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Hemolysis and Septic Shock with Encapsulated Bacteria in Eculizumab Management of Paroxysmal Nocturnal Hemoglobinuria

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Abstract

Diagnosis and management of severe hemolysis in patients taking eculizumab is rather challenging and may indicate an onset of life-threatening conditions such as septic shock or drug resistance. Eculizumab-related sepsis due to *Neisseria meningitides* has been reported in the literature but report of severe infections secondary to other encapsulated organisms is lacking. Vaccinating patients against these bacterial pathogens may help prevent such devastating infections, but the bacterial organisms without vaccinations still pose a problem. Hemolysis related to eculizumab resistance has also been reported, and various hypotheses have been put forward. We present a young patient with paroxysmal nocturnal Hemoglobinuria treated with eculizumab, who subsequently developed severe sepsis, preceded by hemolysis and complicated by IgG-related extra vascular hemolysis.

Keywords: AIHA; PNH; Eculizumab; Resistance

Abbreviations

PNH: Paroxysmal Nocturnal Hemoglobinuria; PIG-A: Phosphatidylinositol N-Acetylglucosaminyltransferase Subunit A; GPI: Glycosyl Phosphatidylinositol; AA-PNH: Aplastic Anemia-Paroxysmal Nocturnal Hemoglobinuria; AA: Aplastic Anemia; CDR: Complementarity-Determining Regions; IgG: Immunoglobulin G; ATG: Anti-Thymocyte Globulin; FACS: Fluorescence-Activated Cell Sorting; FLAER: Fluorescent Aerolysin; CBC: Complete Blood Count; ANC: Absolute Neutrophil Count; TRIUMPH: Randomized, Placebo-Controlled, Phase 3 Study; DAT: Direct Antiglobulin Test; AIHA: Autoimmune Hemolytic Anemia; FcγRs: Fc-Gamma Receptors; RBC: Red Blood Cell; ADCC: Antibody-Dependent Cellular Cytotoxin

Background

Paroxysmal Nocturnal Hemoglobinuria (PNH) is a hematopoietic stem cell disease characterized by complement-induced intravascular lysis of erythrocytes. These cells are abnormally vulnerable to attacks by complement due to the clonal expansion of stem cells with the PIG-A gene mutation, resulting in the deficiency of the endogenous Glycosyl Phosphatidylinositol (GPI)-anchored complement inhibitor protein CD59 on the surface of blood cells [1]. AA-PNH syndrome was first reported in 1967 as a case of AA presenting with PNH-like characteristics of hemolysis, bone marrow failure and thrombosis [2]. The only curative therapy for PNH is hematopoietic stem cell transplantation, but because of severe morbidity and mortality associated with this procedure, patients have been traditionally managed with blood transfusions, steroids, and warfarin.

Eculizumab is a humanized monoclonal antibody directed against the complement protein C5, inhibiting C5 cleavage to C5a and C5b, preventing the generation of the terminal complement complex C5b-9 and thus blocking complement-mediated cell lysis and activation. The antibody is an $IgG_{2/4}$ kappa immunoglobulin comprised of human constant regions and murine Complementarity-Determining Regions (CDRs) grafted onto human framework light- and heavy-chain variable regions. Eculizumab has been shown to inhibit intravascular hemolysis, but may lead to extra vascular hemolysis by inhibiting C5 long enough for C3d fragments to accumulate on the surface of erythrocytes [3].

The most life threatening complication associated with the use of eculizumab is sepsis related to encapsulate bacterial organisms [4]. Phase III clinical trials have shown that patients treated with eculizumab may rarely develop sepsis with *Neisseria meningitides* [5]. The increased risk of meningococcal infection seems to be due to the critical action of the terminal complement in prevention of such infections. Complications related to the use of eculizumab including sepsis are rare but can be preceded by hemolysis. Therefore vigorous search for underlying infection should be considered in the setting of worsening hemolysis.

Case Report

A 25 year-old African American female with a nine-year history of a plastic anemia and two-year history of PNH was admitted for sepsis and severe hemolysis.

She initially presented at age 16 with severe anemia and was diagnosed with aplastic anemia. She did not have a matched sibling donor and her bone marrow function was adequate enough that she was managed for a long time with supportive care measures including cyclosporine, pentamidine, Anti-Thymocyte Globulin (ATG), mycophenolatemofetil and red cell transfusions. She was found to have normal CD59 expression at her initial presentation. However, the disease evolved and she developed a hemorrhagic

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stroke associated with a cerebral venous sinus thrombosis that led to the diagnosis of PNH based on reduced expression of CD55 and CD59 with Fluorescence-Activated Cell Sorting (FACS) and Fluorescent Aerolysin (FLAER). She was then initiated on eculizumab and maintained on 1200mg every 14 days. Complete inhibition of complement activity was seen in vitro per Alexion testing.

Upon admission at age 25, she presented with complaints of abdominal pain, fatigue, and diarrhea. Complete Blood Count (CBC) revealed hemoglobin 7.2 g/dL (normal 11.7-15.7 gm/dl), Absolute Neutrophil Count (ANC) 3268 cells/mm^3, lactate dehydrogenase (LDH) 2144 U/L (normal 313-618 U/L), total bilirubin 1.3 mg/dL (normal 0.1-1.1 mg/dl), platelets 81 (150-450 /mcl), creatinine 1.80 (0.5-1.1 mg/dL)and haptoglobin not detectable. Blood culture grew *Klebsiellaoxytoca* and *Escheriahermannii*. Her sepsis has resolved with fluid resuscitation and intravenous cefepime, vancomycin, and metronidazole. The patient was seen in the outpatient clinic and her labs demonstrated initial improvement followed by a spike in hemolytic markers including LDH 2229 U/L, total bilirubin 1.6 mg/dL, and hemoglobin 8.0 g/dL. Platelets remained stable at 201 /mcl and creatinine stayed at baseline 1.70 mg/dL. A direct antiglobulin test detected warm anti-IgG auto antibodies along with schistocytes on peripheral smear. The patient was treated with prednisone and showed rapid improvement in her hemolysis.

Discussion

Eculizumab is the first and only targeted therapy introduced for the treatment of PNH. Based on the clinical trials, eculizumab has been found to be effective, well tolerated, and relatively safe [4-7]. The efficacy of eculizumab was first demonstrated by the TRIUMPH study in 2006, a double-blinded, randomized, placebo-controlled, phase III trial involving 87 patients, with 86% reduction in intravascular hemolysis [7]. Our patient experienced similar efficacy with decrease in LDH at 16 weeks and further improvement at 26 weeks (Figure 1). However, she developed severe hemolysis during the episode of sepsis.

Sepsis is a major potential complication of eculizumab since the drug inhibits the natural mechanism of terminal complement activation against encapsulated bacteria [4,8]. Infections related to encapsulated bacterial pathogens such as *Klebsiellaoxytoca* and *Escheriahermannii* has not been described in the literature, especially the latter is rarely accountable for sepsis. The patient in this report was vaccinated against *Neisseria meningitidis*, and has received the tetravalent meningococcal vaccine against subgroup A, C, W, and Y at least 14 days prior to receiving the first dose of eculizumab. Currently, there are no vaccinations available against *Neisseria meningitides* serogroup B. Antibiotic prophylaxis has been recommended for patients (penicillin V 500 mg twice a day or erythromycin 500 mg twice a day) in areas where there is a high prevalence of serotype B [9].

Sepsis has been shown to be associated with both intravascular and extra vascular hemolysis [10,11]. The rapid surge in hemolytic markers prior to the episode of sepsis could be an indication of infectious process. However, development of eculizumab resistance contributing to the hemolysis from sepsis is another possibility. With treatment of sepsis, the patient's hemolytic markers (including haptoglobin, LDH and bilirubin) have improved but remained abnormally elevated, further supporting the possibility of ongoing intravascular hemolysis. Concurrently, the patient may have developed IgG-related extra vascular hemolysis, however, Direct Antiglobulin Test (DAT) was not carried out at this point.

The patient improved with treatment of infection, but a few months later worsened due to autoimmune hemolytic anemia (Figure 1 and 2). Positive DAT after the resolution of the septic episode points to a separate hemolytic event as the markers related to intravascular hemolysis have subsequently increased. Intravascular hemolysis could be related to eculizumab resistance, as it cannot be explained by any other mechanism. The possible mechanisms of eculizumab resistance include C3-mediated hemolysis [12], as well as genetic variation in C5 [13]. Eculizumab also increases the percentage of CD55-, CD59- erythrocytes in peripheral circulation and during acute AIHA, these cells may undergo complement-mediated hemolysis and generate schistocytes [6,14]. A previous study showed that erythrocytes from PNH patients on long term treatment with eculizumab were twice as vulnerable as normal erythrocytes to lysis induced by complement activated serum [15].

However, for our patient, DAT was negative for anti-complement, which hints at a unique mechanism separate from the above. Complement-independent hemolysis may result from the formation of soluble immune complexes or cellular-bound antibody with the engagement of cellular effectors. Ligand cross-linking of

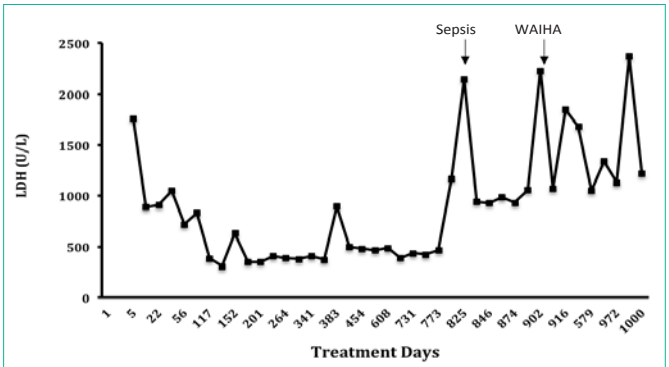

Figure 1: Lactate dehydrogenase levels over time. Treatment with eculizumab was initiated on day 1. Serum concentration has been shown to reach steady state after about 150 days. LDH spikes at day 152 and 383 were clinically asymptomatic. Sepsis corresponds to hospitalization on day 825 and warm AIHA was diagnosed on day 902.

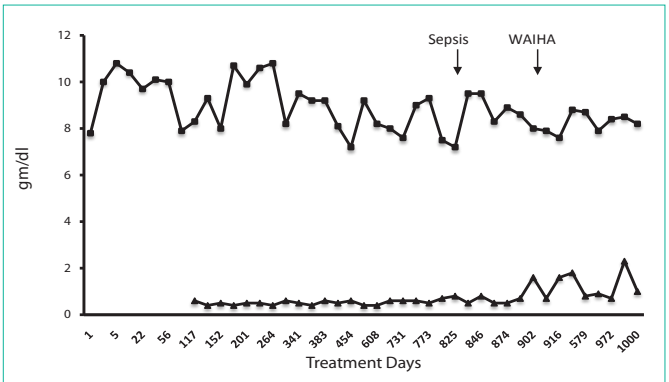

Figure 2: Hemoglobin and total bilirubin levels during treatment. Eculizumab was initiated on day 1. Sepsis corresponds to hospitalization on day 825 and warm AIHA was diagnosed on day 902.

FcγRs on natural killer cells, neutrophils, basophils, eosinophils, and monocyte-derived cells initiates the activation of phagocytosis, Antibody-Dependent Cellular Cytotoxin (ADCC), and the release of inflammatory mediators. This can ultimately lead to cellular destruction and the amplification of hemolysis [16], a mechanism similar to that of drug-induced immune hemolytic anemia [17].

Previously, drugs that bind covalently to RBC membrane components have been shown to stimulate hapten-dependent antibodies [18]. Eculizumab is a high affinity anti-C5 antibody (K_d=120 pmol/L) [6] that may cause a subtle structural change in the cell surface antigens, allowing *in vivo* sensitization and specific binding of the resultant antibodies. The resultant auto antibodies do not activate complement but instead cause Fc-mediated extra vascular hemolysis. To explore this hypothesis, further testing is needed with antibody specificity analysis performed while a patient on eculizumab is actively undergoing hemolysis.

Conclusion

Worsening hemolysis in PNH patients taking eculizumab could be related to sepsis or drug resistance. A prompt and comprehensive workup to rule out sepsis should be initiated to prevent such life threatening complication. Patients who take eculizumab need to be vaccinated and antibiotic prophylaxis should be considered in high-risk areas. A full workup related to eculizumab resistance including direct antigen test for complement, and genetic testing should be undertaken. If extra vascular hemolysis is suspected, further investigations should be initiated and then treated with steroids.

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