Paroxysmal Nocturnal Hemoglobinuria

Indications for Ordering

• Unexplained hemoglobinuria
• Coombs-negative hemolytic anemia
• Unusual thrombotic sites (eg, Budd-Chiari, cerebral)
• Thrombosis combined with intravascular hemolysis or cytopenias
• Aplastic or hypoplastic anemia
• Monitoring of individuals with confirmed paroxysmal nocturnal hemoglobinuria (PNH)

Test Description

Quantitative flow

Tests to Consider

Typical testing strategy

• Initial testing
  o Complete blood count (smear is typically abnormal)
  o Reticulocyte count
  o Lactate dehydrogenase
  o Bilirubin
  o Haptoglobin
  o Direct Coombs

• Secondary testing if suspicion exists based on primary tests
  o Flow cytometry of white blood cells (WBC) and red blood cells (RBC)
  o Ham and sugar water tests are obsolete

Primary test

Paroxysmal Nocturnal Hemoglobinuria (PNH), High Sensitivity, RBC and WBC 2005006

• Preferred test for initial diagnosis of PNH and quantification of PNH clones
  o Includes high-sensitivity WBC and RBC analysis

Related tests

Individual testing by cell type

Paroxysmal Nocturnal Hemoglobinuria, High Sensitivity, RBC 2004366

• Use to monitor subclinical PNH and eculizumab treatment

Paroxysmal Nocturnal Hemoglobinuria, High Sensitivity, WBC 2005003

• Use to quantify or monitor PNH clone

Disease Overview

Incidence – 1.3/million

Age of onset – median 40 years

Symptoms

• Chronic hemolysis and hemoglobinuria
  o Jaundice
  o Dark urine
  o Anemia – fatigue, pallor, weakness
• Thrombophilia – ~40% of individuals
  o Thromboses at unusual sites
    ▪ Hepatic veins (Budd-Chiari)
    ▪ Cerebral veins
  o Leading cause of death in PNH
    ▪ Chronic kidney disease
• Bone marrow (BM) failure
  o May complicate aplastic anemia
• Other symptoms
  o Dysphagia
  o Abdominal pain
  o Smooth muscle dysfunction
  o Male impotence

Physiology

• Rare acquired hemolytic disorder due to nonmalignant clonal expansion of >1 stem cell line
• Acquired mutation of PIGA gene
  o Results in deficiency or absence of GPI-anchored cell membrane proteins on progeny of affected stem cells
  o Causes RBC sensitivity to complement lysis
• Pathophysiology of BM failure and thromboses not known
• ~60% of individuals with acquired aplastic anemia have detectable PNH cells
• Subclinical PNH population in myelodysplastic BM disorders (eg, aplastic anemia or refractory anemia) may correlate with a positive immunotherapeutic response
• Determination of PNH clone size – use WBC test to quantify percentage of PNH neutrophils
• Discrimination of PNH cell type – analyze RBCs
  o Type I – normal levels of CD59
  o Type II – reduced levels of CD59
  o Type III – absent levels of CD59
Test Interpretation

Analytical sensitivity – limit of detection is 0.005% for RBCs, 0.005% for PMNs, and 0.020% for monocytes

Results

- Positive
  - PNH cells ≥1% in RBCs and WBCs indicates PNH
  - RBC PNH cells (≥0.005% to <1%), WBC (PMN) PNH cells (≥0.005% to <1%), and WBC monocyte PNH cells (≥0.020% to <1%) indicate subclinical PNH
  - Often associated with BM failure syndromes

- Negative
  - Reduces probability of PNH, but does not eliminate it

Limitations

- Compromised accuracy
  - Significant neutropenia
  - Gross hemolysis
  - Samples lacking CD15, CD64, or glycophorin A expression
  - Recent RBC transfusions may decrease percentage of PNH cells measured in RBCs