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Case Report

Paroxysmal Nocturnal Hemoglobinuria: Two Reports from Togo

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Abstract

We report two cases of paroxysmal nocturnal hemoglobinuria in a hemolytic form. Deep vein thrombosis was one of the major complications in one of the observations. Vitamin B9 deficiency due to overconsumption must also be detected and treated. The treatment issues were linked to the lack of geographical and economic availability of specific treatments. The transfusion support from to these patients exposes them to additional adverse effects, leading to worse prognosis of the disease despite the current relative stability.

Keywords: Paroxysmal nocturnal hemoglobinuria; Hemolysis; Thrombosis; Lomé-Togo

Introduction

Paroxysmal Nocturnal Hemoglobinuria (PNH) or Marchiafava-Micheli disease is an acquired, clonal disease of one or more hematopoietic stem cells and is due to a mutation in the Phosphatidylinositol Glycane class A (PIG-A) gene. This mutation results in a defect synthesis of the Glycosylphosphatidylinositol (GPI) anchor and a lack of binding to GPI-anchored proteins on the plasma membrane of hematopoietic cells. Deficiency of the GPI-anchored complement regulatory proteins CD55 or DAF (decay-accelerating factor) and CD59 or MIRL (membrane inhibitor of reactive lysis) is central to the pathophysiology of the disease [1-3]. PNH is characterized by the association, to varying degrees, of pancytopenia, type of the bone marrow aplasia, a predominantly nocturnal hemolytic anemia with negative Coombs' tests, and a propensity to develop thrombosis. Diagnosis is based on identification of defective membrane proteins, notably CD55 and CD59, by flow cytometry. Treatment includes hematopoietic stem cell transplantation for severe forms, immunosuppressive therapy for aplastic forms and eculizumab for hemolytic forms [1]. In 1882, Paul Strübing identified the disease as a new pathological entity, reporting in detail on a case of PNH after other authors. He hypothesized intravascular hemolysis with the presence of pigment in the urine (hemosiderinuria) linked to fragility. Similar cases were successively described by other authors, including Marchiafava in 1911 and in 1928 Micheli, whose names were assigned to the disease. Eneking in Holland introduced the term "paroxysmal nocturnal hemoglobinuria" [4]. It's a rare desease, with around 450 cases diagnosed in France over a 40-year period [1]. In Africa, descriptions are rare; in Morocco, one study reported 11 cases of PNH [5] and one case was described in Senegal as the etiology of pancytopenia [6]. In Togo, no description has yet been made to our knowledge. The general aim of this work is to report observations of PNH for the first time in Togo.

Observations

Observation N°1

Mrs LM, O positive rhesus blood group, nulligate, nulliparous, retailer, born on 20th August 1982, single with no children, referred from cardiology department to hematology on 10th August 2021 for chronic aregenerative normocytic normochromic anemia (hemoglobin level 4g/dl). She had consulted cardiology on 08th January 2019 for a warm, painful swelling of the left lower limb. Clinical examination revealed a lack of ballottement of the

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left calf with the presence of Homans' sign. Lower-limb venous echodoppler revealed suropopliteal and external femoro-iliac deep venous thrombosis on the left, and suropopliteal and superficial femoral deep venous thrombosis on the right. She was then admitted to hospital for treatment.

The blood count made in cardiology showed peripheral bicytopenia with aregenerative normocytic hypochromic anemia (hemoglobin 8.2 g/dl, MCV 82.6 fl, MCH 25.4 pg, MCHC 29% and reticulocytes 47300/mm^3) associated with thrombocytopenia 99000/mm^3. The myelogram revealed an erythroblastic marrow.

The work-up for congenital thrombophilia (antithrombin, protein C and protein S) or acquired thrombophilia (anti-nuclear speckled antibodies) was negative. HIV, hepatitis B and C serologies were negative. The patient was put on anticoagulant therapy with low-molecular-weight heparin, followed by anti-vitamin K drugs. The thrombosis progressed very well under treatment. The patient also received several transfusions of packed red blood cells for anemic decompensation. Throughout follow-up, no hemoglobinuria or other signs of hemolytic anemia were reported. Because of persistent anemia with hemoglobin levels between 3.5 and 7 g/dl, she was referred to the clinical hematology department for further management.

Clinical examination on admission to haematology revealed asthenia and pale anicteric conjunctivae. She had a normal body temperature of 37°C, a weight of 68 kg for a height of 1.65 m, body mass index of 24.97 kg/m^2, oxygen saturation 98%, blood pressure 110/80 mmHg, heart rate 80 beats/min. The rest of the physical examination was normal. There was no evidence of hemoglobinuria. The blood count showed normocytic normochromic non-regenerative anemia (hemoglobin=4g/dl, MMV=92.9fl, MCH=28.6pg, MCHC=33%, reticulocytes=75900/mm^3), leukoneutropenia (leukocytes=3100/mm^3, neutrophils=1333/mm^3) and a normal platelet count of 196000/mm^3. The rest of the laboratory work-up showed the anemia to be hemolytic, with LDH elevated to 1750IU/ml, and haptoglobin plummeting (below 0.1mg/l). In contrast, bilirubin fractions were normal. Direct and indirect Coombs tests were negative. Serum vitamin B12 was normal at 772.2 pg/ml (Norms: 200 to 1000 pg/ml).

Mrs LM received transfusions of packed red blood cells and prednisone-based corticosteroid therapy at 1mg/kg/day for a period of three months. This corticosteroid therapy reduced the need for transfusions and enabled a hemoglobin level of 7g/dl to be achieved, but the improvement was short-lived (3 months), with rapid onset of corticosteroid resistance.

The occurrence of a new episode of Deep-Vein Thrombosis (DVT) in May 2022, and the persistence of the hemolytic anemia associated with other fluctuating cytopenias, prompted a search for an PNH clone, which was carried out on 03rd November 2022 at the CERBA laboratory (France). The HPN clone was detected in the leukocyte populations tested, estimated at 85.32% for neutrophils and 84.68% for monocytes, confirming the diagnosis of HPN. Therapeutically, neither eculizumab nor hematopoietic cell transplantation was accessible to the patient (technical platform and financial difficulties). She was therefore given a supportive treatment based on transfusions of packed red blood cells as needed. Anticoagulant therapy with acenocoumarol is ongoing, and a switch to Direct Oral Anticoagulants (DACs) is being considered. The thrombosis has progressed favorably (no new thrombosis episodes), but the anemia is unfavorable, with hemoglobin levels fluctuating between 5.7g/dl and 6.2g/dl.

Observation N2

Mr A.Y., student, born on 11th December 1997, O rhesus positive blood group, was referred from the internal medicine department on 04th June 2020 for anaemia at 4.1 g/dl normocytic normochromic argerative, evolving for 3 years and having required several transfusions of erythrocyte concentrates. He had no family history and carried an AA hemoglobin phenotype.

Clinical examination revealed an altered general condition (asthenia with icteric pale conjunctivae), a normal body temperature of 37.5°C, a weight of 67 kg for a height of 1.72 m, a body mass index of 22.64 kg/m^2. Oxygen saturation was 99%, blood pressure 110/80 mmHg and heart rate 68 beats/min; there was no evidence of hemoglobinuria. Examination of the other systems was unremarkable.The blood count on admission in haematology confirmed normocytic normochromic aregeberative anaemia (haemoglobin = 4.5 g/dl, MCV = 100 fl, MCH = 31.3 pg, MCHC= 34%, reticulocytes = 14494/mm^3) with leukoneutropenia (leukocytes = 2520/mm^3, neutrophils = 640/mm^3) and thrombocytopenia at 80000/mm^3. Myelogram showed erythroblastic marrow with stigmata of vitamin deficiency. The haemolysis work-up revealed elevated LDH at 2954 IU/ml, haptoglobin collapsed to 3 mg/l and then secondarily below 0.01 mg/l; bilirubin fractions were normal (total bilirubin: 12 mg/l; direct bilirubin: 8mg/l). G6PD activity was normal; direct and indirect Coombs tests were negative, and HIV, hepatitis B and C serologies were negative. Folate levels were below the detection limit. Vitamin B12 and ferritinemia were normal. Despite prednisone corticosteroid therapy (1 mg/kg daily for 4 weeks), folic acid supplementation (15 mg/d) and monthly transfusions of at least two (02) adult red blood cell concentrates, Mr. AY's hemoglobin level fluctuated between 4 and 8 g/dl. The onset of morning hemoglobinuria in August 2022, which became nocturnal two months later, prompted the PNH clone test performed on 22nd December 2022 at the CERBA laboratory (France). It resulted in the detection of 91% of cells deficient for FLAER (Fluorescein-Labelled pro-Aerolysin) and for the GPI-linked CD24 molecule on CD15+ neutrophils, and 91% of cells deficient for FLAER and for the GPI-linked CD14 molecule on CD33+ monocytes. The diagnosis of PNH was retained. No specific treatment (eculizumab, hematopoietic stem cell transplant) could be administered to the patient (technical platform and financial difficulties). Supportive treatment based on transfusion of packed red blood cells as needed was instituted. The course was unfavorable, with hemoglobin levels fluctuating between 5.7-8.3 g/dl.

Discussion

The pathophysiology of PNH largely explains the clinical and biological signs of the disease. Chronic hemolysis in the intravascular system is caused by a deficiency of the complement inhibitory proteins CD55 and CD59 on the RBC. The term "nocturnal" refers to the belief that hemolysis is triggered by acidosis during sleep, which activates complement to hemolyze an unprotected cell membrane. In reality, hemolysis occurs throughout the day [7]. In fact, our second patient presented with both morning and nocturnal hemolysis. The mechanism of bone marrow failure in HPN is poorly understood: autoimmunity and activation of T and NK cells directed against normal stem cells are involved. This may be linked to increased expression of human leukocyte antigen (HLA) class II molecules on patients'

hematopoietic stem cells. Reduced apoptosis may also be a major selection factor for GPI-defective hematopoietic cell clones [8]. The release of hemoglobin by intravascular hemolysis leads to a reduction in the pool of nitric oxide (NO) in the blood. NO depletion affects platelet activation and causes an increase in the expression of P-selectin, which is involved in platelet aggregation and can activate the complement system, the thrombin-antithrombin system and the fibrinolytic system, increasing the tendency to thrombosis [2].

Epidemiological Aspects

Epidemiologically, this is a very rare desease, with an estimated frequency of 15.9 individuals per million worldwide [9]. In France, only 450 cases have been reported over a 40-year period [1]. In Morocco, 11 cases of HPN were reported by Habib et al over a period of 09 years [5], and one case in Senegal by Niang et al [6]. This is the first report of this rare pathology in Togo. Because of a lack of awareness of the disease due to its rarity and clinical polymorphism, PNH is not always diagnosed in people presenting with limited symptomatology or comorbidities that confound the diagnosis. These first two cases were identified in young adults of both sexes (a 41-year-old woman and a 25-year-old man). The 41-year age of the first patient was in line with the median age of 42.7 years (interquartile range (IQR) 28.6-59.8 years) of the 2701 subjects diagnosed with a GPI-deficient granulocytic clone in the International PNH registry in 2017 [10]. In fact, the median age of disease onset is earlier, 36.5 years (IIQ 23.8-55.2 years) in the same registry [10] and closer to the age of our second patient. Both men and women are affected by PNH, with a slight female predominance at 52.1% [10]. Of the 11 cases reported by Habib in Morocco, 7 were women [5]. Indeed, the gene defective in PNH, PIG-A, is located on the X chromosome, but random inactivation of one of the female X chromosomes tends to balance the incidence of the disease relative to men [11].

Clinical Aspects

In both cases, the initial manifestations were chronic anemia. Hemoglobinuria appeared secondarily in the male subject, whereas in the female, the secondary manifestations were thrombotic events in the lower limbs. Thromboembolic events are diagnosed in around 29% to 44% of patients at least once during the course of the disease [8]. These usually involve unusual venous localizations in the liver (Budd-Chiari syndrome), intra-abdominal (portal, mesenteric, splenic), and cerebral (sagittal and cavernous sinuses) [2,12]. Lodhi and al in Pakistan described a case of PNH with bilateral renal thrombosis in a 23-year-old boy [13]. The classic localization of deep vein thrombosis is always possible [10], as in our patient's case in the lower limb. The risk of thromboembolic events increases as the proportion of GPI-deficient granulocytes rises above 50-60% [2]. This was the condition of our two observations. The first patient was suffering her first recurrence of deep vein thrombosis. In fact, 4.3% to 31.8% of patients followed for PNH and having had a thrombotic event have a recurrence of their thrombosis during follow-up, i.e. a five-fold increase in risk [14,15].

Biological Aspects

Laboratory tests revealed normocytic normochromic hemolytic anemia with aregeneration in both cases, with bicytopenia in the female and pancytopenia in the male. In the search for the etiology of this hemolytic anemia, direct and indirect Coombs tests were negative, G6PD activity normal and serologies negative. Flow cytometry remains the test of choice for the diagnosis of PNH [1,2]. It has been shown to identify the deficiency in 85.32% of neutrophils and 84.68% of monocytes in women, and 91% of neutrophils and monocytes in men. It is recommended that the deficient clone be identified on at least two cell lines [8], thus distinguishing PNH in which the mutation affects the hematopoietic stem cells from myelodysplastic syndromes in which the mutation involves more committed progenitors [16]. This identification is routinely performed on erythrocytes, neutrophils and monocytes [2]. In our patients, the transfusion history precluded erythrocyte testing. In our patients, hemolysis characterized by elevated LDH (greater than 1.5 times normal) and collapsed haptoglobin is part of bi- or even pancytopenia. Niang et al. in Senegal reported a case of PNH as an etiology of pancytopenia [6]. Habib and al. in Morocco reported regenerative hemolytic anemia in 8 cases, with pancytopenia in 6 and bicytopenia in 3. Bone marrow biopsy revealed regenerative hyperplastic marrow in 5 patients and bone marrow aplasia in 4 [5]. Despite the erythroblastic character of the marrow in our two observations, the anemia, although hemolytic, was aregenerative. This is explained by the folic acid deficiency documented in the second observation and probable in the first, due to a tendency towards macrocytosis on the blood count. PNH is a diagnosis to be considered in the presence of "rich" marrow pancytopenia [1].

Therapeutic and Evolutionary Aspects

The mainstay of current PNH treatment includes eculizumab and allogeneic hematopoietic stem cell transplantation [17]. The development of complement fraction 5 inhibitors was a major turning point in the treatment of PNH. First came eculizumab, associated with a reduction of over 50% in transfusion requirements and a reduction of almost 70% in the risk of thrombotic events and major adverse vascular complications [18]. This was followed by the development of ravulizumab, another C5 inhibitor which, among 441 PNH patients followed from 27 weeks to 2 years, led to an improvement in LDH levels and transfusion avoidance in over 83% of patients [19]. As these inhibitors and allogeneic hematopoietic stem cell transplantation were not geographically or financially accessible to our patients, we were unable to institute this treatment for our patients. So, we just limited ourselves to provide them symptomatic treatment involving transfusion of packed red blood cells, folic acid supplementation and corticosteroid therapy, as well as management of the various complications. Iterative transfusions of erythrocyte concentrates help manage anemia. However, they can lead to iron overload in patients [2], with a potential risk of cardiac and hepatic damage in particular, in a context of difficult access to chelators. However, the increased iron loss through hemoglobinuria and hemosiderinuria characteristic of PNH, estimated at between 2.6 mg and 11 mg per day [20,21], should delay the onset of martial overload in our patients.

Oral prednisone improved hemoglobin levels and reduced transfusion requirements in the first observation. Because of the complications associated with its long-term use, corticosteroid therapy at a dose of 0.25 to 1 mg/day is recommended only for the management of acute episodes of intravascular hemolysis [2,3]. However, its efficacy on hemolysis remains limited [22], which explains the persistence of hemolytic anemia in our two patients.

Thrombotic complication was found in our first observation. As reported in the literature, and found in both patients, the need for blood transfusion, the importance of the PNH clone

(GPI-deficient granulocytes > 50%) and the high (lactate dehydrogenase) LDH level (greater than 1.5 times the upper limit of normal) are risk factors for thrombosis [10,20]. This suggests that, in the absence of specific treatment, long-term anticoagulant therapy should be maintained in the first observation and primary thromboprophylaxis should be discussed for the second observation [2, 3,20], or at least special attention should be paid to screening for signs of thrombotic events. However, the benefit of primary thromboprophylaxis is limited, as the risk of thrombotic events remains. Thromboembolic complications are fatal in 30% of cases of PNH [2]. Despite his recurrence, the evolution was favourable in our first observation, as in the case described by Lodhi et al. where heparin treatment was effective in a 23-year old with renal thrombosis during PNH [13].

Conclusion

Our work has enabled us to describe the epidemiological, clinical, paraclinical, therapeutic and evolutionary characteristics of PNH in two patients. Epidemiologically, these were two young adults of opposite sexes. Clinical manifestations were essentially those of anemia associated with thrombosis in one and hemoglobinuria in the other. Venous thrombosis was controlled with low-molecular-weight heparin. The anemia was aregenerative and at least partly related to folic acid deficiency. The diagnosis was made by flow cytometry performed abroad. Long-term symptomatic treatment of the anemia was poor, in the absence of a complement inhibitor.

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