Report of a case of paroxysmal nocturnal hemoglobinuria (PNH) with complex evolution and liver transplant

Relato de um caso de hemoglobinúria paroxística noturna (HPN) com evolução complexa e transplante hepático

Railene Célia B. Alencar; Andréa M. Guimarães; Lacy C. Brito Junior

Universidade Federal do Pará (UFPA), Pará, Brazil.

ABSTRACT

The paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired disease, with thrombotic episodes and frequent pancytopenia. We report the case of a 32 year-old female PNH patient with bone marrow aplasia, which followed a complex course, diagnosed with aplastic anemia associated with PNH, evolving in three years with Budd-Chiari syndrome and liver transplantation. Post-transplant complications, hepatic arterial thrombosis, graft rejection, liver retransplantation and treatment of PNH with eculizumab. Clinical stabilization and cessation of symptoms were achieved.

Key words: pure red-cell aplasia; paroxysmal hemoglobinuria; liver transplantation.

INTRODUCTION

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal disease of bone marrow stem-cells, genetically characterized by the somatic mutation in the phosphatidylinositol glycan protein A (*PIG-A*) gene. The *PIG-A* gene is located on the short arm of the X chromosome (Xp 22.1), it extends over 17 Kb and contains 6 exons. It is essential for the formation of glycosylphosphatidylinositol (GPI) anchor, responsible for anchorage and fixation of certain proteins that regulate the complement system in the outer cell membrane surface of erythrocytes, leukocytes and platelets, such as complement decay-accelerating factor (DAF/CD55) and membrane inhibitor of reactive lysis (MIRL/CD59), formerly used to establish the diagnosis of PNH. These markers, yet, lost ground to another marker, the fluorescein-labeled proaerolysin (FLAER), much more specific and precise for the diagnosis of PNH because it binds directly to the GPI anchor. Therefore, deficiency of proteins CD55 and CD59 and of the GPI anchor in PNH cells make them sensitive to lysis upon complement, causing chronic intravascular hemolysis, thus cells deficient in PNH will have some different degrees of deficiency for these proteins, either partial (PNH type II cells, with around 10% of normal expression) or total (PNH type III cells, with complete absence of the protein)[1-3].

The world incidence of PNH is still unknown, but it is estimated to be 1-5 cases per million inhabitants in the USA, a much lower incidence than that of bone marrow aplasia, whose prevalence is 5-10 times higher. Still about statistics in the USA, prevalence does not vary by sex or race/ethnicity. The same occurs in some Asian countries, such as Thailand, Japan, and the Far East, where PNH has lower incidence than bone marrow aplasia. As regards sex, in Europe, PNH is more common in women, whereas in Asia it is more common in men. PNH can occur in any phase of life, just because it is an acquired disease, but it generally affects more young adults aged 35 years on average, and it is more commonly diagnosed between the third and the fifth decades of life[4-6].

In children and teenagers, PNH is observed in just 10% of the diagnosed cases, clinically associated with bicytopenia or pancytopenia, while thrombosis can occur with the same frequency in all age groups. A survival time of 10-15 years is estimated for PNH patients, when not adequately treated; thrombosis and progression of pancytopenia are the leading causes of death in 50%-60% of the cases. Spontaneous remission occurs in 10%-15% of PNH cases; and the evolution of PNH into acute myeloid leukemia can occur in 5% of the patients. Association of PNH clones was observed with other pathologies, such as Coombs-negative hemolytic anemia, bone marrow aplasia, myelodysplastic syndromes, and unexplained thrombosis[1, 4, 5].

The diagnosis of PNH is made by means of clinical findings and laboratory tests to confirm the degree of hemolysis (haptoglobin, lactate dehydrogenase [LDH], direct Coombs test, reticulocyte count and total bilirubin and bilirubin fraction) and deficiency of anchored proteins of the complement system (CD55, CD59 and FLAER) in granulocytes (CD15, CD33 and CD24) and monocytes (CD14 and CD64) by flow cytometry, the gold standard method[6-12].

The objective of this study was to report the case of a patient with PNH associated with bone marrow aplasia, with the course complicated by inferior vena cava thrombosis (Budd-Chiari syndrome), need of liver transplantation, rejection and liver retransplantation.

CASE REPORT

A 32-year-old female patient resident in Manaus (AM) had presented in 1999 severe anemia, purpuric spots on the skin, blood count with 10,000/µl platelets and several episodes of respiratory infection associated with low immunity. She was referred to Fundação Hospitalar de Hematologia e Hemoterapia do Estado do Amazonas (HEMOAM) for specialized care, where she underwent bone marrow examination and biopsy, with a diagnosis of aplastic anemia. Treatments were carried out during six months with cyclosporine, corticosteroid and folic acid; polytransfusions with erythrocyte concentrates (EC) and platelet concentrates (PC) were performed whenever necessary; the patient was enrolled at the National Registry of Bone Marrow Receptors (REREME) for possible hematopoietic stem-cell transplantation (HSCT). After this initial period, with the partial improvement of the patient's condition, HSCT was ruled out, and the patient was given folic acid, ferrous sulfate and blood transfusions, these last ones just during crises.

In 2002, with clinical stabilization, and after three years' treatment, the patient was discharged, yet with anemia (8.9 g/dl hemoglobin) and thrombocytopenia (80,000/mm³), thus remaining for the whole year 2003. In 2004, she was transferred to the city of Belém (PA), due to the worsening of her clinical situation, being referred to Hospital Ophir Loyola (HOL). Then she presented ascites, abdominal pain, nausea, vomiting, dyspnea, tiredness and fatigue, besides hepatic vein thrombosis and hepatic nodules shown in abdominal ultrasound. The diagnosis of Budd-Chiari syndrome (hepatic thrombosis) was reached.

In that period she was treated with oral anticoagulants, without success, due to the repeated hemorrhagic events. Then her medication was changed to injectable anticoagulant (60 mg) and diuretics, with the same surgical procedure being recommended for the placement of a transjugular intrahepatic portosystemic shunt (TIPS) and a suprahepatic venous stenting. Still in 2004 there was aggravation of the patient's status with severe pancytopenia, nasal and gingival bleeding, and hemoglobinuria in the first-morning urine. The hypothesis of PNH was suggested, and confirmatory tests for this disease were conducted. At the time: complete blood count (pancytopenia), Han test (positive), direct Coombs test (negative), and LDH (1,599 U/l), confirming the diagnostic hypothesis of PNH. Due to the seriousness of the case, and to the fact that in Belém, at that time, there were no resources for the patient's treatment, she was referred to Hospital das Clínicas (HC) of São Paulo (SP), with abdominal pain, ascites, thrombosis of the suprahepatic veins, and esophageal varices for diagnostic confirmation and provision of the adequate treatment.

At HC/SP, the diagnoses of Budd-Chiari syndrome and PNH were confirmed, the last one by means of flow cytometry immunophenotyping. The patient's clinical status had worsened with the presence of several hepatic nodules and cirrhosis. In the beginning of 2005, liver transplantation was indicated, and the patient was listed at the central for liver transplantation. However, due to the complexity of her comorbidities, bone marrow aplasia associated with PNH clone, there was very high likelihood of her having a large hemorrhage during transplantation.

In the three subsequent years, while she waited for a liver donor, the patient underwent clinical treatment with anticoagulants, diuretics, folic acid, gastric mucosal protective drugs and low-sodium diet. However, her clinical condition was even worse with the rupture of several esophageal varices needing rubber band

ligation, multiple hypervascular hepatic nodules in advanced state, cirrhosis and hepatic vein thrombosis. At that moment, due to the high death risk, the patient was given high priority for liver transplantation.

In September 2008, she was communicated about the possibility of liver transplantation from a deceased donor, to be performed at Hospital Albert Einstein, São Paulo; the transplantation was successful. Nevertheless, 15 days after the procedure, already during supportive care, she presented hepatic artery thrombosis, undergoing another liver transplantation, because of graft failure. In the second transplantation, the patient's condition worsened with the occurrence of severe esophageal hemorrhage, and she was again subject to corrective surgery and was put in induced coma for a week. After the coma period, she had progressive improvement, but was still kept at the intensive care unit (ICU) for a month.

With stabilization of symptoms, after two months' hospitalization, and the new graft responding well to immunosuppression, supportive care and continuous use of anticoagulants, the patient was discharged from hospital. However, as she was still enfeebled, and due to a weak immune system, the episodes of infection by cytomegalovirus and respiratory problems were frequent. She was followed up to the end of 2011 as an outpatient by hematologists and by the liver transplant team.

In the beginning of 2012, after her return to Belém, Pará, she again presented strong pain in abdomen and lower limbs, nausea, vomiting, and weakness due to crises associated with PNH. She searched a hematologist, who ordered new complementary exams and search of PNH clones by flow cytometry. This showed the substantial loss of expression of antigens CD59 in erythrocytes (type III clone), CD24 in granulocytes, CD14 in monocytes, and FLAER (aerolysin) in granulocytes and monocytes (type II clone). Treatment with monoclonal antibody eculizumab was urgently suggested, for she presented imminent risk of a new thrombotic episode. However, just in the middle of 2013, she obtained the right to receive the medication and begin the treatment, through a lawsuit filed at the Brazilian Public Ministry. She used tacrolimus daily and eculizumab fortnightly.

Nowadays, after two years' treatment with eculizumab, and seven years after the second transplantation, the patient, now 32 years old, is stable with significant improvement in anemia (11.2 g/dl hemoglobin), thrombocytopenia (104,000/mm³ platelets) and LDH levels (235 U/l), and reduction in PNH clone size, besides stabilization of liver function. On physical examination, she remains well and asymptomatic, living a normal life and having resumed her daily routine.

DISCUSSION

The first reports on the association between PNH and bone marrow aplasia were made by Dacie *et al*. (1963)[13] and Lewis *et al*. (1967)[14], but only with the adoption of PNH clone assessment by flow cytometry this association became more evident. Nowadays reports on the presence of small to moderate populations of cells associated with PNH clones are frequent in up to 70% of the patients with acquired bone marrow aplasia[14].

Historically, the studies of Valla *et al*. (1987)[15] already demonstrated that around 12% of thrombosis cases of hepatic veins or Budd-Chiari syndrome were associated with PNH patients, this being the leading cause of mortality in these patients when not timely treated.

According to Martens and Nevens (2015)[16], Budd-Chiari syndrome is associated with a thrombotic tendency, generally manifested as episodes of venous thrombosis in great vessels as brain vessels, mesenteric and supra-hepatic veins in PNH patients. It can be clinically recognized, in cases of hepatic thrombosis, by recurrent crises of abdominal pain, ascites, painful hepatomegaly and fever.

Based on literature reports[3, 16, 17], the association of PNH, bone marrow aplasia and Budd-Chiari syndrome, as described in this case report, is a rare condition.

Data by Arruda *et al*. (2010)[1] show that cases of thrombosis can occur in more than half of PNH patients after 15 years of diagnosis, with higher incidences in Latins and Afro-Americans than among Orientals. They also indicate that around 40% of PNH patients will present any thrombotic event during life. This risk is greater in patients that present large PNH clones, mainly due to excessive release of hemoglobin in the plasma, leading to an increase in nitric oxide causing dysphagia (difficulty swallowing), respiratory syndromes, fatigue, esophageal spasm, erectile dysfunction, and thrombosis[7].

However, what makes the current case more intriguing is the fact that it is a young patient with severe thrombosis in portal vein and Budd-Chiari syndrome that culminated in the need for a liver transplantation, graft rejection due to thrombosis in hepatic artery and need for retransplantation; this condition of hepatic artery thrombosis is a rare occurrence[16].

As also demonstrated in this case report, before the appearance of eculizumab, treatment for PNH was more life supportive

than curative, as well as the practice of blood transfusions and replacement of folic acid and iron due to anemia; low-dose corticosteroid therapy to decrease hemolysis; use of anticoagulants in patients with large PNH clones, aimed at reducing the risks of thrombotic complications[1, 2, 17].

Eculizumab was first approved for use in humans by the European Commission and by the Food and Drug Administration (FDA) and made available in the market for treatment of PNH in 2007. This drug acts as a human monoclonal antibody that inhibits terminal complement activation at C5 and avoids formation of C5a and the C5-9 complex of membrane attack, being considered the first effective therapy against PNH clones. Its high efficacy has already been demonstrated in the control of intravascular hemolysis, reduction of need for blood transfusion and consequently in the stabilization of hemoglobin and LDH levels, especially in patients with classic PNH. This reduces thrombosis risks considerably during treatment in around 85% of the cases. However, patients on this drug are not free of adverse events, such as headaches, nasopharyngitis, back pains, and respiratory tract infections, due to, principally, the inhibition of the terminal complement system, a fact that leaves patients more vulnerable to infections by *Neisseria* and with necessity of immunization against *Neisseria meningitidis* two weeks before beginning treatment[7, 18].

Studies also reveal that patients with bone marrow aplasia associated with PNH are less prone to respond to eculizumab, seen that the main mechanism of anemia, in these cases, is bone marrow suppression, instead of complement-mediated hemolysis[7]. However, in the present case, even with bone marrow aplasia and PNH, the patient presented excellent results with eculizumab treatment, with apparent symptom remission and improvement of her quality of life.

Arruda *et al*. (2010)[1] state that the allogenic HSCT (allo-HSCT) is the only curative treatment for PNH patients. However, most patients with PNH do not have a compatible donor, a fact that many times makes this therapy unviable, besides associating it with high rates of morbidity and mortality. Therefore, allo-HSCT is more indicated for patients with bone marrow failure syndrome, severe cytopenias, and as the first treatment for children and teenagers with PNH and aplastic anemia.

Concerning the patient of the current report, nowadays she is clinically stable, in child-bearing age, and with no limitations of her daily activities. But other issues are raised for a 32-year-old patient, as the possibility of pregnancy, which, in this case, is totally inadvisable, mainly because pregnancy in PNH patients represents a high-risk situation, due to the probability of thrombosis, miscarriages and maternal death during and after childbirth. Thromboembolism in PNH patients during pregnancy has an estimated risk of around 8%-10% of the cases[2, 4].

CONCLUSION

Due to the fact that PNH is a rare disease, with clinical evolution so variable that it can be confounded with other pathologies, and the high rate of morbidity and mortality associated with thrombosis, the precise diagnosis of the disease and the early beginning of treatment are crucial for longer survival of the patient.

In the presented case report, several complications happened during the disease course, but the expertise of the medical staff that cared for the patient from diagnosis, transplantation, supportive therapy up to early beginning of eculizumab treatment was important for her to resume normal life.

This study reinforces the necessity and the importance of a multidisciplinary follow-up of patients with PNH.

RESUMO

Hemoglobinúria paroxística noturna (HPN) é uma doença rara, adquirida, com episódios trombóticos e pancitopenia frequente. Relatamos o caso de uma paciente jovem, 32 anos, sexo feminino, portadora de HPN e aplasia de medula, com evolução complexa e diagnóstico de anemia aplástica associada à HPN, evoluindo em três anos com síndrome de Budd-Chiari e transplante hepático. Complicação pós-transplante, trombose arterial hepática, rejeição do enxerto, retransplante hepático e tratamento da HPN com eculizumab. Obtiveram-se estabilização clínica e cessação dos sintomas.

Unitermos: aplasia pura de série vermelha; hemoglobinúria paroxística; transplante de fígado.

REFERENCES

1. Arruda MMAS, Rodrigues CA, Yamamoto M, et al. Hemoglobinúria paroxística noturna: da fisiopatologia ao tratamento. Rev Assoc Med Bras. 2010; 56(2): 214-21.

2. Garcia AA, Franco RF. Trombofilias adquiridas. Hemostasia e trombose. Medicina, Ribeirão Preto. 2001; 34: 258-68.

3. Brodsky RA. Paroxysmal nocturnal hemoglobinuria: stem cells and clonality. Hematology Am Soc Hematol Educ Program. 2008; 111-5.

4. Nomura ML, Surita FGC, Parpinelli MA, et al. Hemoglobinúria paroxística noturna e gravidez. Rev Bras Ginecol Obstet. 2004; 26(7): 579-82.

5. Brito Junior LC, Cardoso MS, Rocha EG, et al. Frequency of paroxysmal nocturnal hemoglobinuria in patients attended in Belém, Pará, Brazil. Rev Bras Hematol Hemoter. 2011; 33(1): 35-7.

6. Modesto TM, Neves MAB, Brito AE, et al. Importância e vantagem da citometria de fluxo frente aos testes de triagem no diagnóstico da hemoglobinúria paroxística noturna. Rev Bras Hematol Hemoter. 2006; 28(4): 275-9.

7. Brodsky RA. Advances in the diagnosis and therapy of paroxysmal nocturnal hemoglobinuria. Blood Rev. 2009; 22(2): 65-74.

8. Höchsmann B, Rojewski M, Schrezenmeier H. Paroxysmal nocturnal hemoglobinuria (PNH): higher sensitivity and validity in diagnosis and serial monitoring by flow cytometric analysis of reticulocytes Ann Hematol. 2011; 90: 887-99.

9. Urbano-Ispizua A, Gaya A, Colado E, et al. Diagnóstico y tratamiento de la hemoglobinuria paroxística nocturna. Med Clin (Barc). 2011; 136(3): 121-7.

10. Sutherland DR, Keeney M, Illingworth A. Practical guidelines for the high-sensitivity detection and monitoring of paroxysmal nocturnal hemoglobinuria clones by flow cytometry. Cytometry B Clin Cytom. 2012; 82(4): 195-208.

11. Battiwalla M, Hepgur M, Pan D, et al. Multiparameter flow cytometry for the diagnosis and monitoring of small GPI-deficient cellular populations. Cytometry B Clin Cytom. 2010; 78(5): 348-56.

12. Borowitz MJ, Craig FE, DiGiuseppe JA, et al; on behalf of the Clinical Cytometry Society. Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry. Cytometry B Clin Cytom. 2010; 78: 211-30.

13. Dacie JV, Lewis SM. Paroxysmal nocturnal haemoglobinuria: variation in clinical severity and association with bone-marrow hypoplasia. Br J Haematol. 1963; 7: 442-57.

14. Lewis SM, Dacie JV. The aplastic anaemia – paroxysmal nocturnal haemoglobinuria syndrome. Br J Haematol. 1967; 13(2): 236-51.

15. Valla D, Dhumeax D, Babany G, et al. Hepatic vein thrombosis in paroxysmal nocturnal hemoglobinuria: a spectrum from asymptomatic occlusion of hepatic venules to fatal Budd-Chiari syndrome. Gastroenterology. 1987; 93: 569-75.

16. Martens P, Nevens F. Budd-Chiari syndrome. United European Gastroenterol J. 2015; 3(6): 489-500.

17. Araújo CJ, Soares FVM, Rocha FD, et al. Hemoglobinúria paroxística noturna: relato de dois casos. Rev Bras Hematol Hemoter. 2002; 24(4): 1-7.

18. Dmytrijuk A, Robie- Suh K, Cohen MH, et al. FDA report: eculizumab (Soliris) for the treatment of patients with paroxysmal nocturnal hemoglobinuria. Oncologist. 2008; 13: 993-1000.

CORRESPONDING AUTHOR

Lacy Cardoso de Brito Junior
Universidade Federal do Pará; Instituto de Ciências Biológicas; Laboratório de Patologia Geral, Imunopatologia e Citologia; Av. Augusto Corrêa, 01; Guamá; CEP: 66075-900; Belém-PA, Brasil; e-mail: lcdbrito@ufpa.br.