Approaches in Hematology

Hematology Made Easy

All what you need to know about PNH.

- Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired disorder in which hematopoietic stem cells and their cellular progeny have reduced or absent glycosylphosphatidylinositol (GPI)-anchored proteins on the cell surface.

- Loss of the GPI-linked complement inhibitors, CD55 andCD59, on red blood cells (RBCs) leads to chronic and/or paroxysmal intravascular hemolysis and a propensity for thrombosis, organ dysfunction, and hypocellular or dysplastic bone marrow.

- A subset of patients with PNH have clinically significant aplastic anemia or myelodysplastic syndrome.

- Diagnosis of PNH may be delayed because of its nonspecific clinical features, variable clinical presentation, and rarity.
  - Age: median age around 30 years
  - Males & females are equally affected.

- PNH is also associated with aplastic anemia (AA)and, less commonly, with myelodysplastic syndromes (MDS) and can have manifestations of thrombocytopenia and/or neutropenia.

Presentation

- Many patients present with the following:
  - Unexplained hemolytic anemia
  - Fatigue
  - Jaundice
  - Red/pink/dark urine
  - Some patients present with venous thrombosis (especially at atypical sites)

Or

- have only nonspecific symptoms associated with smooth muscle dystonia, which can cause abdominal pain, erectile dysfunction, renal insufficiency, or pulmonary hypertension.

- Hematologic-PNH is typically associated with anemia-related findings caused by
  - Hemolysis of red blood cells (RBCs)
  - Bone marrow hypoplasia or dysplasia
  - Folate or iron deficiency may be present.

Details of presentation

- Hemolysis: the degree of hemolysis and associated manifestations generally varies from a person to another.

  - Anemia-related symptoms: Many patients present with:
    - Persistent or episodic fatigue (out of proportion to the degree of anemia)
    - Weakness
    - Jaundice
    - Hemoglobinuria (i.e., red, pink, or "cola-colored" urine)
    - Hemolysis typically occurs at a low level throughout the day and increases at night, as the name of the disease implies.

- Important Note (1): Some individuals have paroxysms of hemolysis that are triggered by infections, inflammatory stimuli, surgery, strenuous physical activity, blood transfusion, or alcohol.
- Important Note (2): Iron repletion in an iron-deficient patient can increase hemolysis by facilitating production of a large population of PNH RBCs that are highly susceptible to complement lysis.

- Vasospasm: Hemoglobin release can cause vasospasm by depleting circulating nitric oxide (NO), leading to smooth muscle dystonia, abdominal or muscle pain, pulmonary hypertension, or renal insufficiency.

- Thrombosis: Hemolysis contributes to the hypercoagulable state associated with PNH

  - Thrombophilia is the leading cause of death in patients with PNH.
  - Thrombosis often involves unusual sites, such as mesenteric, cerebral, or dermal veins; arterial events are less common.
  - The presentation of PNH-associated thrombosis varies with the site of involvement.
    - The onset of clots can be insidious or abrupt and some are discovered incidentally.
    - Thromboses occurred in up to 40 % of patients.
    - Intra-abdominal sites of thrombosis (e.g., hepatic, portal, mesenteric veins) account for2/3 of clots in patients with PNH, followed by intracerebral sites (10 - 20 %) and other locations (e.g., skin, lower extremity).

    - Clinical manifestations include the following:
      - ***Hepatic vein (Budd-Chiari syndrome):*** Hepatic vein thrombosis can develop insidiously or suddenly, the latter often during an episode of brisk hemolysis. Hepatic vein thromboses tend to recur, causing cirrhosis and rerouting blood from the portal circulation, which can be exacerbated by portal vein thrombosis.

      - ***Inferior vena cava, portal, and splenic veins Thrombosis*** can cause splenic congestion and hypersplenism.

- Important Note: Microvascular thrombosis of splanchnic vessels can also occur and produce bouts of abdominal pain and/or mucosal ulceration.

- ***Cerebral veins thrombosis*** can occur as a catastrophic event or with an insidious onset that may be confused with other causes of headache. .

- ***The major venous sinuses*** (e.g., superior sagittal, lateral, cavernous, sigmoid) are most often involved, but thrombosis may also occur in the veins covering the cerebrum (particularly the parietal lobe).

- ***Dermal veins thrombosis*** can present as discrete areas of erythema, swelling, and pain or as a syndrome resembling purpura fulminans. It may occur in areas of trauma or in sites of previous inflammation or allergic reaction.

    - Note (1): Arterial thrombosis: The incidence of arterial thrombosis (e.g., cerebral, or coronary arteries) is increased in patients with PNH, but it is much less common than venous thrombosis.
    - Note (1): Arterial thrombosis tend to occur at an earlier age compared within age-matched population.

- ***Smooth muscle dystonia*** is associated with dysphagia/odynophagia, abdominal pain, and erectile dysfunction in patients with PNH.

    - Mechanism: NO relaxes smooth muscle, but in PNH, intravascular hemolysis releases free hemoglobin into the circulation and the resulting depletion of NO is thought to cause excessive smooth muscle contraction and dystonia.

    - Abdominal pain/dysphagia: Smooth muscle in the gastrointestinal tract appears to be preferentially affected in PNH.

    - The most common manifestation is esophageal spasm; esophageal manometry has noted intense peristaltic waves in association with symptoms.

    - Many patients describe dysphagia, abdominal cramping, or pressure in the chest during episodes of hemoglobinuria.

- ***Erectile dysfunction*** is associated with PNH, particularly during hemolytic episodes, because NO is required for vascular dilatation in the corpora cavernosa.

- ***Pulmonary hypertension*** can result from pulmonary emboli and from NO depletion in the pulmonary circulation, when clinically significant pulmonary hypertension occurs, it is usually associated with pulmonary emboli.

- ***Renal insufficiency***
  - PNH can be associated with acute and chronic renal disease.
  - Chronic intravascular hemolysis can cause renal hemosiderosis (renal iron deposition), which interferes with proximal tubule function and causes interstitial scarring and cortical infarcts.
  - Severe acute hemolytic episodes can cause acute renal failure from direct toxicity of free heme in the kidney.

Evaluation

- Evaluation for PNH should document complement-mediated hemolysis, assess organ function, and determine if there are cytopenia or dysplasia that indicate bone marrow failure.

- Clinical
  - History should include symptoms related to anemia (e.g., fatigue, dyspnea, weakness), hematuria, thrombosis (including abdominal, cerebral, or dermal veins), unexplained abdominal pain, erectile dysfunction, dysphagia, bleeding/bruising, and recurrent infections.
  - Physical examination should seek signs of anemia (e.g., pallor, tachycardia, tachypnea), excessive bleeding/bruising or infection, and evidence of thrombosis (e.g., redness or swelling of an extremity, splenomegaly).

- Laboratory
  - Laboratory findings should document hemolytic anemia, exclude other causes of hemolysis (e.g., immune, microangiopathic, and mechanical), and evaluate organ function.

  - Hematological profile
    - Complete blood count (CBC) with differential count
    - Reticulocyte count
    - Blood smear for red blood cell (RBC) morphology and other abnormalities
    - Direct antiglobulin (Coombs) testing (DAT)
    - Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and D-dimer.
    - Serum chemistries
      - Electrolytes, blood urea nitrogen (BUN), creatinine, and liver function tests, including lactate dehydrogenase (LDH) and direct and indirect bilirubin.
      - Serum haptoglobin, free hemoglobin
    - Iron, transferrin saturation, and ferritin should be tested, as clinically indicated.
    - Urine for hemoglobin and hemosiderin

  - Specific tests for PNH

- Flow cytometry and FLAER
  - Flow cytometry is the preferred method for evaluating and diagnosing PNH. Flow cytometry documents reduction or loss of glycosyl phosphatidyl inositol (GPI)-anchored proteins on blood cells and defines the size of the PNH population of blood cells.

- Reagents:
  - Flow cytometry is performed with fluorescently labeled monoclonal antibodies that bind to GPI-anchored proteins (e.g., CD59, CD55). Most tests for PNH also incorporate Fluorescent Aerolysin (FLAER), a reagent derived from the bacterial toxin aerolysin that directly binds the GPI anchor.

❖ Many laboratories also report the percentage of different types of PNG:

i. Type I PNH cells (normal expression of GPI-anchored proteins)
ii. Type II (partial expression)
iii. Type III (absent expression).

❖ Blood cells: Both granulocytes and RBCs should be evaluated to assess the size of the PNH clonal population. Testing of RBCs alone may underestimate the PNH population because of the short lifespan of PNH red cells and/or their dilution by transfused blood from unaffected donors.

❖ Value of Bone marrow exam
- All patients with significant leukopenia or thrombocytopenia should undergo bone marrow examination to evaluate bone marrow failure as a contributor to the cytopenia.

- Bone marrow microscopy for cellularity and morphology and Giemsa-stained chromosome banding analysis should be performed.

- Patients with classical hemolytic PNH typically have normocellular or hypercellular bone marrow with erythroid hyperplasia; there may also be erythroid dysplasia due to active RBC turnover.

Important Note: PNH, itself, is not associated with chromosomal abnormalities, but an abnormal karyotype may be found in patients with PNH-associated MDS.

Diagnosis:

- PNH should be suspected in individuals with direct antiglobulin testing (DAT; Coombs test) negative hemolytic anemia thrombosis at an unusual site or early age, unexplained abdominal pain, and for unexplained cytopenia, aplastic anemia (AA), or myelodysplastic syndrome (MDS)

- Diagnostic criteria:

- The diagnosis of PNH is established by flow cytometry that demonstrates a population of granulocytes and red blood cells (RBCs) that are deficient in (GPI) glycosylphosphatidylinositol -linked proteins (e.g., CD55, CD59) in an appropriate clinical setting, such as:
  - ✓ DAT-negative hemolytic anemia
  - ✓ Thrombosis
  - ✓ Unexplained abdominal pain
  - ✓ AA (Aplastic Anemia) or MDS

Classification of PNH

- PNH is classified according to:
  - Symptoms:
    - Anemia-related symptoms
    - Transfusion-dependence
    - Thrombosis
    - Pain
    - Or organ dysfunction
  - Bone marrow failure – Findings that meet criteria for Aplastic Anemia or MDS on bone marrow. Examination if performed for leukopenia or thrombocytopenia.
- PNH is a dynamic condition and the category of PNH may evolve over time.

1) Hemolytic (classical) PNH:

- Patients with classical hemolytic PNH have findings of intravascular hemolysis, but mild or no evidence of bone marrow failure.
- Criteria:
  - Hemolysis – Prominent symptoms related to hemolysis (e.g., fatigue, dyspnea, RBC transfusion-dependence, episodic hemoglobinuria, thrombosis, pain, and/or organ dysfunction); LDH is typically >1.5x upper limit of normal (ULN).
  - Leukopenia and/or thrombocytopenia: Normal white blood cell (WBC) count and platelet count or modest, asymptomatic leukopenia and/or thrombocytopenia.
  - Clone size: Granulocyte PNH clone is typically >50 percent; RBC clone size is variable.
  - Bone marrow: is cellular with erythroid hyperplasia and no significant dysplasia.
  - Other findings: Thrombosis, pain that requires hospital admission or opioid analgesia, and organ dysfunction (e.g., renal insufficiency, pulmonary insufficiency or pulmonary hypertension) may be present.

2) Subclinical PNH

- PNH is detected, but there are no substantial clinical findings and no bone marrow abnormalities.

- Criteria:
  - Hemolysis: No substantial anemia-related symptoms, thrombosis, pain, organ dysfunction, or ongoing transfusion-dependence; LDH is typically ≤1.5x ULN.
  - Leukopenia and/or thrombocytopenia: Normal WBC count and platelet count or modest, asymptomatic leukopenia/thrombocytopenia.
  - Clone size: PNH granulocytes are typically ≤20 %; RBC clone size is small, but variable.
  - Bone marrow: has normal or near-normal cellularity and morphology.
  - Other findings – There is no thrombosis, pain, or organ dysfunction.

3) *PNH with bone marrow failure:*

- Bone marrow examination reveals hypoplasia or myelodysplasia that meets criteria for Aplastic Anemia or MDS, with variable levels of hemolysis-associated findings:
  - Hemolysis: Variable anemia-related symptoms, thrombosis, pain, or organ dysfunction and variable level of serum LDH.
  - Leukopenia and/or thrombocytopenia: Prominent severe, symptomatic leukopenia and/or thrombocytopenia.
  - Clone size: Variable size of granulocyte and RBC PNH clones.
  - Bone marrow: cellularity and/or morphology meet criteria for severe Aplastic Anemia or high-risk MDS.

Management

- Treatment is guided by the type and severity of manifestations and complications of PNH

- Symptomatic hemolytic PNH without bone marrow failure (BMF): e.g., thrombosis, organ dysfunction, pain.

  $\mathcal{R}$ a C5 complement inhibitor (C5i) is recommended.

- Subclinical PNH: For patients with no substantial PNH-associated symptoms or Bone marrow failure,

  $\mathcal{R}$ watchful waiting suggested rather than treatment with a C5i or allogeneic hematopoietic cell transplantation (HCT).

- PNH with severe Bone marrow failure: For patients with PNH and severe aplastic anemia or high-risk MDS.
  - ℛ Management is guided by treatment for severe Aplastic Anemia or MDS (see algorithm)

- For less severe Aplastic Anemia or lower-risk MDS
  - ℛ management is guided by PNH-associated symptoms.

Complement inhibitor therapy!

- C5i treatment can relieve PNH-related symptoms, eliminate transfusion-dependence, prevent thrombosis, and relieve pain, but does not mitigate Aplastic Anemia or MDS.

- Choice of agent – For a C5i, we suggest rovelizumab rather than eculizumab, based on comparable efficacy and toxicity, but greater convenience, lower overall expense, and fewer episodes of pharmacokinetic breakthrough hemolysis.
  - ℛ Ravulizumab: trade name is Ultomiris.

- Ravulizumab is the preferred complement inhibitor based on its more convenient treatment schedule and similar efficacy and adverse effects, compared with eculizumab.
  - ℛ Eculizumab: trade name Soliris

    - Administration: The standard schedule for eculizumab in adults is 600 mg IV once per week for the first four weeks, followed by 900 mg IV one week later, followed by 900 mg IV once every two weeks thereafter. Eculizumab is generally continued indefinitely in responding patients.
    - Patients on eculizumab have risk of meningitis.
    - Monitoring the response to eculizumab is done weekly for the first four weeks (including CBC, LDH, reticulocyte count, and serum chemistries) and can be extended to every one to two months in responding patients.

- High risk of meningitis with C5i therapy – All patients who are treated with a C5i should be vaccinated and receive oral antibiotic prophylaxis.

- Breakthrough hemolysis: Pegcetacoplan or a clinical trial with a newer complement inhibitor should be offered to patients who remain transfusion-dependent or continue to have symptomatic extravascular hemolysis.

- Thrombosis or history of thrombosis is an indication for C5i therapy.

- Management and prophylaxis of thrombosis
  - Treatment of deep vein thrombosis (DVT): Acute thromboses should be treated with anticoagulation and/or thrombolysis for life-threatening thromboses, according to the site of the DVT and the severity of clinical findings.

- For patients whose PNH is well-controlled on a complement inhibitor, it appears to be safe to discontinue anticoagulation after three to six months However, patients who developed a thrombosis while receiving a complement inhibitor should be anticoagulated indefinitely.

- For patients with a life-threatening thrombosis in the setting of severe thrombocytopenia (e.g., platelet count <50,000/microL), it may be necessary to transfuse platelets to safely administer anticoagulants and/or thrombolytic therapy.

- No prior DVT: For patients with PNH who have not had a prior thrombosis, we suggest not treating with prophylactic anticoagulation, based on a lack of high-quality evidence that this is effective and to avoid precipitating or exacerbating bleeding in patients with concomitant thrombocytopenia.

- For patients with PNH who are hospitalized with an acute medical illness or for a surgical procedure, short-term prophylactic anticoagulation is appropriate because PNH may exacerbate the known risks of venous thromboembolism.

- Secondary prophylaxis: Thrombosis in a patient with PNH is an indication for complement inhibition, which should be initiated as soon as possible, rather than delaying therapy until completion of anticoagulation.

- Transplantation:
  - Because of the efficacy of complement inhibition for controlling hemolysis and thrombosis, allogeneic hematopoietic stem cell transplantation (HCT) is generally reserved for patients who have PNH with co-existent severe bone marrow failure (BMF). Complement inhibitors are safe and effective for managing hemolysis-associated findings while awaiting identification of a suitable transplant donor.

  - Indications for allogeneic Hematopoietic cell transplantation (HCT) in PNH include:

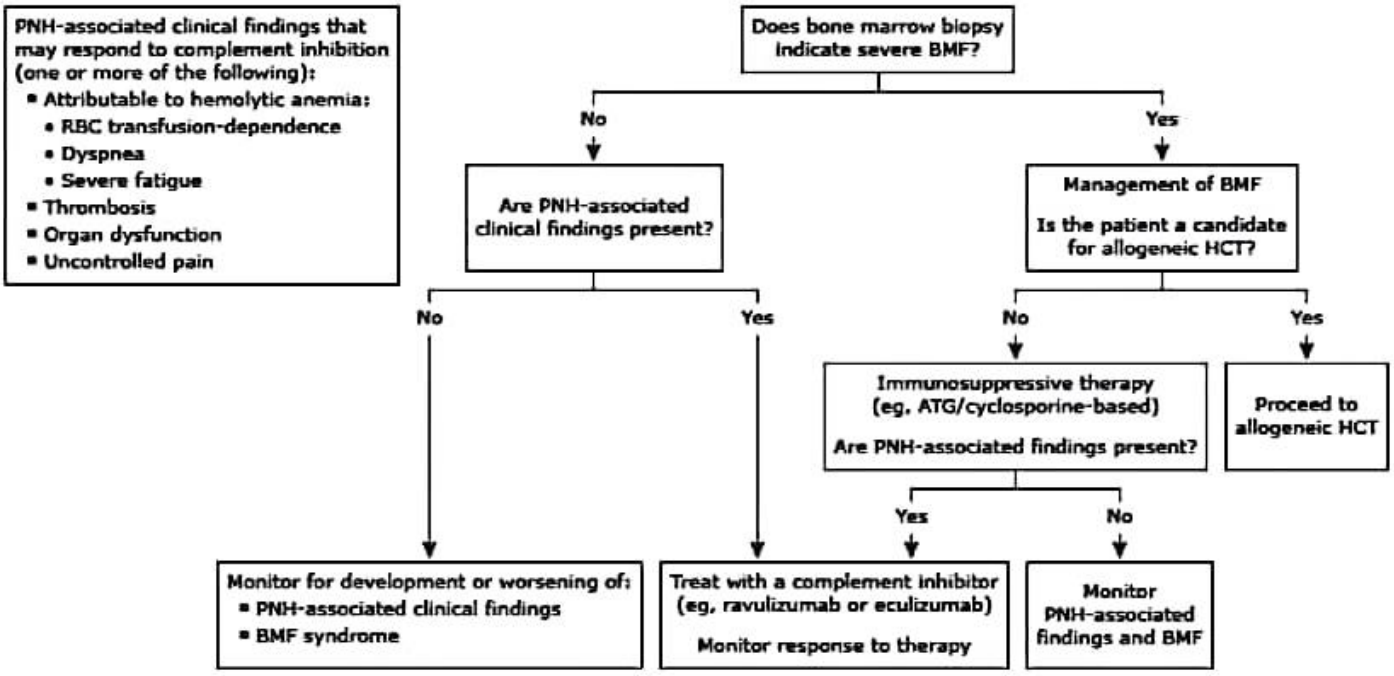
  - Severe aplastic anemia (sAA):
    - Allogeneic HCT is indicated in patients with PNH and sAA who have a suitable donor. A decision to proceed with transplantation should be made on a case-by-case basis with input from clinicians with expertise in managing PNH and transplantation.

    - For patients with PNH and sAA who are not medically fit for transplantation or do not have a suitable donor, we treat with immunosuppressive therapy (e.g., anti-thymocyte globulin and cyclosporine), as described separately.

  - Myelodysplastic syndromes (MDS):

- Some patients with PNH and co-existent high-risk MDS are treated with allogeneic HCT. A decision to proceed with transplantation should be made on a case-by-case basis with input from clinicians with expertise in managing PNH, MDS, and transplantation.
- Candidates for allogeneic HCT must have no severe lung, heart, liver, or kidney disease. He must have a suitable graft source.

Management of paroxysmal nocturnal hemoglobinuria



Diagnosis of PNH is based on absence or marked reduction of cell surface CD55/CD59 on two peripheral blood lineages by flow cytometry and FLAER. Refer to related UpToDate material for details of criteria for PNH diagnosis and severe BMF syndrome, PNH-associated clinical findings, supportive care, other aspects of PNH management, eligibility for allogeneic HCT, and management of BMF.

ATG: anti-thymocyte globulin; BMF: bone marrow failure; FLAER: fluorescent aerolysin; HCT: hematopoietic cell transplantation; PNH: paroxysmal nocturnal hemoglobinuria; RBC: red blood cells.