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Paroxysmal Nocturnal Haemoglobinuria: A Literature Review

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ABSTRACT

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare X-linked clonal disorder of hematopoietic stem cells characterized by the triad of complement-mediated hemolytic anemia, pancytopenia, and thrombophilia. Additional clinical manifestations include renal insufficiency, smooth muscle dystonia, and bone marrow failure in the advanced stages. PNH is caused by an acquired mutation in the PIG-A gene, which is necessary for the synthesis of the glycophosphatidylinositol (GPI) anchor. This pattern of uncontrolled complement activation is responsible for the clinical manifestations of PNH. Flow cytometry is the gold standard diagnostic tool and can be used to ascertain the size and type of the PNH clone, as well as to categorize PNH into one of three types. Terminal complement inhibitors such as eculizumab are the treatment strategy of choice and have been proven to resolve episodes of intravascular hemolysis, reduce the risk of thromboembolic complications, stabilize renal function, lower mortality rates, and significantly improve patient quality of life. The only curative option for PNH is allogeneic bone marrow transplantation; however, this is reserved for refractory cases due to the risk of severe complications.

Keywords: Paroxysmal nocturnal hemoglobinuria (PNH), Allogeneic bone marrow transplantation, Clinical signs, Complications, Treatment/Management.

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1. INTRODUCTION

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare X-linked clonal disorder of hematopoietic stem cells that presents with complement-mediated hemolytic anemia, pancytopenia, and a propensity for thrombotic events. Other clinical manifestations include renal insufficiency, smooth muscle dystonia, and bone marrow failure in the advanced stages. The critical role that complement cascade dysfunction plays in the unique pathophysiology of PNH has long been an area of captivation for hematologists worldwide.

The term paroxysmal nocturnal hemoglobinuria was coined by Enneking in 1925. However, descriptions of the disorder have been documented as early as the 1800s, the most famous of which was that of Dr. Paul Strübing (1882), who reported that an adult male presented with fatigue, abdominal pain, and severe nocturnal episodes of hemoglobinuria. Observation of the patient's plasma turning red following bouts of hemoglobinuria led to the deduction that intravascular hemolysis was responsible. In 1973, the acidified serum (Ham) test became the first diagnostic test for PNH after Thomas Ham discovered that PNH erythrocytes were lysed when incubated with normal, acidified serum. While speculated for years, it was not until 1954 that complemented mediated lysis was formally recognized as the cause of hemolytic episodes in PNH. The specific cell surface protein deficiency affecting PNH cells was identified in subsequent years, which facilitated the identification of the responsible genetic mutation.

PNH has a high mortality rate and a monumental impact on a patient's quality of life. Prior management was mainly supportive in nature. However, recent advances in treatment, such as the introduction of complement inhibition therapy, have drastically improved survival and have been shown to ameliorate instances of hemolysis and thrombosis.

This review explores the pathogenesis of PNH, clinical manifestations and complications of the disease, diagnostic investigations, and management of patients with this disorder. [1]

2. ETIOLOGY

The development of PNH can be attributed to an X-linked genetic mutation in hematopoietic stem cells. Mutation of the phosphatidylinositol glycan class A (PIG-A) gene impairs biosynthesis of the glycosylphosphatidylinositol (GPI) anchor, which is responsible for affixing other proteins to the plasma membranes of blood cells. As a consequence, there is absent or reduced surface expression of various GPI-anchored proteins, the most significant being CD55 and CD59.

These protein moieties regulate the complement cascade. Hence, their deficiency in PNH erythrocytes renders these cells susceptible to complement-mediated attacks, which leads to intravascular hemolysis and other symptoms associated with PNH. Precipitating factors that activate complement and induce hemolytic episodes include vigorous exercise, surgery, pregnancy, infections, and alcoholism. [1][2]

3. EPIDEMIOLOGY

PNH is a rare disease with an estimated incidence of 1–1.5/1,000,000 individuals worldwide. However, the incidence may be greater in certain geographical areas. A potential explanation

for these low numbers may be due to underdiagnoses in patient populations that exhibit limited symptomatology. The presence of comorbidities that conceal PNH diagnosis may also account for some misdiagnosed cases.

Epidemiological data from The International PNH Registry (est. 2003), which aims to gather comprehensive statistical data from PNH patients, confirms that as of June 30, 2012, a total of 1610 patients originating from 273 centers in 25 countries were enrolled in this study. [3 The analysis revealed that European and North American patients had the highest rates (92.5%), with 85% being of Caucasian descent. Of the remaining participants, 5.0% were Asian/Pacific Islanders, 3.5% were of African descent, and a minority of 0.2% were found to be Native/Aboriginal. 3.9 Of the participants, 3.9% were identified as having other/unknown ethnicity. To date, no conclusive biological data explaining this distribution exist.

There are variations in the complications associated with PNH according to ethnic group. For example, the rates of thromboembolism secondary to PNH are higher among Caucasian populations than among Japanese populations. Conversely, bone marrow failure is predominant among residents of Asia, the Pacific Islands, and Latin America. [4]

The International PNH Registry reports patient ages ranging from to 3-99 years, with a median age at enrollment of 42 years and a median duration of disease lasting 4.6 years. According to the study, most patients affected by PNH fell within the age range of 30-40 years. [5]

Despite PNH being caused by an X-linked chromosomal mutation, there was a slight female predominance.

The incidence of paroxysmal nocturnal hemoglobinuria is rare in children. However, the frequency of bone marrow failure in members of the pediatric population is greater than that in adults with this condition, leading to significant morbidity and mortality. Hence, bone marrow transplantation should be considered as a therapeutic option for all pediatric PNH patients with bone marrow failure. [6][7][8][9]

4. IMMUNOPATHOGENESIS

Individuals with paroxysmal nocturnal hemoglobinuria possess clonal blood cells with defective surface expression of various GPI-anchored proteins. The pathogenesis of PNH involves a mutation in the X-linked PIG-A gene, which is necessary for the synthesis of glycophosphatidylinositol (GPI) anchor within the endoplasmic reticulum. Biosynthesis of GPI occurs via the sequential addition of monosaccharide molecules and phospholipids within the endoplasmic reticulum, after which the completed protein is transferred to the cell surface and primarily localized to the lipid rafts. These are microdomains abundant in glycosphingolipids and cholesterol. Somatic mutations of the PIG-A gene interrupt the first step of GPI biosynthesis, and incomplete bioassembly leads to the absence of the GPI anchor. Consequently, the surface expression of various GPI-anchored proteins was absent or reduced. The *PIG-A gene* is located on Xp22.2. The monoallelic gene expression of PIG-A on the X chromosome indicates that a single somatic mutation is sufficient to disrupt GPI assembly. This also explains why all cases of acquired PNH present with this specific mutation, as only one allele is functional in both males and females. The other genes involved

in the GPI biosynthesis pathway are autosomal, so both alleles would need to be mutated in the same cell to affect GPI production to the same extent as a PIG-A mutation. A fundamental component of the innate immune system is the complement cascade, in which a series of reactions have a cytolytic effect either through opsonization and cell phagocytosis, or through the initiation of a membrane attack complex (MAC) that generates pores in the cell membrane. The proteins CD55 (decay accelerating factor, DAF) and CD59 (membrane inhibitor of reactive lysis, MIRL) are universally expressed on hematopoietic cells and have complementary inhibitory properties. The primary function of CD55 involves the inactivation of C3 convertases via dissociation into their constituent proteins, as well as preventing their assembly. This reduces the frequency of C3 cleavage, which prevents the formation of the membrane attack complex. CD59 plays an essential role in protecting cells against self-destruction by complement-mediated lysis. When complement activation leads to the deposition of C5b, C6, C7, and C8 on host cells, CD59 can inhibit the polymerization of C9 and formation of MAC. Absent or reduced expression of CD55 and CD59 on PNH erythrocytes renders them susceptible to complement-mediated attacks, which leads to intravascular hemolysis. The majority of the symptoms associated with PNH can be attributed to this destruction. Red blood cells in PNH can be classified into three groups depending on their degree of sensitivity to complement. Type I erythrocytes have normal expression of GPI-anchored proteins, Type II cells have a partial deficiency, and Type III cells have a complete deficiency. The extent to which individuals suffer from hemolytic complications of PNH is proportional to the quantity of type II and type III erythrocytes present. As a rule of thumb, individuals with a higher percentage of type III cells tend to experience more severe hemolytic episodes. GPI-deficient clonal cells can arise from many other cell lines, including platelets, monocytes, B and T lymphocytes, and natural killer cells. However, the lack of a nucleus renders erythrocytes more susceptible to lysis. [10]

Extravascular haemolysis in PNH:

Patients with PNH may have varying degrees of extravascular hemolysis. Accumulation of C3 on the surface of erythrocytes allows recognition and phagocytosis by resident macrophages in the spleen and liver. Flow cytometry demonstrated the presence of C3 fragments in the PNH erythrocytes. However, these findings were absent in normal RBCs from the same patient. [11]

Bone marrow failure in PNH:

Bone marrow failure due to PNH is thought to be the result of autoimmune destruction of hematopoietic stem cells. The reduction in hematopoietic progenitors and impaired hematopoiesis leads to other cytopenias that accompany PNH-associated hemolytic anemia. Marrow failure can vary from subclinical to severe aplastic anemia (AA). This phenomenon has not been proven to be a direct consequence of PIG-A mutations in PNH. Paradoxically, it appears that the underlying pathophysiological process of bone marrow failure confers a relative survival advantage to mutant PIG-A stem cells. It has been suggested that loss of the GPI anchor in PNH cells also results in a deficiency of stress-inducible GPI-linked membrane proteins (ULBP1 and ULBP2) in PNH cells. These proteins are responsible for the activation

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of natural killer and T cells, so it can be deduced that their absence can contribute to the survival and immunoselection of PNH erythrocytes over normal cells in the setting of immune-mediated bone marrow destruction. These surviving cells then undergo clonal expansion, which surpasses normal hematopoiesis. [12]

5. HISTOPATHOLOGY

Renal:

Renal biopsy in PNH patients with acute kidney injury revealed acute tubular necrosis due to hemosiderin deposition secondary to hemoglobinuria. Deposits occur primarily in the proximal convoluted tubules and, to a lesser extent, the loop of Henle and the interstitium. The nephrotoxic effect of the incorporated iron and free hemoglobin induces highly reactive hydroxyl radicals, which results in tubular atrophy and interstitial fibrosis. Biopsy findings in AKI also revealed a diminished brush border, cytoplasmic vacuolation, and cellular engorgement due to extensive tubular necrosis. Regenerative changes include flattening of the lining, basophilic cytoplasm, an increased nuclear-to-cytoplasmic ratio, and cellular mitoses. [13]

Chronic renal failure in PNH presents with renal infarcts, cortical thinning, papillary necrosis, interstitial tissue hyalinization, and scarring. These are thought to result from recurring episodes of microvascular thrombosis, which lead to microinfarctions and diffuse vascular damage. Kidneys in PNH are also notably larger owing to venous congestion and sludging. [14]

Bone Marrow:

In instances of hemolytic PNH, normocellular-to-hypercellular bone marrow is often seen alongside erythroid hyperplasia. As a consequence of rapid erythrocyte turnover, erythroid dysplasia is common and results in bone marrow, assuming the appearance of that observed in myelodysplastic syndromes. Generally, myeloid and megakaryocyte lineages are morphologically normal. The possibility of karyotypic abnormalities exists in up to 25% of patients with PNH. Conversely, in PNH secondary to aplastic anemia, hypocellular bone marrow is a distinguishing feature. There is relative erythroid hyperplasia, meaning there are mild elevations in erythrocyte precursor counts, but this increase is amplified by the absence of other hematopoietic elements. Subtle erythroid dysplasia with drastically reduced megakaryocyte counts is typical, along with a significantly reduced hematopoietic stem cell ($CD34^+$) count. Karyotypic abnormalities are rare. [2] [15] [16]

6. SIGNS AND SYMPTOMS

Hematologic manifestations:

Intravascular hemolysis in PNH results in moderate to severe anemia, and patients present with constitutional signs and symptoms of excessive fatigue, exertional dyspnea, dizziness, weakness, conjunctival pallor, and tachycardia. Intermittent episodes of jaundice often present as a consequence of unconjugated hyperbilirubinemia due to RBC hemolysis. In patients with aplastic anemia, bone marrow failure is the primary cause of anemia, and

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pancytopenia may be apparent. In addition to anemia, these patients present with purpura, petechial, mucosal bleeding, and recurrent infections, which are characteristic of thrombocytopenia and leukopenia, respectively. [1]

Thrombosis:

Venous thrombosis in atypical locations remains a classic finding in PNH and accounts for the highest mortality rate among patients. Thrombosis can be arterial in origin, but this is infrequent. Budd-Chiari syndrome is the most frequent site of thrombosis, followed by abdominal (mesenteric, splenic, portal) and cerebral (sagittal, cavernous sinus) veins. Deep vein thrombosis, pulmonary emboli, and dermal thrombosis are not infrequent, and renal vein thrombosis has been reported. [17] [18]

Although the exact pathophysiological mechanism underlying thrombotic events in PNH remains uncertain, several theories have been explored. One of these is that complement-mediated intravascular hemolysis results in platelet and thrombin activation with subsequent endothelial dysfunction. Additionally, platelets that lack GPI-linked proteins are predisposed to complement-mediated lysis, leading to their activation, along with the formation of MAC containing prothrombotic microparticles. It has also been suggested that free hemoglobin has a toxic effect, resulting in chronic vascular endothelial activation. This is supported by the development of thrombophlebitis in healthy volunteers following the administration of free haem. Furthermore, nitric oxide depletion from binding free hemoglobin also exacerbates platelet aggregation and activation. Moreover, the heightened thrombotic risk in PNH is multifactorial, as opposed to being attributed to a single underlying mechanism. [18] [19] [20]

Renal manifestations:

Hemoglobinuria episodes have been reported in almost 50% of patients with PNH. The quantity of free hemoglobin passing through the glomeruli exceeds the renal threshold for reabsorption, causing urine that ranges in color from pink to red to dark brown. This usually occurs in the morning due to nocturnal urine concentration and the mild respiratory acidotic state that accompanies shallow breathing during sleep. Precipitating factors that activate complement and induce hemolytic episodes include vigorous exercise, surgery, pregnancy, infections, and alcoholism. A common finding in patients with PNH is renal impairment, which can result from renal cortical hemosiderosis, microvascular thrombosis, and acute injury due to the toxic effect of free heme released during hemolytic episodes. [20]

Smooth muscle dystonias:

Abdominal pain, esophageal spasm, dysphagia, and erectile dysfunction are common complaints in patients with PNH and are a direct manifestation of the free hemoglobin released during hemolytic episodes. Free hemoglobin is normally eliminated from circulation by haptoglobin, along with other clearing mechanisms. However, in the setting of PNH, chronic intravascular hemolysis overpowers the usual operations, resulting in elevated free plasma hemoglobin levels. As a compensatory response, hemoglobin irreversibly binds nitric oxide to produce methemoglobin and nitrate, leading to a depletion in the NO supply. Nitric

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oxide maintains vasodilation and smooth muscle relaxation, resulting in abnormal smooth muscle tonicity and spasms in the gastrointestinal tract. Vascular constriction of the corpora cavernosa results in impotence in male patients. [21]

7. INVESTIGATIONS

Basic Laboratory Investigations:

When a patient presents with signs and symptoms of PNH, such as anemia and hemoglobinuria, laboratory tests, including a complete blood count with differential, reticulocyte count, peripheral blood smear, basic metabolic panel (BMP), and urinalysis. A differential CBC would reflect evidence of anemia through a reduction in hemoglobin (Hb) concentration, hematocrit (Hct), or RBC count. The reticulocyte count was elevated as a compensatory response. Evidence of leukopenia and thrombocytopenia may also be apparent. Increases in serum creatinine and blood urea nitrogen (BUN), as well as electrolyte abnormalities, can indicate renal dysfunction, as seen in acute kidney injury and chronic kidney disease secondary to PNH. Substantially elevated LDH, low haptoglobin, and unconjugated hyperbilirubinemia are markers of hemolysis that are expected from repeated episodes of intravascular hemolysis. Urinalysis can demonstrate evidence of hemoglobinuria and hemosiderosis. Exploration of these basic laboratory parameters can then determine the need for flow cytometry. [5]

Flow cytometry:

The gold standard diagnostic test for PNH is flow cytometry, which is capable of assessing numerous GPI-anchored proteins with high sensitivity and specificity; CD55 and CD59 are the most notable. This test detects GPI-anchored proteins after the cells are labeled with monoclonal antibodies (e.g. anti-CD55, and anti-CD59) or the FLAER reagent (fluorescein-labeled proaerolysin), which binds directly to the glycan portion of the GPI anchor. Therefore, flow cytometry identified all GPI-negative erythrocytes, monocytes, and granulocytes. A clinical diagnosis of paroxysmal nocturnal hemoglobinuria can be made when a minimum of two or more lineages of blood cells exhibit an absence or severe deficiency of GPI-anchored proteins. PNH erythrocytes are further classified into types 1, 2, or 3. Type 1 signifies cells demonstrating normal expression of GPI anchor proteins, type 2 indicates a partial deficiency, and type 3 indicates a complete absence. False negatives are possible with this testing method, owing to rare congenital deficiencies of single antigens (e.g. CD55, and CD59) or polymorphisms of individual antigens (e.g., CD16) that render them undetectable by monoclonal antibodies. To combat this, two or more GPI-linked proteins must be studied. [1]

PNH can be classified into three types: classic PNH (hemolytic), PNH with a concomitant bone marrow disorder (e.g., aplastic anemia, primary myelofibrosis, MDS), and subclinical, which is asymptomatic. Both low-sensitivity and high-sensitivity flow cytometry tests exist, and while the former is sufficient for a PNH diagnosis, the latter is more appropriate in instances of PNH in the setting of a bone marrow disorder. Subclinical PNH presents with no clinical or laboratory markers of hemolysis. It is therefore imperative that asymptomatic patients are

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reassessed at 6- to 12 month intervals to determine the progression of the disease and monitor the size of the PNH clone. [22]

Other diagnostic tests:

Patients with a granulocyte clone of greater than 20% on flow cytometry should be assessed with supplementary tests for hemolysis, silent thrombosis, and end organ damage. These include D-dimer, liver function tests, iron, and brain natriuretic peptide (BNP). Bone marrow aspiration and biopsy are not essential for PNH diagnosis. However, in instances of severe pancytopenia, it will aid in the classification of PNH when an associated bone marrow abnormality is suspected. Imaging studies are also crucial in these investigations. For example, pulmonary hypertension can be assessed using echocardiography. Hepatic blood flow and the presence of thrombosis can be evaluated using Doppler ultrasonography. Abdominal CT can identify cases of hepatic vein thrombosis (Budd-Chiari syndrome), and MRI of the head is useful in detecting cerebral vein thrombosis. [5]

8. COMPLICATIONS

Thrombotic events:

Thrombosis remains the most significant cause of morbidity and mortality, with Budd Chiari syndrome (hepatic vein thrombosis) being the leading cause. Thrombotic emboli are also commonly observed in the intra-abdominal and cerebral vessels.

Renal dysfunction:

Patients with PNH have a substantially increased risk of developing chronic kidney disease, owing to the effects of chronic intravascular hemolysis and tubulointerstitial inflammation. [23]

Acute kidney injury is possible due to the noxious effect of free hemoglobin on the renal parenchyma and tubules.

Pulmonary Hypertension and reduced right ventricular function:

Although infrequent, instances of pulmonary hypertension and dyspnea have been described. These events may be related to diminish nitric oxide levels and accompanying vasoconstriction of the pulmonary vessels. Some echocardiographic studies have demonstrated elevated pulmonary artery pressure in patients with PNH. Reduced right ventricular function is an under-recognized complication, and it has been reported that patients with PNH have high levels of N-terminal pro-brain natriuretic peptide (NT-proBNP), which is associated with cardiac ventricular dysfunction. [24]

Increased risk of acute leukaemias:

Paroxysmal nocturnal hemoglobinuria is associated with leukemic progression in some patients. Studies have demonstrated that up to 15% of PNH patients develop acute myeloid leukemia as a secondary hematopoietic disorder, likely due to dyscrastic cells that evolve from PNH clones. [25][26]

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9. DIFFERENTIAL DIAGNOSIS

When considering differential diagnoses, other conditions that cause hemolytic anemia, atypical venous thrombosis, thrombocytopenia, pancytopenia, and bone marrow failure should be evaluated.

Haemolytic anemias:

Other causes of hemolytic anemia include autoimmune hemolytic anemia, paroxysmal cold hemoglobinuria, and microangiopathic hemolytic anemia (MAHA). AIHA is an immune-mediated hemolysis characterized by a positive direct antiglobulin test (DAT). However, this test was negative for the PNH. Paroxysmal cold hemoglobinuria (PCH) is associated with a biphasic antibody against the P antigen in erythrocytes, leading to rare complement-mediated intravascular hemolysis. Consequent haemoglobinuria is a feature that overlaps with that of PNH. Individuals with this condition will have a positive Donath-Landsteiner test, which is diagnostic for PCH. MAHAs such as thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) can also mimic PNH with clinical manifestations of excessive fatigue, pallor, mucosal bleeding, easy bruising, jaundice, and evidence of impaired renal function (elevated BUN, creatinine, and hematuria). Laboratory parameters also revealed hemolytic markers, such as elevated LDH, schistocytes on peripheral smear, and decreased haptoglobin levels. An accurate diagnosis can be made based on careful history taking and additional laboratory studies. The etiology of HUS is due to an offending microorganism, most commonly *enterohemorrhagic Escherichia coli*) and is associated with gastrointestinal manifestations, including nausea, vomiting, and bloody diarrhea, which are not characteristic of PNH. Similarly, an elevated WBC count is typical of HUS, but not habitual in PNH. ADAMTS13 activity assay should be ordered if TTP is suspected, and would demonstrate decreased activity in this enzyme if positive. If DIC is suspected, labs would reveal prolonged PT and APTT and markers of fibrin breakdown, including elevated D-dimer levels and decreased fibrinogen levels. These parameters were not expected in the PNH. [27][28][29][30][31][32].

Pancytopenia:

Additional pathologies to consider are those that result in pancytopenia, etiologies of which can be broadly classified into central type, which involves diminished production of cell lines, or a peripheral type involving increased consumption/destruction. Central pancytopenia may result from aplastic anemia, ineffective hematopoiesis due to nutritional deficiencies, bone marrow suppression from exposure to drugs or toxins, bone marrow infiltration in hematological malignancies, and infections such as tuberculosis and HIV. In aplastic anemia, the reticulocyte count will be low, reflecting bone marrow insufficiency and poor compensation. This is in contrast with PNH, where the reticulocyte count is elevated as a compensatory response to red cell destruction. [33][5]A bone marrow biopsy in instances of aplastic anemia would display a hypocellular, fat-filled marrow, which is not usually characteristic of classic PNH. [34]

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However, it must be noted that PNH can also occur in patients with concomitant aplastic anemia, in which case a dry bone marrow tap can be expected. Other primary bone marrow disorders such as primary myelofibrosis and myelodysplastic syndrome should also be considered. Nutritional deficiencies related to alcoholism, eating disorders, malnutrition, or malabsorption can impair hematopoiesis, and clinicians must be meticulous when eliciting history and examination, which can be used to confirm or rule out PNH as the etiology of pancytopenia. For instance, RBC macrocytosis and hypersegmented neutrophils on a peripheral smear and a positive Romberg test suggest subacute combined degeneration of the spinal cord secondary to vitamin B12 deficiency. Physical examination may reveal peripheral stigmata of alcoholic liver disease such as spider naevi or caput medusae, and labs may reveal elevated AST and ALT levels, none of which are characteristic of PNH. [35]

A proper history would enable clinicians to determine whether the etiology of pancytopenia is due to drugs (e.g., anticonvulsants) or toxins (e.g., benzene, radiation) evoked bone marrow suppression, in which case symptoms should resolve when the offending drug is stopped. Bone marrow infiltration by malignancies such as lymphoma, multiple myeloma, and hairy cell leukemia can also present with pancytopenia. However, in addition to the clinical features of pancytopenia, hematological malignancies may also present with lymphadenopathy, non-specific symptoms of fever, weight loss, night sweats, and peripheral blood film features unique to the malignancy, such as the presence of rouleaux formation in multiple myeloma or lymphoblasts, as in acute lymphoblastic leukemia. None of these features are regarded as distinguishing features of paroxysmal nocturnal hemoglobinuria. Peripheral pancytopenia may result from hypersplenism, autoimmune diseases such as SLE, infections with viruses, bacteria, fungi, or certain drugs. Hypersplenism presents with signs of anemia, hemolysis, and hematological features of pancytopenia, similar to PNH. To determine whether these findings reflect PNH or hypersplenism, the underlying etiology must be determined. However, it is worth noting that PNH itself can lead to hypersplenism as a consequence of mesenteric vein thrombosis and subsequent splenomegaly. [36] Autoimmune diseases can lead to peripheral cytopenia, but usually present with other systemic manifestations of the specific disease that would aid in establishing the diagnosis. Additionally, an autoimmune panel would reveal parameters such as elevated CRP, ESR, and positive autoantibodies, such as ANA, which further exclude the diagnosis of PNH. Laboratory diagnostic methods, such as blood cultures, serological testing, and nucleic acid detection, can be used to identify the causative microorganism in cases of suspected infection. [37]

Atypical venous thrombosis:

The presence of venous thrombosis in atypical locations (e.g., mesenteric vein and portal vein) can also be attributed to thrombophilia, the most common of which is inherited Factor V Leiden. [38] Other worthy of investigation include protein C and S deficiency, antithrombin III deficiency, prothrombin mutation, and antiphospholipid syndrome. However, with these disorders, the incidence of thrombosis in unusual locations is rare, in contrast to PNH, where atypical blood clots are a common cause of mortality. Laboratory studies, including

hypercoagulability panels, coagulation studies, and antiphospholipid antibody panels, can be used to determine the etiology of thrombosis in patients. [39][40]

10. TREATMENT/MANAGEMENT

Prior management of PNH involved supportive measures, including regular blood transfusions and iron supplements for hemolysis and associated anemia, together with anticoagulant prophylaxis against thrombosis. This has since been replaced in favor of complement inhibition therapy and allogeneic hematopoietic stem cell transplantation.

Complement Inhibition Therapy (Eculizumab, Ravulizumab):

Complement inhibition therapy is the current mainstay of treatment for paroxysmal nocturnal hemoglobinuria. Loss of the CD55 and CD59 anchoring proteins and chronic dysregulation of the alternative complement pathway are the fundamental grounds behind complement-mediated hemolysis in PNH. Thus, the C5 inhibitors Eculizumab and Ravulizumab, which block the alternative complement pathways, have yielded considerable improvements in the quality of life of patients with PNH. These drugs are monoclonal antibodies that bind with high affinity to the complement protein C5, inhibiting its cleavage to the proinflammatory anaphylatoxin C5a, and the initiating subunit of the terminal complement complex, C5b. The generation of the membrane attack complex (MAC) is prevented, and with terminal complement activity suppressed, complement-mediated intravascular hemolysis was eliminated. [41]

Treatment with eculizumab and rivalizumab resolves episodes of intravascular hemolysis, reduces the rate of thrombosis, stabilizes renal function and pulmonary artery pressures, and lowers the overall morbidity and mortality of the disease. It must be noted that terminal complement blockade is associated with an increased risk of meningococcal infections; hence, all patients receiving C5 inhibitor therapy must be vaccinated against *Neisseria meningitides.* [42]

Bone Marrow Transplant:

Allogeneic hematopoietic stem cell transplantation is the only curative therapeutic option for PNH. Despite this, there is a considerable risk of graft-versus-host disease. Hence, this procedure is second-line to complement inhibition therapy and reserved for refractory cases where the benefits outweigh the risk, such as instances of severe bone marrow failure in PNH or in those with a suboptimal response to eculizumab. It may also be considered in eligible patients in countries where terminal complement inhibitors, such as eculizumab, are unavailable. [1]

Iron and folate supplementation:

Folic acid is imperative in PNH to sustain increased demand and red cell turnover due to recurrent hemolytic episodes. Iron supplementation is also required to treat anemia associated with this condition. [2]

Thrombotic events

Acute thrombotic events must be promptly treated with anticoagulation therapy, first using heparin or low-molecular-weight heparin. Within 24 hours of any new thrombotic event, complement inhibition therapy must be initiated to prevent the propagation of thrombosis and long-term sequelae. [43]

In patients with Budd-Chiari syndrome, anticoagulants are unreliable in the restoration of hepatic blood flow, and thrombolytic therapy poses a risk of hemorrhage. However, complement inhibitors, such as eculizumab, have proven effective in reducing mortality. PNH patients with Budd-Chiari syndrome are at increased risk of hepatocellular carcinoma; therefore, routine liver ultrasound and monitoring of serum α-fetoprotein are recommended. [44]

Acute Kidney Injury

Acute renal damage in PNH is best managed with continuous renal replacement therapy (CRRT), which provides greater hemodynamic stability while avoiding rapid fluid and electrolyte shifts in intermittent hemodialysis. Doppler ultrasonography was used to rule out renal vein thrombosis. [13]

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