World Journal of Medical Sciences 5 (2): 36-40, 2010
ISSN 1817-3055

Paroxysmal Nocturnal Hemoglobinuria: Case Report and Review of the Literatures

[1]F. Al-Sayes, [2]F. Azhar and [3]S. Hindawi

[1]Consultant Hematologist, Faculty of Medicine,
King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia
[2]Registrar at Department of Hematology, Faculty of Medicine,
King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia
[3]Consultant Hematologist, Transfusion Medicine, Faculty of Medicine,
King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia

Abstract: Paroxysmal nocturnal hemoglobinuria is a rare disease. It is an acquired clonal stem cell disorder caused by mutation in the X-linked PIG-A gene, that manifests itself with intravascular hemolysis, bone marrow failure, thrombosis and smooth muscle dystonias. In this study, we report a young male who presented with a history of easy fatigability, dizziness, jaundice and pallor. He was diagnosed as having severe hemolytic anemia on clinical and laboratory basis. The diagnosis of Paroxysmal nocturnal hemoglobinuria was confirmed by immunophenotyping of granulocytes and red cell which were negative for CD59 and CD55 in 52% of the cells. He is currently on supportive transfusions of Packed Red Blood Cells folic acid and iron supplement. He has been started on oral anticoagulation with warfarin as he suffered from pulmonary embolism followed by hepatic vein thrombosis (Budd Chiari Syndrome) despite therapeutic anticoagulation.

Key words: PNH · (PRBCs) transfusion · Budd Chiari syndrome

INTRODUCTION

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hemolytic anemia caused by the expansion of a hematopoietic progenitor cell that has acquired a mutation in the X-linked PIG-A gene. The *PIG-A* gene product is required for the biosynthesis of glycophosphatidylinositol anchors, a glycolipid moiety that attaches dozens of proteins to the plasma membrane of cells. Consequently, the PNH stem cell and all of its progeny have a reduction or absence of glycosyl phosphatidylinositol (GPI)–anchored proteins. Two of these proteins, CD55 and CD59, are complement regulatory proteins; the absence of these proteins is fundamental to the pathophysiology of the disease [1]. Bone marrow failure and the presence of the abnormal cells account for the clinical phenotype of patients with PNH, including haemolysis, cytopenia and thrombophilia [1].

The pathogenesis of the disease involves CD55 that inhibits C3 convertases and CD59 that blocks formation of the membrane attack complex (MAC) by inhibiting incorporation of C9 into the membrane attack complex. The loss of complement regulatory proteins renders PNH erythrocytes susceptible to both intravascular and extra-vascular haemolysis, but it is the intravascular haemolysis that contributes to much of the morbidity and mortality from the disease. Intravascular haemolysis releases free haemoglobin into the plasma. Free plasma haemoglobin scavenges nitric oxide and depletion of nitric oxide at the tissue level contributes to numerous PNH manifestations, including oesophageal spasm, male erectile dysfunction, renal insufficiency and thrombosis [2]. PNH can arise de novo or in the setting of aplastic anemia (AA) [3].

The natural history of PNH is that of a chronic disorder but highly variable, ranging from indolent to life-threatening. The diagnosis of PNH is most frequently made in young adults; however, PNH occurs also in the elderly and in children. The disease affects both genders equally. The median survival has been estimated to be about 10 to 15 years [4,5]. Thrombosis is the most frequent cause of death, but others may die of complications of bone marrow failure, renal failure,

Corresponding Author: F. Al-Sayes, Consultant Hematologist, Faculty of Medicine,
King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia,
P.O. Box 80215 Jeddah 21589, Saudi Arabia, E-mail: fatinsayes@yahoo.com

myelodysplastic syndrome and leukaemia [1]. About 10% to 15% of patients show spontaneous remission, which may occur even after many years of disease.

Therapy is to treat and prevent complications. Supportive transfusions for treatment of anaemia's, anticoagulation for prevention and treatment of thrombosis or immunosuppressant therapy for bone marrow failure. Hemopoietic stem cell transplantation (HSCT) and complement inhibition with eculizumab are the only proven effective therapies [1].

Here we report a young male who is diagnosed as PNH and developed pulmonary embolism, followed by hepatic vein thrombosis (Budd-Chiari syndrome) despite being warfarin with therapeutic anticoagulation.

Case Report: A previously known healthy 36 year old Palestinian male presented to haematology clinic in November 2007, with few months history of easy fatigability, dizziness, shortness of breath on minimal exertion, yellowish discolouration of sclera and history of passing coffee coloured urine on and off associated with abdominal pain. There was no history of travel or use of medication, no history of fever, weight loss. He gave history of similar complaints 2 years back for which he presented in other hospital and discovered to have low haemoglobin 4g/dl and received supportive transfusions and corticosteroid at 1 mg/kg/day devided doses for 3 weeks with no improvement what so ever.

On examination he was significantly pale, jaundiced. chest and CNS examination were unremarkable, cardiovascular examination showed mild tachycardia and ejection systolic murmur. Abdominal examination showed hepatomegaly of 2 cm below costal margin.

Laboratory Examination Disclosed the Following Findings: WBC: 4.1 x 10^9/L (4,l00/mm); Hb: 5.7g/dl; MCV: 93fl.; MCH 31 pg; RDW: 31.4% Platelets: 104 x 10^9/L; Reticulocytes 10%; Peripheral blood film revealed: polychromasia; otherwise no significant finding; DCT: negative; G6PD quantitation: normal; Hb electrophoresis:A/A; Serum iron: 14 umol; serum ferritin: 12.39 ng/m (NR 30-400 ng/ml); serum folate: 29.58 nmol/L (NR 7-40 nmol/L); Serum B12 276pmol/L; Biochemical studies showed normal electocyte and renal function. Liver function tests were normal except for high AST: at 105µ/L (NR 15-37 µ/L) and Total Bilirubin: 83µmol/L (NR 0-17 umol/L); and was mainly indirect. Flowcytometry from peripheral blood showed lack of CD_{55}, CD_{59} on 52% of RBC's, Bone marrow aspiration and trephine biopsy showed hypercellular reactive marrow with moderate erythroid Hyperplasia, no dysplastic changes in all three cell lines; Immunophenotyping on bone marrow cells revealed deficient CD_{55} and CD_{59} cells. Antinuclear antibody, DS DNA were negative; Urine analysis showed brown turbid colour with RBC of 3/HPF microscopically and 4+ urobilinogen; Ultrasound abdomen revealed mildly enlarged liver measuring 15.9 cm with coarse Echotexture; Splenomegaly measuring around 13 cm in largest longitudinal and transverse diameter. No focal lesion seen. His kidneys, pancreas and other organs were within normal. Echocardiogram was normal. Patient has been reviewed in the outpatient. He was counselled and educated about his rare disease and treatment options. He was started on folic acid 5 mg orally with iron supplement. He received supportive packed rbc's transfusion after red blood cell phenotype. HLA typing of patient and his sibling was done.

In April 2008 patient presented to our ER with a complaint of chest pain shortness of breath associated with right flank pain of 4 days duration. There was no history of fever or cough. The right flank pain was dull aching in nature with no associated symptoms. On examination, patient were in pain with 0_2 saturation 98% at room air, with jaundice and pallor. Chest examination showed decreased air entry on right side of chest. No added sounds. Heart assessment was unremarkable. Abdominal examination showed mild hepatosplenomegaly and Rt. Flank tenderness

CBC: WBC: 6.7 x 10^9/L; Hb: 9.3g/dl; Platelet count: 79 x 10^9/L Peripheral blood film revealed dimorphic features

Coagulation Profile: PT-12.2 sec.; APTT 28.0 sec. INR-1; Fibrinogen: 70/mg/dl; D-Dimer: 643.5µg/L _(0-170µg/L) Cardiac enzymes were normal. ABG:0_2 98%. CT pulmonary angiogram revealed filing defect in right interlobar artery confirming the diagnosis of pulmonary embolism. CT scan abdomen and pelvis revealed left cortical nephrocalcinosis and lung window showed bilateral pleural effusion with right sided basal consolidation.

Initially patient received anticoagulation with therapeutic dose of heparin followed by warfarin with target of INR of 2.5.

Urology team was consulted for flank pain for which he received conservative management with hydration and analgesia.

Patient has been followed up in hematology clinic for anticoagulation monitoring and has been receiving supportive PRBC transfusions to maintain Hb level >7g/dl and was well anticoagulated.

In June 2008 patient presented again to our hospital emergency room with increasing abdominal pain and abdominal distension, lower limb edema involving scrotum for past 10 days. He gave history of passing dark brown colour urine and increasing jaundice. There was no shortness of breath. Chest pain, cough or hemoptysis.

On examination, he was pale, jaundiced, bilateral lower limb pitting edema extending up scrotum. Chest heart, CNS examination were normal abdominal examination revealed ascites with hepatosplenomegaly.

Lab Investigation Disclosed the Following Findings: CBC: WBC: 3.2 x 10^9/L; Hb: 6.8g/dl; Platelet 121 x 10^9/L

Coagulation Profile: PT:38.7 sec.; APTT 45.1 sec. INR: 3.2; D-Dimer: 7.14 ng/L (0-0.55)

Electrocytes and renal functions were normal; Liver function test showed albumin of 32g/L (NR 34-50 g/L) AST: 115μ/L (NR 15-37 μ/L); ALT: 172 μ/L (NR 30-65 μ/L); GGT: 139 μ/L (NR 5-85 μ/L).

Total Biliburin: 102 μmol/L mainly indirect: direct bilirubin was 22 μmol/L.

CT of abdomen and pelvis revealed enlarged and swollen liver with heterogenous patchy abnormal parenchymal enhancement. The intra-hepatic inferior vena cave is compressed and slit like in appearance with obliteration and non visualization of hepatic vein. The patient was diagnosed clinically and radiologically as hepatic vein thrombosis (Budd-Chiari syndrome).

He was treated with diuretics and received anticoagulation with warfarin to increase his target INR to 3.5.

DISCUSSION

PNH is an acquired stem cell disorder with protein manifestation of hemolytic anemia. Pancytopenia, smooth muscle dystonia and thrombosis. It is classified into classic (hemolytic), PNH in the setting of another bone marrow disorder and subclinical [1].

Classic PNH presents with clinical manifestation of intravascular hemolysis in the form of anemia, hemoglobinuria, fatigue, headache, cholelithiasis, recurrent urinary tract infection, esophagospasms and erectile dysfunction. Diagnosis of PNH classically was by Ham's test. Current well established diagnostic tools include flowcytometry analysis to show negative CD_{55} and CD_{59} on peripheral blood red cells and granulocytes. It has big advantage which is determining the degree of glucoprotein 1-anchor deficiency. Other diagnostic method is FLEAR (fluorescently labeled inactive toxin areolysin) off course laboratory evidences of hemolysis are usually supportive of the diagnosis [2].

Our patient presented with the features of classic PNH. He presented with pancytopenia with evidence of hemolysis like high LDH, increase unconjugated bilirubin. Reticulocytosis cholelithiasis and erythroid hyperplasia in the bone marrow. Flocytometric analysis confirmed the lack of CD_{55} and CD_{59} on 52% of RBC's and granulocyte. The management of PNH is by supportive packed red blood cell transfusion to a defined Hb-level suitable for the patient clinical condition and style of life as well as prevention of thrombosis by prophylactic anticoagulation. Corticosteroids can be used for both chronic hemolysis and acute hemolytic exacerbation. Response can be seen within 24 hours of initiating therapy, which could be as a direct result of inhibition of some component of alternate pathway or indirectly by decreasing inflammation [2]. Hemopoietic stem cell transplantation is curative but considered only for patients with bone marrow failure and major complications of PNH. PNH specific transplant related issues should be considered like appropriate conditioning regimen depending on type of PNH and type of donor. Overall survival for unselected PNH patients who undergo transplantation using HLA-matching sibling donor is in the range of 50% -60% [2].

Our patient received supportive treatment by packed RBC transfusion to increase hemoglobin concentration and to ameliorate hemolysis by suppressing erythropoiesis. Iron supplementation and folic acid to compensate for increased erythropoiesis and excessive iron loss, however, iron supplementation was given cautiously as it increases the rate of hemolysis.

Natural history of PNH is that median survival is 10-15 years and the prognosis is worse in patient with history of thrombosis or severe pancytopenia.

Thrombosis is the leading cause of death which occurs in one third of patient and about 5% of cases evolve to MDS/AML [2].

Patient with PNH have increased propensity for the development of life threatening venous thrombosis, particularly cerebral, hepatic, portal mesenteric, splenic and renal veins. About 40% of patient experience venous thrombotic event during the course of their disease [3,4]; more than 50% in the patient with a large PNH clone. Pathogenesis of thrombosis is multifactorial like increase procoagulant and fibrinolytic activity suggesting increase fibrin generation and turnover [5], deficiency of

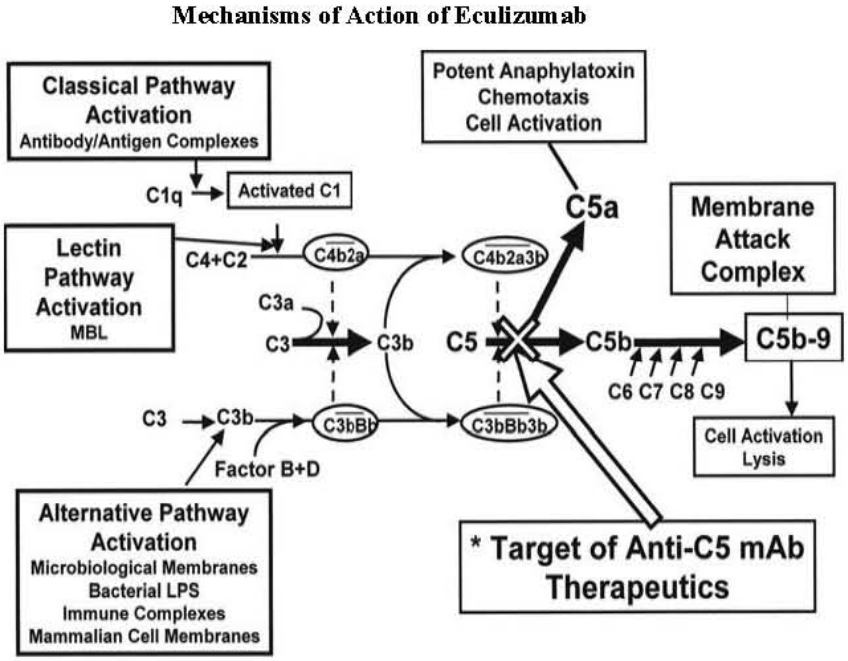

Fig. 1: The complement cascade and eculizumab

fibrinolytic system including deficiency of urokinase-plasminogen activator receptor [6], nitric oxide deficiency secondary to scavenging affecting of the plasma hemoglobin etc [7]. Interestingly thrombosis in PNH patient frequency follows episode of acute hemolysis suggesting that inadequate complement inhibition or hemolysis might be an important underlying factor contributing to thrombophilia [1]. Results from the international trials using complement inhibitors for treatment of hemolysis, demonstrated that inhibition of intravascular hemolysis reduced thrombosis in PNH patient [8].

Our patient developed pulmonary embolism for which he received anticoagulation with a target INR of 2.5. Then he developed hepatic vein thrombosis (Budd-Chiair syndrome), despite being well anticoagulated. He has been treated with raised target INR to 3.5. This has been reported in many literatures, patients with PNH appears to be especially at risk for Budd-Chiari syndrome more than other form of thrombophilia. Data from a recent review report hepatic vein thrombosis, leading to Budd-Chiari syndrome as the most frequent (40.7%) thrombotic complication in PNH accounting for the majority of death, followed by cerebral vein and sinus thrombosis [9]. Even reoccurrence of Budd-Chiari syndrome has been reported after liver transplant in PNH [10].

Eculizumab is humanized monoclonal antibody against complement C5.

The complement cascade culminating in the production of the membrane attack complex that results in the lysis of the cell. The cleavage of C5 is the pivotal point of the pathway. Inherited deficiencies prior to C5 result in recurrent pyogenic infections and autoimmune disorders, whereas deficiencies after C5 have remarkably little effect except for an increased risk of infection by encapsulated organisms. The site of blockage of eculizumab is demonstrated [14].

Results from randomized clinical trials like (TRIUMPH) [15] showed improved anemia as measured by stabilized hemoglobin levels and reduced transfusion requirement over 6 months of treatment. The effect on chronic intravascular hemolysis was demonstrated by an immediate and sustained decrease in LDH level. Other clinical symptoms such as abdominal pain, dyspepsia, erectile dysfunction, rate of thrombosis have also been reported to improve during eculizumab therapy with improvement in quality of life. These results are also supported by another study (SHEPARD'S) [11, 12].

Adverse effects of eculizumab include increase risk of infection by encapsulated organisms particulary nesserie meningitides [13]. Patients have to be vaccinated before commencement of eculizumab but the risk will not be completely abolished and therefore vigilance for the symptoms of meningitis or septicaemia is essential. This occurs in approximately 0.5 cases per 100-patient years on eculizumab [16] There is also theoretical risk of potential

intense hemolysis if eculizumab treatment is stopped, as the number of PNH red cell increase because of their protection from complement mediated destruction. Patient who elect to discontinue eculizumab treatment should be carefully monitored and possibly transfused with normal red cells depending on clinical circumstances [14].

The main problem is that the treatment is indicated for long term probably for life and it is costly. Currently eculizumab is indicated for patients with moderate to severe PNH. Those who are at risk or developed complications like thrombosis, renal failure, significant fatigue or poor quality of life [14].

Novel agents under study are replacement of complement regulatory proteins on PNH cells like recombinant transmembrane form of CD_{59} (CD_{59}TM), artificial glycolipid anchor (prodaptin) to anchor CD_{59} (prodaptin CD_{59}) into the cell membrane as well as gene therapy.

Spontaneous remission is known to occur in around 25% of patient during the course of their disease, making the decision to treat radically by HBMT very difficult one. The new modality of treatment (eculizumab) is quite expensive and available only in certain reference centers.

CONCLUSION

This case is unusual because it presents a young man with a diagnosis of classical PNH who developed deep vein thrombosis at two different sites. Hepatic thrombosis (Budd-Chiari syndrome) emerged while on therapeutic anticoagulation.

REFERENCES

1. Bessler, M. And J. Hiken, 2008. The Pathophysiology of Disease in Patients with Paroxysmal Nocturnal Hemoglobinuria. ASH.
2. Parker, C., M. Omine and S. Richards, 2005. Diagnosis and management of paroxysmal nocturnal hemoglobinuria. Blood, 106: 12.
3. Ware, R.E., S.E. Hall and W.F. Rosse, 1992. Paroxysmal nocturnal haemoglobinuria with onset in childhood and adolescence. N. Engl. J. Med., 325: 991-996.
4. Socie, G., J.Y. Mary, A. de Gramont, *et al*., 1996. Paroxysmal nocturnal haemoglobinuria: long-term follow-up and prognostic factors. French Society of Haematology. Lancet., 348: 573-577.
5. Grunewald, M., A. Siegemund, A. Grunewald, *et al.,* 2003. Plasmatic coagulation and fibrinolytic system alterations in PNH: relation to clone size. Blood Coagul Fibrinolysis., 14: 685-695.
6. Gao, W., Z. Wang, X. Bai, Y. Li and C. Ruan, 2002. Diagnostic significance of measurement of the receptor for urokinase-type plasminogen activator on granulocytes and in plasma from patients with paroxysmal nocturnal hemoglobinuria. Int. J. Hematol., 75: 434-439.
7. Lancaster, J.R., Jr, 1997. A tutorial on the diffusibility and reactivity of free nitric oxide. Nitric Oxide, 1: 18-30.
8. Hillmen, P., P. Muus, U. Duhrsen, *et al.,* 2007. Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria. Blood, 110: 4123-4128.
9. BCS in PNH patient, 2009. Antonella Tufano, Nicola Macrone Palieri. Int. Emerg. Med., 4: 75-77.
10. Reoccurrence of BCS after liver transplant in PNH, 2005. Mathias J. Bahr jlrg Schubert jorg s Bleck, Transplant International, 16: 12.
11. Brodsky, R.A., N.S. Young, E. Antonioli, *et al.,* 2008. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. Blood, 111: 1840-1847.
12. Schubert, J., P. Hillmen, A. Röth, *et al*., 2008. Eculizumab, a terminal complement inhibitor, improves anaemia in patients with paroxysmal nocturnal haemoglobinuria. Br J. Haematol., 142: 263-272.
13. Ross, S.C. and P. Densen, 1984. Complement deficiency states and infection: epidemiology, pathogenesis and consequences of neisserial and other infections in an immune deficiency. Medicine (Baltimore), 63: 243-273.
14. The role of complement inhibition in PNH peter Hillmen, ASH, 2008.
15. Hillmen, P., Hallc, J.C.W. Marsh, *et al*., 2006. Effect of Ecluzimab on hemolysis and transfusion requirement in PNH New England J. Med., 355: 1233-1243.
16. Socie, G., P. Hillnen, Muusp, *et al.,* 2007. Sustained improvements in transfusion requirements, fatigue and thrombosis with eculizumab treatement in PNH (abstract) blood 110. Abstract #3672.