DELAYED ERYTHROID AND PLATELET RESPONSE TO ECULIZUMAB IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA - A CASE REPORT AND LITERATURE REVIEW

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ABSTRACT

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired clonal disorder of haemopoiesis characterised by haemolytic anaemia, thrombophilia and variable cytopaenias. Complement-mediated blood cell damage leads to the main clinical features of PNH, including anaemia, haemoglobinuria, other haemolysis-related symptoms, thrombosis, and thrombocytopaenia. The treatment of PNH has remained supportive until the development of the first complement inhibitor, eculizumab. This antibody efficiently blocks terminal complement activity, quickly halting intravascular haemolysis. However, both the time course and the magnitude of erythroid and platelet responses to this drug are highly variable. Here, we report a case illustrating both delayed erythroid and platelet responses to eculizumab, and review mechanisms and therapeutic options for partial responses.

Keywords: Paroxysmal nocturnal haemoglobinuria, intravascular haemolysis, anaemia, thrombocytopaenia, bone marrow failure, eculizumab.

INTRODUCTION

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare clonal haematological disorder caused by an acquired deficiency of the glycosylphosphatidylinositol (GPI) anchor in a haematopoietic stem cell. The defect is transmitted to all its progeny with consequent absence or reduction in all plasma membrane GPI-anchored proteins in a subpopulation of every blood cell type.[1,2] The absence of the GPI-anchored complement inhibitory proteins, CD55 and CD59, renders affected blood cells susceptible to complement attack, which causes intravascular haemolysis and damage to other blood cells. The consequences are haemolytic anaemia, haemoglobinuria, thrombosis, dysphagia, abdominal pain, and other PNH clinical symptoms.[1,2]

A clinically evident or subclinical bone marrow failure is frequently the background where a PNH clone appears and expands, sometimes to become responsible for a meaningful proportion of haemopoiesis.[2] Thus, besides haemolytic anaemia, PNH is also characterised for cytopaenia, thrombocytopaenia and/or neutropaenia of variable severity. These cytopaenias may involve multiple physiopathological pathways, such as bone marrow failure and/or complement-mediated cell damage.

Classically supportive measures are employed to treat cytopaenias, including red blood cell (RBC) transfusions and iron and folic acid supplementation.[3] 6 years ago, both the US Food and Drug Administration and European Medicines Agency (FDA and EMA), American and the European medical regulatory agencies, approved eculizumab to treat PNH. It is a humanised monoclonal antibody that binds to complement fraction 5, blocking its cleavage to C5a and C5b, mediated by the C5 convertases. The assembly of the terminal complement complex is thus inhibited.[1] Eculizumab treatment results in haemolysis blockade, stabilisation of haemoglobin

Table 1. Haematological responses to 4 months corticosteroids (03/2011) and to long-term eculizumab (06/2011).

Date	Hb (g/dL)	Reticulocytes /μL	WBC /μL	Platelets /μL	I. Bilirubin (mg/dL)	LDH (⊥: ≤480 U/L)	RBC (units transfused)*
10/2008	9.4	-	4,260	138,000	0.82	1,725	0
03/2009	9.2	-	4,130	161,000	1.79	1,777	0
09/2009	9.3	84,500	3,750	144,000	1.7	3,247	0
07/2010	9	53,460	3,500	100,000	1.01	4,380	0
12/2010	6.6	121,800	2,700	45,000	2	2,593	0
02/2011	4.7	84,500	2,650	30,000	3.1	2,944	4
03/2011	6.1	475,000	3,240	16,000	3.5	4,380	4
04/2011	7.3	179,250	4,100	41,000	1.3	1,281	7
06/2011	7.5	258,700	5,030	49,000	1.4	2,234	0
10/2011	7.6	62,140	2,440	31,000	1.5	544	6
02/2012	7.2	157,560	3,470	68,000	2.2	520	6
06/2012	7.1	184,240	3,050	89,000	2.2	504	2
10/2012	8.2	389,480	3,720	98,000	2.7	479	0
02/2013	8.7	201,840	3,910	141,000	2	380	0
04/2013	10.6	183,040	5,960	157,000	2.5	373	0

* RBC units transfused in the interval from the previous control date until the following one.

levels, resolution or decrease in transfusion requirements, improvement in quality of life,[4] and sometimes an increase in platelet counts.[5] However, the time and magnitude of maximal erythroid and platelet response to the drug is variable in every patient.

We report here a case with a delayed bilineal response to eculizumab, showing a long-term effect of complement blockade.

CASE REPORT

A 20-year-old woman was diagnosed in July 2002 with infectious mononucleosis. She later experienced more episodes of jaundice. In March 2005, a haemolytic crisis with fever, back pain, and dark urine took place. Her initial blood analysis revealed bicytopaenia, haemolysis and haemoglobinuria.

An abdominal ultrasound showed normal suprahepatic and portal veins, as well as a normal spleen. A flow cytometry of peripheral blood was positive for PNH (October 2005), with clone sizes of 68.5% in granulocytes, measured as CD66b negative neutrophils, and 27.02% in RBC, as shown by CD59 negative erythrocytes.

The patient was treated with folic acid and iron. Her clinical course was characterised by chronic haemolysis, with acute exacerbations every 1 to 3 months, with or without an identified trigger. She also experienced episodic dysphagia and abdominal pain, and subsequently her cytopaenias worsened (Table 1 and Figure 1). In January 2011, a disabling fatigue compelled her to leave work, with RBC transfusions required. A new peripheral blood flow cytometry showed a PNH clone size of 92% in granulocytes and 22% in erythrocytes.

A bone marrow biopsy showed a 20% cellularity with relative erythroid hyperplasia and without fibrosis (Figure 2). The cytogenetic study was normal. On March 2011, severe thrombocytopaenia with gingival bleeding and menorrhagia appeared. Eculizumab was prescribed and corticosteroids were administered. A more immunosuppressive therapy was postponed until the response to eculizumab could be evaluated.

Corticosteroid treatment (meprednisone 40 mg daily) was associated with both a decrease in transfusion requirements and increases in leukocyte and platelet counts, but at the expense of a pharmacological Cushing's syndrome.
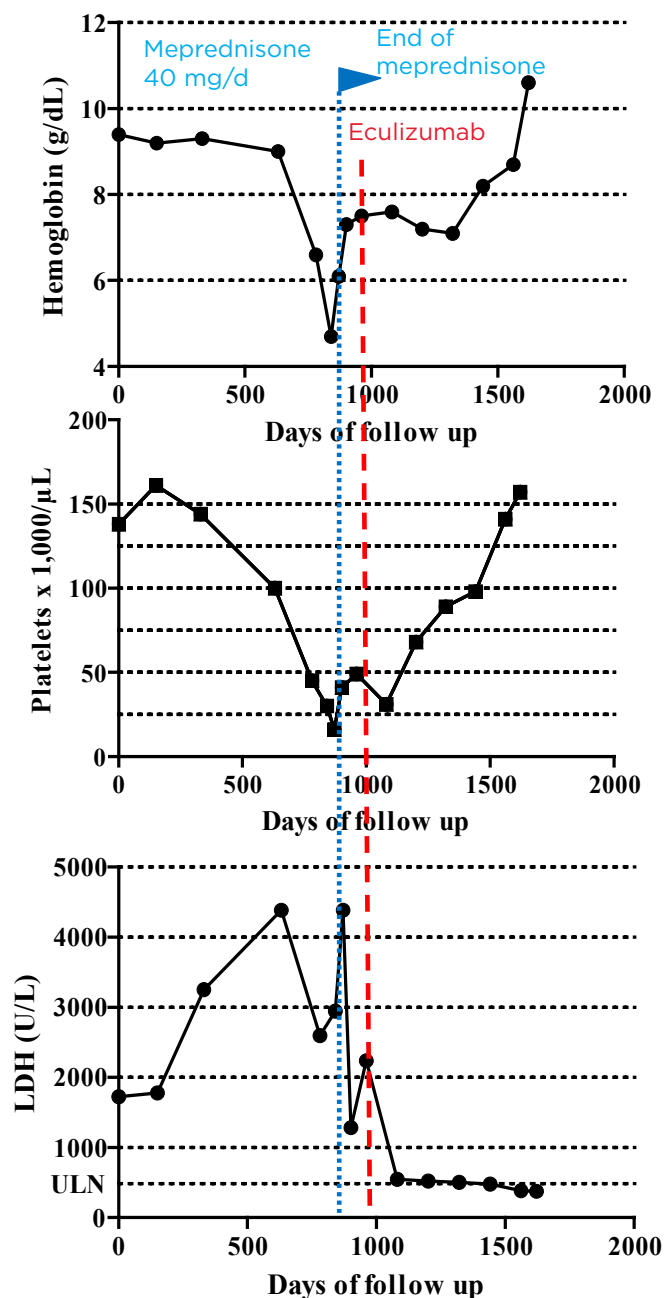
Figure 1. Graphs showing spontaneous and treatment-related changes in haemoglobin, platelet counts and LDH levels, start of meprednisone and eculizumab treatments and end of corticosteroids. ULN: upper limit of normal of LDH levels.

In June 2011, eculizumab treatment was started at the prescribed doses: 600 mg weekly for 4 doses, 900 mg at the fifth week, and then 900 mg every 14 days. Doses of meprednisone were progressively lowered and halted, lactate dehydrogenase (LDH) values sharply decreased, but RBC transfusion requirements persisted.

An erythrocyte survival study showed two RBC populations differing in their half-lives, seen at 12.5 and 23 days respectively, with a normal RBC lifespan range of 25-31 days for this study, with erythrocyte destruction taking place mainly in the spleen. Erythropoietin levels were very high at >750 mU/mL (normal range 9-19 mU/mL), as were ferritin levels (between 761 and 1,275 ng/mL), attesting to iron overload secondary to RBC transfusions. A liver Magnetic Resonance Imaging (MRI) scan confirmed an increase in hepatic iron.

Three therapeutic options were considered to address the ongoing transfusion requirements:

a) Low doses of corticosteroids to reduce extravascular haemolysis.

b) Splenectomy to remove the main site of PNH RBC destruction.

c) Erythropoietin or danazol to stimulate erythropoiesis. However, as reticulocytes counts and erythropoietin levels were high, their chance to achieve transfusion independence was judged very low.

An alternative option was to continue with RBC transfusions and to begin iron chelation.

The patient refused low doses of corticosteroids and chose to continue with eculizumab and supportive treatment. In the following months, her transfusion requirements diminished progressively and her platelet counts increased (Table 1). After 1 year on eculizumab, she required no more transfusions and her platelet counts increased above 80,000/μL in the last year and above 100,000/μL in the last 4 months (Table 1 and Figure 1). A new flow cytometry performed in March 2013 showed a PNH clone size of 95.72% in granulocytes and 56.84% in erythrocytes.

DISCUSSION

Eculizumab treatment blocks intravascular haemolysis in PNH patients. The results are an increase of PNH RBC counts and a consequent improvement of anaemia.[6] In the present case, PNH red cell counts increased in a 24 month period (March 2011 to March 2013 respectively) from 651,000/μL to 1,415,316/μL, accounting for both transfusion independence and improved haemoglobin levels.

The persistence of anaemia in PNH patients treated with eculizumab is mainly due to two mechanisms:

a) Activation of the proximal alternative pathway of complement, not blocked by eculizumab, leading to the deposition of C3b and its catabolites on PNH RBC surface. These C3 peptides are opsonines,
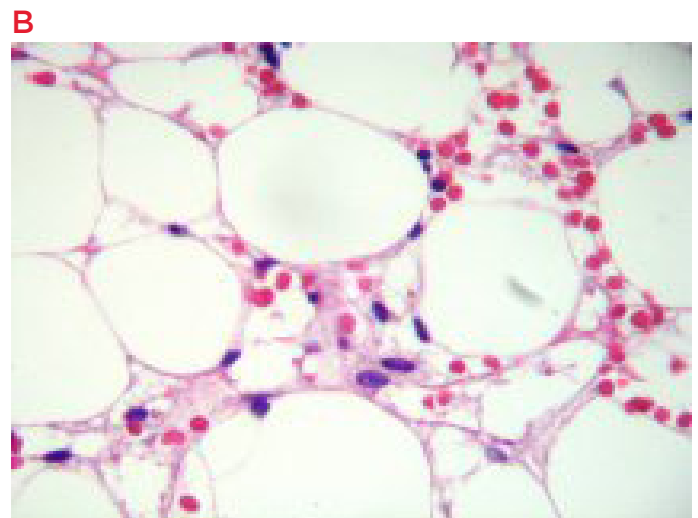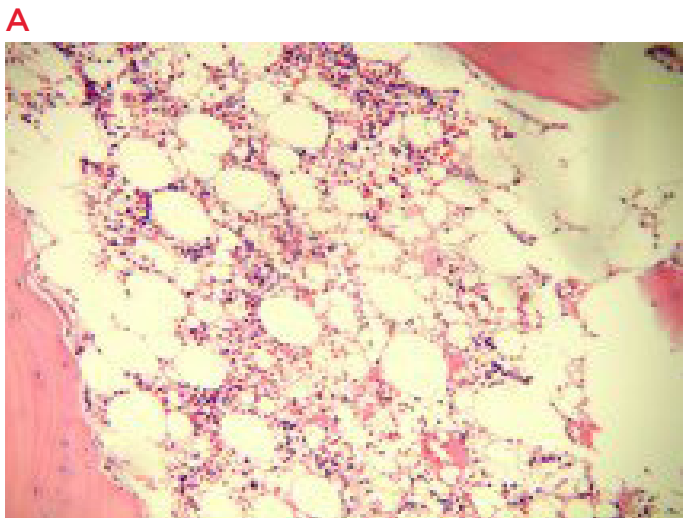
A

B

Figure 2. Bone marrow biopsy shows a 20% cellularity with relative erythroid hyperplasia (HE A: 100x B: 400x).

recognised by complement receptor 2 present in the surface of reticuloendothelial system macrophages. PNH erythrocyte phagocytosis then takes place, accounting for the extravascular haemolysis associated to eculizumab treatment.[7,8]

b) Bone marrow failure, present in nearly every patient with PNH, with deficient erythropoiesis, inadequate to fulfill patient requirements.[2]

In every case, the therapeutic options will be different. In the setting of bone marrow failure, erythropoietin or danazol may be tried to stimulate erythropoiesis. With predominant extravascular haemolysis, options include splenectomy, corticosteroid use, or blood transfusions associated with iron chelation. Transfusions with packed RBCs do not stop haemolysis, only transiently improve anaemia-related symptoms, cause iron overload, and may transmit multiple infections. Corticosteroids and androgen therapy, on the other hand, may lead to well-known adverse effects. Splenectomy has halted transfusion requirements in a case with significant extravascular haemolysis, taking place mainly in the spleen.[9] However, the risks (thrombosis, life-long immunosuppression) and benefits should be carefully balanced before this surgery is prescribed.

As shown by the present case, the deadline to deciding whether to remove a spleen as a rescue therapy to avoid transfusions in an eculizumab-treated patient should extend to more than a year (between 12 and 16 months). Due to corticosteroidal side effects (obesity, acne, hirsutism) the patient refused to receive them, which in turn allowed a

late erythroid and platelet response to eculizumab to appear.

Thrombocytopaenia in PNH also has several physiopathological mechanisms: bone marrow failure, ineffective thrombopoiesis, platelet activation mediated by complement, with or without haemostatic consumption (microthrombosis), or platelet margination in an enlarged spleen or in the vascular bed.[10] In some, but not all, PNH patients with thrombocytopaenia, platelet survival is reduced.[10,11] Furthermore, in eculizumab-treated patients a study showed an inverse relationship between blood levels of both thrombosis and inflammation markers, on one hand, and platelet counts on the other.[12] So, in some PNH patients, thrombocytopaenia may be partially due to thrombin-mediated consumption of platelets, as attested by the quick rise in platelets seen in a previous case.[13]

Eculizumab treatment does not globally produce an increase in platelet levels. However, PNH patients with severe thrombocytopaenia experienced a highly significant rise in platelet counts, at odds with what happened to the less affected ones. This platelet response in a subgroup of patients was not paralleled with an increase in marrow blood cell production.[5] Although more research is required, all these findings suggest that terminal complement activity results in PNH-platelet activation, as has been shown *in vitro*,[12] frequently causing platelet consumption with thrombocytopaenia and thrombosis.

This case also shows that, instead of a shorter platelet half-life as compared to RBC, a platelet response to

eculizumab may also be a delayed phenomenon, requiring months, or even more than a year, to show its full magnitude. The mechanisms underlying such a late improvement are not clear. A spontaneous improvement in marrow hypoplasia may have played a role, but the initial response of both RBC and platelets to corticosteroids and subsequently to eculizumab suggests a PNH-related reduced survival as the main cause of bicytopaenia.

CONCLUSION

In conclusion, this case illustrates that both erythroid and platelet responses to eculizumab can be delayed phenomena. Therefore, many months may be required before undertaking alternative, more toxic, or invasive treatments.

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