Presentation and management of paroxysmal nocturnal hemoglobinuria: a single-center experience

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Abstract

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired disorder characterized by intravascular hemolysis. Real-world experience of PNH management is largely unreported. A retrospective analysis was undertaken based on medical records from six patients with PNH [two with aplastic anemia (AA)] treated at our center, Dicle University, Turkey. Diagnosis was based on granulocyte PNH clones, ranging from 93% to 66%. All patients had symptoms consistent with PNH. One patient was managed adequately with supportive measures only. Five were treated with the complement inhibitor eculizumab. Follow-up data (<1 year) were available in four cases (the fifth had received only three infusions by final follow-up). Hemoglobin level in these four patients increased from 4.1-7.2 g/dL to 8.3-13.0 g/dL. Lactate dehydrogenase, a marker for hemolysis, decreased profoundly in the two non-AA patients, with more minor improvements in the two AA patients. Weakness and fatigue improved in all eculizumab-treated patients. Four of the five treated patients became transfusion independent, including the patient given only three infusions. In the remaining case, a patient with AA, transfusion requirement decreased, and abdominal pain and dysphagia resolved. No adverse events occurred. PNH can be successfully managed in routine practice.

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired disorder with an estimated incidence of 1.5 to 2 cases per million of the population per year.[1] It can develop in the absence of any other bone marrow disorder or secondary to conditions such as aplastic anemia (AA). PNH is characterized by somatic mutations in hematopoietic stem cells.[2] These mutations inhibit synthesis of the glycophosphatidylinositol (GPI) anchor, leading to a deficit of GPI-linked complement regulatory proteins on the cell surface of erythro-

cytes.[3] As a consequence, the erythrocytes become susceptible to uncontrolled complement-mediated intravascular hemolysis. Symptoms include anemia, dyspnea, fatigue, headache, dysphagia, erectile dysfunction and abdominal pain, and quality of life is compromised. Rates of hospitalization for PNH-related complications such as renal dysfunction and pulmonary hypertension are high,[4] and approximately 25% of patients die within 10 years of diagnosis,[5] most frequently due to thromboembolic events.[4]

Allogeneic stem cell transplantation offers a potential cure but the associated morbidity and risk of mortality usually precludes its use in cases of PNH. Instead, the management of PNH has historically been based on supportive measures, with frequent use of red blood cell transfusions and anticoagulation therapy.[4] Since 2007, the humanized monoclonal antibody eculizumab has been licensed for the treatment of PNH. It acts by binding to complement protein C5, thereby inhibiting terminal complement formation,[6] and in clinical trials has significantly reduced hemolysis, transfusion requirements, the incidence of thrombotic events and improved fatigue and quality of life, with an acceptable safety profile.[7-11]

The rarity of PNH means that reports of its presentation and treatment outside the confines of a clinical trial, or real-world experience with eculizumab therapy in this setting, are extremely sparse.[12]

We report here six cases of PNH who were identified and managed at our center under routine conditions.

Materials and Methods

The Department of Hematology of Dicle University in southeast Turkey is the specialist hematology referral center for a population of approximately six million people. All patients presenting with Coombs-negative non-immune hemolytic anemia, refractory anemia myelodysplastic syndrome, AA or atypically-located thrombosis are screened for PNH. Patient records from January 2011 to November 2015 were reviewed to identify all cases of PNH.

Diagnostic procedures were performed according to the International Clinical Cytometry Society guidelines.[13] Diagnosis of PNH requires that at least two different GPI protein deficiencies be detected within two different cell lines from granulocytes, monocytes or erythrocytes. At our center we used fluorescently-labeled aerolysin (FLAER) flow cytometry, the most sensitive and reliable diagnostic method available, to assess GPI protein deficiency in granulocytes and monocytes since previous transfusions or massive hemolysis

can bias data from erythrocytes.

Autoimmune hemolytic anemia was ruled out by the direct Coombs test. Diagnostic bone marrow aspiration and biopsy were performed in cases of bone marrow failure.

Red blood cell transfusions were given to patients in whom hemoglobin (Hb) level was <7 g/dL or in whom symptoms of anemia were present. Thrombocyte transfusions were given if thrombocyte level decreased below 20.000 mm[3] or if bleeding occurred in patients with AA. In patients with a confirmed diagnosis of PNH, eculizumab was initiated in response to presentation with fatigue, with or without other symptoms, in transfusion-dependent patients. Eculizumab was administered by intravenous infusions, dosed as per the manufacturer's recommendations i.e. 600 mg weekly for the first four weeks, followed by a dose of 900 mg one week later, and 900 mg every two weeks thereafter.[14] Patients were assessed prior to the start of eculizumab therapy and every two weeks thereafter, including complete blood count and measurement of lactate dehydrogenase (LDH), indirect bilirubin, creatinine and reticulocytes to evaluate the extent of hemolysis. FLAER testing was repeated every six months to evaluate clone size.

Due to the increased risk of *Neisseria*

meningitidis meningococcal infection in patients treated with eculizumab,[15] all patients treated with eculizumab were vaccinated with a polyvalent meningococcal vaccine. Patients were closely monitored for early signs and symptoms of meningococcal infection.

Data collection from the medical records included demographics at the time of diagnosis, presence or absence of AA or other bone marrow conditions, clinical presentation, transfusion requirements, use of medication to treat PNH or AA, and the dose and number of eculizumab infusions (if used). Information on PNH clone sizes in both granulocytes and monocytes was collected but data from granulocytes were used to establish clone sizes. Hematology and biochemistry results were captured at the time of diagnosis and at the last follow-up point prior to analysis in November 2015.

Results

In total, approximately 600 patients were screened for PNH during January 2011 to September 2015. Small asymptomatic PNH clones were detected in 14 patients, ranging in size from 0.1% to 12%. A diagnosis of symptomatic PNH was confirmed in six cases, which are described here. Last follow-up of these six

patients took place in November 2015.

The six patients with PNH ranged from 17 to 46 years of age (Table 1). Five were male, one was female. In two patients with bone marrow failure, the presence of AA was confirmed by bone marrow aspiration and biopsy (Patients #3 and #4). All six patients presented with fatigue, with or without other symptoms typical of PNH (Table 1). No patient had any co-morbid disease and there was no evidence of organomegaly. No patient had experienced PNH-related complications, including thrombosis. Granulocyte PNH clone sizes ranged from 93% to 66%, and were generally mirrored by monocyte clone sizes (Table 1). The highest granulocyte clone sizes were seen in three of the non-AA patients (Patients #1, #2 and #6). All patients had a negative direct Coombs test.

All patients were severely anemic (range 4.1-7.2 g/dL), particularly the two patients with AA (4.1 and 4.8 g/dL). Mean corpuscular volume (MCV) values at baseline were within or above the normal reference range. Counts of white blood cell counts, neutrophils and platelets were low in the patients with AA, as expected. Levels of LDH, a marker for hemolysis, were dramatically elevated in three of the patients with PNH and no AA (Patient #1, 1687 IU/L; Patient #2, 1995 IU/L; Patient #6, 1136 IU/L), less severely raised in the other non-AA patient (706 IU/L), mildly elevated in one of the patients with AA (464 IU/L) and within nor-

mal range for the second patient with AA (262 IU/L), consistent with the observed differences in granulocyte clone sizes. Compatible with this, reticulocyte counts and bilirubin levels were higher in the patients without AA, indicative of high erythrocyte turnover. Both of the patients with PNH and AA were receiving immunosuppressive treatment with horse antithymocyte globulin and cyclosporine at presentation (Patients #3 and #4). Two patients, one without AA (Patient #2) and one with AA (Patient #4), were receiving steroid therapy. In both patients, cyclosporine therapy was ongoing at last follow-up, after a minimum of two years' treatment.

All six patients were offered eculizumab. One patient (Patient #6) who had Hb 11 g/dL and only moderately increased LDH (706 IU/L), was offered eculizumab to reduce the risk of thrombotic complications. He declined, and was instead managed using red blood cell transfusions, with short-term steroid therapy during hemolytic attacks. At last follow-up, more than four years after diagnosis, he remains free of PNH-related complications but continues to experience fatigue.

Four patients received eculizumab as planned, being given a total of 8, 18, 25 and 23 doses during the analysis period, respectively, with a duration of 11, 31, 47 and 41 weeks between start of therapy and last follow-up. A further patient (Patient #5) had received only

Table 1. Patient characteristics.

	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Patient #6
Age, years	46	24	31	17	45	30
Gender	Male	Male	Female	Male	Male	Male
Symptoms of PNH	Fatigue, dyspnea	Fatigue	Fatigue, abdominal pain, dysphagia	Fatigue, easily tired	Fatigue, dark urine	Fatigue
Date of diagnosis*	June 2015	March 2015	July 2014	September 2013	January 2011	September 2015
Diagnosis	PNH	PNH	PNH, aplastic anemia	PNH, aplastic anemia	PNH	PNH
PNH clone size,° granulocytes (CD24), %	91	93	84	66	75	93
PNH clone size,° monocytes (CD14), %	89	88	78	61	77	91

PNH, paroxysmal nocturnal hemoglobinuria. *Last follow-up November 2015. °Fluorescently labeled aerolysin assay.

Table 2. Hematology and biochemistry values before eculizumab treatment (baseline)# and at last follow-up.

	Patient #1 B.	Patient #1 L.	Patient #2 B.	Patient #2 L.	Patient #3 B.	Patient #3 L.	Patient #4 B.	Patient #4 L.	Patient #5 B.	Patient #5 L.	Patient #6 B.	Patient #6 L.	Normal range
Hb, g/dL	7.2	10.6	6.2	12.0	4.8	8.3	4.1	13.0	11.0	-	7.2	-	14-18 (M), 12-16 (F)*
White blood cell count, /mm³	12,800	8800	8100	5900	2700	2600	1100	5600	2400	-	7900	-	5000-10,000*
Absolute neutrophil count, /mm³	8200	4800	5700	3400	480	700	850	1280	900	-	4800	-	1500-8000*
Platelet count, ×10³/mm³	727	546	99	125	22	14	14	21	70	-	295	-	200-500*
Reticulocytes, % RBC	11	3.5	4.4	2	1.1	1	0.8	0.9	5.6	-	5.1	-	0-1%*
Lactate dehydrogenase, IU/L	1687	233	1995	216	464	204	262	238	706	-	1136	-	105-333°
Total bilirubin, mg/dL	2.2	3.0	2.8	2.3	1.1	1.1	0.6	0.5	2.2	-	0.7	-	<1.0*
Direct bilirubin, mg/dL	0.7	0.8	0.7	0.6	0.5	0.3	0.2	0.2	0.6	-	0.4	-	<0.4*

#Values recorded at clinic visit immediately prior to start of eculizumab therapy . B, baseline; L, last follow-up; Hb, hemoglobin; RBC, red blood cells. M, male; F, female. *Food and Drug Administration Investigations Operations Manual 2015; °US National Library of Medicine. Time from start of eculizumab therapy to last follow-up was 11, 31, 47 and 41 weeks in Patients #1, #2, #3 and #4, respectively. Follow-up data were not available for Patient #5 due to short follow-up. Patient #6 declined eculizumab therapy.

three doses of eculizumab therapy by the end of the follow-up period, such that meaningful post-treatment laboratory results were not available. All patients remained on eculizumab treatment at last follow-up. In each case, symptoms of fatigue and weakness resolved. Four of the five treated patients became transfusion-independent, including the patient who had received only three doses, and the fifth patient (who had AA) showed a reduced requirement for transfusion. No PNH-related symptoms have been reported during eculizumab therapy. Each patient showed a pronounced increase in Hb levels after starting eculizumab, although none reached the normal range (Table 2). Hb at last follow-up was lowest in Patient #3 (8.3 g/dL), who had severe AA, but abdominal pain and dysphagia stopped completely after starting eculizumab therapy. Allogeneic hematopoietic stem cell transplantation was planned from an HLA-matched sibling donor for this patient but she refused the procedure. Patient #4, who also had AA, had been receiving immunosuppression for a year prior to the start of eculizumab but Hb increased from 4.1 g/dL at baseline to 13.0 g/dL at last follow-up despite LDH in the normal range when eculizumab was started. The physiological basis for this is unclear.

The two non-AA patients in whom follow-up data were available (Patients #1 and #2) both showed a decrease from highly elevated levels of LDH to normal levels, indicating a profound reduction in hemolysis. LDH declined to within normal range in one patient with AA (Patient #3), and remained in normal range in the second patient with AA (Patient #4).

No thrombotic episodes or other PNH-related complications were observed in any patient during follow-up, and there were no cases of *N. meningitidis* infection.

There were no serious adverse events associated with eculizumab therapy. During follow-up, one patient experienced an upper respiratory tract infection (*Cryptic tonsillitis*) and another patient developed herpetic conjunctivitis, both of which were treated successfully with penicillin and acyclovir.

Discussion

The six patients with PNH described here had relatively severe disease compared to the worldwide PNH population[4], as indicated by granulocyte clone size, LDH levels and clinical symptoms. The patients without AA generally had higher clone sizes than the two patients with AA, as reported in the literature,[4] and higher levels of LDH. This indicates the dominance of hemolysis as the driver for anemia and clinical symptoms in these non-AA individuals. In the two patients for whom PNH was secondary to AA, LDH levels were only moderately above normal or within normal range.

Symptoms of PNH, most notably fatigue and weakness, entirely resolved under eculizumab. Follow-up data from two non-AA patients given eculizumab therapy demonstrated a dramatic decline in elevated LDH levels, reaching the normal range. In the two patients with PNH secondary to AA, hemolysis was less pronounced prior to treatment, but addition of eculizumab to long-term immunosuppressive therapy was nevertheless associated with Hb increases of 3.5 and 8.9 g/dL, respectively. No patient experienced a thrombotic event or developed renal dysfunction during eculizumab therapy, and no adverse events occurred.

Dosing of eculizumab according to the manufacturer's recommendations was practicable under routine conditions. At final follow-up, all patients remained on eculizumab therapy at the full recommended dose. One patient, aged 17 years and weighing 80 kg, received the standard adult dose instead of the recommended pediatric dose (for patients <18 years) due to his height and weight.

Both the patients with AA were receiving immunosuppression with horse antithymocyte globulin and cyclosporine at the point when PNH was diagnosed. This regimen achieves response rates of 50-60% even in severe AA,[16,17] but in these two patients the baseline Hb remained below 5 g/dL despite ongoing immunosuppressive therapy. Immunosuppression does not affect PNH clone size,[18] and here the clone sizes (>50%) indicated a need for eculizumab to minimize the risk for PNH-associated complications.[19] Case reports of eculizumab therapy in PNH with AA are rare. Hill *et al.* described a 48-year-old transfusion-dependent male who developed AA and was diagnosed with PNH five years later.[20] In the absence of immunosuppressive therapy, six months' treatment with eculizumab reduced hemolysis and lowered the need for transfusions by 44%, with stable Hb.[20] Asano and colleagues have reported the case of a 65-year-old woman in whom a hemolytic attack, triggered by sepsis, was diagnosed as PNH with AA.[21] Eculizumab therapy successfully controlled the hemolytic attack, after which horse antithymocyte globulin and cyclosporine were introduced. The patient was able to stop blood transfusions and there was no recurrence of hemolysis. In the two patients with PNH and AA in our series, Hb improved markedly but in one case (Patient #4) baseline LDH was in the normal range and it is not clear what drove the improvement in Hb. He had been receiving immunosuppressive therapy for a year, so this is not likely to account for the changes observed after starting eculizumab. Further research is required to elucidate the effects of eculizumab in this setting.

The rarity of PNH means that even special-ist referral centers see few cases. In this series of six patients, diagnosis was readily established based on FLAER analysis of granulocytes. Despite the relative severity of PNH in our series, symptoms resolved and elevated LDH levels normalized following eculizumab-mediated inhibition of complement-mediated intravascular hemolysis. Eculizumab was administered successfully outside the confines of a clinical trial with good tolerability. Although no patient was followed for more than a year under eculizumab, long-term data from clinical trials (up to 5.5 years) suggest that the benefits of eculizumab are sustained.[22–24]

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