Paroxysmal Nocturnal Haemoglobinuria

Patient: A 45 year old female

Presentation: Pancytopenia, severe anaemia and abdominal pain.

Action taken: A paroxysmal nocturnal haemoglobinuria (PNH) screen is requested on a peripheral blood sample.

Q1. Discuss the principles of diagnosing PNH by flow cytometry

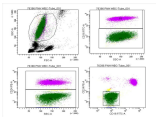
Open all sections

Answer to Q1

PNH is a rare disease. The acquired defect in haematopoietic stem cells is due to a somatic mutation in the PIGA gene. This leads to a deficiency in proteins linked to the cell membrane by GPI (glycophosphatidylinositol) anchors. The deficiency may be partial or absolute.

Flow cytometry is the method of choice for diagnosis and monitoring of PNH.Principle of the test is to examine peripheral blood populations for the presence or loss of GPI-anchored proteins.

Q2. From the dotplots below, report the findings in this case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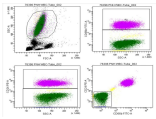
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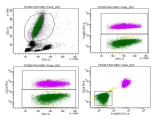
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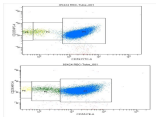
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Answer to Q2

RBC analysis – recognize and quantify red cells that are lacking GPI-anchored proteins CD55 (DAF) and CD59 (MIRL)Cells may be completely lacking these antigens (type III) or partially deficient (type II).

WBC analysis –Leucocyte analysis is the best method for assessing the size of a PNH clone. Granulocytes and monocytes are the best target for assessing PNH, granulocytes used most commonly.GPI-linked antigens assessed on granulocytes include CD16, CD24 and CD66b.FLAER (fluorescent aerolysin) is a bacterially derived protein that binds specifically to GPI-anchors. Its staining is reliably absent in PNH and FLAER is used to detect WBC PNH clones.

The flow cytometry in the first 3 slides has been gated on granulocytes, based on scatter (FSC/SSC).

It shows that there is a major PNH clone within the granulocyte population that is CD16, CD24, CD66b and FLAER deficient (58% of leucocytes).

The 4th slide is a RBC analysis (the sample has not undergone RBC lysis, which is routine prior to leucocyte immunophenotyping).

Red cells gated on CD235a (glycophorin A) are costained for CD55 and CD59. There is small but clearly deficient RBC clone (the patient had been heavily transfused).

Q3. What are the clinical indications for PNH testing?

Clinical indications

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