Paroxysmal Nocturnal Hemoglobinuria Assessment by Flow Cytometric Analysis

Mike Keeney, ART, FCSMLS(D)[a,*],
Andrea Illingworth, MS, H(ASCP), SCYM[b], D. Robert Sutherland, MS[c]

KEYWORDS

• PNH • FLAER • Aplastic anemia • MDS • Flow cytometry

KEY POINTS

- Paroxysmal nocturnal hemoglobinuria (PNH) is a serious life-threatening disease that is difficult to recognize clinically.
- PNH clones may be found in other bone marrow failure-related states, for example, aplastic anemia and myelodysplastic syndrome.
- Small PNH clones may develop into clinical PNH disease over time.
- White blood cell clone size (neutrophil or monocyte) reflects actual clone size because of the impact of hemolysis or previous transfusions on red blood cell clone size.
- Flow cytometry is ideally suited to recognize both small and large clones in PNH.

INTRODUCTION

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired hematopoietic stem cell disorder resulting from the somatic mutation of the X-linked phosphatidylinositol-glycan complementation class A (PIG-A) gene.[1] In PNH, a mutation in the PIG-A gene leads to nonmalignant clonal expansion of cells with a partial or absolute loss in glycosylphosphatidylinositol (GPI)-anchored proteins, the consequences of which include intravascular hemolysis (that leads to hemoglobinuria) and thrombosis, with the latter

Disclosure: M. Keeney and the laboratory have received funding from Alexion Pharmaceuticals, Canada; A. Illingworth is a consultant for and has received speaker fees from Alexion Pharmaceuticals; the laboratory has received funding from Alexion Pharmaceuticals; R. Sutherland has received funding from, is a consultant for, and has received speaker fees from Alexion Pharmaceuticals, Canada.

[a] Pathology and Laboratory Medicine, London Health Sciences Centre, 800 Commissioners Road East, ON N6A 5W9, Canada; [b] Dahl Chase Diagnostic Services, Bangor, ME 04401, USA; [c] Laboratory Medicine Program, Toronto General Hospital, University Health Network, Toronto, 200 Elizabeth Street, ON M5G2C4, Canada
* Corresponding author.
E-mail address: Mike.keeney@lhsc.on.ca

Clin Lab Med ■ (2017) ■–■
http://dx.doi.org/10.1016/j.cll.2017.07.007 **labmed.theclinics.com**

being a major cause of morbidity and mortality.[2] Before the advent of the complement C5 inhibitor eculizumab, 35% of patients with PNH died within 5 years of diagnosis even with the best available treatment.[2] Eculizumab is a humanized monoclonal antibody approved for the treatment of patients with hemolytic PNH. This drug significantly reduces hemolysis, transfusion requirements, and thrombosis and has been shown to improve both quality of life and life expectancy.[3] There is also a well-documented relationship between PNH and other bone marrow failure syndromes, such as aplastic anemia (AA)[2,4] and low-grade MDS. With modern, high-sensitivity assays, up to 60% of patients with AA have PNH clones,[5] 10% to 25% of which may exhibit clonal expansion and progression to clinical PNH. Small populations of GPI-deficient PNH clones have been reported in patients with early stage myelodysplastic syndrome (MDS).[4]

PATIENT GROUPS WHO SHOULD BE SCREENED FOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

PNH is a very rare disease with a prevalence of about 16 cases per million and only about 1.3 new cases diagnosed per million per year. As such, PNH testing should not be used as a front-line screening test and more common causes of hemolysis should always be ruled out first. Nevertheless, the life-threatening and progressive nature of PNH warrants testing of appropriate patient populations at risk of PNH with early diagnosis essential for improved patient management and prognosis.[6,7] Such groups include:

1. Unexplained cytopenias with evidence of hemolysis, that is, increased levels of lactate dehydrogenase, low haptoglobin, or elevated reticulocyte count.
2. All patients with AA and those with low-grade refractory-anemia MDS with evidence of hemolysis.
3. Unexplained thrombosis despite anticoagulation; in patients with cytopenia; less than 50 years of age; in unusual sites, for example, cerebral, hepatic portal, dermal vein; with evidence of hemolysis; or with other clinical manifestations of PNH (abdominal pain, chest pain, dyspnea, dysphagia, severe fatigue).
4. All patients with unexplained direct antiglobulin test–negative hemolytic anemia, hemoglobinuria, and/or hematuria.

HISTORY OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA TESTING

PNH was initially recognized as a hemolytic anemia; therefore, assays to detect the disease focused on red blood cells (RBCs). These tests included the Ham test and the sugar-hemolysis test, both of which demonstrated the increased sensitivity of PNH RBC to complement-mediated hemolysis. These tests were laborious, difficult to standardize, and neither specific nor sensitive. Since the early 1990s, flow cytometric detection of cells lacking expression of GPI-anchored surface molecules has become the method of choice for diagnosing and monitoring PNH. Early methods to detect PNH relied on detecting the loss of CD55 and CD59, 2 GPI-linked complement-regulatory structures expressed on normal RBCs and white blood cells (WBCs).[8]

Although the ability to rapidly detect GPI-deficient cells by flow has led to improved diagnosis, patient management, and prognosis in PNH and related disorders, simple CD55/CD59-based approaches were neither accurate nor sensitive at less than the 1% to 4% clone size, rendering them inadequate to detect small PNH clones present in PNH+ AA and MDS cases.[9]

Because 40% of samples positive for the presence of PNH cells contain GPI-deficient cells at a level of 1% or less (Illingworth A, unpublished observations), the