Flow cytometric testing for GPI-deficient populations and Paroxysmal Nocturnal Haemoglobinuria (PNH)

Introduction

The name Paroxysmal Nocturnal Haemoglobinuria (PNH) itself is a misnomer, as it is not paroxysmal or nocturnal, haemolysis is constant and only around a quarter of patients present with haemoglobinuria.[1]

PNH is an acquired haematopoietic stem cell disorder in which somatic mutation of the X-linked phosphatidylinositol glycan complementation class A (PIG-A) gene results in a partial or absolute deficiency of all proteins normally linked to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor.[1,2] Clonal expansion of this cell population also frequently occurs in patients with aplastic or hypoplastic anaemia in which normal haematopoiesis has failed.

Classical clinical features of this condition are intravascular haemolysis, bone marrow failure and a thrombotic tendency, though patients show a wide spectrum of clinical presentation,[3,4] including abdominal pain, dysphagia, erectile dysfunction, renal failure and extreme fatigue or lethargy which is disproportionate to their anaemia and patient presentation is highly variable. The disease is rare with a reported incidence of 1.3/million/year and prevalence of 15.9/million.[5] Median age of diagnosis is around 30 years and median survival is around 10 years.

As PNH is a rare disease patients may go undiagnosed for many months. Consequently, the availability of a diagnostic flow cytometry assay means that in patients with suspected PNH, a definitive diagnosis can be rapidly established resulting in improved patient management and prognosis.

Requests for testing should be sent to:

Flow Cytometry Laboratory, Heamatology Department,
Laboratory Medicine, Birmingham Heartlands Hospital,
Bordesley Green East, Birmingham, B9 5SS.
Tel. 0121 424 0704

Recommended sample requirements:
3mL of EDTA blood received within 24 hours of collection[6]

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ALEXION

Test for GPI-deficient populations in the following groups:[6]

Coombs-negative haemolytic anaemia[1,6]

Non-schistocytic, non-infectious haemolytic anaemias[6]

Or haemolytic anaemias with concomitant iron deficiency[1]

Absence of other RBC abnormalities (sickle cells, spherocytes etc.)

Unexplained thrombosis (venous or arterial)

Venous or arterial thrombosis accounts for >40% of deaths[7,8]

- Evidence of haemolysis
- Unusual sites
- Any cytopenia
- Nonresponsive to anticoagulation

Haemoglobinuria and/or haemosiderinuria

26% of patients reported haemoglobinuria at diagnosis[1]

Unexplained cytopenias[6]

Consider PNH after adequate work-up

Hypoplastic or difficult diagnosis MDS

IPIG and NCCN guidelines recommend PNH testing in MDS[1,9]

Aplastic anaemia

British Journal of Haematology (BJH) guidelines recommend screening for PNH at the diagnosis of aplastic anaemia

If negative:

- Repeat test every 6 months for 2 years
  - Repeat annually if negative

If positive:

- Repeat test every 3 months for 2 years
  - If clone size is stable, reduce testing frequency[10]

Renal dysfunction

With signs of haemolysis (↑ LDH or ↑ reticulocyte count or ↓ haptoglobin)[1,11,12]

Also, suspect PNH in patients with the following:

Laboratory analysis	Clinical features
	Unexplained:
Indicators of haemolysis: ↑ LDH,[1,13] ↓ Haptoglobin,[1,13] ↑ Indirect bilirubin[1]	Thrombosis[6]
Indicators of renal dysfunction: ↑ Creatinine,[14] ↓ eGFR,[14] ↑ BUN[13]	Blood transfusions[6]
Granulocytopenia and/or thrombocytopenia[6]	Renal dysfunction[6]
Abnormal reticulocyte count[1]	Abdominal pain[6]
↑ D-Dimers[15]	Oesophageal spasm[6]
↓ Haemoglobin[1]	Pulmonary hypertension[6,16]
N-terminal pro-B-type natriuretic peptide (NT-proBNP) ≥160pg/ml[16] predictive of pulmonary hypertension and independent mortality risk	Erectile dysfunction[6]
	Severe fatigue[8]

Patients with any clone size detected are eligible for referral to the national PNH Service at St James, Leeds, or Kings College Hospital, London, for further assessment/evaluation. Your nearest outreach clinics are at the Peterborough City Hospital, Queen Elizabeth Hospital, Birmingham and the Oxford Churchill Hospital.

References: 1. Parker C *et al. Blood* 2005; 106:3699-709. **2.** Rother RP *et al. Nat Biotechnol* 2007; 25:1256-64. **3.** Hillmen P *et al. NEJM* 1995; 333:1253-8. **4.** Socié G *et al. Lancet* 1996; 348:573-7. **5.** Hill A *et al. Blood* (ASH Abstract). 2006;108: Abstract 985. **6.** Borowitz M *et al. Clinical Cytometry* 2010; 78b:211-30. **7.** Hill A *et al. ASH* Annual Meeting 2006: Abstract 979. **8.** Hillmen P *et al. Blood* 2007; 110:4123-28. **9.** NCCN Guideline. Version 2; 2010 National Comprehensive Cancer Network. **10.** Guidelines for the Diagnosis and Management of Adult Aplastic Anaemia. British Committee for Standards in Haematology. *Brit J Haematol* 2016; 172(2):187-207. **11.** Sharma VR *Clin Adv Hematol Oncol.* 2013; 11: 1-12. **12.** Ballarin J *et al. Nephrol Dial Transplant.* 2011; 26: 3408-3411 **13.** Rother R *et al. JAMA* 2005; 293:1653-62. **14.** Hillmen P *et al. Am J Hematol* 2010; 85: 553-9. **15.** Helley D *et al. Hematalogica* 2010; 95(4):574-81. **16.** Hill A *et al.* ASH Annual Meeting Abstract 2008; 112:486.

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