

Paroxysmal Nocturnal Haemoglobinuria

Paroxysmal Nocturnal Haemoglobinuria (PNH) is an acquired haematopoietic stem cell disorder. Clinical symptoms include chronic haemolytic anaemia, thrombosis and bone marrow failure. The true incidence of PNH is not yet known, but as it is such a rare condition and shares a close relationship with Aplastic Anaemia it is likely that up to 6 people per million will be affected.^{1,2}

The discovery of PNH was made over a century ago by Strubing³ who described a haemolytic anaemia with an accompanying night time haemoglobinuria. Ham and Dingle⁴, half a century later, demonstrated that PNH red cells had increased susceptibility to lysis in acidified serum. This discovery led to the development of the Ham test for the diagnosis of PNH, which even today remains widely used. Biochemical studies of PNH red cells demonstrated a deficiency in the complement regulatory protein known as “decay acceleration factor” (DAF)⁵. This protein inhibits the formation of complement C3 convertase and is attached to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor as a posttranslational processing step. Studies showed that PNH cells were deficient in all proteins combined to the cell membrane by the GPI anchor.⁶ This implicated the failure to synthesise the GPI anchor as the cause of PNH and there was a marked increase in activity to identify the underlying biochemical and molecular defect. The genetic abnormality was discovered in 1993 by Miyata et al⁷ who demonstrated that the introduction of the phosphatidylinositol glycan complementation class A (pig-a) gene into PNH cell lines restored the expression of GPI-linked proteins. Further studies have revealed that all patients with PNH have a somatic mutation within the pig-a gene that are all different and occur throughout the gene coding region.⁸⁻¹⁰ Most of the mutations are small deletions or insertions resulting in frameshifts that generate a total absence of the GPI anchored proteins (i.e. Type III cells). Less common mutations are misense, point mutations that lead to the synthesis of small amounts of GPI anchor and hence the cells exhibit partial expression of GPI anchored proteins (i.e. Type II cells).

Flow Cytometry and PNH

The use of monoclonal antibodies and flow cytometry has made a significant contribution to defining phenotypes of PNH. In 1985

2 groups used flow cytometry and an antibody to DAF (CD55) to investigate patients with PNH.¹¹⁻¹² The studies showed DAF-deficient populations of red cells, granulocytes, lymphocytes, monocytes and platelets confirming the multi-lineage nature of PNH and provided further evidence that the abnormality was a clonal stem cell disorder. As a consequence flow cytometry has now replaced the Ham test as the gold standard technique for diagnosing patients with PNH.

There are a number of considerations when flow cytometry is the analysis of choice, not only in terms of gating strategy and antibody choice but also interpretation of results in which a detailed knowledge of the cellular distribution of GPI-linked antigens and their expression at different stages is essential. Flow cytometry for PNH is curious in that all the diagnostic antigens are actually absent from PNH cells, it is therefore crucial to include at least 2 GPI antigens on any cell type to exclude a congenital deficiency of single antigens and to exclude technical problems. It is also imperative to include a transmembrane antigen as a positive control.

It has been found that analysis of red cells gives the clearest definition of type III (complete deficiency) type II (partial deficiency) and type I (normal expression) populations (Fig. 1). For routine screening of red cells it is recommended that 2 GPI linked antigens be examined (CD55 and CD59) using directly conjugated monoclonal antibodies. If negative or partially deficient cells are detected, then this must be for both GPI-linked antigens for a diagnosis of PNH to be made.

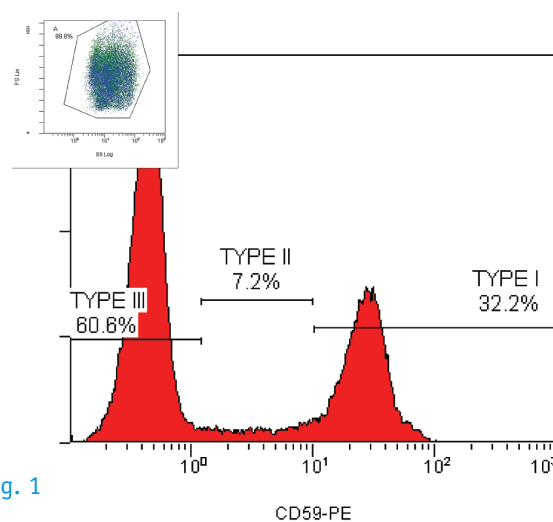


fig. 1

For granulocyte and monocyte analysis (Fig. 2 and 3) an optimal 3 colour staining procedure involving sample preparation with minimal cell loss and the use of side scatter versus a GPI-linked antigen is recommended. Leaving a further 2 fluorescent channels to examine an absence of expression in GPI-linked antigens for the diagnosis of PNH, as can be seen in the figures below showing a large PNH clone in the granulocytes and monocytes using CD55, CD66b, CD64 and CD14 respectively.

Once diagnosis has been confirmed, monitoring of the PNH clone by flow cytometry can take place giving an accurate indicator of

disease activity at any one time. It has been shown that clone size is directly linked with the clinical phenotype of the patient allowing the prediction of a prognosis.¹³ Flow cytometry is now playing an essential role in new developments of PNH management with advances in new therapeutic drugs designed to target complement inhibition. This highlights the significant and critical role that flow cytometry now plays in management and diagnosis of this singular condition.

fig. 2

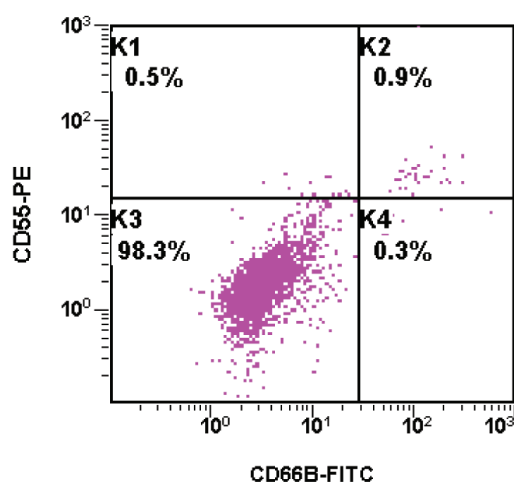
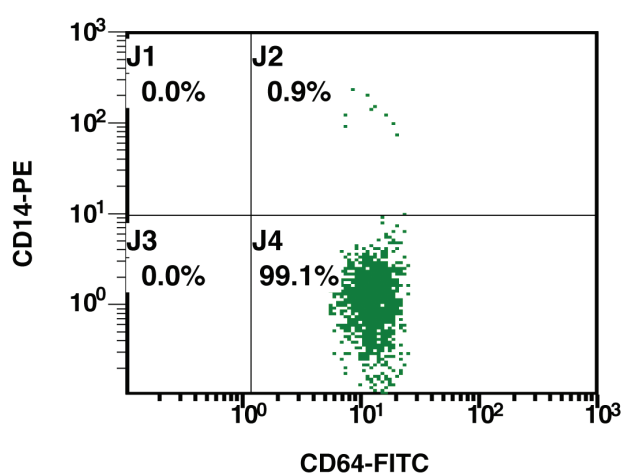


fig. 3



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