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## Paroxysmal Nocturnal Hemoglobinuria

Paroxysmal nocturnal hemoglobinemia (PNH) is an acquired hematopoietic stem cell disorder in which somatic mutation of the X-linked *PIG-A* gene results in partial or absolute deficiency of all proteins normally linked to the cell membrane by a glycoposphatidylinositol (GPI) anchor. The abnormal gene occurs in somatic cells, especially hematopoietic stem cells, but not in germ cells, making PNH an acquired disorder. So far, *PIG-A* gene mutations have not been detected in somatic cells outside of the hematopoietic system. All hematopoietic cell lineages are affected including erythrocytes, platelets, granulocytes and monocytes. Approximately 174 somatic mutations in the *PIG-A* gene have been identified. The initial mutagenic event remains unknown.

PNH is rare; the estimated incidence is 1.3 new cases per one million individuals per year. Development of clinical disease requires the combination of a hypoplastic or dysplastic bone marrow, somatic mutation in the *PIG-A* gene, and clonal expansion of PNH stem cells. Clinical features vary greatly from patient to patient during the course of the disease but there are three main pathophysiologic features:

- Intravascular hemolysis
- Venous thrombosis
- Bone marrow aplasia or dysplasia

Hemolysis is a consequence of abnormal erythrocyte sensitivity to complement-mediated lysis. PNH erythrocytes are abnormally sensitive to complement because they are deficient in two complement regulatory proteins, decay accelerating factor (DAF, CD55) and membrane inhibitor of reactive lysis (MIRL, CD59). DAF regulates the early portion of complement activation including C3-C5 while MIRL inhibits the incorporation of C9 into the membrane attack complex. The absence of CD59 is primarily responsible for red cell hemolysis and may contribute to thrombosis.

Deficiency of CD59 on platelets is also thought to play a role in thrombosis. CD59 deficient platelets are more readily activated by complement, leading

to greater procoagulant activity and thrombin generation. Venous thrombosis often occurs in unusual anatomic locations such as mesenteric, hepatic portal (Budd Chiari Syndrome), splenic, dermal veins and cerebral sinuses. Thrombosis, which is often recurrent and resistant to treatment, is the major cause of death in Western patients with PNH while pancytopenia is the major manifestation of PNH in younger and Asian patients.

The International PNH Group recommends that patients with the following conditions should be tested for PNH:

- Coombs-negative, non-schistocytic hemolytic anemia
- Unexplained hemoglobinuria
- Venous thrombosis involving unusual sites
- Dysphagia with elevated LDH
- Aplastic Anemia (AA)
- Myelodysplasia (MDS)

The frequent association of PNH clones in patients with AA supports the theory that a hypocellular aplastic marrow may be conducive to the development of PNH. Recent data showed that small PNH clones can be detected in a relatively high percentage of cases of aplastic anemia and myelodysplastic disorders.

The defining phenotypic feature of PNH cells is their deficiency of proteins that require a GPI anchor for attachment to the cell membrane. A partial list of GPI-linked proteins includes CD14, CD16, CD24, CD55, CD56, CD58, CD59, C8-binding protein, alkaline phosphatase, acetylcholine esterase, and a variety of high frequency human blood group antigens.

Flow cytometric analysis of peripheral blood is the current gold standard for laboratory detection of PNH. Flow cytometry uses fluorescently labeled monoclonal antibodies and FLAER to detect the presence or absence of GPI-linked proteins on granulocytes, monocytes, and erythrocytes. FLAER is a fluorescently labeled variant of the channel forming protein, aerolysin that binds specifically to GPI anchors. Individuals with PNH have decreased or absent expression of CD14 on

monocytes, CD 16 on neutrophils and NK cells, CD24 on neutrophils, CD59 on red blood cells and FLAER on neutrophils and monocytes. The Ham's and sugar water tests are now considered obsolete.

Patients with established diagnoses of PNH should have their PNH clone size monitored at regular intervals. If the disease is stable, annual monitoring may be sufficient. Any change in clinical or hematologic parameters requires more frequent monitoring. Regular monitoring is useful in patients receiving eculizumab therapy, but a consensus has not been reached regarding frequency of testing.

### **Weak D Testing**

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Historically, weak D red blood cells were defined as having decreased D antigen levels that required the indirect antiglobulin test for detection. Weak D phenotype most commonly results from a single point mutation in the RHD gene. Fifty four different weak D types have been reported so far. Since the difference in D antigen expression is quantitative and not qualitative, the majority of patients with a weak D phenotype can be safely transfused with Rh positive red blood cells and do not form anti-D antibody. However, a couple of weak D phenotypes (4.2, 11 and 15) have been classified as partial D because patients with these phenotypes may produce anti-D after exposure to Rh positive red blood cells. In the United States, approximately 1% of patients have weak D and 1% have partial D variants.

Today, the anti-D blood group reagents that have been approved by the FDA contain both monoclonal IgM and IgG antibodies. The former detect D antigen during immediate spin and the latter detect D antigen in the antiglobulin phase of testing. Weak D phenotypes are detected with these reagents. Many weak D variants that previously were only detected in the antiglobulin phase using older polyclonal reagents are now detected by routine typing.

Today, weak D antiglobulin testing is required for blood donors and newborns of D negative mothers to detect potentially immunogenic weak D red blood cells. Weak D typing is not required, nor encouraged, for transfusion recipients and pregnant women. By eliminating the antiglobulin phase of testing for these patients, some partial D variants at risk of forming anti-D will be classified as Rh negative. Therefore, these patients will be

candidates for Rh immune globulin during pregnancy and transfused with Rh negative red blood cells.

Controversy still exists regarding whether pregnant women who were previously identified as weak or partial D should be given Rh immune globulin prophylaxis. Arguments against giving RhIG include the relatively low risk of anti-D formation and the lack of evidence supporting its efficacy. Arguments in favor of RhIG administration include the possibility of a partial D phenotype and the unknown risk anti-D formation. In these situations, the medical director of the transfusion service should consult with the obstetrician.

Occasionally, discrepancies in D typing occur between laboratories because of the various methods and reagents used for testing. An obstetrical patient may have been previously tested for weak D in another laboratory or as a blood donor. Laboratories following current guidelines will not perform weak D testing and classify this patient as Rh negative. Another possibility is that an obstetrical patient, who really has a weak D phenotype but was classified as Rh negative, may have a falsely positive postpartum fetal rosette test due to reaction with maternal red blood cells.

### **CMV Virus Testing**

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Cytomegalovirus (CMV) is a major cause of morbidity in organ transplant recipients and other immunocompromised patients. Distinguishing between CMV latent infection and clinical disease is important, as early administration of antiviral therapy can ameliorate the severity of CMV disease. Saint Luke's Regional Laboratories recently implemented an automated real-time PCR method for CMV quantitation that targets a 240 bp fragment of the UL54 DNA polymerase gene. The new assay requires a minimum 2mL of sample for either quantitative or qualitative CMV testing. Quantitative plasma testing can be ordered using test code CMV PCR QT; a minimum 5mL of blood is required to provide 2 mL plasma. Qualitative testing on bronchoscopy, urine, amniotic fluid and CSF can be ordered using test code CMV PCR QL; a minimum 2 mL of sample is required. Lower limit of quantitation for the new assay is unchanged at 400 copies/mL.