CASE REPORT

Haemolytic anaemia—PNH type II cells presenting a diagnostic dilemma

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SUMMARY

A 28-year-old man presented with a history of intermittent haematuria over the past 10 years usually following fever episodes and requiring blood transfusions during the episodes. History of any thrombotic complications, chest pain or erectile dysfunction was not forthcoming. Examination revealed severe pallor with mild icterus and mild splenomegaly. His blood picture showed pancytopenia with elevated reticulocytes and indirect hyperbilirubinaemia. Indirect Coombs test was positive but direct was negative, serum lactate dehydrogenase was elevated and agglutinins were found to be of IgG type. Bone marrow showed a hypercellular marrow with myeloid and megakaryocytes suppressed. Donath-Landsteiner antibodies were found to be negative ruling out paroxysmal cold haemoglobinuria. Flow cytometry was performed with a suspicion of paroxysmal nocturnal haemoglobinuria (PNH) and was shown to be partially negative for CD59 but positive for CD55, a pattern consistent with type II PNH.

BACKGROUND

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired clonal stem cell disorder characterised by intravascular haemolysis, increased susceptibility to infections, deep venous thrombosis and bone marrow failure.¹ Presentation may vary from a primary haemolytic form with no evidence of bone marrow involvement to overt bone marrow failure disorders such as aplastic anaemia (AA) or myelodysplastic syndrome (MDS). International PNH Interest Group (I-PIG) proposed a classification scheme for PNH.² (1) Classic PNH, which includes haemolytic and thrombotic patients; (2) PNH in the context of other primary disorders, such as AA or MDS and (3) subclinical PNH, in which patients have small PNH clones but no clinical or laboratory evidence of haemolysis or thrombosis. The clinical indication of PNH testing relies heavily on the presence of thrombosis with unusual features but the presence of thrombosis occurred only in 5% of patients in one series.³ However in the absence of thrombosis the diagnosis is rarely suspected especially in a resource-limited country like India. Inadequate knowledge of this rare condition and the lack of availability of flow cytometry compounds the problem; as a result patients with this condition are routinely undiagnosed and hence inadequately treated.

CASE PRESENTATION

A 28-year-old man presented with a history of intermittent haematuria over the past 10 years. At

the age of 18, he developed haematuria during an episode of enteric fever that persisted for 6–8 weeks after the fever subsided. He required repeated transfusions during the episode and as per the available record direct and indirect Coombs tests were positive and a diagnosis of autoimmune haemolytic anaemia was made by the treating physician on the basis of splenomegaly and positive Coombs test and steroids in the form of prednisolone were started at a dose of 1 mg/kg. The patient took treatment for 4 weeks and as haematuria did not subside and development of steroid facies, he defaulted on treatment. The patient improved spontaneously and did not follow-up.

The patient continued to intermittently have haematuria and would go to local doctors and received blood transfusions for it. His episodes initially were associated with fever and would usually last for 2–3 weeks on average. But subsequently the episodes occurred spontaneously even without fever. Another evaluation performed elsewhere some years previously showed only indirect Coombs being positive and direct being negative. When he presented to our hospital the patient's main symptoms were tiredness, passing dark coloured urine for the past 1 week and slight icterus of eyes, as noticed by his brother.

There was no history of fever so no medications were taken for it. There was no family history of any similar disorder or any history suggestive of haemolytic anaemia. Urine output was normal and there was no history of chest pain, difficulty in swallowing or early satiety.

INVESTIGATIONS

Investigations revealed a haemoglobin level of 5.4 g/dL, total leucocyte count 2000 cells/ μ L and platelet count 60 000/ μ L. He had an uncorrected reticulocyte count of 20.4% and serum lactate dehydrogenase level was elevated. Bone marrow showed a hypercellular marrow with erythroid-to-myeloid ratio of 5:1 and no iron stores (figure 1). Serum B₁₂ levels were normal and urine was positive for haemosiderin in three separate samples. Antinuclear antibodies were negative and a Donath-Landsteiner (DL) antibodies test was negative ruling out paroxysmal cold haemoglobinuria (PCH). Ultrasound abdomen was performed which showed mild splenomegaly with normal-sized liver. There was absence of IgM agglutinins in his blood and glucose-6-phosphate dehydrogenase deficiency (G6PD) levels were normal. Ham's test could not be performed in absence of suitable control and flow cytometry was ordered for CD55 and CD59

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Figure 1 (A) Leishman's staining of bone marrow sample aspirated from iliac crest of the patient. The current picture demonstrates hypercellular marrow particles as viewed in $\times 10$ magnification. (B) Leishman's staining of bone marrow sample aspirated from iliac crest of the patient. The current picture clearly shows the erythroid islands indicating an erythroid hyperplasia with suppression of myeloid lineage cells as viewed in $\times 20$ magnification.

antigens which showed a partial deficiency of CD59 antigen consistent with a diagnosis of type II PNH ([figure 2](#)).

DIFFERENTIAL DIAGNOSIS

Haemolytic anaemia is a diagnostic dilemma but the presence of haemosiderinuria and twice negative Coombs test with absence of significant splenomegaly suggested intravascular haemolysis.

G6PD deficiency was ruled out with normal levels. Cold agglutinins and PCH were the other closest differentials.

TREATMENT

Financial constraints prevented us from giving the option of eculizumab but an option of allogeneic bone marrow transplant has been offered to the patient.

Figure 2 CD59 analysis showing two different cell populations of patient and normal values in control.

DISCUSSION

PNH is an acquired clonal stem cell disorder characterised by chronic intravascular haemolysis that is punctuated by episodes of paroxysms which may present with macroscopic haematuria. These paroxysms may occur spontaneously or be precipitated by events like infection. The haemolysis usually occurs at night and is manifested as frank or microscopic haematuria in the morning. The haemolysis is complement dependent on the surface of red blood cells (RBCs) due to the absence of CD55 (decay accelerating factor) and CD59 (membrane inhibitor of reactive lysis). In a given patient, the severity of haemolysis depends on the number of RBCs which are abnormal and the population of cells in each subgroup of PNH, where the more the number of abnormal cells the more the chances of them being lysed and type III, having complete absence of GPI (glycosylphosphatidylinositol)-linked proteins and having a higher risk of getting lysed.

The case presented a unique diagnostic challenge and hence remained undiagnosed for a period of 10 years. The presence of splenomegaly with an elevated reticulocyte count pointed towards a haemolytic anaemia with the positive direct Coombs test performed elsewhere suggesting an immune-mediated extravascular haemolysis, but Coombs test once performed elsewhere and twice in our hospital was negative. A history of frank haematuria, elevated lactate dehydrogenase and urine haemosiderin positivity also proved the haemolysis to be intravascular. The rarity of PNH made us suspect cold agglutinin as the cause but there was absence of IgM agglutinins and PCH was excluded due to the absence of DL antibodies. The patient received two transfusions before coming to our hospital and as a result the flow cytometry showed only 9% (abnormal) RBCs with an absence of GPI-linked CD59 and no abnormal white cells. Analysis of the abnormal RBCs showed PNH types II and III morphology. However, the testing of RBCs alone in a routine assay is not adequate for evaluation of patients with PNH, because haemolysis and transfusion may greatly underestimate the size of the PNH clone.⁴ But leucocytes have to be processed within 24–48 h for a reliable report which is difficult in a resource-limited setting and in our patient a total leucocyte count of 2000 makes it even more difficult to get a reliable report.

The inadequate samples processed in flow cytometry centres in developing country like India as well as the ignorance of the diagnosing physicians to the possibility of this condition makes patients with this rare condition go a long time without a diagnosis. In this case the patient has a brother who can possibly be a donor for bone marrow transplant, the potentially curative option for this condition.

Learning points

- ▶ Paroxysmal nocturnal haemoglobinuria being a rare disorder is usually missed as a diagnostic consideration in cases of haemolytic anaemia.
- ▶ Availability of flow cytometry and immaculate processing of samples which can be analysed accurately by haematologists is required.
- ▶ Eculizumab and bone marrow transplant are the treatment options available in this formerly untreatable condition.

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