Am J Blood Res 2016;6(2):19-27
www.AJBlood.us /ISSN:2160-1992/AJBR0031541

Review Article

Pesg PNH diagnosis, follow-up and treatment guidelines

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Received May 1, 2016; Accepted June 28, 2016; Epub August 5, 2016; Published August 15, 2016

Abstract: PNH Education and Study Group (PESG) have been established in December 2013 as a non-profit, independent, medical organization www.pesg.org. Paroxysmal Nocturnal Hemoglobinuria *(PNH)* is a multi-systemic disease that should be treated with a multidisciplinary approach. Patients may apply to the clinics other than the hematology due to variability and diversity of clinical findings which lower the rate of diagnosis due to low awareness about PNH. PNH might be overlooked and diagnosis might be delayed. Regarding these, PESG was established with the collaboration of Immunology, Cardiology, Thorax Diseases (Pulmonology), Neurology, Gastroenterology, General Surgery and Urology specialists in addition to hematologists dealing with PNH. The PESG study group aims to increase the awareness about PNH, including training activities about PNH, strengthening the relations between clinics and planning of clinical studies as a goal. It is the first professional organization focusing on PNH, in Turkey. In this guideline, we want to facilitate the diagnosis attributes of physicians from all specializations that deal with PNH and its systemic complications. One can perceive this as a tailor made guideline of international guidelines but not a compilation.

Keywords: PNH, guideline, diagnosis, treatment

Introduction to the disease

Paroxysmal Nocturnal Hemoglobinuria (PNH) is a chronic, progressive, life-threatening, rare, multi-systemic disease, developing as a result of somatic mutation of hematopoietic stem cell, and characterized by clonal, complement-mediated intravascular hemolysis.

Large-scaled epidemiological studies have shown that 35% of PNH patients lose their lives within 5 years from the date of diagnosis and their 10 years mortality reaches approximately 50% [1]. The clinical case that is actually responsible for mortality is thrombosis. Thrombotic events are responsible for 40-67% of deaths [2-5]. Other causes of morbidity and mortality can be listed as renal failure, pulmonary hypertension, erectile dysfunction and dysphagia [5-8].

PNH is a disease whose diagnosis may be delayed due to variable clinical findings and this delay increases the risk of mortality and morbidity. Therefore, early diagnosis is important for both to prevent morbidity and to reduce the risk of mortality.

There are few data regarding the prevalence and incidence of PNH, because it is a rare disease. In the most large-scaled UK study, its prevalence was published as 15.9/1 million, its

Table 1. PNH Laboratory Findings

Laboratory findings of hemolysis
Hemogram: Anaemia ± Leukopoenia ± Thrombocytopenia
Reticulocytosis
Peripheral Smear*
Elevated Indirect Bilirubin Level
Elevated LDH Level
Decrease in Haptoglobin Level
Direct Coombs negativity
Flow Cytometric Findings
Deficiency of GPI anchored proteins (such as CD55, CD59)
Bone Marrow Aspiration-Biopsy (BMAB) Findings**
Aplastic Anemia (AA)
Myelodysplastic Syndrome (MDS)

*There is not any finding specific to PNH in peripheral smear, however, as well as hemolysis is observed as non-schistocytic, indirect findings that will remind MDS or IMF might be observed. **BMA is not necessary for PNH diagnosis. However, it is recommended to be performed in order to investigate association with diseases such as MDS, AA or IMF and to give direction to the treatment.

incidence has been published as 1.3/1 million [1, 9]. There is not yet available epidemiological data on the prevalence and incidence of PNH in Turkey.

PNH is classified into three different clinical forms [10, 11]:

Classic PNH (Hemolytic PNH): Classic type PNH that consists of clinical and laboratory findings of intravascular hemolysis, does not include conditions associated with bone marrow deficiency such as Aplastic Anemia *(AA)* or Myelodysplastic Syndrome *(MDS)*.

PNH on the base of AA, MDS (PNH/AA, PNH/MDS): PNH that consists of clinical and/or laboratory findings of intravascular hemolysis in the presence of conditions associated with bone marrow deficiency such as AA, MDS.

Subclinical PNH (Non-Hemolytic PNH): Patients with PNH have no clinical or laboratory evidence of hemolysis. Small populations of GPI-AP-deficient hematopoietic cells *(peripheral blood erythrocytes, granulocytes, or both)* are detected by very sensitive flow cytometric analysis.

Diagnosis of PNH

Following the clinical evaluation, the laboratory findings for the diagnosis of PNH can be grouped under 3 main titles (**Table 1**):

Laboratory findings of hemolysis: Hemogram, peripheral blood smear, absolute reticulocyte count, indirect bilirubin, LDH, Haptoglobin, Direct and Indirect Coombs tests.

Displaying lack of Glycosyl Phosphatidyl Inositol *(GPI)* protein with Flow Cytometry: It is demonstrated by showing lack of GPI anchored proteins *(CD55, CD59)* in peripheral blood sample with flow cytometry method. High Precision Flow Cytometry is a Golden Standard in PNH diagnosis. FLAER *(fluorescently labeled aerolysin)* which binds directly to the GPI anchor protein and is consequently reliably absent from GPI anchor deficient granulocytes and monocytes, has become the best way for determining leukocyte PNH clones [10, 12, 13].

Bone Marrow Analysis: Bone marrow analysis (BMA) is not a direct indicator of PNH but it is necessary for elaborating whether there is bone marrow deficiency *(such as AA, MDS, IMF)* or not *(with bone marrow aspiration and biopsy)* [8].

The fundamental diagnostic criteria that confirms the presence of PNH clone is High Precision Flow Cytometry. As antibodies associated with GPI are connected to GPI, FLAER which is fluorescently labeled with inactive bacterial toxin targets GPI binders on cell surface [10, 13-16]. Flow cytometry is the most sensitive and reliable diagnostic method for the diagnosis of PNH. Test and monitoring should be conducted according the International Clinical Cytometry Society *(ICCS)* guide in 2010. All the technical details are described in the relevant literature [13].

At least 2 different GPI protein deficiencies within 2 different cell lines from granulocytes, monocytes or erythrocytes are need to be shown with flow cytometry for diagnosis *(2 × 2 rule)* [10, 13, 17]. Leucocytes *(granulocytes and monocytes)* denote more reliable results than the erythrocytes because of previous transfusions or massive hemolysis may reveal incorrect results [12].

PNH erythrocytes are named according to characteristics of bearing GPI anchored proteins [10, 13, 18, 19]:

Table 2. Clinical indications for PNH Screening [13, 22, 23]

Intravascular Hemolysis
Hemolysis accompanied by any of the following items
Abdominal Pain
Esophageal Spasm
Cytopenia
Iron Deficiency
Coombs negativity
Non-schistocytic, non-infectious hemolytic anemia
Thrombosis presence having any of the following characteristics
Young patient (especially younger than 45 y.o.)
Unusual localization
Hepatic vein (Budd-Chiari Syndrome)
Other intra-abdominal veins (portal, splenic, etc.)
Cerebral sinus
Dermal veins
If it is accompanied by hemolysis
If it is accompanied by cytopenia
If there is resistance to anticoagulation therapy
Bone Marrow Deficiency Findings
Aplastic/Hypoplastic Anemia
MDS-with any of the followings:
any subtype showing evidence of hemolysis
hypoplastic
refractory cytopenia
Unexplained cytopenia (s)

Type I Erythrocytes: Erythrocytes bearing GPI anchored proteins in a normal way.

Type II Erythrocytes: Erythrocytes bearing decreased proportion of GPI anchored proteins.

Type III Erythrocytes: Erythrocytes not bearing GPI anchored proteins.

A certain threshold value has been identified for CD55 and CD59 expression level in PNH. Determination of PNH clone is sufficient for diagnosis.

Sampling for flow cytometry

Flow cytometric analysis should be done with peripheral blood samples taken into EDTA tubes within 24-48 hours. These samples should be kept in room temperature for 24 hours (+4°C for 48 hours) however, it is recommended to process at the shortest possible time interval for accurate results. As the expression of GPI anchored proteins in hematopoietic cells is low, bone marrow examination is not recommended.

Screening for PNH?

Clinical recommendations for PNH screening are elaborated in **Table 2**. Some particular points should be emphasized:

At the beginning of the disease, hemoglobinuria can be observed approximately in 25% of patients therefore all individuals with this finding should be examined. However, absence of hemoglobinuria cannot be a predictive reason to eliminate PNH. Almost all patients with classic PNH develops hemoglobinuria in a period of their lives however due to the small PNH clones, they might be AA/PNH and MDS/PNH patients.

Intravascular hemolysis is typical for PNH and blood tests almost always show an increased activity of LDH (LDH > 1.5 ULN). For this reason, examination of PNH should begin with intravascular hemolysis findings and increased LDH level for each patient. Patients with high PNH clone size and especially with a majority of type II PNH cells should be considered that LDH activity may increase in a small amount or may be within reference values [10, 13, 21-23].

PNH screening for all patients with AA is necessary even without hemolysis [10]. Individuals with AA and MDS that are considered as low risk in terms of PNH should be screened for PNH with high sensitivity flow cytometry [13].

Thrombosis is the most common complication of PNH [1, 2] and 7% of patients experiencing thrombotic events is diagnosed with PNH [11]. For this reason, in the presence of atypical location of thrombosis (Budd Chiari Syndrome, Cerebral vein thrombosis, dermal vein thrombosis, intra-abdominal thrombosis), thrombosis and intravascular hemolysis and/or cytopenia, PNH screening is highly recommended. In addition, PNH screening is performed for thrombosis that can't be explained with general reasons (**Figure 1**). Also one should bear in mind that

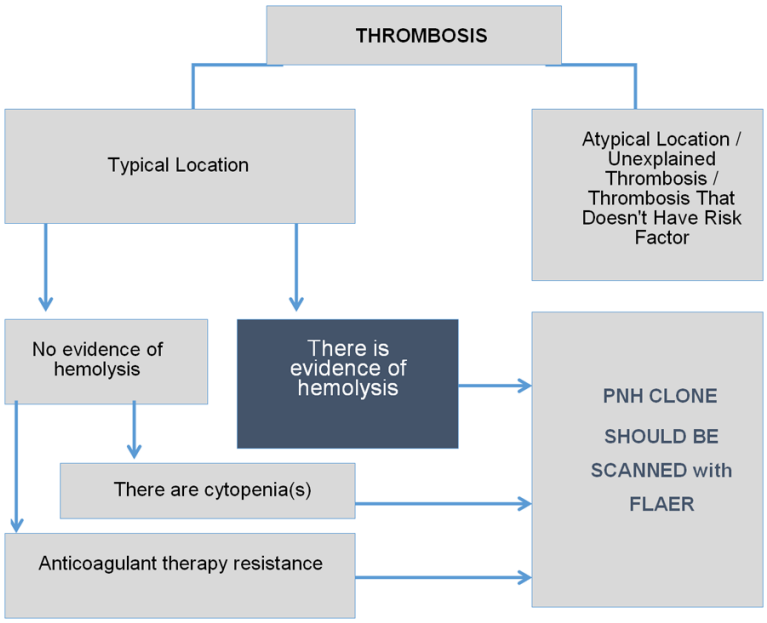

Figure 1. Characteristics of Thrombosis for which **PNH Clone is Recommend**ed (Adapted from www.pesg.org).

Table 3. Risk Assessment in PNH Patients

Hemolysis findings
LDH ≥ 1.5 × ULN
Reticulocyte count
Hemoglobin value
Haptoglobin level
Direct and Indirect Bilirubin
Hemoglobinuria
Symptoms of kidney dysfunctions
Glomerular Filtration Rate
Serum creatinine level
Signs and symptoms of thrombosis
Thromboembolism history
D-dimer
Platelet count
Abdominal pain
Chest pain
Dyspnea
Neurological symptoms
Pulmonary Hypertension
Increased NT-proBNP
Echocardiography
Factors of Quality of Life
Fatigue/Weakness
Pain
Dysphagia
Erectile dysfuntion

PNH screening is not indicated for thrombosis other than these [10, 13].

During diagnosis, 10% to 20% of patients with PNH present abdominal pain and other gastrointestinal disorders (dysphagia). These symptoms are more common for classical PNH patients and its rate reaches 33% [11]. Routine PNH screening is not mandatory for all patients with these symptoms, unless clinical and laboratory intravascular hemolysis findings are observed in patients with abdominal pain, dysphagia, chest pain, dyspnea and erectile dysfunction.

Flow cytometric follow-up

Patients with full or partial GPI anchored protein deficiency together with the PNH clone size of more than 0.01% should be monitored. For patients with clone size of less than 1% and do not manifest clinical symptoms of hemolysis, test relating to determine GPI anchored protein from peripheral blood should be performed every year. As clone size may be increased in time, for patients with clone size of more than 1%, test should be performed at least once in 6 months.

PNH clone size may decrease or become undetectable within time, for this reason, in case of deterioration of clinical symptoms, thromboembolic event and increase in hemolysis, immediate testing is recommended [10, 13, 20, 24].

There are no clinical symptoms associated with hemolysis and laboratory findings for subclinical patients with PNH. Taking this into consideration one can say that 15-17% of patients with AA/subclinical PNH develop hemolytic anemia in a certain time interval and close follow-up should be carried out in order to determine hemolysis findings and clonal expansion of PNH cells [20, 25].

Clone analysis with flow cytometry is recommended for patients diagnosed with AA or MDS-RA in the time of diagnosis and annually, follow-up should be performed once in every 6 months for patients with positive results, whereas annual follow-up is recommended for patients with negative results [10].

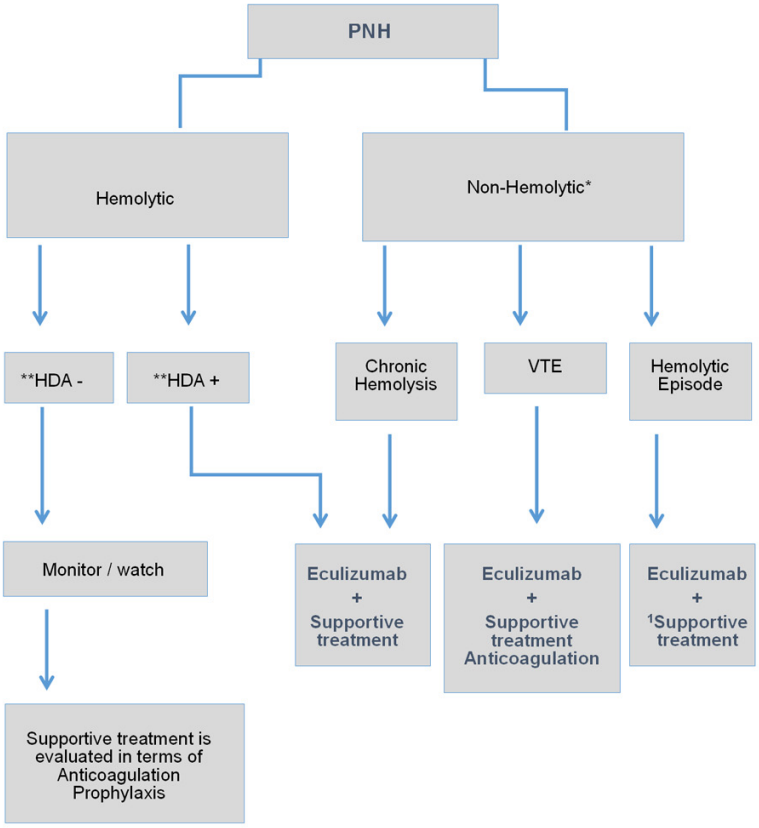

Figure 2. PNH Treatment Algorithm. VTE: Venous Thromboembolism. *In case of bone marrow deficiency in non-hemolytic PNH, treatment of the underlying disease has priority. **High Disease Activity. Supportive treatment: folic acid, iron replacement and transfusion. [1]Short-term steroid therapy.

Risk assesment for progression/complications of PNH

Risk classification at the time of diagnosis and clinical and laboratory parameters used in follow-up are both elaborated in **Table 3**. Disease risk assessment is important to determine whether there is high disease activity or not in order to determine treatment options and frequency of follow-up.

High disease activity

PNH Registry study (M07-001) evaluated the effectiveness of Soliris® *(Alexion Pharmaceuticals, Cheshire, CT)* in PNH patients with no history of transfusion and clinical symptoms associated with increased hemolysis *(LDH > 1.5 ULN).* These symptoms were determined as; weakness, fatigue, hemoglobinuria, abdominal pain, dyspnea, anemia *(Hb < 10 g/dl),* major vascular event *(such as thrombosis),* dysphagia or erectile dysfunction. European Medicine Agency defined high disease activity in PNH based on this data in March 2015. According to this, LDH which is equal to one and a half of the normal limit and more than this and presence of at least one or more of the above-defined symptoms at the same time indicate High Disease Activity *(HDA)* [26].

Treatment

PNH treatment algorithm is shown in **Figure 2**. PNH treatment can be grouped under three main titles:

Supportive Treatments; Treatment changing the course of the disease; Potential Curative Treatment.

Supportive treatments and immunosuppressive treatments

Blood/erythrocyte suspension transfusion: It should be considered in the presence of symptomatic anemia [7].

Folic acid, vitamin B12 support: It can be used in order to support increased erythropoiesis in the bone marrow [27].

Oral Iron supplementation: If eculizumab treatment is initiated effectively, ferritin and hemolysis will be controlled and iron deficiency will also be prevented. Due to this transferrin saturation analyzed and intravenous iron therapy should be avoided as it may cause hemolytic crisis [28].

Steroids: Utilization of steroids in modern PNH treatment can only be considered in hemolytic episodes, and only for a short time. Long-term steroid treatment is not recommended [29].

Anticoagulant treatment: Anticoagulant treatment alone is not sufficient to prevent progressive thrombotic events. In patients who are not treated with eculizumab, consideration of primary prophylaxis should be given to reduce the risk of thrombosis if there is no contraindica-

Pretreatment		Induction Period					Maintenance Period			
≥ 2 weeks before induction treatment: Neisseria meningitidis vaccine	Week	1	2	3	4	5	6	7	8	Once in two weeks
	Eculizumab dose	600 mg	600 mg	600 mg	600 mg	900 mg	x	900 mg	x	900 mg

Figure 3. Eculizumab Treatment Scheme in PNH.

tion, such as thrombocytopenia or other bleeding risk. It has been shown that eculizumab treatment decreases thrombotic events at the rate of 92% and also eculizumab use in patients with thrombosis has a protective efficacy against recurrent thrombotic events [30].

Long-term (lifetime) anticoagulation (coumarin derivatives and heparin) can be considered in the course of life-threatening thrombotic events.

Immunosuppressive treatment: It doesn't have indication for PNH treatment apart from cases such as PNH/AA accompanied by bone marrow deficiency findings.

Treatment changing the course of the disease

Eculizumab: Eculizumab *(Soliris®, Alexion Pharmaceuticals, Cheshire, CT)* is a humanized monoclonal antibody which blocks decomposition of C5 into C5a and C5b by binding to C5-complement [31-33].

Previous research on eculizumab proved that it reduces the risk of organ damages in PNH caused by hemolysis, transfusion dependence, incidence of thrombosis, renal failure and pulmonary hypertension. Eculizumab significantly heals symptoms such as weakness, dyspnea at the same time, improves renal functions and quality of life.

Long-term use of eculizumab increases survival up to the level of general population in the same age group [32-35]. Eculizumab is not a curative treatment but increases survival [35]. Long-term eculizumab treatment has also a good safety profile and reduces complications and the risk of death significantly [8].

For patients diagnosed with PNH, Eculizumab treatment indications are available in following cases [4, 35]:

Presence of thrombotic event, Presence of organ damage due to chronic hemolysis, If PNH patient is pregnant, (only if clearly needed, the pregnancy category is C and risk benefit ratio should be analyzed), Transfusion dependence, For patients with high LDH activity (one and a half of the normal limit), PNH complications including life-threatening cases increased significantly. For this reason, eculizumab should be used in treatment of patients having increased LDH activity, which is associated with chronic hemolysis-for example; thrombosis, anemia, acute and chronic renal failure, pulmonary hypertension, smooth muscle dystonia (for example; abdominal pain, dysphagia, erectile dysfunction etc.) - and clinical symptoms [8, 26].

Eculizumab administration: Eculizumab should be administered within 25-45 minutes of intravenous infusion. A dose of 600 mg/week dose is given in the first 4 weeks, 900 mg in 5th week and then it is continued with 900 mg dose once in 14 days (**Figure 3**) [26]. Complement mediated hemolysis can be inhibited in > 90% of patients with this treatment scheme [32]. *"Break-through-hemolysis"* might be seen in some patients before the next dose of eculizumab *(12th-14th Days).* Eculizumab dosage intervals can be reduced to 12 days in these patients and/or can be increased to 1200 mg [35].

Including patients with spontaneous remissions, eculizumab treatment should be continued throughout lifetime [1, 35, 37].

Meningococcal prophylaxis: The risk of meningococcal infection *(Neisseria meningitidis)* increases in association with blockage of terminal portion of the complement by eculizumab. For this reason, all patients should be vaccinated against meningococcus at least 2 weeks before initiating eculizumab treatment. At the same time, H. influenza type b and pneumococcal vaccines are recommended [26]. If eculizumab is to be started immediately and there is no 2 weeks period, antibiotic prophylaxis can be initiated and continued for the next two weeks after vaccination.

Potential curative treatment

Allogeneic bone marrow transplantation: Allogeneic bone marrow transplantation is the only potential curative treatment for PNH. However, it should be considered in selected patient groups due to high morbidity and mortality. It is especially recommended for PNH/AA, PNH/MDS patients with prominent bone marrow deficiency and patients resistant to thromboprophylaxis and eculizumab treatment, experiencing recurrent thromboembolic events. After allogeneic bone marrow transplantation from donors having full HLA compatibility, 2-year survival rate is 56% and 10-year survival rate is 42%. The average incidence of graft versus host disease has been observed as 40-50%. Venous-occlusive disease was observed in half of the patients [38-40]. Bone marrow transplantation and its complications also effects quality of life of patients negatively [41, 42]. Retrospective analysis by the French group has shown that survival rate of PNH patients with thrombosis history after allogeneic bone marrow transplantation is significantly lower than the group without thrombosis [43]. In the light of all this data, one can say that allogeneic bone marrow transplantation for classic PNH is not the first choice and it should be brought into question for mentioned special patient groups.

In patients that allogeneic bone marrow transplantation is planned, reported eculizumab use is very low with a small number of case examples. Eculizumab treatment can be used to control hemolysis concomitantly with transplantation and is to be ceased within 2-4 weeks before stem cell transplantation. In case of post-transplant hemolytic attacks additional dose of eculizumab is appropriate. However, since there are no comprehensive studies, process should be initiated with individual decision by considering the high morbidity and mortality risks. After allogeneic stem cell transplantation for PNH, testing should be done once in every 3 months until GPI anchor deficient cell population is not determined, and then, remission should be checked annually [44, 45].

Pregnancy and PNH

The risk of pregnancy complications such as thrombotic events, maternal and fetal mortality increases in patients with PNH. For this reason, eculizumab treatment is indicated for all PNH patients considering pregnancy (*only if clearly needed, the pregnancy category is C and risk benefit ratio should be analyzed*) For low disease activity patients it is recommended to start eculizumab before pregnancy, continue treatment during pregnancy and post-partum at least 3 months. Treatment termination decision should be made by performing risk assessment again.

Although few, there are case reports related to the use of eculizumab treatment without occurrence of teratogenicity during successful pregnancy. When pregnancy is detected, eculizumab treatment should not be stopped. (Eculizumab does not pass into milk and umbilical blood).

Eculizumab dose should be increased in third trimester of pregnancy and in case of *"breakthrough hemolysis" (Up to 900 mg in a week).* Preventive anticoagulant treatment *(LMWH)* might be used when folic acid and iron supplements, erythrocyte and platelet transfusions are needed [46, 47].

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