

CASE REPORT

Assessing donor chimerism using flow cytometry in paroxysmal nocturnal haemoglobinuria after stem cell transplantation - a case report

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Abstract

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired haemopoietic stem cell disorder arising from somatic mutation of the X-linked PIG-A gene which leads to deficiency of the glycosylphosphatidylinositol (GPI) membrane anchor proteins such as CD 59 (MIRL: membrane inhibitor of reactive lysis) and CD 55 (DAF: decay accelerating factor). Allogeneic peripheral blood stem cell transplant (PBSCT) is a curative mode of treatment in symptomatic PNH patients. Assessment of donor chimerism for PBSCT can be performed by various methods including short tandem repeat loci (STR) and variable number of tandem repeats (VNTR). Flow cytometry, which is much cheaper and faster, also can be used to assess engraftment in patients with PNH. Engrafted patients will show the presence of CD 55 and CD 59 on their red cells and white cells. We describe here the usefulness of flow cytometry in the assessment of donor chimerism following allogeneic PBSCT, in a case of PNH.

Key words: Flow cytometry, allogeneic peripheral blood stem cell transplant, donor chimerism.

INTRODUCTION

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare disorder of insidious onset, characterized by attacks of intravascular haemolysis and early morning haemoglobinuria. It is an acquired anaemia caused by complement-mediated haemolysis. It arises as a result of somatic mutation of the X-linked PIG-A gene that affects the synthesis of the glycosylphosphatidylinositol (GPI) membrane anchor required for several membrane proteins such as CD 59 (MIRL: membrane inhibitor of reactive lysis) and CD 55 (DAF: decay accelerating factor). The mutation arises in an abnormal clone of haemopoietic stem cells and affects GPI anchored molecules on all haemopoietic lineages. Patients with PNH have abnormal red blood cells that are sensitive to complement due to the deficiency of cell surface complement regulatory proteins CD59 and CD55.¹ The absence of these proteins is the most reliable diagnostic criterion of the disease and is responsible for many of the clinical manifestations of PNH. GPI-anchor proteins

are also missing from white cells and platelets. There is a close association with aplastic anaemia (AA) and patients may present with a disease having characteristics of both AA and PNH.² The thrombophilia of PNH is characterized by venous thrombosis occurring at unusual sites such as cerebral or mesenteric veins. The diagnosis of PNH using immunophenotyping is more sensitive and specific even after blood transfusion. After stem cell transplantation, donor chimerism can also be assessed using flow cytometry.

Case report

A 34-year-old Chinese man presented with severe anaemia for one year. He also had history of haemoglobinuria early in the morning. He was admitted to a private hospital and required multiple blood transfusions. He had a splenectomy but his anaemia did not improve and he was referred to UKM Hospital for further management. On admission, he was very pale and jaundiced. There was a splenectomy scar. His full blood counts at admission showed that

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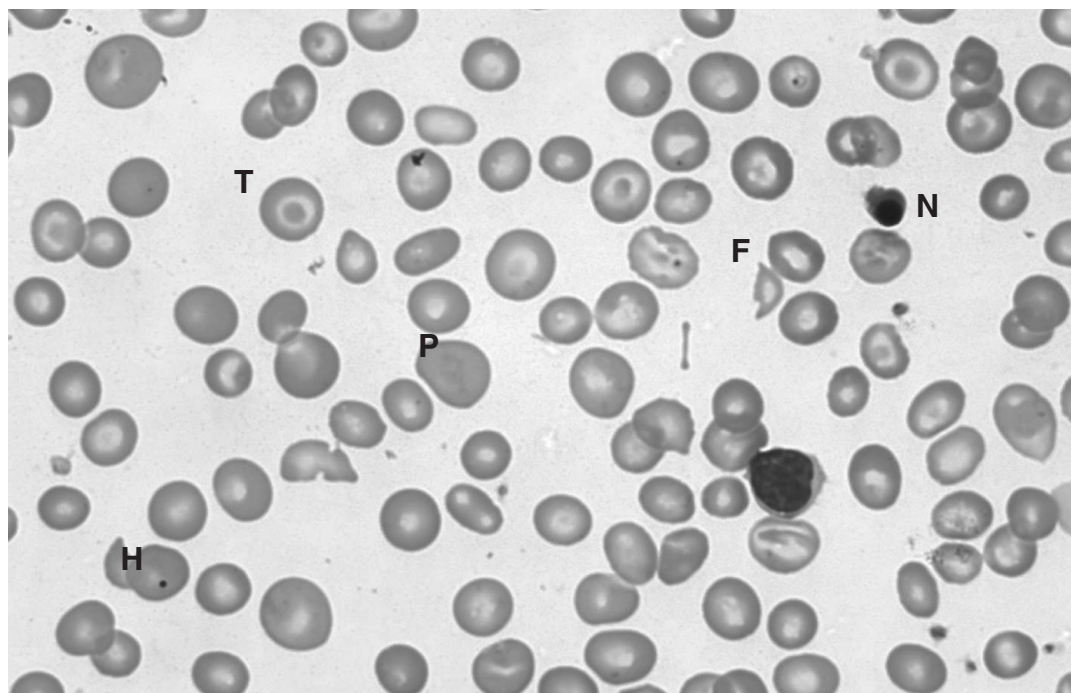


FIG. 1: Peripheral blood film (Wright stain x 40) showing polychromatic cells (P), nucleated red cells (N), target cells (T), Howell-Jolly body (H) and fragmented red cells (F)

he was anaemic with high reticulocytosis. His haemoglobin level was 7.7g/dl, the platelet count was $221 \times 10^9/l$ and the total white cells count was $10.8 \times 10^9/l$. The reticulocyte count was 14.4%. The full blood picture (Figure 1) showed anisopoikilocytosis with polychromasia. There was spherocytosis with microspherocytes seen. Numerous target cells were noted. There were numerous nucleated red cells with a few Howell Jolly bodies seen. The platelet count was more than $193 \times 10^9/l$ with many giant platelets seen. Most neutrophils were hypersegmented. The white cell differential count was: neutrophils 36%, lymphocytes 28%, nucleated red cells 22%, monocytes 14%. Renal profile was normal (sodium 139 mmol/l, potassium 4.0 mmol/l, urea 3.7 mmol/l, creatinine 74 $\mu\text{mol/l}$). Random blood sugar was normal (4.8 mmol/l). His liver function test was abnormal; he had high bilirubin however other parameters were within normal limits (total protein 82 g/L, albumin 48 g/L, total bilirubin 61 $\mu\text{mol/l}$, alkaline phosphatase 57 U/L, alanine transaminase 14 U/L). G6PD screening was normal. Direct and indirect Coombs' tests were negative. Urine hemosiderin was positive. Ham's test showed lysis in acidified serum. Immunophenotyping showed that the population of red cells and granulocytes were negative for CD 55 and CD 59 (Figures 2 and 3). He

was diagnosed to have paroxysmal nocturnal haemoglobinuria. Warfarin was started but was later changed to aspirin as he developed allergic reactions towards the drug. A nonmyeloablative allogeneic PBSCT was performed for him and the donor was his brother. On the 18th day post transplantation, he developed an urticaria rash. On the same day peripheral blood sample for short tandem repeat loci (STR) was done and the results showed that full chimerism was achieved (Figure 4). One-month post transplant, repeated urine haemosiderin was negative. Ham's test showed no lysis in acidified serum. Serum bilirubin level was normalized (Figure 5). Bone marrow aspiration sent for immunophenotyping showed that both red cells and granulocytes were positive for CD55 and CD 59. His haemoglobin level was normalized. STR was done and the results showed that full chimerism was achieved (Figure 4). On day 36 post transplantation he developed more erythematous rashes over the inner thighs, trunk and shoulders. At the same time his liver enzymes started to rise. The ALT and ALP were 59 U/L and 152 U/L respectively. His cyclosporin dosage was increased to 125mg bd. In view of the acute GVHD, prednisolone was later added while on cyclosporin to control the GVHD.

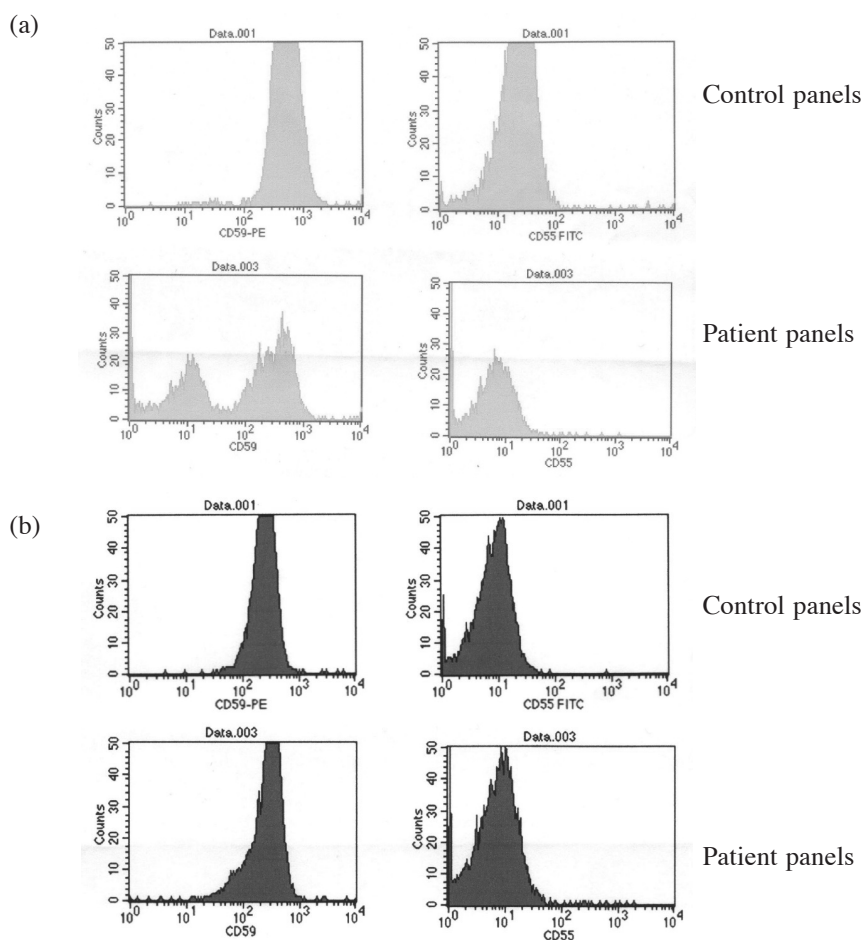


FIG. 2: Immunophenotyping for CD55 and CD59 in patient's red cells: (a) Before PBSCT: Flow cytometry showed negative expression of both proteins on patient's red cells. However, some population of red cells had positive expression for CD59 and CD55 (most likely contaminated with transfused red cells). (b) After PBSCT: both CD55 and CD59 are expressed on patient's red cells, indicating complete donor chimerism, control red cells were from the donor.

DISCUSSION

Paroxysmal nocturnal haemoglobinuria is an acquired disorder of the red cell membrane. There are two proteins, DAF (CD55), which accelerates the conversion of C3b to the inactive C3d and MRL (CD59), which protects the cell from lysis by the membrane attack complex (MAC), that are attached to the membrane by the GPI-anchor. Absence of these proteins leads to an increase in susceptibility to complement lysis by several folds. GPI-anchor proteins are also missing from white cells and platelets, and the lack of CD59 on platelets may account for the thrombotic tendency in patients with PNH.³

The diagnosis of PNH in this patient was confirmed after full blood investigations were done at Hospital UKM. The Ham's

test showed lysis in acidified serum and his immunophenotyping analysis showed a population of red cells and granulocytes, which were negative for CD 55 and CD 59. The flow cytometric assay is more sensitive and more specific than the Ham's or sucrose lysis tests, and is particularly helpful for diagnosis in the early stage of the disease, when only a small part of the total blood cell population may be affected. It allows estimating the proportions of PNH type I, II and III erythrocytes reflecting changes in the proportion of abnormal hematopoietic bone marrow cells. In our patient (Figure 2), it showed two populations of cells: normal (type I) and partial deficiency (type II) of CD59 expression, and those PNH type II cells were no longer present following transplantation. However,

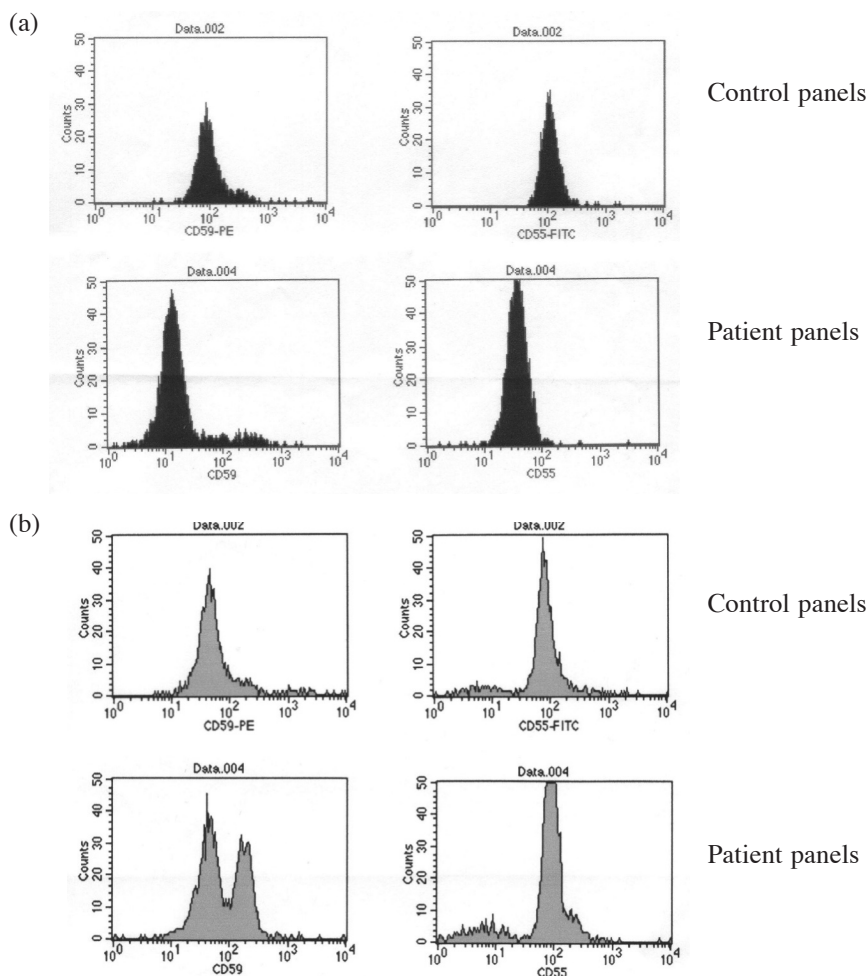


FIG. 3: Immunophenotyping for CD55 and CD59 in the patient's white cells: (a) Before PBSCT: Flow cytometry showed negative expression of both proteins on the patient's white cells. (b) After PBSCT: Both CD55 and CD59 were expressed on the patient's white cells, indicating complex donor chimerism, control granulocytes were from the donor.

the patient had received multiple packed cells transfusions before transplantation, thus the normal red cells were actually from the transfused blood. The white cells showed only PNH type II cells (partially deficient of CD55) and those cells were also absent following transplantation (Figure 3).

This patient was having symptomatic chronic haemolytic anaemia and required regular blood transfusion. In PNH, the treatment is directed either at the symptoms that arise as a result of the defective cells or at the hematopoietic defect. As we have seen in this patient, he received multiple blood transfusions due to severe anaemia. He also had splenectomy done before he was admitted to HUKM; however the operation was not helpful in controlling the haemolysis.

Immunosuppression with antithymocyte globulin (ATG) may induce a remission in bone marrow depression in some patients with PNH. Studies by Paquette *et al*, showed that ATG was able to improve peripheral blood cytopenias in patients with a hypoproliferative form of PNH⁴ but not in haemolytic PNH. Bone marrow transplantation remains an effective alternative for symptomatic patients. With the availability of suitable donors, allogeneic stem cells transplantation (SCT) is the only curative treatment for PNH. The haemoglobin level in our patient improved after PBSCT (Figure 6) and he did not require any more blood transfusion. His bilirubin level was also normalized (Figure 5) indicating that there was no more underlying haemolysis of red cells. Flow cytometry (Figures 2 & 3), which

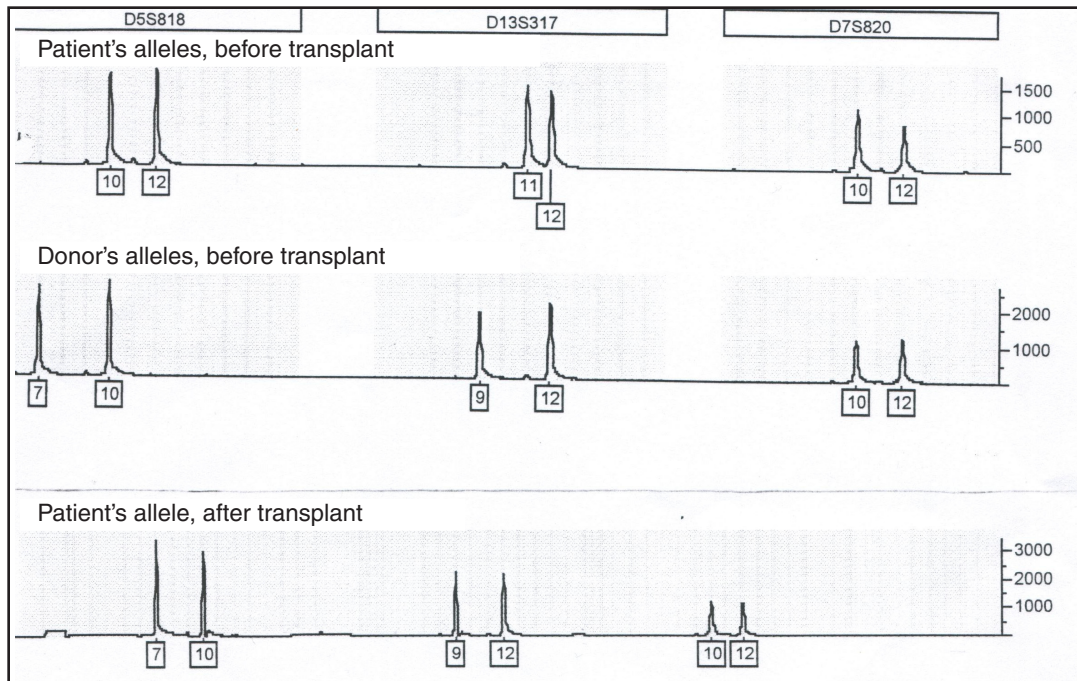


FIG. 4: Short tandem repeat loci done at 1 month post PBSCT showed complete donor chimerism.

was done at 18 days after PBSCT showed CD55 and CD59 were positive for both red cells and granulocytes. The median survival of patients with PNH is 10 – 15 years. Without SCT, since it is not a malignant condition, spontaneous remission can occur in about 15% of patients.⁵

Donor chimerism can be assessed by many methods. In early studies, red cell antigens were

used to demonstrate the presence of donor cells after PBSCT such as ABO, Rhesus, MNSs, Duffy, Kidd or Kell. However, this method only can be done if both donor and recipient have different red cell antigens and assay results can be confounded by red cell transfusions before and after PBSCT. Other methods such as erythrocyte enzymes also can be used for analyses of chimerism after

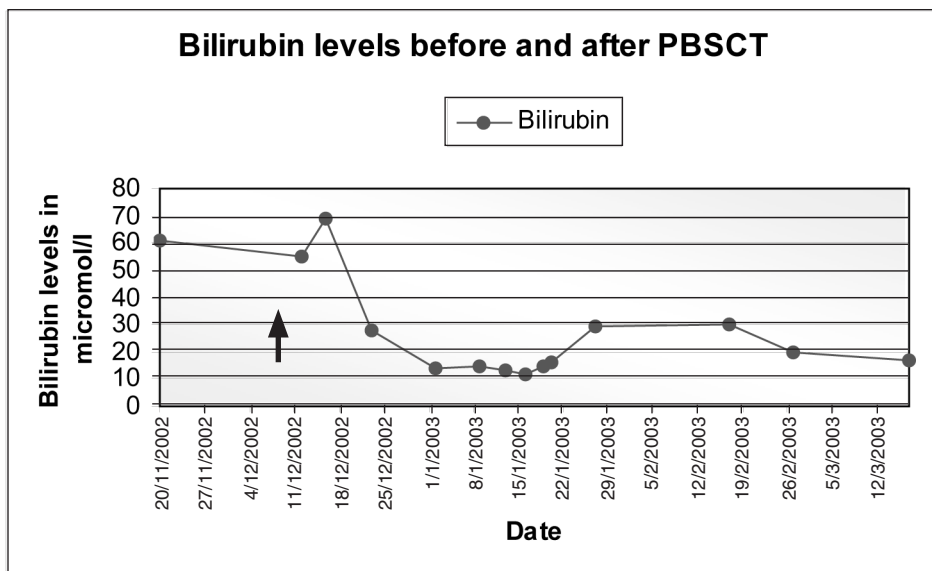


FIG. 5: Bilirubin levels of the patient before and after PBSCT (↑)

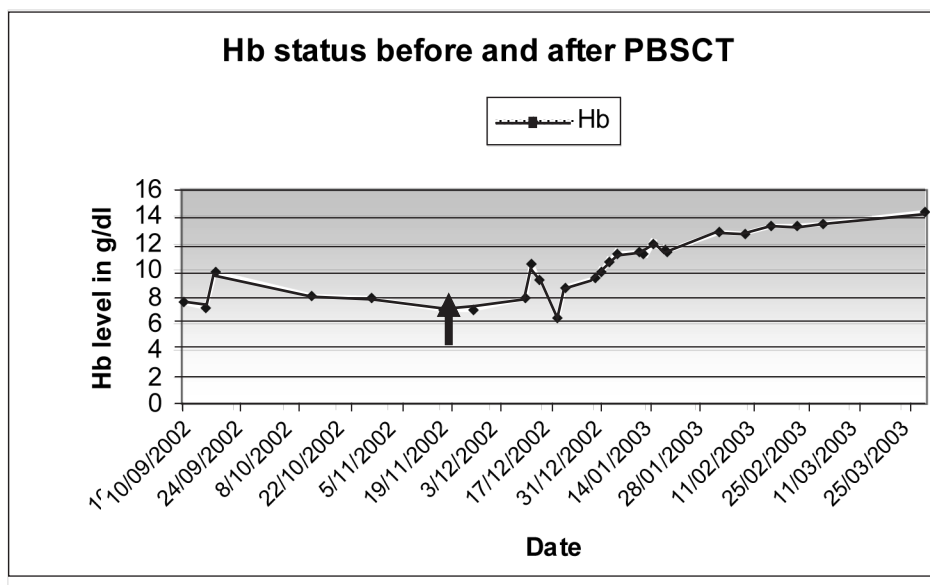


FIG. 6: Haemoglobin levels of the patient before and after PBSCT (↑).

PBSCT by looking at enzyme polymorphisms identified as electrophoretic mobility variants. However it also suffers the same problems as erythrocyte antigens. HLA antigens also can be used as informative genetic markers only when there is HLA disparity between the donor and recipient. Conventional cytogenetics can be done if there is gender disparity between the donor and recipient. However methods such as short tandem repeat loci or variable number tandem repeat polymorphisms using DNA amplification are the most sensitive and specific methods. There are varieties of allele present in a population, so a high degree of discrimination among individuals in the population may be obtained when multiple STR loci are examined. Thus, even when donor and recipient are of the same sex, same HLA types or blood group antigens, by using multiple STR loci examination, the presence of donor haematopoietic cells can be assessed accurately. Unfortunately, the molecular methods are very expensive. As illustrated by this case, immunophenotyping can be used to assess chimerism. The recipient red cells and granulocytes are negative for CD 55 and CD 59. If donor chimerism occurs, population of red cells and granulocytes with positive CD 55 and CD 59 can be seen. The tests are much cheaper and the patient can be assessed more frequently. Besides, the chimerism status can be quantified. If the patient has recurrent red cell transfusion, assessment still can be done by using granulocytes instead of red cells.

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