Clinical features and responses to eculizumab of paroxysmal nocturnal hemoglobinuria patients: A single-center experience

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Abstract
Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal hematopoietic stem cell disease classically characterized by chronic intravascular hemolysis, bone marrow failure, and thrombosis. Eculizumab is an anti-C5 monoclonal antibody proven to reduce hemolysis and thrombotic attacks in the treatment of PNH. We aimed to present our data on PNH, a rare disease, and to share our experiences treating PNH with eculizumab. Demographic data, clinical features, history of thrombosis, responses to eculizumab treatment, and survival rates of 9 patients diagnosed with PNH at our hematology center were retrospectively analyzed. The median follow-up period was 46 months (range: 25-62). Five of the patients were female (55.5%), and 4 were male (44.5%), with a median age of 33.5±12.3 years. The mean hemoglobin level was 8.2 g/dL (5.7–10.1 g/dL), mean leukocyte count was 5.80x10³/μL (1.72x10³/μL–8.30x10³/μL), mean platelet count was 96.6x10³/L (42x10³/L–214x10³/L), mean lactate dehydrogenase level was 1312 U/L (423–2690 U/L), and mean reticulocyte level was 3.76% (1.1–6.3%). In our study, all cases received eculizumab therapy, of which 8 exhibited full or partial responses, while one was unresponsive to treatment. All patients were alive after the follow-up period. In 8 of the nine patients treated with eculizumab, hemolysis decreased following treatment and blood transfusion was not necessary. The quality of life experienced by all patients was improved. Following determination of the pathogenesis of the PNH, besides the classical treatment methods, eculizumab is a popular treatment option.

Keywords: Paroxysmal nocturnal hemoglobinuria, eculizumab, anti-C5 monoclonal antibody

Introduction
Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired hematopoietic stem cell disease characterized by chronic intravascular hemolysis, bone marrow failure, and thrombosis. It results from a somatic mutation in the PIG-A (phosphatidylinositol glycan-complementation class A) gene [1]. This mutation in the PIG-A gene disrupts the normal structure of glycosylphosphatidylinositol (GPI), which binds various proteins to the erythrocyte cell membrane, thus leading to an absence of CD55 and CD59 proteins on the cell surface [2]. As a result, erythrocytes become a clear target for complexation, and uncontrolled intravascular hemolysis occurs [3-4]. The prevalence of PNH is 2 – 5 per 1,000,000, with a mean age of 33 and equal frequency in both genders [5]. The most commonly used method of diagnosis is flow cytometry [5,6]. Screening for PNH in the presence of venous thrombosis is recommended in cases where recurrent hemoglobinuria, Coombs negative intravascular hemolysis, aplastic anemia, and myelodysplastic syndrome (refractory anemia or multilineage dysplasia) are not expected [7]. Additionally, PNH should be considered and screened for in cases of recurrent miscarriages, and renal insufficiency is concomitant with unexplained abdominal pain, fatigue, dysphagia, dyspnea, erectile dysfunction, iron deficiency, and cytopenia accompanied by hemolysis [8,9].

The treatment of PNH patients depends on the clinical form from which they suffer: classic PNH, PNH associated with aplastic anemia, or subclinical PNH. In recent years, apart from classical treatment options like bone marrow transplantation and corticosteroids, eculizumab, the development of which was oriented toward etiopathogenesis, has come to the forefront in the treatment of PNH. Eculizumab is a monoclonal antibody proven to reduce hemolysis and thrombotic attacks in the treatment of PNH, also used in atypical hemolytic uremic syndrome [10]. Despite the limitations common side effects of eculizumab, like headache, described above, eculizumab is highly effective in treating PNH and has changed the natural history of the disease [11]. This study also aimed to contribute to the literature on PNH, a rare disease, by presenting data on our cases and sharing our experiences treating PNH with eculizumab.
Material and Methods

Nine patients diagnosed with PNH were enrolled in the present study, which was conducted at our hematology center between the years 2012-2017. The study protocol conforms to the ethical guidelines of the 1975 declaration of Helsinki, and it was approved by the ethics committee of faculty of medicine (date/number: 10.01.2018/10). Demographic and clinical characteristics, hemogram values, reticulocyte count, LDH levels, history of thrombosis, antiagulant use, eculizumab treatment and responses, PNH clone percentages, and overall survival (OS) ratios of the study PNH patients were retrospectively analyzed. Diagnosis of PNH was obtained using the fluorescence aerosol (FLAER) test using peripheral blood flow cytometry. In this test, a diagnosis of PNH was made by detecting the absence of GPI proteins in at least two of three different cell lines consisting of granulocytes, monocytes, or erythrocytes. Due to previous transfusions, to obtain more sensitive and reliable data, the FLAER results in granulocytes and monocytes were taken into account.

Statistical Analysis

The Statistical Package for Social Sciences software (version 22.0) was used for the statistical evaluation of the research data. Quantitative variables were expressed as mean ± standard deviation, while qualitative variables were presented as number (n) and percentage (%).

Results

Five of the patients were female (55.5%), and 4 were male (44.5%), and the median age at the time of diagnosis was 33.5±12.3 (range 19–68). Seven of the patients were diagnosed with classical PNH (77.8%), while 2 (22.2%) had PNH accompanied by bone marrow failure (aplastic anemia) (PNH/AA). Patient characteristics at the time of diagnosis are presented in Table I. Upon referral, the following symptoms and findings were reported for the 9 patients: 6 cases of fatigue, 2 cases of dyspnea, 3 cases of abdominal pain, 1 case of dysphagia, 1 case of recurrent low-level tumors, and 2 cases of erectile dysfunction. Also, at the time of diagnosis, hemoglobinuria and iron deficiency were detected in 5 patients, thrombosis in 1 patient, renal failure in 1 patient, and cytopenia without anemia (leukopenia and thrombocytopenia) in 5 patients (Table II). Results of the laboratory analyses were as follows: the mean hemoglobin level was 8.2 g/dL (5.7-10.1 g/dL), mean leukocyte count was 5.80x10³/μL (1.72x10³/μL–8.30x10³/μL), mean platelet count was 96.6x10³/μL (42x10³/μL-214x10³/μL), mean lactate dehydrogenase level was 1312 U/L (423-2690 U/L), and the mean reticulocyte level was 3.76% (1.1 – 6.3).

PNH clone size was also examined at the time of diagnosis. The mean granulocyte clone size was 72.5% (19.3 – 98.1), the mean monocyte clone size was 75.4% (18.4 – 99.3), and the mean erythrocyte clone size was 42.8 (6.8 – 90.6) (Table III). Clone size of patients with PNH accompanied by bone marrow failure was markedly lower than that of patients with classical PNH. The mean clone size in erythrocytes was lower than the clone size in monocytes and granulocytes for all patients.

### Table 2. Symptoms at time of diagnosis

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Number of patients</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>6</td>
<td>(67%)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>2</td>
<td>(22%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>3</td>
<td>(33%)</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>1</td>
<td>(11%)</td>
</tr>
<tr>
<td>History of recurrent miscarriages</td>
<td>1</td>
<td>(11%)</td>
</tr>
<tr>
<td>Erectile dysfunction</td>
<td>2</td>
<td>(22%)</td>
</tr>
<tr>
<td>Hemoglobinuria</td>
<td>5</td>
<td>(55%)</td>
</tr>
<tr>
<td>Iron deficiency</td>
<td>5</td>
<td>(55%)</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>1</td>
<td>(11%)</td>
</tr>
<tr>
<td>Renal failure</td>
<td>1</td>
<td>(11%)</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>0</td>
<td>(0%)</td>
</tr>
<tr>
<td>Anemia-free cytopenia</td>
<td>5</td>
<td>(55%)</td>
</tr>
</tbody>
</table>

### Table 3. PNH clone size of patients at diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Total (n=9)</th>
<th>Classical PNH (n=7)</th>
<th>PNH/AA (n=2)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>median % (range)</td>
<td>median % (range)</td>
<td>median % (range)</td>
</tr>
<tr>
<td>Granulocyte clone size</td>
<td>72.5 (19.3 – 98.1)</td>
<td>83.4 (65.9 – 98.1)</td>
<td>21.8 (19.3 – 24.3)</td>
</tr>
<tr>
<td>Monocyte clone size</td>
<td>75.4 (18.4 – 99.3)</td>
<td>85.2 (55.8 – 99.3)</td>
<td>27.1 (18.4 – 35.8)</td>
</tr>
<tr>
<td>Erythrocyte clone size</td>
<td>42.8 (6.8 – 90.6)</td>
<td>52.7 (20.3 – 90.6)</td>
<td>10.2 (6.8 – 13.6)</td>
</tr>
</tbody>
</table>

Discussion

In classical PNH cases, hemolysis and elevated serum LDH levels are almost always the expected findings. Therefore, in the presence of direct anti-globulin (Coombs) test-negative hemolytic anemia, whether or not accompanied by hemoglobinuria, PNH should be suspected. In such cases, findings of thrombosis, iron deficiency, and cytopenia increase the likelihood of PNH [6].

In our study, two patients had findings of bone marrow failure at the time of admission, and 7 had clinical hemolytic anemia. One of the patients with hemolytic anemia was diagnosed with thrombosis (portal vein thrombosis), and one received the diagnosis of PNH while being examined for a history of recurrent miscarriage. One patient had portal vein thrombosis at the time of diagnosis, and another developed pulmonary artery thrombosis after diagnosis. Anticoagulant therapy was given to both patients following thrombosis. In the patient experiencing renal failure, kidney function returned to normal in the third month of treatment with eculizumab. In 2 patients with bone marrow failure, the presence of AA was confirmed via bone marrow aspiration and biopsy. These patients initially received anti-thymocyte globulin (ATG)+cyclosporine+steroid treatment for aplastic anemia. Partial response was obtained for both patients following ATG treatment. Six patients with PNH who had symptomatic anemia and required transfusion at the time of diagnosis were treated with folic acid and iron and underwent erythrocyte transfusion.
A patient with recurrent miscarriages had a history of anemia that remained unchanged despite undergoing replacement therapy for approximately four years; 3 consecutive pregnancies had resulted in miscarriages. Of the patients who underwent treatment with eculizumab, 8 exhibited a full or partial response, and one showed no response to treatment. The unresponsive patient required frequent transfusions, and alloergic hematopoietic stem cell transplantation was planned for this patient. Patients who had previously required erythrocyte transfusion and responded fully or partially to eculizumab treatment did not require transfusion. At the end of the median follow-up period of 46 months, all patients were still living.

One study found that aplastic anemia developed over eight years in 8% of PNH patients, while patients with aplastic anemia may also develop PNH. In another study, it was determined that the PNH clone observed in aplastic anemia increased in 17% of cases and disappeared in 24% of cases [12,13]. During the follow-up of our aplastic anemia cases, PNH clone showed an increase. Although cases of clones disappearing following treatment for aplastic anemia have been reported in the literature, none of our cases resulted in clone negativization.

Renal problems are an important factor in PNH cases and are the second most common cause of death after thrombotic events. One study reported renal insufficiency in 64% of patients, of whom 21% experienced end-stage renal failure after the 10-year follow-up [14]. One of the cases in the present study had renal dysfunction at the time of diagnosis, but renal function returned to normal following treatment with eculizumab.

In a clinical series study of 80 cases diagnosed with PNH, the following symptoms were observed at the time of first referral: anemia (35%), hemoglobinuria (26%), aplastic anemia (13%), gastrointestinal symptoms (10%), hemolytic anemia and jaundice (9%), iron deficiency anemia (6%), and thrombosis or embolism (6%). In our case series, symptoms at the time of diagnosis were as follows: anemia (100%), aplastic anemia (22.2%), iron deficiency (55.5%), gastrointestinal symptoms (33%), and thrombosis (11%). Two of the patients who developed thrombosis received oral anticoagulants following diagnosis, and subsequently, their thrombosis did not recur.

Treatment is usually planned according to the level of clone positivity in PNH. In cases where the clone level is <10%, symptoms are generally faint, and treatment is not necessary; however, the PNH clone should be monitored at 6-12-month intervals. Basic supportive care for PNH patients includes iron supplementation and folic acid replacement to offset its deficiency [15,16]. All our cases were followed up, and those who were initially deficient were given iron and folic acid support. After the study, all patients continued to receive folic acid supplements.

Eculizumab, an anti-C5 monoclonal antibody in transfusion-dependent patients who develop PNH complications (thrombosis, attacks of paroxysmal pain, organ damage), is the primary treatment option. It is administered via intravenous infusions for the first four weeks at a dose of 600 mg per week, 900 mg the following week, and after that 900 mg every two weeks. Eculizumab controls the hemolytic process, reduces thrombotic attacks, decreases the need for transfusion, prevents organ damage, and improves quality of life [17]. Monitoring patients being treated with eculizumab should be performed by direct antiglobulin testing if there is complete blood count, reticulocyte level, serum bilirubin and LDH levels, and extravascular hemolysis (anemia and increased reticulocyte count) [18]. The most important point to be aware of in patients due to be treated with eculizumab is that to prevent N. meningitis infections, vaccination should be scheduled at least two weeks before treatment. The most important risk of terminal complement blockade is life-threatening Neisserial infections (5% after ten years) [19].

We had all the cases that started with Eculizumab, and we had N. meningitis vaccines before treatment. None of the patients in the present study experienced any complications related to this pathogenic microorganism. In all of the patients diagnosed with classical PNH treated with eculizumab and in one patient with PNH accompanied by aplastic anemia, there was no need for transfusion. The quality of life of the eight patients who continued to receive eculizumab also increased significantly.

In the study, the patients who receive significant benefit from Eculizumab treatment are still receiving this treatment. Information about when this treatment should be discontinued is still unclear.

**Conclusion**

In conclusion, the only curative treatment of PNH is allogenic stem cell transplantation, which is recommended for all suitable patients. In treating PNH, eculizumab is a good treatment option for patients who cannot undergo a transplant, are transfusion-dependent, and at risk of thrombosis. Patients should be managed by the literature and, regarding the use of eculizumab, the proper treatment should be initiated without delay. Cases receiving eculizumab treatment should be monitored appropriately.

**Competing interests**

The authors declare no potential conflicts of interest concerning the research, authorship, and publication of this article.

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**Ethical approval**

All Authors declare that originality of research/article etc. and ethical approval of research, and responsibilities of research against local ethics commission are under the Authors responsibilities. The study was conducted due to defined rules by the Local Ethics Commission guidelines and audits (date/number: 10.01.2018/10).

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