Paroxysmal Nocturnal Hemoglobinuria Testing

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hemolytic disorder caused by nonmalignant clonal expansion of one or more stem cell lines due to an acquired mutation in the *PIGA* gene. PNH is associated with intravascular hemolysis, thrombotic complications, and bone marrow failure.[1]

Typical Testing Strategy

- Initial testing includes:
  - Complete blood count with peripheral smear
  - Reticulocyte count
  - Direct Coombs test
  - Serum lactate dehydrogenase
  - Indirect bilirubin
  - Serum haptoglobin
- Diagnostic testing (if suspicion exists based on primary tests) should include flow cytometry of both white blood cells (WBCs) and red blood cells (RBCs)
- Flow cytometry testing of WBCs and/or RBCs may be used in therapeutic monitoring
  - Ham and sugar water tests are no longer used; do not order

Disease Overview

Incidence

1.3/million[1]

Symptoms

- Hemolysis[1]
  - Symptoms include dysphagia, lethargy, renal failure, anemia, hemoglobinuria, male impotence, pulmonary hypertension
- Thrombophilia[1]
  - Potentially life-threatening
  - Thromboses located at unusual sites (eg, hepatic portal)
- Bone marrow (BM) failure[1]
  - May present as severe aplastic anemia

Physiology

- PNH is caused by a somatic mutation of *PIGA* gene which results in deficiency or absence of glycosylphosphatidylinositol (GPI)-anchored cell membrane proteins on progeny of affected stem cells[1]
  - Lack of CD55 and CD59 causes RBC sensitivity to complement lysis
  - Pathophysiology of thrombophilia and bone marrow failure in PNH is unknown
- Percentage of RBCs or WBCs that entirely or partially lack GPI-linked antigens is referred to as PNH clone size[1,2]
  - WBC testing is most accurate in the determination of PNH clone size
  - RBC testing is most appropriate for detection of cells only partially lacking GPI-linked antigens
    - Type I: normal levels of CD59
    - Type II: reduced levels of CD59
    - Type III: absent levels of CD59

Tests to Consider

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

Method: Quantitative Flow Cytometry

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

Indications for Ordering

Diagnose PNH in patients with

- Unexplained hemoglobinuria
- Coombs-negative hemolytic anemia
- Unusual thrombotic sites (eg, Budd-Chiari, cerebral)
- Thrombosis combined with intravascular hemolysis or cytopenias
- Aplastic or hypoplastic anemia

Monitor individuals with confirmed PNH

Related Tests

Paroxysmal Nocturnal Hemoglobinuria, High Sensitivity, RBC 2004366

Method: Quantitative Flow Cytometry

Use to monitor subclinical PNH and eculizumab treatment

Paroxysmal Nocturnal Hemoglobinuria, High Sensitivity, WBC 2005003

Method: Quantitative Flow Cytometry

Use to quantify or monitor PNH clone

Test Interpretation

Analytical Sensitivity

Limits of detection:

- RBCs: 0.005%
- Polymorphonuclear neutrophils (PMNs or granulocytes): 0.005%
- Monocytes: 0.020%

Results

Results	Cells Detected	Interpretation
Positive	PNH cells: ≥1% in RBCs and WBCs	Indicates PNH
	RBC PNH cells: ≥0.005% to <1%	Indicates subclinical PNH
	WBC (PMN) PNH cells: ≥0.005% to <1%	
	Monocyte PNH cells: ≥0.020% to <1%	Often associated with symptoms of bone marrow failure
Negative	PNH cells: not detected	Reduces, but does not eliminate the probability of PNH

Limitations

- Conditions that may compromise accuracy include significant neutropenia, gross hemolysis, and specimens that lack expression of CD15, CD64, or glycophorin A
- Recent RBC transfusions may decrease percentage of PNH cells measured in RBCs

References

1. Borowitz MJ, Craig FE, Digiuseppe JA, et al. Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry. Cytometry B Clin Cytom. 2010;78(4):211-230. PubMed

2. Sutherland DR, Acton E, Keeney M, et al. Use of CD157 in FLAER-based assays for high-sensitivity PNH granulocyte and PNH monocyte detection. Cytometry B Clin Cytom. 2014;86(1):44-55. PubMed

Related Information

Paroxysmal Nocturnal Hemoglobinuria - PNH

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