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ORIGINAL ARTICLE

Clinical characteristics and therapeutic outcomes of paroxysmal nocturnal hemoglobinuria patients in Turkey: a multicenter experience

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Abstract
The aim of this study is to collect paroxysmal nocturnal hemoglobinuria (PNH) patient data from hematology centers all over Turkey in order to identify clinical features and management of PNH patients. Patients with PNH were evaluated by a retrospective review of medical records from 19 different institutions around Turkey. Patient demographics, medical history, laboratory findings, and PNH-specific information, including symptoms at the diagnosis, complications, erythrocyte, and granulocyte clone size, treatment, and causes of death were recorded. Sixty patients (28 males, 32 females) were identified. The median age was 33 (range; 17–77) years. Forty-six patients were diagnosed as classic PNH and 14 as secondary PNH. Fatigue and abdominal pain were the most frequent presenting symptoms. After eculizumab became available in Turkey, most of the patients ($n=31/46$, 67.4%) were switched to eculizumab. Three patients with classic PNH underwent stem cell transplantation. The median survival time was 42 (range; 7–183 months) months. This study is the first and most comprehensive review of PNH cases in Turkey. It provided us useful information to find out the differences between our patients and literature, which may help us understand the disease.

Keywords Eculizumab · Hemolysis · Paroxysmal nocturnal hemoglobinuria · Thrombosis

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare (incidence is 1.5–2 cases per million of the population per year) but life-threatening disease characterized by chronic hemolysis leading to thrombosis, renal impairment, pain, severe fatigue, and eventually death [1]. Thrombosis has been considered as a significant risk factor for mortality and the leading cause of death in PNH patients [2].

PNH arises from somatic mutations of the phosphatidylinositol glycan, class A gene (PIGA) in one or more hematopoietic stem cell (HSC) lines [3–5]. The mutation leads to disruption to glycosylphosphatidylinositol (GPI) anchor biosynthesis [6], and thus a deficiency of all GPI-anchored proteins on the cell membrane [7]. The lack of synthesis of the GPI anchor leads to under expression of two important complement regulatory proteins, CD55 and CD59 [8], resulting in increased complement sensitivity of PNH cells, intravascular hemolysis, elevated levels of inflammatory mediators, and high concentration of free hemoglobin scavenges in the plasma. Elevated free hemoglobin consequences with nitric oxide (NO) scavenge and NO depletion causes majority of symptoms of PNH such as abdominal pain, erectile dysfunction, and dysphagia [9].

Although PNH is an HSC disorder, it is a chronic multisystem disease. It shows frequent recurrences and spontaneous long-term remissions are rare. The median survival is about 10 years with supportive treatment such as transfusions, steroids, and immunosuppressive therapy [10]. However, survival can be significantly shortened in some cases with severe thrombosis, renal deficiency, or bone marrow failure. Currently, C5-blockade with monoclonal antibodies became available as a treatment option. Eculizumab is

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a humanized monoclonal antibody directed against the terminal complement protein C5 [11]. It has had a significant impact on the management of PNH. It has been shown to reduce hemolysis and improve symptoms and quality of life (QoL) of PNH patients [12].

There are several articles regarding etiology, pathogenesis, clinical characteristics, and management of PNH from different countries. However, there is no published data about PNH patients in Turkey. From this point of view, we aimed to collect PNH patient data from hematology centers all over Turkey in order to identify clinical features and management of PNH patients in our country. By this way, we will be able to shed light into the natural course and management approaches of PNH cases in Turkey.

Subjects and methods

Sixty patients with PNH diagnosed between January 2001 and September 2016 were evaluated by a retrospective review of medical records from 19 different institutions in Turkey. Ethics committee approval was obtained from Eskişehir Osmangazi University Faculty of Medicine Ethics Committee and the tenets of the Declaration of Helsinki were followed. Patients were divided into two groups: classic PNH and PNH in the setting of another bone marrow disorder, as previously described by Parker et al. [13]. Classic PNH patients had clinical evidence of intravascular hemolysis but had no evidence of another defined bone marrow abnormality. Paroxysmal nocturnal hemoglobinuria patients in the setting of another bone marrow consists of patients with clinical and laboratory evidence of hemolysis but also had concomitantly, or have had a history of, underlying bone marrow biopsy proven aplastic anemia (AA) or myelodysplastic syndrome (MDS).

Patient demographics, medical history, laboratory findings, and PNH-specific information, including symptoms at the diagnosis, complications, erythrocyte, and granulocyte clone size, past/current treatment, and causes of death were recorded. Clinical symptoms related with PNH such as fatigue, abdominal pain, chest pain, dyspnea, and hemoglobinuria were evaluated based on the patient charts after diagnosis of PNH. All laboratory parameters such as hemoglobin, creatinine, and lactate dehydrogenase (LDH) were recorded at the time of diagnosis, before eculizumab treatment, and at the last visit of patient. Clinically significant hemolysis was defined as LDH levels of 1.5 times or more the upper limit of normal (ULN) based on previously published, multinational, registration clinical trials for the treatment of PNH [14, 15].

In 56 patients, PNH was diagnosed by using flow cytometric method based on the analysis of expression of CD55 and CD59 on erythrocytes described previously [1, 16]. In addition, fluorescein-labeled proaerolysin (FLAER) method was used in 42 patients. Four out of 60 patients were diagnosed before the establishment of flow cytometry. In these patients, positive Ham or sucrose lysis test had been performed.

Overall survival (OS) time was calculated from date of diagnosis to date of last follow-up or death.

Statistical analysis

Statistical analysis was performed using commercially available software (IBM SPSS Statistics version 21). We performed the Shapiro–Wilk test for normality. Because some of the variables did not show normal variance, we used non-parametric Kruskal–Wallis variance analysis. Pearson correlation analysis was performed to evaluate relationship between variables. $P < 0.05$ was considered as statistically significant.

Results

Patient characteristics

A total of 60 patients were identified. There were 28 males and 32 females. The median age was 33 (range; 17–77) years. Forty out of 60 (66.6%) patients were under 40 years old. Forty-six patients were diagnosed as classic PNH and 14 as secondary PNH (13 patients had PNH and aplastic anemia (PNH + AA), 1 patient had PNH and myelodysplastic syndrome (PNH + MDS)). Baseline demographic characteristics, hematological parameters, symptoms, and signs are summarized in Table 1. Fatigue and abdominal pain were the most frequent complaints during the first admittance to the hospital. Organomegaly was present only in 7 patients (4 out of 7 had splenomegaly, 3 out of 7 had hepatomegaly). The median platelet count and LDH levels were significantly higher in the patients with classic PNH. The flow cytometric analysis performed at the time of diagnosis was shown in also Table 1. Median granulocyte and monocyte PNH clone sizes were 75% (23.1–99.5) and 77% (14.2–99) in classic PNH, respectively and 70.5% (13.8–95) and 61% (14–95.5) in PNH + AA patients, respectively.

Risk factors for thrombosis

Regardless of treatment, thrombosis incidence was 1.8% per observation year. Three out of 60 patients presented with thromboembolic events (TE) at the time of diagnosis (deep vein thrombosis in two patients and portal vein thrombosis in one patient). During follow-up in pre-eculizumab era (mean follow-up time 19.7 months), 15 out of 60 patients developed TE. Of these 15 patients, 12 were diagnosed with classic PNH and 3 with PNH + AA. Eight out of these 15

Table 1 Baseline demographic characteristics and hematological parameters of the study population

Parameters	Classic PNH	PNH + AA/PNH + MDS	*P* value
Number of patients (*n*)	46	14	-
Median age, years (range)	36 (17–77)	32 (18–63)	0.252
Gender (male/female)	24/22	4/10	-
Hemoglobin (gr/dL) (12–16 g/dL)	8.2 ± 2.17	6.6 ± 2.65	0.42
MCV (femtoliter) (80–96 fL)	101.5 ± 10.1	97.9 ± 14.3	0.23
White blood cells* ($\times 10^3$/μL) ($4.4–11.3 \times 10^3$/μL)	4.5 (1.0–12.8)	2.9 (0.7–8.2)	0.07
Platelets* ($\times 10^3$/μL) ($150–400 \times 10^3$/μL)	89.5 (10–727)	26 (7–252)	**0.002**
Creatinine (mg/dL) (0.7–1.2 mg/dL)	1.51 ± 1.2	1.17 ± 1.3	0.665
Lactate dehydrogenase* IU/L (135–225 IU/L)	1018 (205–5568)	444 (151–1567)	**0.003**
Corrected reticulocyte (%)	4.4 ± 2.7	1.8 ± 1.4	0.158
Haptoglobin (mg/dL) (14–258 mg/dL)	2.4 ± 2.6	36.8 ± 44.1	**0.00**
D-dimer (ng/mL) (< 500 ng/mL)	374.5 ± 124.6	470.1 ± 142.8	0.617
Loss of CD59 (%)	57.9 ± 26	59.5 ± 41.4	0.065
Loss of CD55 (%)	50.8 ± 33.3	30.7 ± 36	0.872
Granulocyte clone with FLAER* % (range)	75 (23.1–99.5)	77 (14.2–99)	0.215
Monocyte clone with FLAER* % (range)	70.5 (13–95)	61 (14–95.5)	0.188
Fatique (*n*) (%)	38 (82)	12 (85.7)	
Epistaxis/gingival bleeding (*n*) (%)	7 (15.2)	3 (21.4)	
Abdominal pain (*n*) (%)	9 (19.5)	1 (7.1)	
Haemoglobinuria (*n*) (%)	4 (8.6)	0	
Organomegaly (*n*) (HM/SM)**	7 (3/4)	0	

Note that platelet count was lower in PNH with AA/MDS patients. On the other hand, hemolysis parameters were statistically significant in classical PNH patients (lactate dehydrogenase and haptoglobulin were found higher and lower respectively). *P* value < 0.05 was considered as statistically significant

*Median values were provided because these parameters did not show normal distribution. All remaining values were shown as mean values with standard deviation

***HM/SM*, hepatomegaly/splenomegaly

patients had venous thrombosis of the lower extremities (2 patients), portal vein thrombosis (3 patients), Budd-Chiari syndrome (2 patients), and mesenteric vein thrombosis (one patient). Renal failure was described in four patients, while three others showed gastrointestinal bleeding, transfusion hemosiderosis, and erectile dysfunction, respectively. TEs were reported 17.3% and 14.2% in patients with classic PNH and PNH + AA respectively. There was no significant difference between these two patient groups in the prevalence of stated TE ($p = 0.57$).

At the time of diagnosis, median LDH level was found 945 IU/L (range; 484–2991 IU/L) and 597 IU/L (range; 151–5568 IU/L) in patients with TE and without TE, respectively ($p = 0.021$). Age, hemoglobin, platelet count, and granulocyte clone size did not show significant differences in patients with or without TE in our study group. On the other hand, 38 of 58 patients (65.5%) (29 patients with classic PNH and 9 patients with PNH + AA) with recorded LDH levels had values $\geq 1.5 \times$ ULN at the time of diagnosis. Chi-square test showed patients with LDH $\geq 1.5 \times$ ULN had a significantly increased incidence of TE (9 of 38 (23.6%)) compared with patients with LDH $\leq 1.5 \times$ ULN ($p = 0.021$).

We analyzed association between LDH levels and granulocyte clone size. Correlation analysis showed a statistically significant positive correlation between LDH levels and granulocyte clone size ($r = 0.520$, $p < 0.01$) (Fig. 1). Instead, granulocyte clone size was not a risk

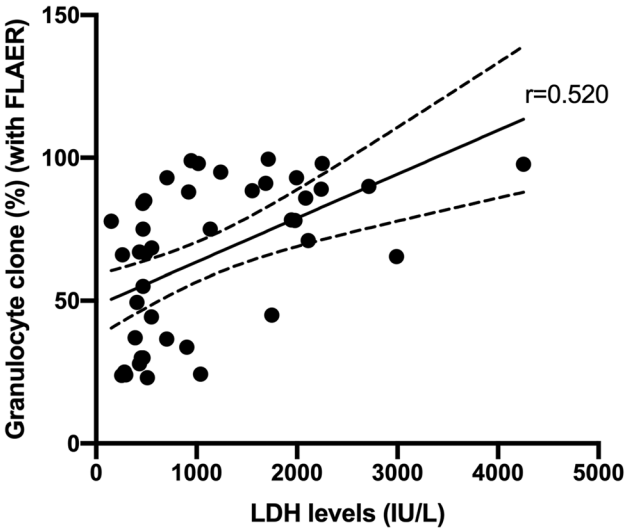

Fig. 1 Correlation analysis showing a significant correlation between serum LDH levels and granulocyte clone size

factor for thrombosis in our study group. Median granulocyte clone size was 85% and 67% in patients with TE and without TE, respectively ($p = 0.35$). While number of patients with granulocyte clone size > 50% were 9 (81.1%) in patients with TE, it was 20 (60.6%) in patients without TE.

Treatment of PNH

Pre-eculizumab era treatments are summarized in Table 2. Because eculizumab was not commercially available before 2009 in Turkey, almost all of the PNH patients diagnosed before 2009 were started on immunosuppressive therapy at the time of diagnosis. After eculizumab became available in Turkey, patients were switched to eculizumab if they had indications for C5-blockade with monoclonal antibodies such as PNH-related thrombosis, transfusion needed hemolytic PNH, symptomatic PNH, and presence of organ damage due to PNH such as kidney failure and pulmonary hypertension. When all patients were evaluated (Table 2), the most frequently used previous therapies were prednisolone (45.6%) and cyclosporine (28.2%) for classic PNH patients and cyclosporine (85.7%) with/without anti-thymocyte globulin (ATG) for PNH + AA patients. Eight out of 14 (57.1%) patients with PNH + AA were treated with anti-thymocyte globulin (ATG) for bone marrow hypoplasia. Three underwent allogeneic bone marrow transplantation and 7 patients received eculizumab therapy after ATG treatment.

Current treatment approaches as of March 2016 were shown in Table 3. As shown in Table 3, the total number of patients receiving eculizumab was 43 out of 60 (71.6%). Patients who got eculizumab were 78.2% and 50% in classic PNH and PNH + AA groups, respectively. Six patients underwent allogeneic stem cell transplantation. Seven patients assigned as "no treatment." There are reasons why some patients cannot receive eculizumab therapy such as patient refusal to treatment (2 patients), lack of frequent visits to the hospital due to economic reasons (1 patients), and health insurance problems (1 patient). Moreover, 3 patients were recruited as asymptomatic, and they were being followed up by the clinician without treatment.

Given the fact that eculizumab is the first-line treatment, we further analyzed transfusion frequencies, LDH values, renal functions, and granulocyte clone sizes before and after eculizumab treatment in patients with classic PNH (31 patients) separately. We found that after starting eculizumab treatment number of transfusions (Fig. 2),

Table 2 Table showing past treatment (pre-eculizumab era) approaches for paroxysmal nocturnal hemoglobinuria patients (PNH) in our study group. Please note that these percentages have been calculated for the use of each drug alone and/or in combination therapy

Past treatments	Classic PNH ($n = 46$)	PNH+AA/ PNH+MDS ($n = 14$)
Oxymetholone (%)	-	4 (28.5)
Prednisolone (%)	21 (45.6)	7 (50)
Danazol (%)	3 (6.5)	3 (21.4)
Cyclosporine (%)	13 (28.2)	12 (85.7)
Azathioprine (%)	2 (4.3)	-
ATG (%)	3 (6.5)	8 (57.1)
HSCT (%)	3 (6.5)	3 (21.4)

AA, aplastic anemia; *MDS*, myelodysplastic syndrome; *ATG*, anti-thymocyte globulin; *HSCT*, hematopoietic stem cell transplantation

Table 3 Current treatment approaches for paroxysmal nocturnal hemoglobinuria patients (PNH) in our study group. Please note that eculizumab is the most common used drug in line with current literature

Current treatments	Classic PNH ($n = 46$)	PNH+AA/ PNH+MDS ($n = 14$)
Eculizumab alone	31	7
Corticosteroid alone	2	-
Eculizumab + cyclosporine	5	-
Corticosteroid + cyclosporine	1	1
HSCT	3	3
No treatment	4	3

AA, aplastic anemia; *MDS*, myelodysplastic syndrome; *HSCT*, hematopoietic stem cell transplantation

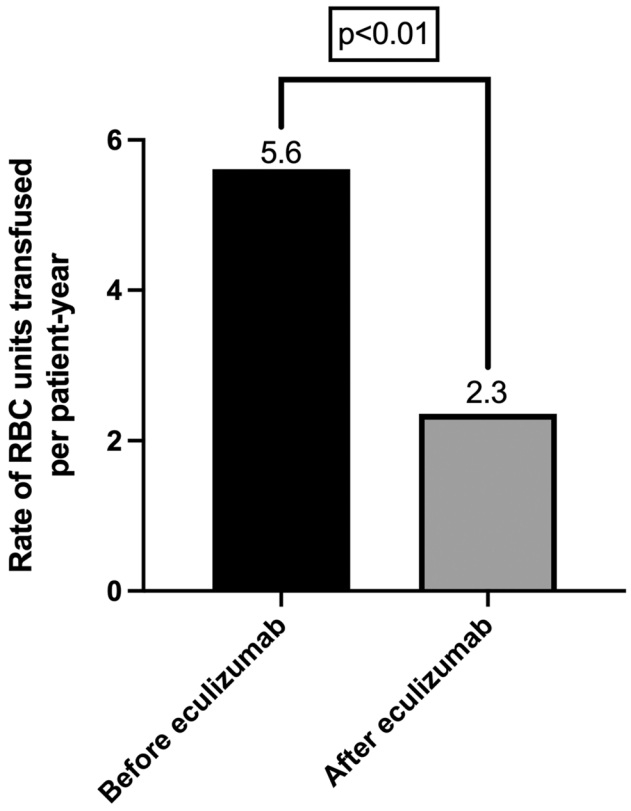

Fig. 2 Figure showing rate of the red blood cell units transfused in patients with PNH. After eculizumab treatment, transfusion need was significantly decreased

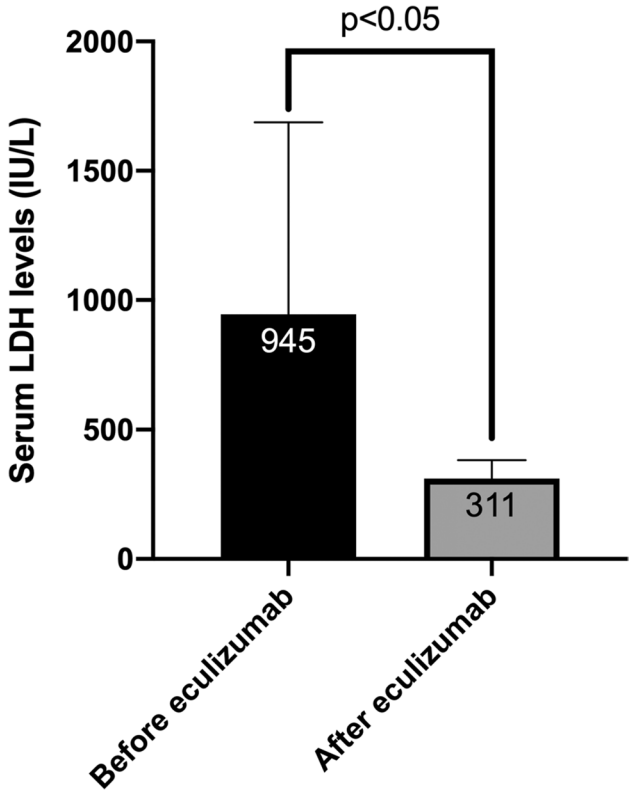

Fig. 3 Figure showing median LDH levels before and after starting eculizumab treatment

LDH values (Fig. 3) and creatinine levels (Fig. 4) were decreased significantly. Twenty out of 31 patients had a history of receiving red blood cell (RBC) transfusions before eculizumab initiated. It was observed that transfusion need was decreased by 58.9% after eculizumab treatment. Mean number of RBC units transfused were 5.6 (± 6.2) and 2.3 (± 2.4) during the 12 months before and after eculizumab treatment was started (Fig. 2). On the other hand, median LDH levels were 945 IU/L and 311 IU/L, before and after eculizumab treatment, respectively (Fig. 3). Thromboembolic events were observed in 3 out of 31 patients (two patients had deep venous thrombosis and one patient had cerebrovascular event) followed up under eculizumab treatment. All of these 3 patients had LDH levels ≥ 1.5 × ULN. Also, mean creatinine levels were 1.5 (± 1.3) and 1.0 (± 0.4) mg/dL before and after eculizumab therapy respectively ($p < 0.05$) (Fig. 4). Moderate-to-severe renal failure which is accepted as glomerular filtration rate ≤ 60 ml/dk was identified in four patients, and two out of four patients were receiving dialysis at treatment entry. After eculizumab, it was observed that these two patients became dialysis-free. Besides, multiple stepwise backward linear regression analysis was performed in order to find effect of certain parameters (age, sex, hemoglobin, white blood cells, platelets, LDH, and PNH clone size) on creatinine levels in our study cohort. In patients with classic PNH, only pre-eculizumab LDH levels was found be statistically significant positive correlated with creatinine levels ($r^2 = 0.513$, $p = 0.019$). There was no significant correlation between certain parameters mentioned above and creatinine levels in PNH + AA pat ients.

Survival outcomes

The median survival time was 42 (range; 7–183 months) months. Four out of 60 (6.6%) patients died due to infections during follow-up. Two of these 4 patients were diagnosed as classical PNH, whereas other two patients were in PNH + AA group. All 4 patients had received ATG therapy in the past. Only one of them was able to receive eculizumab after ATG therapy. Two died as a result of fungal pneumonia, one died due to bacterial sepsis after a long history of recurrent bronchopneumonia before starting eculizumab, and one died because of cytomegalovirus infection after allogeneic stem cell transplantation.

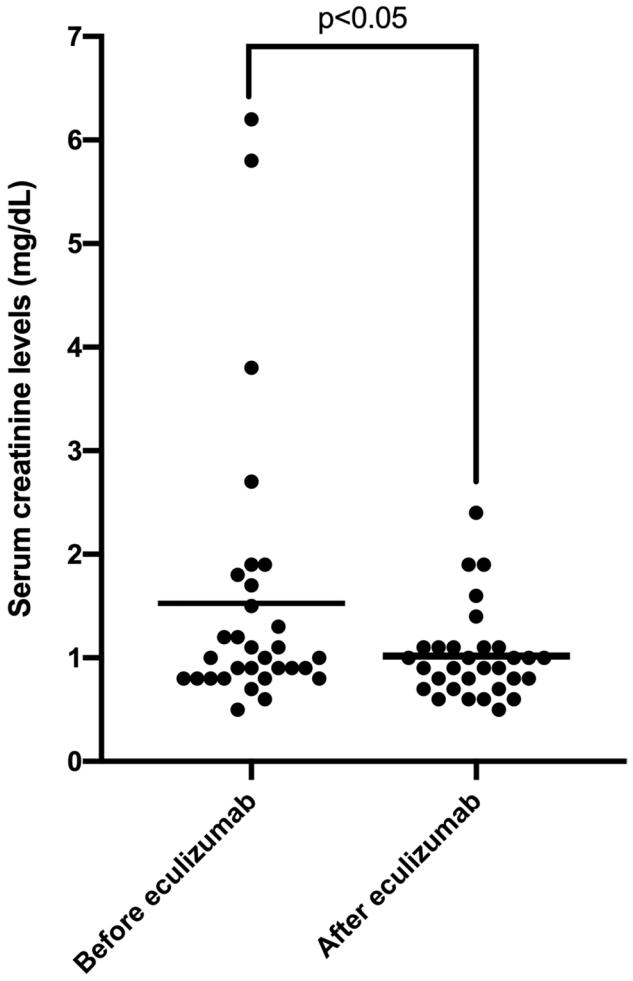

Fig. 4 Scatter dot plot graph showing significant decreased of mean creatinine levels after eculizumab treatment in PNH patients. Mean creatinine levels were 1.5 (±1.3) and 1.0 (±0.4) mg/dL before and after eculizumab therapy respectively ($p < 0.05$)

Discussion

PNH is a rare acquired disorder of hematopoietic stem cells, which mainly presents as a disease of adults and, to a less extent, of childhood and adolescence. The peak incidence is in the third and fourth decades of life. Our study showed that the median age of PNH patients at the time of diagnosis was 33 years, which was similar to previous reports [10, 17]. Both sexes can be affected; our series revealed a slight female preponderance of PNH. Also, regarding baseline laboratory parameters, patients with PNH + AA had significantly lower platelet count and LDH levels which was similar to reported by Lee JW et al. [18].

Thrombotic complications in PNH patients can arise in venous site (85%) such as hepatic, cerebral, and deep limb veins, but arterial thrombosis is not so rare (15%) [19]. Our study showed that the incidence of thrombotic events as 35%, which was comparable to previous reports from European population [10, 20, 21]. Prior to initiation of eculizumab, 18 out of 60 (30%) patients experienced TE in our study. Only 3 patients had TE after eculizumab treatment. Likewise, Hillmen et al. reported that TE incidence was 32.3% (63 out of 195 patients) in pre-eculizumab era, and they observed an 81.8% reduction in the incidence of TEs with long-term eculizumab treatment [22]. There are other reports showing that eculizumab has a protective effect against thrombosis [23–25].

In our study, we found that the median LDH level was significantly higher in patients with TE than in patients without TE. We also did see the elevated hemolysis (LDH ≥ 1.5 ULN) was an increased risk factor for TE. In a study by Lee et al. [14], it showed that, at the diagnosis, PNH patients with LDH ≥ 1.5 ULN had an increased risk of TE compared with PNH patients with LDH ≤ 1.5 × ULN. These findings also supported with a report by International Paroxysmal Nocturnal Hemoglobinuria Registry [26]. Elevated LDH levels (≥ 1.5 ULN) is a well-known marker for hemolysis and should be considered as a predictive factor of increased risk for TEs. On the other hand, clinicians should remember that significant hemolysis is not always necessary for thrombosis, especially in the presence of high PNH clone [27]. Although mechanism for thrombosis in patients with non-hemolytic PNH is not fully understood, it has been shown that platelet derived microparticles, endothelial activation, and the formation of neutrophil extracellular traps may play a key role [28–30].

Renal failure is one of the leading causes of death in PNH patients. Our study demonstrated that creatinine levels positively correlated with pre-eculizumab LDH levels, and it significantly decreased after initiation of eculizumab. In a recent study from Spanish PNH Registry, it was shown that, PNH patients with acute/chronic renal failure, all patients treated with eculizumab, creatinine levels were significantly improved [31]. The authors concluded that clearance of iron from the kidney, inhibition of the production of anaphylatoxin C5a, together with decreased intravascular hemolysis and normalization of nitric oxide levels, were the most relevant benefits of eculizumab treatment on renal functions.

In our study, major cause of death was infection. Overall, four out of 60 patients were deceased. This finding is not consistent with the previous reports, because thrombotic complications are the leading cause of death and occur in approximately 40% of PNH patients in the published literature [9, 13, 20, 32]. There is no single possible explanation of this inconsistency. However, we speculate that routinely performed primary thrombosis prophylaxis with warfarin in patients with granulocyte clone size > 50% and > 100,000 platelets may have led to a significant decrease in death events in our series. Likewise, Hall et al. [33] showed that

TE rate was assessed at 3.7 events per 100 patient-years in 67 high-risk patients with PNH not taking prophylactic anticoagulants, while none TE events in 117.8 patient-years was seen in 39 PNH patients treated with anticoagulants as primary prophylaxis. We also believe that, in addition to warfarin prophylaxis, good control of intravascular hemolysis may contribute to a reduction in the incidence of thrombosis. Additionally, hemolysis is an important clinical manifestation of PNH and most likely contributes to thromboembolic events. Eculizumab itself also reduces intravascular hemolysis and this may help decrease TE incidence in PNH patients [25].

There are some limitations of our study that must be addressed. First, this is a retrospective study. Second, there is limited number of patients when Turkish population was taken into consideration. One of the possible explanations is there are limited number of hematologists in Turkey, given the fact that Turkey's population is over 80 million. Also, necessary measures can be taken to increase accessibility to hematologists and eculizumab treatment. Another reason could be the internists are overlooking PNH patients because of their low awareness against this rare disease. However, given the fact that PNH is a rare disease, 60 patients can be considered sufficient to draw any conclusions from this data set.

In conclusion, our study is the first and most comprehensive review of PNH cases in Turkey. It provided us useful information to find out the differences between our patients and literature, which may help us to understand the disease. Further studies are needed to set up individually therapeutic regimens for Turkish PNH patients in the near future.

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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