Paroxysmal Nocturnal Hemoglobinuria

Subjects: Urology & Nephrology | Pathology

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Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal disease that presents an estimated incidence of 1.3 cases per million per year, with a prevalence of 15.9 cases per million. It is characterized by hemolysis, bone marrow dysfunction with peripheral blood cytopenia, hypercoagulability, thrombosis, renal impairment and arterial and pulmonary hypertension. Hemolysis and subsequent hemosiderin accumulation in tubular epithelium cells induce tubular atrophy and interstitial fibrosis.

Paroxysmal Nocturnal Hemoglobinuria | Complement Inhibition | Thrombosis | Membrane Attack Complex | Complement Dysregulation

1. PNH Genetic Mutation

The cause of PNH is a somatic mutation in the X-linked phosphatidylinositol glycan class A (PIG-A) gene on Xp22 [1], coding for one of the several enzymes involved in the generation of glycosyl phosphatidylinositol (GPI) anchors in the endoplasmic reticulum. Deficiency in *PIG A* leads to a lack of the expression of approximately 150 cell surface proteins [2]. The common core structure of GPI is made up of a molecule of phosphatidylinositol and a glycan core consisting of three mannoses, an ethanolamine phosphate and glucosamine [3].

At least 10 reactions and more than 20 different genes are implicated in the biosynthesis of GPI anchors [4]. In PNH, this mutation results in the production of clonal blood cells with a deficiency in those surface proteins that protect against damage caused by the complement system [5]. In this way, such a disorder makes these cells excessively susceptible to the lytic action of activated complement.

PNH features are closely connected with a deficit in or complete absence of CD55 (a decay accelerating factor) and CD59 (a membrane inhibitor of reactive lysis), from the family of GPI-anchored proteins. These proteins modulate solid phase complement activity; CD55 inhibits alternative pathway C3 and C5 convertases, and CD59 prevents the creation of the membrane attack complex (MAC) [6] (Figure 1).

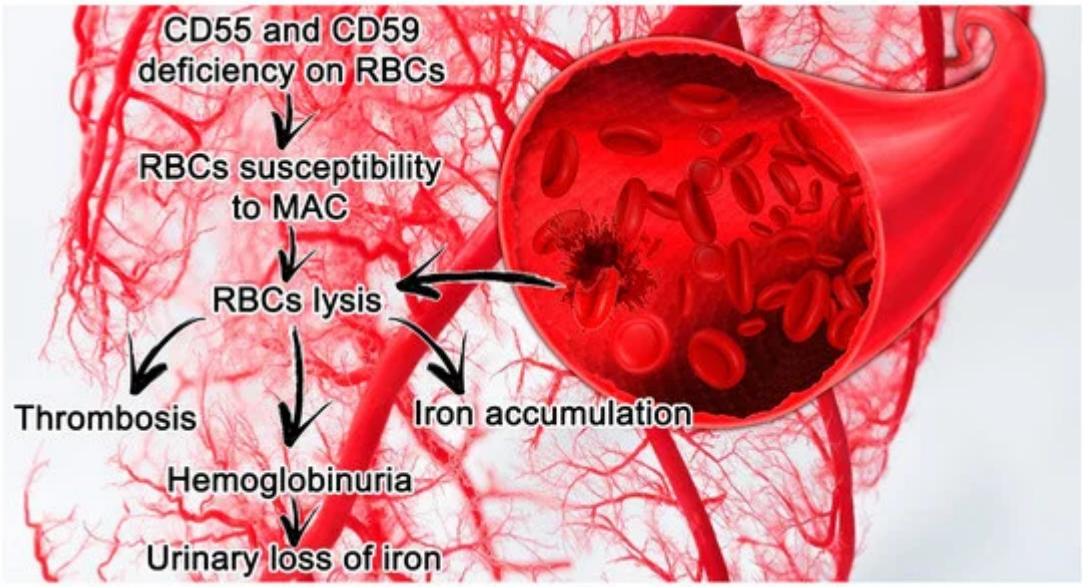

Figure 1.[7] Paroxysmal nocturnal hemoglobinuria (PNH) features in small vessels and its consequences. Erythrocytes lacking CD55 and CD59 are more susceptible to hemolysis mediated by MAC, which leads in turn to thrombosis and the release of hemoglobin and free iron. Abbreviations: CD55, a decay accelerating factor; CD59, a membrane inhibitor of reactive lysis; RBC, red blood cells; MAC, membrane attack complex.

Other proteins have a minor role in PNH pathophysiology, the most common of which are monocyte differentiation antigen (CD14), low-affinity immunoglobulin gamma Fc region receptor III-B (CD16b) and CD48 antigen [8][9][10].

2. PNH Laboratory Findings and Clinical Manifestations

The major clinical manifestations and complications of PNH are associated with hemolytic anemia, thrombosis and bone marrow failure [11].

Laboratory results in PNH patients highlight anemia with negative Coombs tests and hemoglobinuria with dipsticks positive for heme but negative sediments for red blood cells, increased reticulocyte counts, elevated levels of lactate dehydrogenase (LDH) and bilirubin, diminished levels of haptoglobin, and iron deficiency. Diagnosis is based on the demonstration of the PNH phenotype in a substantial proportion of red cells and granulocytes [12].

PNH patients show various degrees of anemia and other bone marrow-related disorders such as granulocytopenia and thrombocytopenia. The incidence of anemia ranges from 88% to 94% and that of leukopenia ranges from 41% to 72%, while that of thrombocytopenia ranges from 51% to 80%[13][14].

Anemia derives from both hemolysis and bone marrow dysfunction that is mostly intravascular[15]. Even if hemolysis is continuous during the day, in the night-time, the decline in the blood pH triggers an activation of complement components. This mechanism may be also dependent on an increased nocturnal absorption of lipopolysaccharide (LPS), a major component of the outer membrane of bacteria that stimulates the complement system and is normally bound by monocytes through a GPI-linked protein, CD14, which is lacking in PNH

patients[16][17][18]. The urine is black when the patient awakens, because the amount of free hemoglobin passing through the glomeruli exceeds the absorptive ability of renal tubules[19].

Thrombosis represents one of the major determinants of morbidity and mortality in PNH patients[20]. In particular, thromboembolism is the principal cause of mortality in this population, leading to 40%–67% of deaths for which the cause is known [[21]]. PNH venous thrombosis often occurs at uncommon sites such as hepatic veins, causing Budd–Chiari syndrome, which represents the principal (40.7%) thrombotic event in these subjects. The second most frequent type of thrombosis is represented by cerebral vein and sinus thrombosis. Pulmonary embolism represents a high-risk site of thrombosis and has been reported in 26 cases, 10 of which were fatal [[22]].

Arterial thrombosis has been described in a minority of subjects, mostly of young age, supporting the hypothesis that it may occur de novo without significant predisposing atherosclerotic disease [[23]].

The pathophysiology of the pro-thrombotic state in PNH is still debated. Ploug et al. [[24]] supposed a failure of the fibrinolytic system, such as a deficiency in urokinase type plasminogen activator receptor on leucocytes. A significant role can be attributed to dysregulated complement activity on GPI anchor-deficient platelets, granulocytes, monocytes and endothelial cells [[25]]. Complement-mediated attack on CD55 and CD59 deficient platelets stimulates the production of factors Va, Xa and prothrombin complex [[26]].

The most-adopted PNH classification was proposed in 2005 by the International PNH Interest Group [[27]], which identified three different kinds of clinical manifestation: classic PNH, which includes patients who have evidence of PNH without bone marrow disorder; PNH associated with another bone marrow disorder [[28]]; and subclinical PNH, in which patients have defective PNH clones without clinical or laboratory signs of hemolysis or thrombosis. De Latour et al. [[29]] added a fourth subgroup defined as intermediate PNH, underlining the fact that many PNH patients do not perfectly fit into these categories because they may suffer from cytopenia or signs of a still-undiagnosed underlying bone marrow failure.

3. PNH Therapy

In PNH, hemolysis is mostly mediated by the alternative pathway of complement, so complement inhibition therapy is the best strategy against this disease. The choices among the different kinds of complement inhibitor or modulator will be soon enriched by several molecules currently under investigation or recently approved. The activation of complement factor C5, that is at the basis of PNH, generates the potent anaphylatoxin C5a, leading to pathogen lysis, inflammation and cell damage. The first C5 inhibitor was h5G1.1.mAb, later named eculizumab. Eculizumab improved the management of and clinical outcomes in patients with PNH, becoming a proof of concept for other complement-mediated diseases.

Eculizumab inhibits the formation of the proinflammatory metabolite C5a and construction of the MAC via C5b, through a mechanism of C5-binding aimed at limiting its cleavage by C5 convertases[7]. Before the approval of eculizumab, the therapeutic alternative for PNH was only supporting the patients by blood transfusion, iron

supplementation, anti-thrombosis prophylaxis or therapy and allogeneic bone marrow transplantation. A recent eculizumab-like monoclonal antibody engineered to have a longer half-life is proposed to guarantee the same effects of eculizumab but with a more advantageous and effective dosing schedule: Ravulizumab (ALXN1210) [30]. This drug represents a new promising instrument for the treatment of PNH, permitting longer dosing intervals of 8 weeks[31]. Despite the increased knowledge of this syndrome, the most appropriate strategy and choice of therapies for PNH are still up for debate. The landscape of combination PNH therapy has markedly progressed over the last few decades, and it will continue to expand with the introduction of long-acting complement inhibitors and the development of complement-modulating drugs. The choice of the right drug is a key component in the improvement of personalized medical care for patients with such a complex disease.

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