Paroxysmal nocturnal haemoglobinuria

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SUMMARY

Paroxysmal nocturnal haemoglobinuria is a rare, acquired haematological disease that manifests with haemolytic anaemia, thrombosis and impaired bone marrow function. The absence of two glycosylphosphatidylinositol-anchored proteins, CD55 and CD59, leads to uncontrolled complement activation that accounts for haemolysis and other paroxysmal nocturnal haemoglobinuria manifestations. Patients may present with a variety of clinical manifestations, such as anaemia, thrombosis, kidney disease, smooth muscle dystonias, abdominal pain, dyspnoea, and extreme fatigue. Delayed recognition of this condition is common due to the variable clinical presentation. This delay in diagnosis confers an increased risk of mortality and morbidity. Therefore, the purpose of this review is to raise awareness about this potentially life-threatening disease among haematologists and to provide a guide to diagnosis and treatment.

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INTRODUCTION

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired, life-threatening haematological disease. It is characterised by haemolytic anaemia, thrombosis and impaired bone marrow function. PNH is rare and affects approximately 1.13 persons per million in the general population.[1] It is primarily a disease of younger adults, with a median age at diagnosis of 35-40 years.[2] The prognosis of PNH patients in the pre-eculizumab era was poor, with a median survival between ten and fifteen years, but has dramatically changed with the advent of anti-complement therapy.[3]

PNH is an X-linked haematopoietic stem cell disorder in which uncontrolled complement activity leads to systemic complications, principally through intravascular haemolysis and platelet activation. It arises from acquired mutations in the *phosphatidylinositol glycan A (PIG-A)* gene whose protein product is a glycosyl transferase. This enzyme is essential in the biosynthesis of the glycosylphosphatidylinositol (GPI) anchor. The GPI anchor attaches different proteins to the cell surface, among which the complement regulatory proteins CD55 and CD59 are the most important. Lack of these complement inhibitors on the red blood cell (RBC) surface in PNH patients results in complement-mediated haemolysis (*Figure 1*), with release of free haemoglobin and depletion of nitric oxide (NO).[4,5] Despite being an X-linked disorder, PNH appears to affect males and females equally. This can be explained by the fact that PNH arises from somatic, rather than germline, mutations and that in female somatic cells only one X-chromosome is used.[6]

Since PNH is a rare blood disorder with a heterogeneous clinical presentation, diagnosis is often delayed resulting in increased morbidity and mortality. The aim of this article is to raise awareness of PNH among haematologists and to present an overview of its various clinical presentations, the diagnostic tools available and its treatment options.

DIAGNOSIS

PNH is a disease with a wide constellation of clinical signs and symptoms, making the diagnosis extremely challenging. The clinical characteristics of PNH are summarised in *Table 1*. Symptoms are mainly due to haemolysis with release of free haemoglobin, which in turn acts as scavenger for NO. Nitric oxide depletion inhibits smooth muscle relaxation, causing PNH symptoms such as abdominal pain, oesophageal

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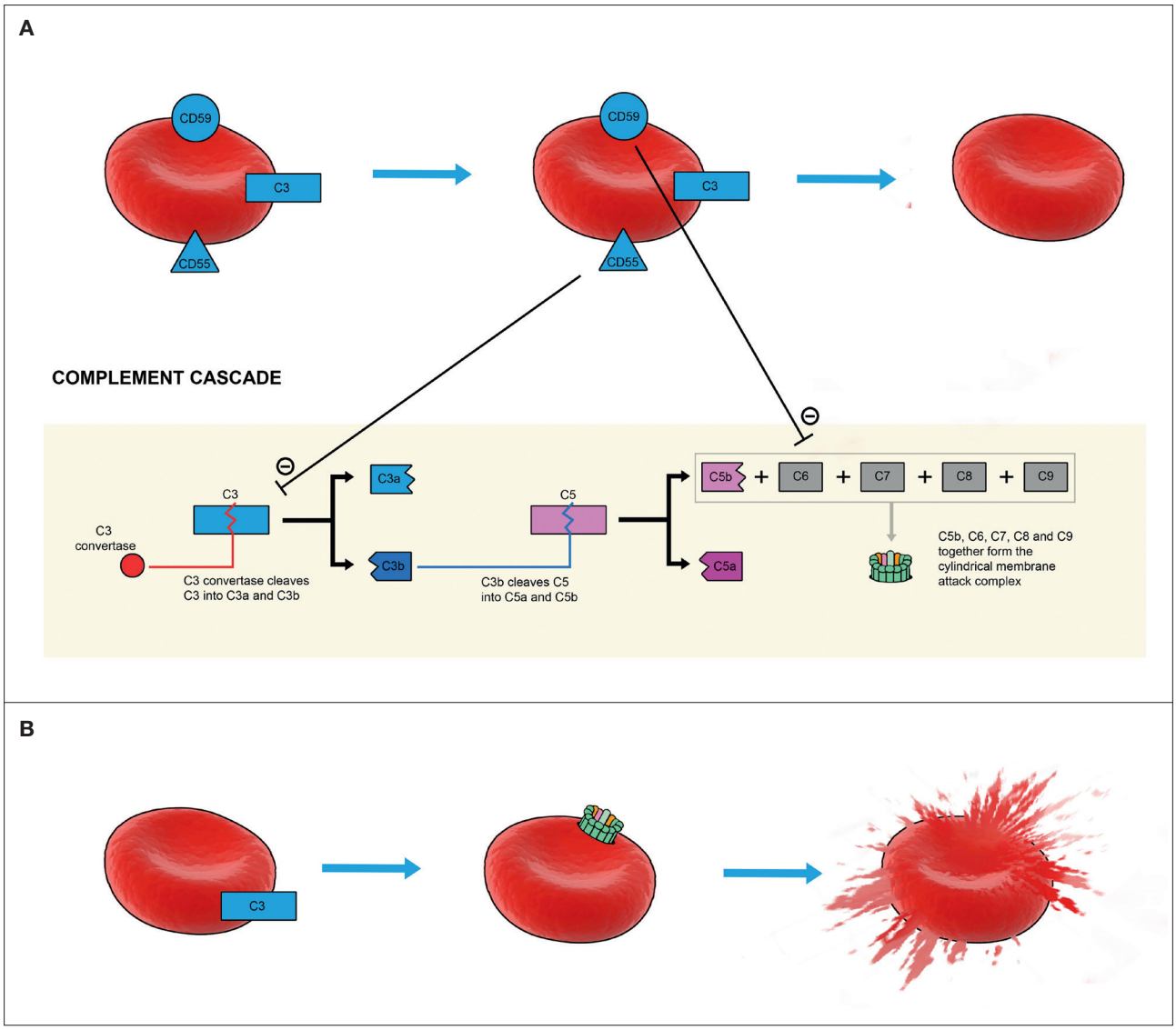

FIGURE 1. Action of complement in healthy subjects **(A)** and paroxysmal nocturnal haemoglobinuria (PNH) patients **(B)**. **(A)** Due to the presence of GPI-anchored membrane proteins CD55 and CD59, a normal erythrocyte is protected from complement activation. **(B)** CD55 and CD59 deficiency makes the erythrocyte sensitive to complement attack, resulting in haemolysis. CD55 regulates activation of C3 by C3 convertase. Subsequently, C5 is cleaved into C5a and C5b. C5b begins the terminal pathway of complement and the assembly of the membrane attack complex (MAC). The formation of the MAC is regulated by CD59, another GPI-anchored protein.

spasms, erectile dysfunction and pulmonary hypertension. The complement and coagulation systems are closely linked, explaining why thrombosis is another hallmark finding of PNH (*Table 1*).[2,7,8]

Following the clinical suspicion, the laboratory findings for the diagnosis of PNH can be evaluated. There are several clinical situations in which testing for PNH can be appropriate: in the work-up of a Coombs-negative haemolytic anaemia and/or haemoglobinuria; in the setting of venous or arterial thrombosis at unusual sites (e.g., Budd-Chiari syndrome or cerebral veins) and/or in young patients and/or in case of resistance to anticoagulation therapy; and in patients presenting with bone marrow failure (e.g. unexplained cytopaenias).

The laboratory diagnosis of PNH is based on the following three criteria (*Table 1*):[4,5,9,10]

1. Evidence of haemolysis (non-immune): Complete blood count showing anaemia with positive haemolysis parameters: reticulocytosis, elevated lactate dehydrogenase level, elevated indirect bilirubin level and decreased haptoglobin level. The direct Coombs will be negative (non-immune haemolysis).

2. Evidence of a population of peripheral blood cells deficient in GPI-anchored proteins by routine flow cytometric analysis (e.g. using monoclonal antibodies against CD55 and CD59). At least two different GPI protein deficiencies within two different cell lines from granulocytes, monocytes or erythrocytes are needed to confirm diagnosis.[11] An additional flow-based assay is the FLuorescent AERolysin (FLAER) assay. FLAER is a reagent derived from the bacterial toxin aerolysin, which binds directly to the GPI anchor. As such, this assay can be used to directly demonstrate the presence of a PNH clone.
3. Bone marrow aspirate, biopsy and cytogenetics: Bone marrow analysis is not required for the diagnosis of PNH but is important for classification purposes to prove or exclude the presence of an associated bone marrow syndrome (e.g. myelodysplastic syndrome or aplastic anaemia).

These diagnostic studies will allow classification of patients into three categories based on the recommendations of the International PNH Interest Group (IPIG): classic PNH, PNH associated with another bone marrow syndrome and subclinical PNH (*Table 2*).[9] These three categories are dynamic and overlapping.

TABLE 1. Signs and symptoms of PNH.

Haemolysis
Sign/symptoms caused directly by anaemia/red blood cell lysis • Fatigue • Jaundice • Red urine due to haemoglobinuria
Signs/symptoms caused indirectly by haemoglobin release via NO release • Smooth muscle dystonia: abdominal pain, dysphagia, erectile dysfunction • Pulmonary hypertension • Renal insufficiency • Hypercoagulability
Thrombosis
Laboratory abnormalities
Haemolysis
Evidence of a population of peripheral blood cells deficient in GPI-anchored proteins (CD55, CD59) by flow cytometric analysis
Bone marrow dysfunction (cytopaenias)

TREATMENT

HEMATOPOIETIC STEM CELL TRANSPLANTATION AND ECULIZUMAB

For patients with classic PNH, allogeneic hematopoietic cell transplantation and complement inhibition with eculizumab are the only established therapies. Allogeneic hematopoietic stem cell transplantation remains the only curative therapy. It should not be offered as initial therapy for patients with PNH, since there is an important risk of transplant-related morbidity and mortality. Only patients who have a refractory transfusion-dependent haemolytic anaemia, recurrent life-threatening thromboembolic complications or severe aplastic anaemia/myelodysplastic syndromes should be selected for allogeneic stem cell transplantation. A myeloablative-conditioning regimen is not required to eradicate the PNH clone.[12] Eculizumab is the first treatment of choice in classic PNH.[4,9,10,13] Eculizumab is a humanised monoclonal antibody directed against complement factor C5, preventing its cleavage and activation to C5b. C5b is required for the formation of the membrane attack complex. Red blood cells are normally protected from the membrane attack complex by the GPI-linked protein CD59. Lack of CD59 on the red blood cell surface in PNH patients explains their haemolytic propensity. Eculizumab interferes with this step and thus reduces intravascular haemolysis. The efficacy of eculizumab was evaluated in the TRIUMPH study (randomised trial), the SHEPHERD study (observational study) and an extension study.[14-16] Treatment of patients with classic PNH with eculizumab reduces transfusion requirements, ameliorates the anaemia and improves quality-of-life.[14-16]

Historical studies have shown the median survival in PNH patients to be between ten and fifteen years from the time of diagnosis.[3] With the use of eculizumab, the overall survival of PNH patients has consistently shown improvement, approaching that of the general population.[17]

In Belgium, eculizumab (Soliris®) is reimbursed when flow cytometry analysis shows a PNH clone type III (i.e. complete deficiency of GPI-anchored proteins) of >10% and when the patient has had at least four blood transfusions over the last two years. Eculizumab is administered intravenously. The initial treatment phase consists of weekly administrations of 600 mg eculizumab for four weeks. In week five, the dose is augmented to 900 mg. The recommended maintenance dose of eculizumab thereafter is fixed (900 mg every two weeks +/- two days). In case of an inadequate response, there is a possibility to increase either the dosage or the

TABLE 2. Classification of PNH according tot the International PNH Interest Group.

1. Classic PNH: clinical and laboratory findings consistent with the diagnosis of PNH; no evidence of an associated bone marrow failure syndrome (myelodysplastic syndrome, aplastic anaemia or primary myelofibrosis)
2. PNH in the setting of another bone marrow failure syndrome (myelodysplastic syndrome, aplastic anaemia or primary myelofibrosis)
3. Subclinical PNH: demonstration of a PNH clone but no clinical or laboratory findings of haemolysis

frequency of administrations. Adverse effects of eculizumab are headache due to increasing NO levels and meningococcal infections (Neisseria meningitidis). Meningococcal vaccination is mandatory in all patients treated with eculizumab and should be administered two weeks prior to initiation of therapy.[18] A conjugated tetravalent vaccine against serotypes A, C, Y and W is recommended (Menveo® and Nimenrix®). In Belgium, the Superior Health Council also advises vaccination against serotype B (Bexsero®). At the same time, vaccination for Haemophilus influenza type b (Act-Hib®) and pneumococcal vaccination one dose PCV13 [Prevenar 13®] for primo-vaccination, and at least eight weeks later one dose PPV23 [Pneumovax®] are recommended. Patients should be revaccinated every three to five years.[18,19]

In subclinical PNH, no specific therapy is required to eradicate the PNH clone. In PNH in the setting of another bone marrow failure syndrome (e.g. myelodysplastic syndrome or aplastic anaemia), the focus lies on treating the underlying bone marrow syndrome.

SUPPORTIVE CARE

1. Red blood cell transfusions in case of severe anaemia, as per standard transfusion guidelines.
2. Vitamin supplementation with folic acid, vitamin B12 or oral iron to support erythropoiesis. Intravenous iron administration should be avoided as this can trigger the PNH clone resulting in increased haemolysis.
3. In case of iron overload (e.g. polytransfused patients), iron depletion should be initiated.
4. Steroids (0.3-0.5mg/kg) can be used for severe haemolytic exacerbations. Side effects are considerable and their use should be restricted for a limited period of time.
5. Stimulation of the bone marrow with erythropoietin and/or granulocyte colony-stimulating factor in case of cytopaenia.
6. Immunosuppressive treatments (e.g. cyclosporine or anti-thymocyte globulins) are not indicated, except in the case of PNH associated with aplastic anaemia.
7. Contraception: Patients with PNH should avoid oestrogen-based oral contraceptives because of the increased thrombotic risk opting for progesterone only or barrier methods instead.
8. Anticoagulant treatment: discussed below.

PREVENTION AND TREATMENT OF THROMBOSIS IN PNH

Thromboembolism is the most common cause of mortality in patients with PNH.[21] The risk of thrombosis is correlated with the size of the PNH clone. Therefore, primary thrombosis prophylaxis with vitamin K antagonists should be considered in patients with a large (i.e. >50%) granulocyte PNH clone.[21] Nevertheless, severe thromboses can also occur in patients with smaller PNH clones, but the indication for thrombosis prophylaxis in these patients is not established.[20,21]

Given the rarity of PNH and the fact that arterial thromboses are far less common than venous thromboses, there are no data on the efficacy of anti-aggregants (e.g. acetylsalicylic acid) in the prevention of arterial thrombosis in PNH.[21]

In case of an acute thrombosis in an eculizumab-naive PNH patient, management consists of immediate start of treatment with eculizumab and full dose anticoagulation.[21] As discussed above, eculizumab is only reimbursed in Belgium in case of PNH clone of >10%. Hence, patients with a smaller PNH clone and thrombosis are not entitled to receive eculizumab.

A question of clinical importance remains whether life-long anticoagulation is necessary for PNH patients with a history of thrombosis who are well controlled on eculizumab therapy. Although randomised controlled clinical trials are lacking, there is some evidence that it may be safe to withdraw anticoagulation in such patients.[22]

In case of a thrombosis occurring in a patient who is already on eculizumab-treatment, immediate anticoagulant treatment is advised in combination with an additional dose of eculizumab. Since eculizumab itself has anti-thrombotic properties, a dose escalation of eculizumab may be considered.

KEY MESSAGES FOR CLINICAL PRACTICE

1 **PNH is characterised by haemolytic anaemia, thrombosis, and impaired bone marrow function.**

2 **PNH is a disease whose diagnosis may be delayed due to variable clinical findings. This delay increases risk of mortality and morbidity.**

3 **Once suspected, diagnosis of PNH needs to be confirmed by flow cytometric analysis.**

4 **Therapy with eculizumab, a humanised monoclonal antibody directed against complement factor C5, has improved patient survival and quality-of-life.**

5 **The only curative treatment option remains allogeneic hematopoietic stem cell transplantation, but its use is restricted to specific situations.**

6 **Special attention is needed for the management of thrombosis in patients with PNH.**

MANAGEMENT OF PNH DURING PREGNANCY

PNH confers an increased morbidity and mortality risk, both for the mother and for the foetus. However, successful pregnancies have been reported in the literature.[23] Pregnant women with PNH should receive adequate folate and iron supplementation. Given the increased risk of thrombosis in pregnant PNH patients, prophylaxis with low molecular weight heparins is recommended if no contraindication to anticoagulation. Anticoagulation should be continued during the puerperal period.[24]

Eculizumab can be used during pregnancy and lactational period and is associated with improved maternal and foetal outcome. This is confirmed by an observational study of 75 pregnancies in women enrolled in the PNH registry.[23]

PERSPECTIVES: NEW ANTI-COMPLEMENT THERAPIES ARISING

The most obvious unmet clinical need when treating patients with PNH remains residual anaemia. Approximately one third of patients remain transfusion-dependent despite treatment with eculizumab. This is due to persistent activation of the early phases of complement activation; PNH erythrocytes in patients on eculizumab often accumulate C3 fragments because eculizumab blocks complement at C5 (upstream of CD59 but downstream of CD55) (*Figure 1*). CD55 shortens the half-life of C3 by acting on the C3 convertases (*Figure 1*). Because PNH erythrocytes are missing CD55, these C3 fragments accumulate and lead to opsonisation and destruction of the PNH red cells in the spleen.[25] This makes C3-mediated extravascular haemolysis the next challenge of PNH treatment. Several new complement-targeted therapeutics are currently under investigation.[26]

Novel anti-C5 agents

There are a number of novel anti-C5 agents under investigation, including Coversin™ (Akari Therapeutics). Coversin is a subcutaneous C5 inhibitor which is currently under investigation in a phase II clinical trial. Alexion Pharmaceuticals has developed a long-acting variant of eculizumab, called ALXN1210, which has recently received orphan drug designation by the US Food and Drug Administration.

Anti-C3 agents

By acting earlier in the complement cascade, C3 inhibitors might possess superior anti-haemolytic activity over eculizumab and other C5 inhibitors. One example is compstatin, a peptide drug that blocks complement at the C3 level. APL-2 (Apellis Pharmaceuticals), a pegylated derivative of compstatin, has also recently been granted orphan drug designation for the treatment of PNH patients. Safety is a concern with these novel anti-C3 agents; indeed, inherited C3 deficiency is associated with a risk of life-threatening infections and autoimmune diseases.

Inhibitors of the alternative pathway, targeting complement factor B or D

Such inhibitors are anticipated to be highly efficacious, given the consideration that the alternative complement

pathway accounts for 80% of complement activation products.[27] ACH-4471 (Achillion Pharmaceuticals), an oral small molecule factor D inhibitor, has recently entered the clinical trial stage.

CONCLUSIONS

PNH is characterised by haemolytic anaemia, thrombosis, and impaired bone marrow function. PNH is caused by a mutation in *PIGA* that leads to a marked deficiency or absence of the complement regulatory proteins CD55 and CD59. It is a disease with a wide constellation of clinical signs and symptoms, making diagnosis extremely challenging. Once suspected, diagnosis of PNH needs to be confirmed with flow cytometric analysis. Complement inhibition using the C5 monoclonal antibody eculizumab has led to dramatic clinical improvement of overall survival, haemolysis and quality-of-life in PNH. Special attention is needed for treatment and prevention of thrombosis in PNH and pregnancy in PNH.

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