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History, Etiology, and Treatment of Paroxysmal Nocturnal Hemoglobinuria

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ABSTRACT

Paroxysmal nocturnal hemoglobinuria is an acquired, rare, nonmalignant hematological disease that is characterized by uncontrolled complement activation and thus intravascular hemolysis. It is caused by somatic mutations in the PIG-A gene that leads to a deficiency in necessary GPI-anchored proteins, which leads to uncontrolled complement activation and thus the clinical manifestations that PNH is often associated with. It is generally accepted that Dr. Paul Strübing first described the disease in 1882, albeit it was Thomas Hale Ham who designed the first diagnostic test (Ham test or acidified serum test) to diagnose the rare disease. Since then, Ham's test has become obsolete and PNH is detected via flow cytometry. PNH is divided into 3 categories: classical, presence of another bone marrow failure syndrome, and subclinical. Classic PNH is often associated with bone marrow failure, intravascular hemolysis, and/or thrombophilia. A thromboembolic event is the most common cause of mortality for patients with PNH. Before the introduction of anti-complement drugs, allogeneic hematopoietic stem cell transplantation was a common therapy for patients with PNH, although it carried significant risk and has declined drastically as the preferred treatment option of patients. Presently, there are 3 FDA-approved drugs, two terminal complement inhibitors (eculizumab and ravulizumab) and one proximal complement inhibitor (pegcetacoplan). Eculizumab remains the mainstay of treatment as it has since 2007. There is ongoing research and clinical trials in other proximal complement inhibitors to relieve patients of extravascular hemolysis and other common side effects.

Abbreviations

APC: Alternative Pathway of Complement; C3: Component 3 of Complement Cascade; C5: Component 5 of Complement Cascade; FLEAR: Fluorescent Aerolysin; GPI: Glycosylphosphatidylinositol; GVHD: Graft versus Host Disease; HSCT: Hematopoietic Stem Cell Transplantation; LDH: Lactate Dehydrogenase; MAC: Membrane Attack Complex; PIG-A: Phosphatidylinositol Glycan Class A; PNH: Paroxysmal Nocturnal Hemoglobinuria; RBC: Red Blood Cell; TE: Thromboembolism.

Keywords

Paroxysmal nocturnal hemoglobinuria, Hemolysis, Red blood cell, Clinical trial.

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired, rare, nonmalignant hematological disease that is characterized by uncontrolled complement activation and thus intravascular hemolysis [1]. The disease is a hematopoietic stem cell disorder that results in thrombosis, hemolytic anemia, and bone marrow failure [2].

Dr. Paul Strübing described the earliest case of PNH in 1882. A patient presented to Dr. Strübing with numerous symptoms that included abdominal pain, fatigue, and nocturnal paroxysms of hemoglobinuria [2]. Dr. Strübing was able to conclude that the hemolysis occurred intravascularly and not due to dysfunction in the kidneys because the patient's plasma appeared red after episodes of hemoglobinuria [2]. Attempting to describe the

discoloration in the urine, he used technology available at the time to prove that discoloration was due to the presence of hemoglobin [3]. He was also able to conclude that erythrocytes were not present in the urine during an attack, which distinguishes hemoglobinuria from hematuria, still a common cause in the delay of diagnosis of PNH today. Strübing further concluded that sleep played a critical role in the hemolytic process of PNH by awakening the patient at night, which caused the initial episode of hemoglobinuria, compared to no hemoglobinuria at other times when urination was avoided upon awakening [3]. Due to the slowing of blood circulation during sleep, there is a buildup of carbon dioxide and lactic acid in the blood, which creates an acidic environment. Dr. Strübing hypothesized that PNH red blood cells (RBCs) were destroyed during sleep because they were abnormally sensitive to this acid environment [3].

Research on PNH and what caused the disease lapsed for years as Dr. Strübing took a different job. However, Dutch physician A. A. Hijmans van den Berg showed in 1911 that when RBCs of PNH *in vitro* were suspended in serum and introduced to an atmosphere of carbon dioxide, they would hemolyze [3]. He compared these results to RBCs of normal individuals under the same experimental conditions and noted that the cells from normal individuals did not lyse [3]. Due to Jules Bordet's landmark experiments on *vibrio cholera* in 1894 that identified the complement system, the following characteristics of complement were generally accepted at the time of Hijmans van den Berg's study: if the serum were heat inactivated activity would be lost, and this activity could be restored if a small, fresh amount of serum were subsequently added [4]. Hijmans van den Berg correctly observed that the PNH erythrocyte serum no longer underwent hemolysis when incubated for 30 minutes at 50 degrees Celsius. However, consistent with the Strübing's aforementioned hypothesis about erythrocytes being acid-sensitive, Hijmans van den Berg also concluded that hemolysis was due to fragility of the of PNH RBCs in carbon dioxide and not by cytolytic activity of the complement system [4].

In 1937, Thomas Hale Ham published findings that were similar to Strübing's. He too noticed a correlation between sleep and hemolysis, which led him to propose: "Because of the elevation in the carbon-dioxide content of the arterial blood and the decrease in pH known to occur during sleep, it was suspected that a change in acid-base equilibrium was related to the increased hemoglobinemia of the patients during sleep" [5]. This paper previously cited was a landmark research paper on PNH that influenced further study for the next 50 years. Ham and his coauthor Dingle theorized that PNH abnormal erythrocytes lysed by an immune system mechanism independent of antibodies [5]. Contrary to Strübing, the authors stated that "The serum factor essential for hemolysis was closely associated with, if not indistinguishable from complement," which suggests that Ham and Dingle were closing in on the notion that the complement system did indeed play a role in the lytic substance of serum [3]. Ham's two papers in 1939 led directly to the first specific diagnostic test known as the acidified serum lysis and Ham test for diagnosis of PNH[5]. This test remained the foremost diagnostic test for diagnosis of PNH for over 50 years. Acidified serum lysis test (Ham test) is performed by washing erythrocytes from a patient suspected to have PNH in an acidified serum. If the patient is positive for PNH, their RBCs will hemolyze; normal RBCs will not hemolyze. The sample is centrifuged, and the supernatant fluid is evaluated to calculate the percentage of lysis using spectrophotometric quantitation of the amount of hemoglobin present in the fluid.

Limited progress was made in the field in the next decade until Louis Pillemer identified the alternative pathway of complement (APC) in 1954 [2]. Pillemer called this the properdin pathway, based on his partial purification of the plasma protein properdin and its subsequent capacity to activate a complement system without antibodies [6]. The serendipitous discovery of properdin in 1954 by Pillemer and his colleagues came when they were attempting to isolate one of the components of the complement [7]. In his paper, Pillemer describes properdin acting "only in conjunction with complement and magnesium and participates in such diverse activities as the destruction of bacteria, the neutralization of viruses, and the lysis of certain red cells" [7]. The "certain red cells" that Pillemer notes are from PNH patients [4]. His team isolated properdin by eluting it from zymosan that had been incubated with, which is an insoluble residue made from yeast when digested with trypsin [4]. In a paper published the previous year, Pillemer reported that magnesium and serum components that resembled complement at optimal pH of 7 were the mechanisms by which PNH erythrocytes could lyse and zymosan could inactivate the complement [8]. A few years after Pillemer published his landmark paper; he teamed up with a couple other prominent researchers on the properdin system at the time and published findings that supported their conclusion that his properdin system was indeed involved in the lysis of PNH RBCs in an acidified serum. They showed that serum without properdin had no hemolytic activity of PNH erythrocytes, but when they repleted the serum with properdin it reestablished the ability to lyse PNH erythrocytes [9]. Furthermore, the team found that in antibody-dependent systems, serum depleted of properdin was fully able to lyse PNH erythrocytes, which provided substantial evidence for Pillemer's proposed APC [9].

Table 1: A reconstructed table from Hinz et al. (1956) that shows how properdin is required for lysis of PNH erythrocytes. RP indicates serum without properdin. This table also illustrates a concentration-dependent effect of properdin on hemolytic activity of PNH erythrocytes [9].

Serum	Properdin (units/mL)	Hemolytic of PNH (%)
Normal – Number 1	4-8	33
Normal – Number 2	1-2	14
RP	0	0
RP + 3 units properdin	3	23
RP + 5 units properdin	5	36

Pillemer's discovery was of serious consideration as it proposed the first example of natural immunity, and in doing so it attracted deniers. One of which being Robert Nelson, who in 1958 constructed a strong argument that Pillemer's discovery, was contaminated by antibodies in the lab [6]. In his argument, Nelson postulated that Pillemer's observed complement activation was

actually driven by antibody-antigen reactions [6]. At a conference in 1958 where Nelson presented his findings, Pillemer became depressed and subsequently overdosed on barbiturates shortly after, leaving the properdin system and APC without its principal investigator. In 1963, John Dacie, a prominent British hematologist, published an insightful review on PNH where he reinforced Pillemer's conclusions on the properdin system being responsible for lysis of PNH erythrocytes in an acid-environment [10]. Dacie went on to state that it was possible the abnormal cell surface of PNH RBCs that causes adsorption of the properdin complex, which leads to enzymatically driven hemolysis [10]. Like Ham and Dingle, Dacie had also hypothesized that PNH erythrocytes were abnormally sensitive to lysis when the complement was activated. Working with Wendell Rosse in 1966, they modified an assay technique invented by Manfred Mayer at Johns Hopkins University to assay for serum complement. Their results indicated a significant difference in sensitivity to complement for PNH and normal erythrocytes [4]. In fact, the assay, termed the complement sensitivity lysis assay, separated PNH erythrocytes into two quantitatively distinct populations [4]. These populations were termed the complement-sensitive and complement-insensitive. The complement-sensitive population required only 4% of the amount of serum that normal RBCs required for hemolysis, while complement-insensitive population required ~50% as much serum [11]. This work by Rosse and Dacie provided the foundation that PNH erythrocytes are indeed a mosaic. Their paper provided many of the foundational concepts understood of PNH, in particular that the main underlying cause of the disease manifested in the complement-sensitive population.

Discussion

Moving on to etiology and pathophysiology, PNH has an extremely low incidence. Its incidence is estimated to be 0.1-0.2/100,000 persons per year [12]. As aforementioned, the disease is an acquired disorder of hematopoietic stem cells and thus can affect numerous cell lines: erythrocytes, thrombocytes, leukocytes. The hematopoietic stem cells have a somatic mutation in the x-linked gene phosphatidylinositol glycan class A (PIG-A), whose job is the biosynthesis of a glycosylphosphatidylinositol (GPI) anchor [12]. GPI anchors over a hundred different types of proteins to the cell surface [13]. Resultant under-expression of necessary proteins follows for hematopoietic stem cells and all lines they could possibly differentiate into. Two important complement regulatory proteins are severely under produced on the cell surface: CD55, also called delay-accelerating factor, and CD59, also called membrane inhibitor of reactive lysis [12]. A lack of these proteins thus leaves RBCs more susceptible to complement-mediated intravascular hemolysis [12].

Paroxysmal Nocturnal Hemoglobinuria

As discussed earlier, the APC is part of the innate immunity designed to be continuously active to protect our bodies from foreign invaders. The other part of the complement, termed the classical pathway, is a system of acquired immunity, which means it requires an antibody present to initiate activation. The alternative pathway does not require antibody-mediated activation and its cascade can be divided into two subunits: the C3 and C5 convertases (enzymes) that serve for amplification purpose and the membrane attack complex (MAC) [14]. There are self-recognition protocols in place to defend the host from attack by its own APC.

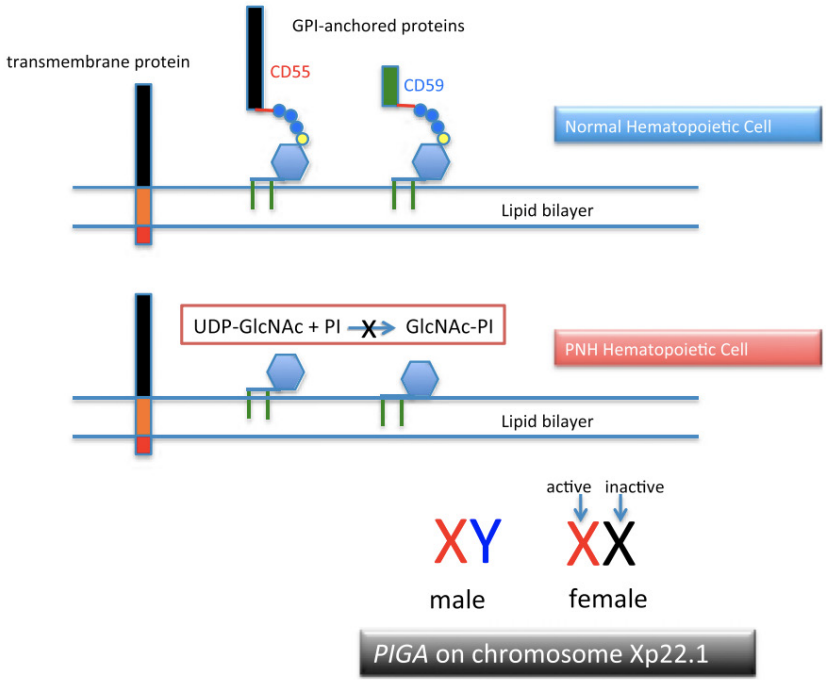

Figure 1: A schematic of the molecular basis of PNH. (Top) A normal hematopoietic stem cell that expresses both transmembrane and GPI-anchored proteins. (Bottom) A PNH hematopoietic stem cell that fails to express GPI-anchored proteins because of a mutation in the PIG-A gene that codes for an enzyme required for transfer of a sugar to phosphatidylinositol. Mutation is located on the X chromosome of individuals [14].

Table 2: A reconstructed schematic of diagnostic criteria for patients with PNH. LDH (lactate hydrogenase) is a biochemical marker of hemolysis [14].

Category	Rate of intravascular hemolysis	Bone marrow	Flow cytometry	Benefit from eculizumab
Classic	Florid (markedly abnormal LDH w/ macroscopic hemoglobinuria)	Cellular marrow from erythroid hyperplasia and normal morphology	Large population (>50%) of GPI-anchor protein-deficient polymorphonuclear cells	Yes
PNH in the setting of another bone marrow failure syndrome	Mild (minimal abnormalities of biochemical markers of hemolysis)	Evidence of concomitant bone marrow failure syndrome	Variable, but relatively small (<50%) percentage of GPI-anchor protein-deficient polymorphonuclear cells	Typically no, but some patients benefit
Subclinical	No clinical or biochemical evidence of intravascular hemolysis	Evidence of concomitant bone marrow failure syndrome	Small (<10%) population of GPI-anchor protein-deficient polymorphonuclear cells	No

The previously aforementioned CD55 and CD59 complexes protect normal human erythrocytes [14]. CD55 controls the formation and stability of the C3 and C5 convertases while CD59 inhibits formation of the MAC [14].

A deficiency of these two protein complexes on PNH erythrocytes leads to the intravascular hemolysis that is a hallmark of the disorder. CD55 is a 68,000-Da glycoprotein whose regulatory capabilities lie in its inhibition of formation of C3 of complement via an accelerated rate of destruction of the membrane-bound C3 convertase [2]. Thus, when CD55 is not present, there is a greater binding affinity of C3 to PNH erythrocytes. CD59 is a 19,000-Da glycoprotein that functions to block complement-mediated lysis by inhibiting the formation of the MAC by blocking aggregation of C9 [2]. As discussed previously, the Ham test (acid test) became the first diagnostic test available for PNH. This test has since become obsolete, as the introduction of flow cytometry has afforded the ability to detect an absence of GPI-anchored proteins in ≥2 cell lineages. It is necessary to confirm an absence serious deficiency in more than just one hematopoietic stem cell lineage as this could be attributed to false negative tests [12]. As discussed earlier, PNH blood cells are a mosaic that can present three different types of cells: PNH type 1 cells, where GPI-anchored proteins are present at normal density, PNH type 2 cells, where cells are slightly deficient in GPI-anchored proteins, and PNH type 3 cells, where no GPI-anchored proteins are present. Flow cytometry can distinguish all these cell types completely [12. RBCs are stained with monoclonal antibodies specific for CD55 and CD59 as well as a reagent known as fluorescent aerolysin (FLEAR) [2]. Aerolysin is a variant of proaerolysin that binds with high affinity to the glycan portion of the GPI anchor [12]. Diagnosis of PNH by flow cytometry has its problems, however. Due to the rare nature of the disease, flow cytometry on a variety of GPI-linked antigens has varied and has been infrequently used in labs until recent years [12]. In conjunction with flow cytometry, complete blood counts and reticulocyte counts should also be assessed. These tests will provide diagnosticians with the effects of the disease on a variety of important markers that include but are not limited to: biochemical markers of hemolysis, production of leukocytes, platelets, and erythrocytes, determination of iron stores, and bone marrow aspirate, biopsy, and cytogenetics [14]. The International PNH interest group has created a schematic for classifying patients with PNH based on a variety of criteria (Table 2).

It is important to note that this classification scheme has resulted in confusion in the prognosis of PNH because all forms of PNH carry a varying degree of bone marrow failure [2]. Classic PNH primarily affects younger people who suffer from chronic intravascular hemolytic anemia, which is caused by a continuous state of complement activation [12]. Due to younger individuals' inclination for strenuous activity and higher alcohol intake, hemolysis could be heightened [12]. Patients in the category of PNH with another bone marrow syndrome typically show evidence of hemolysis but have an underlying marrow abnormality. Identification of said abnormality is attempted via cytogenetics to determine if PNH arose from aplastic anemia (bone marrow stops producing blood cells), myelodysplastic syndrome (group of disorders where blood-forming cells in marrow are damaged), or another type of myelopathy, such as myelofibrosis [15]. Finally, patients in the subclinical category have little or no clinical evidence of hemolysis [15]. There are small populations of GPI-anchored-deficient cells observed via flow cytometric analysis [15]. However, range of bone marrow failure associated with PNH is markedly similar to those in the category of PNH with an underlying bone marrow failure syndrome [14]. Of course, as mentioned earlier, individuals with a mutation in the x-linked PIG-A gene causes a deficiency of GPI-anchored proteins, the hallmark of PNH. Thus, confirmation of mutations in PIG-A would confirm laboratory diagnosis of PNH.

Patients with PNH usually present with a variety of symptoms including but not limited to: fatigue, dysphagia, abdominal pain, shortness of breath, headache, and erectile dysfunction in males [16]. Other possible signs or symptoms, such as neutropenia, renal failure, smooth muscle dystonia, arterial and pulmonary hypertension, and thrombocytopenia, are also common [17].

Thromboembolism (TE) is perhaps the most serious symptom of PNH and is the most significant cause of death [16]. Thromboembolism refers to an obstruction in a blood vessel caused by a clot in that has become dislodged from somewhere else in blood circulation. Anemia and thrombosis are the two most common clinical manifestations of the disease [17]. Thrombosis occurs in about 40% of PNH patients and is the leading cause of morbidity [12]. Thrombosis can occur anywhere, but a venous thrombotic event is far more common than an arterial one (85% compared to 15%, respectively) [12]. Although unknown why, the most common sites include intraabdominal veins (mesenteric,

hepatic, portal, splenic) and cerebral veins (sagittal and cavernous sinus), while hepatic vein thrombosis (also known as Budd-Chiari syndrome) being the most common site [2]. There are many factors leading to thrombosis in PNH patients, high levels of free hemoglobin leading to circulating nitric oxide is important because of nitric oxide's implication in platelet activation and aggregation [2]. Continuous complement activation also contributes: it is possible C5a may contribute to prothrombotic events by activation inflammatory cytokines such as interleukin-6 and interleukin-8, as well as tumor-necrosis factor-α [2]. Finally, a defective ability in patients to break down fibrin in blood clots (fibrinolysis) resulting from deficiency or absence of GPI-linked anchor proteins is another common contributor to the thrombophilia state of PNH patients [2]. Patients that experience a thromboembolic event should take anti-coagulants perpetually. Although a thromboembolic event can occur in any patient with PNH, recent clinical studies support the hypothesis that there is a direct relationship between PNH clone sizes and probability of an event [15]. One study showed that in patients with over 50% of GPI-anchor protein deficient granulocytes, risk of a thromboembolic event was 44% compared to just 5.8% for those with less than 50% of GPI-anchor protein deficient granulocytes [18]. In the study, the researchers used prophylaxis with warfarin for patients with more than 50% GPI-AP–deficient granulocytes. The International PNH Interest Group still debates the use of prophylaxis against thromboembolic events because of a high risk of complications [12].

Chronic anemia is another multi-factorial clinical manifestation of PNH. It is extremely detrimental to organ function, as it causes a deficiency in circulating RBCs to carry oxygen [19]. Anemia typically develops from loss of RBCs in circulation and an imbalance between production and release of RBCs in bone marrow [19]. Anemia is a major cause of morbidity in patients that have PNH with an underlying bone marrow failure syndrome, especially myelodysplastic syndrome [19]. Mortality from anemia in patients with PNH is typically mediated through cardiovascular disease, specifically instances of left ventricular hypertrophy, cardiac enlargement and/or cardiac remodeling [19]. Anemia is usually treated with blood transfusions, and patients with chronic anemia thus develop transfusion dependence, which exacerbates complications like iron overload and refractory anemia [19]. Iron overload can cause damage to the liver, brain, joints, heart, and endocrine system. In one retrospective analysis of patients with refractory anemia, 13 transfusion-dependent patients with chronic or refractory anemia were evaluated for liver function and heart failure [20]. The study found that 10/13 had abnormal liver function and 4/13 experienced heart failure [20].

Women diagnosed with PNH who are pregnant or plan to become pregnant are at serious risk of morbidity. During pregnancy for women with PNH, anemia from intravascular hemolysis and bone marrow failure significantly worsens. There are varieties of risks due to toxicity of treatment to the fetus, so transfusions are the safest treatment option [15]. There are currently a limited number of case studies and reports on women pregnant with PNH. One group of researchers conducted a literature review in 2020 on case studies of women that were pregnant with PNH [21]. The review was comprised of four different women diagnosed with PNH while pregnant. Three of these women had three pregnancies during the review, and one woman had one pregnancy [21]. The group discussed the use of eculizumab, which is one of two currently accepted treatment options for PNH (that will be discussed later) to treat pregnant women with PNH. Only eculizumab was used because the other treatment therapy (ravulizumab) has not yet been investigated for its efficacy and safety in pregnant women with PNH [21]. The report found that in the antepartum period, mild symptoms (such as vaginal bleeding and epistaxis) and life-threatening symptoms (thrombosis) occurred [21]. Transfusions of RBCs and platelets were required in four pregnancies because of hemolysis [21]. The group determined that the safety of eculizumab use during pregnancy was inconclusive and there is need for more prospective studies on the matter with long-term follow up [21].

There are limited treatment options for paroxysmal nocturnal hemoglobinuria. Currently, there are three FDA-approved drugs on the market: eculizumab, ravulizumab, and pegcetacoplan. Both eculizumab and ravulizumab have their differences, but ultimately work in the same therapeutic way: inhibition of the terminal complement subunit C5 [22]. There is preliminary data on some other lesser-known agents that work on the proximal complement subunit to inhibit its continuous activation [23]. Eculizumab is a humanized monoclonal antibody that works on component 5 (C5) of the complement cascade [23]. Inhibition of formation of the MAC is primarily eculizumab's method of treatment, which accounts for deficiency of protein CD59, but not protein CD55, leaving patients still partially susceptible to extravascular hemolysis [2]. Eculizumab is delivered intravenously once a week for the first four to five weeks and then biweekly after that [2]. Headache is the most common side effect presented, which could be attributed to increasing nitric oxide levels [2]. Infection from *Neisseria* is the most life-threatening consequence of terminal complement inhibition, so vaccination against the bacteria should be required before treatment is administered. Eculizumab is very expensive, and administration is indefinite for a sustained response, so patients must make an informed decision based on their insurance and financial security [2]. In the 2006 phase III clinical trial TRIUMPH, researchers established the efficacy of eculizumab as a treatment option for patients with PNH [24]. The study found that the overall rate of transfusion was reduced by 73% for those in the eculizumab group, compared to those in the placebo group [24]. Furthermore, stabilization of hemoglobin levels was nearly half (49%) in the eculizumab group compared to none (0%) in the placebo group [24]. In terms of safety, no one in the trial died, but there were twice as many serious adverse events in the placebo group compared to the eculizumab group (9 vs. 4, respectively) [24]. The groups final conclusion was that eculizumab reduces intravascular hemolysis, reduces or eliminates the need for transfusion, and improves anemia, fatigue, and overall quality of life in patients with PNH [24].

Ravulizumab is a relatively new drug that also is a humanized monoclonal antibody for complement C5 inhibition. In a 2019 phase III clinical trial, ravulizumab was compared to eculizumab for its efficacy in adult patients with PNH naïve to all complement inhibitors [25]. The groups' two primary endpoints were proportion of patients remaining transfusion-free and lactate dehydrogenase normalization, which as discussed earlier is a biochemical marker of hemolysis [25]. Transfusion avoidance was 73.6% in the ravulizumab group compared to 66.1% in the eculizumab group, and LDH normalization was 53.6% compared to 49.4%, respectively [25]. The two patient groups exhibited similar safety and tolerability profiles. In conclusion, the researchers promoted ravulizumab as a safe, tolerable, cost-effective alternative to eculizumab for patients with PNH [25].

Table 3: Reconstructed table of results of primary endpoints and secondary efficacy endpoints from phase III trial on ravulizumab vs. eculizumab [25].

	Ravulizumab (N=125)		**Eculizumab (N=121)**	
Transfusion avoidance rate (%)	73.6		66.1	
LDH normalization (%)	53.6		49.4	
Clinical Manifestations of PNH	Baseline	Day 183	Baseline	Day 183
Fatigue	80	36	76	36
Abdominal pain	17	6	15	6
Dyspnea	42	18	38	17
Dysphagia	13	3	16	1
Chest pain	5	3	17	7
Hemoglobinuria	71	13	56	11
Erectile dysfunction	16	10	21	5

Dr. Robert Brodsky, a professor of medicine at the esteemed Johns Hopkins School of Medicine, published a report in 2021 on the use of eculizumab and ravulizumab to treat patients with PNH. His findings included the use of these two drugs to treat four patients with PNH, and he ultimately recommended ravulizumab for patients requiring perpetual therapy due to its lower treatment costs, more convenient administration, and more reliable C5 blockade [22]. Dr. Brodsky put specific emphasis on the use of penicillin prophylaxis to protect against *Neisseria* infection, a risk discussed previously. Ravulizumab only requires intravenous dosing every eight weeks due to its longer half-life [22].

As discussed earlier, complement inhibition can occur terminally or proximally. So far, only terminal inhibition has been discussed. One such drug that acts via proximal inhibition is pegcetacoplan. Pegcetacoplan is administered twice weekly as a subcutaneous infusion [26]. It is a PEGylated peptide that binds and inhibits C3 and its cleavage fragment C3b, which helps limit C3-mediated extravascular hemolysis [26]. In the phase 3 PEGASUS trial conducted recently, researchers compared pegcetacoplan to eculizumab in a randomized clinical trial. The study found significant differences in transfusion independence, 85% for the group that received pegcetacoplan vs. 15% for the group that received eculizumab. Furthermore, an average increase in hemoglobin levels from baseline (+2.3 g/dL) was observed in the pegcetacoplan group compared to an average decrease (-1.5 g/dL) in the eculizumab group [26]. However, a higher rate of common adverse events and breakthrough hemolysis was observed in the pegcetacoplan group, which could be attributed to a higher PNH red cell clone for the pegcetacoplan group [26].

Although uncommon today because of severe risk and the introduction of complement inhibitors eculizumab and ravulizumab, allogeneic-hematopoietic stem cell transplantation (HSCT) still plays a role in treatment of PNH for patients with aplastic anemia as well. Before eculizumab was approved for use in 2007, allogeneic HSCT was considered the sole potentially curative therapy for patients with an identical human leukocyte antigen sibling and severe PNH [27]. Due to the introduction of eculizumab, allogeneic HSCT has decreased dramatically for patients with PNH [27]. One group reviewed literature published in recent years on the use of allogeneic HSCT for PNH patients with aplastic anemia or severe hemolysis or thrombosis. They reviewed 8 major articles on the use of hematopoietic stem cells from another donor to treat PNH and found several serious side effects were present in all trials [27]. In particular, graft versus host disease (GVHD) remained a significant adverse event associated with allogeneic HSCT [27]. However, overall survival was observed to be at least 68% in 7 of the 8 trials reviewed [27]. The group-determined allogeneic-HSCT should be considered with great caution for patients with severe PNH with thrombosis or another bone marrow failure [27]. A 2021 retrospective study by a Turkish group evaluated the efficacy of allogeneic HSCT for PNH patients with and without aplastic anemia [28]. In the study, 35 patients (19 PNH with aplastic anemia, 16 classical PNH) were included and retrospectively evaluated for the efficiency and safety of allogeneic-HSCT. These transplantations occurred over a 15-year period in ten different transplantations across Turkey [28]. A conditioning regimen of reduced intensity chemotherapy was given to 28 of the 35 patients. Two-year overall survival was observed in 81.3% and 79.9% of classical PNH and aplastic anemic PNH patients, respectively, which was not statistically significant [28]. In the study, 88.5% of patients had undetectable PNH clones and the ones that did have detectable PNH clones were transfusion-independent [28]. In conclusion, the researchers noted that allogeneic-HSCT is a good option for some patients with classical PNH and aplastic anemic PNH patients. They note that the mortality and morbidity rates associated with allogeneic-HSCT remain an issue.

Conclusion

PNH is an acquired, rare, nonmalignant hematological disease that is characterized by uncontrolled complement activation and thus intravascular hemolysis. It is caused by somatic mutations in the PIG-A gene that leads to a deficiency in necessary GPI-anchored proteins which leads to uncontrolled complement activation and thus the clinical manifestations that PNH is often associated with. It is generally accepted that Dr. Paul Strübing first described the disease in 1882, albeit it was Thomas Hale Ham who designed the first diagnostic test (Ham test or acidified serum test) to diagnose the rare disease. Since then, Ham's test has become obsolete and PNH

is detected via flow cytometry. PNH is divided into 3 categories: classical, presence of another bone marrow failure syndrome, and subclinical. Classic PNH is often associated with bone marrow failure, intravascular hemolysis, and/or thrombophilia. A thromboembolic event is the most common cause of mortality for patients with PNH. Presently, there are 3 FDA-approved drugs, two terminal complement inhibitors (eculizumab and ravulizumab) and one proximal complement inhibitor (pegcetacoplan). Eculizumab remains the mainstay of treatment as it has since 2007. There is ongoing research and clinical trials in other proximal complement inhibitors to relieve patients of extravascular hemolysis and other side effects of the drugs.

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