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ONE HAEMOLYTIC ANAEMIA MAY HIDE ANOTHER: PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA MASQUERADING AS *PLASMODIUM FALCIPARUM* INFECTION

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ABSTRACT

Background: Paroxysmal nocturnal haemoglobinuria (PNH) is a rare, genetic and acquired haematologic disease that causes complement-mediated intravascular haemolytic anaemia, thrombosis and bone marrow failure.

Case description: A 27-year-old migrant patient attended the emergency department in a context of fever and chills over the previous few days as well as chronic fatigue, dyspnoea and chest pain. His medical history included chronic anaemia and erectile dysfunction. Initial biology showed a haemoglobin of 6.3 g/dl, platelets of 25,000/µl, total leucocytes of 3,500/µl with 1,500 neutrophils. B12 vitamin, folic acid, ferritin and thyroid stimulating hormone were normal. Lactate dehydrogenase levels were high and haptoglobin was non-measurable. C-reactive protein was 46.1 mg/l. A thick blood smear revealed *Plasmodium falciparum* infection with 0.1% parasitaemia. The patient was treated with an oral combination of artemether and lumefantrine. Three weeks later, the patient consulted the infectious disease department given the lack of clinical improvement. The cytopenias worsened, and lactate dehydrogenase (LDH) and reticulocytes increased. Tests for schistocytes, a thick blood smear for malaria and a direct Coombs test were negative; a myelogram was reassuring. An abdominal, pelvic and thoracic CT scan showed a mild hepatomegaly with no focal lesion and no splenomegaly or adenomegaly. A 12-colour flow cytometry unveiled a PNH clone on 90.9545% of neutrophils and 80.7371% of monocytes.

Discussion: PNH patients can be vulnerable to parasites infection (such as *P. falciparum*) as it may trigger breakthrough haemolysis through uncontrolled resurgence of activity of the complement system. In our patient, *P. falciparum* infection was a confounding factor, as it commonly causes haemolytic anaemia and thrombocytopenia, and patients living in malaria-endemic regions can carry low parasitaemia while being slightly symptomatic or asymptomatic.

KEYWORDS

Paroxysmal nocturnal haemoglobinuria, breakthrough haemolysis, haemolytic anaemia, thrombocytopenia, *Plasmodium falciparum*

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LEARNING POINTS

- *Plasmodium falciparum* infection can cause breakthrough haemolysis in patients with paroxysmal nocturnal haemoglobinuria.
- Low *P. falciparum* parasitemia in patients living in malaria-endemic regions is not always significant as these patients often carry acquired immunity.
- Patients from malaria-endemic regions presenting with severe sickness and low *P. falciparum* parasitemia must be assessed for other diseases, as it cannot explain heavy illness.
- Patients presenting with haemolytic anaemia, no schistocytes, a negative direct Coombs test and other unexplained cytopenia such as thrombocytopenia/neutropenia and other unexplained clinical manifestations such as dyspnoea, chest pain or erectile dysfunction should be assessed for paroxysmal nocturnal haemoglobinuria.

INTRODUCTION

Plasmodium falciparum is the most virulent parasite causing malaria in sub-Saharan African countries, responsible for hundreds of thousands of deaths each year in endemic regions. Its biological features often include haemolytic anaemia and thrombocytopenia.

Paroxysmal nocturnal haemoglobinuria (PNH) is a haematologic orphan disease that causes complement-mediated haemolytic anaemia, thrombosis and bone marrow failure. Many viral and bacterial agents commonly cause breakthrough haemolysis (BTH) in PNH patients through uncontrolled complement resurgence. Whether parasites such as *P. falciparum* can cause breakthrough haemolysis is unknown, as we found no previously described case of BTH induced by *P. falciparum*.

CASE DESCRIPTION

We present a 27-year-old migrant patient from the Union of the Comoros (south-western Indian Ocean) who was admitted to the emergency department in a context of fever and chills over the previous few days, as well as chronic fatigue, dyspnoea and episodes of chest pain. His medical history included chronic anaemia without a precise diagnosis, for which he received multiple red-cell transfusions over the last few months, and he reported chronic erectile dysfunction. He had no other medical condition and had no daily treatment.

At admission, his biology showed a haemoglobin of 6.3 g/dl, reticulocytosis of 68,000/µl (3%), platelets of 25,000/µl, and total leucocytes of 3,500/µl with 1,500 neutrophils. Coagulation and renal function were normal; B12 vitamin, folic acid, ferritin and thyroid stimulating hormone were normal. Lactate dehydrogenase (LDH) was high (801 UI/l), total bilirubin was 26.3 µmol/l (N < 20) with a non-conjugated bilirubin of 21 µmol/l (N < 17), and haptoglobin was non-measurable (< 0.30 g/l); C-reactive protein was 46.1 mg/l. Troponin and brain natriuretic peptide levels were normal; ECG was unremarkable. A thick blood smear was performed in the context of recent travel in a malaria-endemic region and revealed *P. falciparum* infection with 0.1% parasitaemia. The patient received oral treatment with artemether and lumefantrine. A few days later, he was discharged from hospital after a control thick blood smear had proved negative and his haemoglobin level had remained stable.

Three weeks later, in the absence of clinical improvement, the patient was referred to the infectious disease department. His biology showed a further decreased haemoglobin of 5.3 g/dl, reticulocytes of 115,000/µl, LDH 1,432 UI/l, haptoglobin < 0.30 g/l. Platelet count was 54,000/µl, total leucocytes 3,500/µl, neutrophils 1,200/µl. Schistocytes and thick blood smear for malaria were negative. A larger work-up for haemolytic anaemia was conducted. A direct Coombs test (antiglobulin) and an irregular agglutination test were negative. Glucose-6-phosphate dehydrogenase and pyruvate kinase enzymatic dosages were normal. Haemoglobin electrophoresis showed no special features. HIV, hepatitis B and C, syphilis, Epstein–Barr virus, cytomegalovirus and parvovirus B19 were excluded by serological tests. Bone marrow aspiration showed a mild hyperplasia on the erythrocyte line without dysplasia.

There was no abnormal lymphoid population on flow cytometry. Bone marrow mycobacterial cultures and *Mycobacterium tuberculosis* polymerase chain reaction tests were negative. An abdominal, pelvic and thoracic CT scan showed a mild hepatomegaly without focal lesion, and no splenomegaly or adenomegaly. Blood testing using 12-colour flow cytometry revealed a PNH clone on 90.9545% of neutrophils and 80.7371% of monocytes *(Fig. 1)*. Eculizumab, an anti-C5 monoclonal antibody, was started as first-line therapy given the necessity of red-cell transfusions and the active intravascular haemolysis. The patient was vaccinated against *Streptococcus pneumoniae*, *Haemophilus influenzae B* and *Neisseria meningitidis*, and a prophylaxis treatment with oral amoxicillin (500 mg daily) was started. Bone biopsy disclosed a rich bone marrow with no sign of fibrosis or aplastic anaemia. Medullar karyotype was normal, and fluorescence in situ hybridisation analysis showed no abnormality on chromosome 7. Three months after starting eculizumab, his haemoglobin reached 7.8 g/dl, with LDH at 357 UI/l and reticulocytes at 207,000/µl. On his latest evaluation, five months after starting eculizumab and more than four weeks after his last red-cell transfusion, his haemoglobin was 8.9 g/dl *(Fig. 2)*. Platelets increased at 120,000/µl.

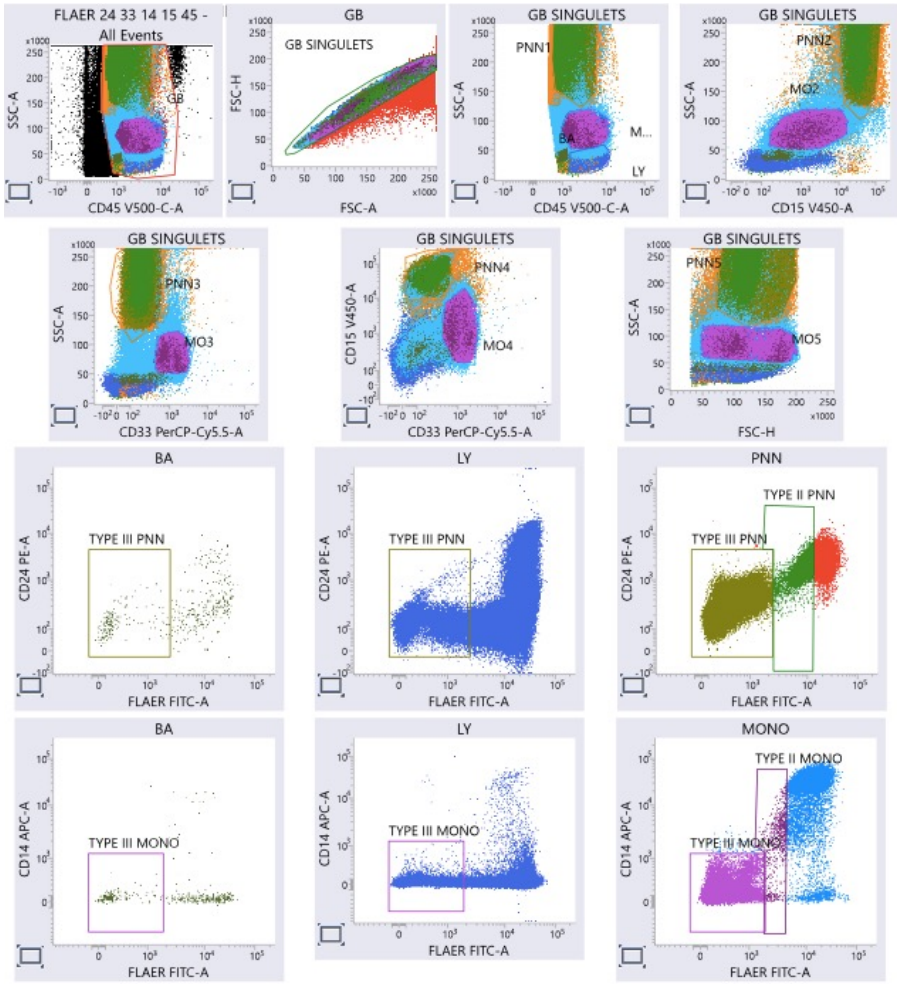


Figure 1. 12-colour flow cytometry unveiling a paroxysmal nocturnal haemoglobinuria clone on 90.9545% of neutrophils and 80.7371% of monocytes.

DISCUSSION

Also known as Marchiafava-Micheli syndrome, PNH is a rare, genetic and acquired haematologic disease that causes complement-mediated haemolytic anaemia. Clinical manifestations typically include intravascular haemolytic anaemia, thrombosis in atypical sites and bone marrow failure in cases of associated aplastic anaemia. Other symptoms include fatigue, abdominal pain, renal failure, pulmonary hypertension, oesophageal spasm, thoracic pain, miscarriage and erectile dysfunction[1]. Our patient presented most of these manifestations together with a symptomatic intravascular haemolysis, thrombocytopenia and mild neutropenia, which led to the diagnosis of PNH.

PNH patients often suffer from a delayed diagnosis as in the case of many orphan diseases. In our case, *P. falciparum* infection caused a delayed diagnosis as it is often responsible for haemolytic anaemia and thrombocytopenia. It is important to specify that low parasitaemia in patients living in malaria-endemic regions is not always significant, as these patients can chronically carry low parasitaemia while being slightly symptomatic, or asymptomatic. In certain countries such as Malawi, the prevalence of *P. falciparum* in asymptomatic patients can be as high as 30%[2]. Our patient presented with fever and chills, headaches and heavy fatigue, together with a biological inflammatory syndrome, and thus was treated as having a *P. falciparum* infection even though he initially presented with a very low 0.1% parasitaemia. Looking back at the patient's story, it seems unlikely that such a low parasitaemia was the only responsible factor for his profound anaemia and thrombocytopenia, together with his chronic symptoms of dyspnoea, chest pain, fatigue and erectile dysfunction, which is why we looked for other diseases. Thus, patients from malaria-endemic regions presenting with such severe sickness and low *P. falciparum* parasitaemia must be assessed for other illnesses, such

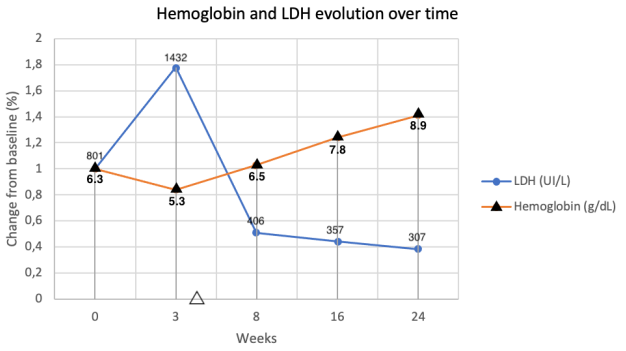


Figure 2. Haemoglobin and LDH evolution over time. Week 0 matches the biology at admission. Week 3 represents the time of PNH diagnosis. △ symbolises the start of eculizumab. Week 8 is one month after starting eculizumab. Week 16 is three months after starting eculizumab. Week 24 is the time of the patient's latest evaluation, five months after the start of eculizumab.

as PNH in our case. On the contrary, in patients who do not come from malaria-endemic regions, any level of *P. falciparum* parasitaemia should be considered significant and dangerous, as these patients do not carry acquired immunity. PNH results from a somatic loss-of-function mutation on the PIG-A gene which causes a loss of expression of glycosylphosphatidylinositol-anchored proteins such as CD55 (also known as decay accelerating factor) and CD59 (protectin or membrane inhibitor of reactive lysis). These surface membrane proteins naturally control and repress complement activation on the surface of mature blood cells through inhibition of C3- and C5 convertases and inhibition of insertion of C5b-9 complexes on the phospholipid membrane, thereby blocking the formation and activation of the membrane attack complex.

A severe complement-mediated complication of patients with PNH is BTH, which can be triggered by immunological stress (such as surgery, pregnancy, vaccines or infections). The complement is a complex and sophisticated system of innate immunity with the aim to eliminate pathogens that intrude into our body. Thus, PNH patients are known to be vulnerable to infections as it may trigger BTH through resurgence of activity of the complement system and uncontrolled activation of the terminal complement pathway. We believe BTH was caused by *P. falciparum* infection in our patient; it is widely described with bacterial and viral pathogens and is commonly observed in viral respiratory tract infections[3].

Whether parasites, such as *P. falciparum*, can be held responsible for BTH is an unanswered question. We found no previously described case of BTH caused by *P. falciparum* in a PNH patient. Several mechanisms can explain BTH in malaria through the complement system activation. Dasari et al. showed that digestive vacuoles that are released in the bloodstream after the lysis of *P. falciparum* parasitised erythrocytes, activate intrinsic clotting and alternative complement pathways[4]. In their study, intravenous injection of digestive vacuoles in rats caused alternative pathway complement activation, which may explain the BTH trigger in PNH patients. They later showed that these digestive vacuoles promote erythrocyte destruction through other mechanisms such as C3-dependent macrophage erythrophagocytosis. Interestingly, they showed that erythrocytes lacking CD55 and CD59 were even more susceptible to the various haemolysis processes[5]. Rathnayake et al. described mechanisms of complement activation in malaria[6]. They explained that the classical pathway activation is mediated by antibodies directed against Plasmodium-derived antigens and immune complexes formation. Increased levels of C4d, C5b-9 and Bb factors have been observed in *P. falciparum* infected patients, showcasing both the alternative and classical pathways activation. *P. falciparum* antigens cause the activation of the three complement pathways, although they cause greatest activation of the alternative pathway. On the other hand, Egan et al. showed that CD55 is an essential host receptor for *P. falciparum* infection and that CD55-null erythrocytes were refractory to *P. falciparum* invasion because of the lack of attachment to the erythrocyte surface[7]. This may explain why PNH and *P. falciparum* are a rare association. *Figure 3* illustrates the different mechanisms of complement activation caused by *P. falciparum*.

Standard-of-care treatments consist of anti-C5 therapies such as eculizumab, an anti-C5 humanised monoclonal antibody. Eculizumab showed haemoglobin stabilisation as well as significant improvement in disease symptoms, and reduction of thromboembolic risk and mortality in PNH patients. However, some patients do not respond sufficiently to eculizumab. Unmet clinical needs such as residual anaemia led to the development of second-generation complement inhibitors, through optimisation of anti-C5 therapy, such as ravulizumab and crovalimab, and through inhibition of

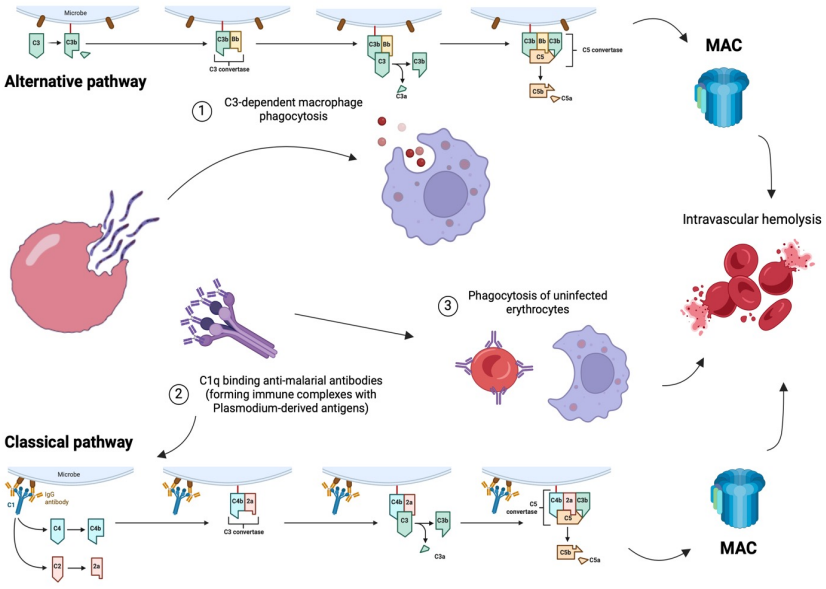


Figure 3. Mechanisms of complement activation induced by P. falciparum. 1) P. falciparum digestive vacuoles released into the circulation during the lysis of parasitised erythrocytes activate the alternative complement pathway. C3-convertase deposition induces bystander attack on non-infected host cells via C3-dependent macrophage phagocytosis. 2) Immune complexes that are formed by anti-malarial antibodies linking Plasmodium-derived antigens recruit C1q (binding the Fc constant region of anti-malarial antibodies) therefore activating the classical complement pathway. 3) Immune complexes and complement deposition on uninfected circulating erythrocytes promote erythrophagocytosis via macrophages.
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early phases of complement activation, using a C3-inhibitor compstatin such as pegcetacoplan. Recent strategies such as factor D inhibitor danicopan and factor B inhibitor iptacopan have shown meaningful improvement of haemoglobin levels through inhibition of MAC-mediated intravascular haemolysis and C3-mediated extravascular haemolysis[8].

Inhibiting the complement system significantly increases the risk of disseminated infection with encapsulated bacteria such as *Neisseria meningitidis*. These patients should be adequately vaccinated and receive antibiotic prophylaxis. Because PNH patients are at high risk of developing aplastic anaemia and myelodysplastic syndrome, they should regularly be assessed by flow cytometry and bone marrow biopsy.

In conclusion, we describe a rare case of *P. falciparum*-induced BTH in a patient with undiagnosed PNH. This case highlights the susceptibility of PNH patients to parasites infections such as *P. falciparum*.

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