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
- Flow cytometry is the gold standard for diagnosis and monitoring, and diagnostic testing (if suspicion exists based on primary tests) should include flow cytometry of both white blood cells (WBCs) and red blood cells (RBCs)2
- 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/million1

Symptoms

- Hemolysis1
  - Symptoms include dysphagia, lethargy, renal failure, anemia, hemoglobinuria, male impotence, pulmonary hypertension
- Thrombophilia1
  - Potentially life-threatening
  - Thromboses located at unusual sites (eg, hepatic portal)
- Bone marrow (BM) failure1
  - 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 cells1
  - 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 size1,3
  - 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
Method: Quantitative Flow Cytometry

Preferred test for initial diagnosis of PNH and quantification of PNH clones
- Use to 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, or aplastic or hypoplastic anemia
- Use to monitor individuals with confirmed PNH
- Includes high-sensitivity WBC and RBC analysis
- If >1% PNH RBCs are detected, then PNH RBC type will be added to report Type II and Type III PNH RBC's.

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: 0.008%
- Polymorphonuclear neutrophils (PMNs or granulocytes): 0.008%
- Monocytes: 0.2%

Limitations of quantification:

- RBCs: 0.02%
- Polymorphonuclear neutrophils (PMNs or granulocytes): 0.02%
- Monocytes: 0.5%

Results

<table>
<thead>
<tr>
<th>Results</th>
<th>Cells Detected</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>% PNH cells: detected, ≥1% in RBCs and WBCs (PMN and monocytes)</td>
<td>Indicates PNH</td>
</tr>
<tr>
<td></td>
<td>% PNH cells: minor population of PNH cells</td>
<td>Indicates subclinical PNH</td>
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<tr>
<td></td>
<td>% PNH RBC: 0.1% to 1%</td>
<td>Often associated with symptoms of bone marrow failure</td>
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<tr>
<td></td>
<td>% PNH PMN: 0.1% to 1%</td>
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<tr>
<td></td>
<td>% PNH monocytes: 0.5% to 1%</td>
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<tr>
<td></td>
<td>% PNH cells: rare cells with PNH phenotype</td>
<td></td>
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<tr>
<td></td>
<td>% PNH RBC: 0.02% to 0.1%</td>
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<tr>
<td></td>
<td>% PNH PMN: 0.02% to 0.1%</td>
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<tr>
<td></td>
<td>% PNH cells: rare cells with PNH phenotype detected below limit of quantification</td>
<td></td>
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<tr>
<td></td>
<td>% PNH RBC: 0.008% to 0.02%</td>
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<tr>
<td></td>
<td>% PNH PMN: 0.008% to 0.02%</td>
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<tr>
<td></td>
<td>% PNH monocytes: 0.2% to 0.5%</td>
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<tr>
<td>Negative</td>
<td>% PNH cells: not detected</td>
<td>Reduces, but does not eliminate the probability of PNH</td>
</tr>
<tr>
<td></td>
<td>% PNH RBC: &lt;0.008%</td>
<td></td>
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<tr>
<td></td>
<td>% PNH PMN: &lt;0.008%</td>
<td></td>
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<tr>
<td></td>
<td>% PNH monocytes: &lt;0.2%</td>
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</tr>
</tbody>
</table>

Limitations

- Conditions that may compromise accuracy include significant neutropenia, gross hemolysis, and specimens that lack expression of CD15, CD64, or glycoporphin A.
- WBC assay sensitivity is much lower for patients with severe pancytopenia.
- Recent RBC transfusions may decrease percentage of PNH cells measured in RBCs.

References


Related Information

Paroxysmal Nocturnal Hemoglobinuria - PNH