance

**Positive Feedback Loop**
- Hyperhemolysis
- Multi organ failure
- Organ damage

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<tr>
<td>Baseline endothelial dysfunction</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Hemolysis and exacerbations</td>
<td>During infections</td>
<td>Chronic and worsened during acute crises</td>
</tr>
<tr>
<td>Complement susceptible RBCs</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>MAHA + thrombocytopenia and organ dysfunction during crises</td>
<td>Yes</td>
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<td>Yes</td>
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