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
  - Complete blood count (smear is typically abnormal)
  - Reticulocyte count
  - Lactate dehydrogenase
  - Bilirubin
  - Haptoglobin
  - Direct Coombs
- Secondary testing if suspicion exists based on primary tests
  - Flow cytometry of white blood cells (WBC) and red blood cells (RBC)
  - 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
  - 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
  - Jaundice
  - Dark urine
  - Anemia – fatigue, pallor, weakness
- Thrombophilia – ~40% of individuals
  - Thromboses at unusual sites
    - Hepatic veins (Budd-Chiari)
    - Cerebral veins
  - Leading cause of death in PNH
    - Chronic kidney disease
- Bone marrow (BM) failure
  - May complicate aplastic anemia
- Other symptoms
  - Dysphagia
  - Abdominal pain
  - Smooth muscle dysfunction
  - Male impotence

Physiology

- Rare acquired hemolytic disorder due to nonmalignant clonal expansion of ≥1 stem cell line
- Acquired mutation of *PIGA* gene
  - Results in deficiency or absence of GPI-anchored cell membrane proteins on progeny of affected stem cells
  - 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
  - Type I – normal levels of CD59
  - Type II – reduced levels of CD59
  - 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