CASE REPORT

Coombs-positive Paroxysmal Nocturnal Haemoglobinuria

Sabrina Kalam¹,*, Rupert Beale², Derralynn Hughes³, Austin Kulasekararaj⁴ and Umasuthan Srirangalingam⁵

¹Department of Neurology, Charing Cross Hospital, London, W6 8RF, UK, ²Department of Nephrology, Royal Free Hospital, London NW3 2QG, UK, ³Department of Haematology, Royal Free Hospital, London NW3 2QG, UK, ⁴Department of Haematology, King’s College Hospital, London SE5 9RS, UK, ⁵Department of Endocrinology, University College London Hospital, London NW1 2BU, UK

*Correspondence address. Charing Cross Hospital, London W6 8RF, UK. Tel: 07460118283; Email: sabrina.kalam@nhs.net

Abstract

Autoimmune haemolytic anaemia (AIHA) and paroxysmal nocturnal haemoglobinuria (PNH) are two distinct causes of haemolytic anaemia. They have different mechanisms that underpin their pathogenesis and, therefore, require different treatment strategies. The direct antiglobulin test (DAT) or Coombs test is positive in cases of immune-mediated haemolytic anaemia and, thus, is positive in AIHA but negative in PNH. We report a case of a woman presenting with a haemolytic anaemia who was found to have concomitant evidence of AIHA and PNH. The case highlights the importance of carrying out a comprehensive haemolysis work-up in patients who present with haemolytic anaemia.

INTRODUCTION

Haemolytic anaemia is defined as anaemia due to haemolysis of red blood cells (RBC) and its causes are subdivided into intracorpuscular or extracorpuscular aetiologies. The hallmark of the autoimmune haemolytic anaemia (AIHA) and paroxysmal nocturnal haemoglobinuria (PNH) is shortened red blood cell survival. Despite this, their pathophysiological mechanisms are distinct. AIHA results from an extracorpuscular defect in which the generation of autoantibodies against antigens on the surface of red blood cells leads to their premature destruction. PNH is a rare, acquired genetic condition caused by the non-malignant clonal expansion of haemopoietic stem cells with somatic mutation of phosphatidylinositol glycan class A (PIGA), which results in red cells being extremely sensitive to complement mediated lysis [1]. This results in a syndrome characterized by intravascular haemolysis, haemoglobinuria, thromboembolic episodes and bone marrow failure. A spectrum of forms has been identified from haemolytic-type PNH through to an aplastic anaemia-PNH form (AA-PNH) [2]. We report the case of a woman who initially presented with an intravascular haemolytic anaemia and was subsequently found to have concomitant AIHA and PNH.

CASE REPORT

A 65-year-old Caucasian female presented to the emergency department with a 3-day history of haematuria, exertional dyspnoea, abdominal pain, myalgia and profound fatigue. She reported profuse non-bloody diarrhoea the day before onset of symptoms, having eaten at a seafood restaurant. Her past medical and drug history are shown in Table 1. Upon clinical examination, she had marked conjunctival pallor, scleral icterus and marked tachypnoea with a respiratory rate of 24 breaths per
Table 1: Past medical history, drug history and blood results

| Past medical history                              | Medications          |
|--------------------------------------------------|-----------------------|
| Chronic obstructive pulmonary disease            | Levothyroxine        |
| Ovarian carcinoma (bilateral oophorectomy)       | Bumetanide            |
| Epilepsy                                         | Aspirin               |
| Aortic valve replacement (porcine valve)         | Phenytoin             |
| Hypothyroidism                                   | Phenobarbital         |
| Fatty liver disease                              |                       |

Blood results

|                  | Units          | Day 1 | Day 2 | Day 3 | Post-eculizumab |
|------------------|----------------|-------|-------|-------|-----------------|
| Hb (g/l)         | 115            | 95    | 67    | 111   |
| WCC (< 10^9/l)   | 10.88          | 13.05 | 10.16 | 7.24  |
| Platelets (< 10^9/l) | 339         | 408   | 315   | 312   |
| MCV (fl)         | 88             | 84    | 84.9  | 100   |
| Reticulocyte count | 6.2          | 7.3   | 3.1   | 3.1   |
| Urea (mmol/l)    | 8.7            | 20.0  | 24.2  | 6.0   |
| Creatinine (µmol/l) | 124         | 307   | 352   | 79    |
| eGFR (ml/min/1.73 m²) | 39           | <15   | <15   | 63    |
| LDH (<240) (IU/l) | 989           | 1100  | -     | 209   |
| CK (IU/l)        | 611            |       |       |       |

Table 2: Autoimmune haemolytic anaemia

| Autoimmune haemolytic anaemia                      |
|---------------------------------------------------|
| Group                                             |
| A Rh D positive                                   |
| C + c + E + e + K                                 |
| Antibody report                                   |
| No atypical antibodies detected                   |
| DAT (short profile)                               |
| Positive                                          |
| Probable due to auto D and Auto E.                |
| Anti-IgG                                          |
| 3                                                |
| Anti C3b-C3d                                     |
| 0                                                |
| DAT control                                      |
| 0                                                |
| Flow cytometry                                    |
| Positive                                          |
| Acidified serum                                   |
| lysis                                             |
| Acidified serum lysis—% lysis                     |
| CD55 (DAF)                                        |
| 83%                                              |
| CD59 (MIRL)                                       |
| 83%                                              |
| FLAER/CD24                                       |
| 22% positive                                      |
| PNH screen Interpretation                        |

78% doubly negative FLAER/CD24 granulocytes detected. 17% RBC showing double negativity for CD55/59 Consistent with patient having PNH in both granulocyte and RBC populations.

and malignancy. Initial management included IV fluids and fluid balance monitoring in the context of the AKI.

Subsequent blood tests were noted to haemolysis and her haemoglobin rapidly fell to 6 g/dl. No clotting samples could be processed. The peripheral blood smear revealed a few spherocytes, mild polychromasia, no fragments and toxic looking neutrophils (Fig. 1). The lack of red cell fragments and normal platelet count made HUS an unlikely differential. Thransthoracic ECHO revealed a functioning porcine aortic valve without evidence of infective endocarditis and renal ultrasound showed normal kidneys.

A haemolysis screen noted a raised LDH, undetectable haptoglobin levels and a positive direct antiglobulin test (DAT) (3+ IgG), suggesting an autoimmune haemolytic anaemia (AIHA) (Table 2). Cold agglutinins and a viral screen were negative. A paroxysmal nocturnal haemoglobinuria (PNH) screen was requested given the possibility of haemoglobinuria (urinalysis positive for blood but absence of red cells on microscopy) and an intravascular source of the haemolysis. This identified marked clonal expansion of GPI-anchor deficient blood cells by flow cytometry, with 17% red cell clone and 78% monocyte and granulocyte clones. A bone marrow aspiration and trephine (BMAT) showed no evidence of an underlying myelodysplastic syndrome or aplastic anaemia.

A diagnosis of concurrent AIHA and PNH, precipitated by a presumed acute gastrointestinal infection was formulated. The patient received packed red cell transfusions and was commenced on oral prednisolone (at a dose of 1 mg/kg) for AIHA. The patient was transferred to PNH National Service centre and subsequently commenced on ecukizumab. She was also treated with thromboembolic prophylaxis (following resolution of the AKI) and was vaccinated against meningitis, due to increased risk conferred with ecukizumab treatment.

She made a good recovery with ecukizumab therapy and was under surveillance. At 3 years following her initial presentation, the PNH clone had persisted. Despite her good recovery and haematological stability, our patient died unexpectedly at 3 years following complications of her underlying (bio-prosthetic) valvular heart disease.
DISCUSSION

We report a rare case of concomitant AIHA and PNH. The patient presented with haemolysis and a positive DAT test, suggesting a diagnosis of AIHA. However the presence of persistent haemoglobinuria also raised the possibility of intravascular haemolysis and prompted a comprehensive haemolysis screen which included flow cytometric analysis, which confirmed an additional diagnosis of PNH.

In PNH, the complement regulatory proteins CD59 and CD55 are unable to attach to the cell membrane via GPI, rendering haemopoitetic cells susceptible to complement mediated intravascular haemolysis and reduced bone-marrow production of white cells and platelets. The cell surface GPI deficiency in PNH is responsible for both the clonal expansion of these PIGA mutant cells, by allowing them to evade immune/apoptotic regulation, but is also the cause of the associated haemolysis \[3\]. Flow cytometry using antibodies against GPI to detect percentages of GPI deficient cells is the gold standard for diagnosis.

The flow cytometry in our patient identified the marked clonal expansion of GPI anchor deficient blood cells. The red cell clone was noted to be smaller (17%) than the granulocyte clone (78%). This is a result of a portion of the red cells being lost to haemolysis and highlights the importance of granulocytes, as they are not lysed like red cells and therefore provide a more accurate reflection of the true PNH clone size.

PNH is linked to aplastic anaemia (AA) and myelodysplastic syndrome (MDS) where cellular autoimmunity is directed against hematopoietic cells. CD55 and/or CD59 deficient cells are not specific to PNH and can be found in other autoimmune mediated haematological conditions including AA, MDS and AIHA \[4\]. PNH and AIHA occasionally occur concomitantly and it has been suggested that the inflammatory/thrombotic process in PNH may cause a conformational change on the erythrocyte surface, which triggers an immune antibody response. Alternatively, autoantibodies in AIHA may facilitate clonal expansion of the GPI deficient cell \[5\]. AIHA is characterized by extravascular haemolysis in contrast to the intravascular haemolysis of PNH. Additionally the presence of a positive DAT could have been incidental and not the primary reason for the patient's haemolysis, as our patient had a persistent PNH clone 3 years after diagnosis but her DAT became negative.

There has only been one other case report of possible coexisting AIHA and PNH reported in the literature \[6\]. This patient had presented with recurrent deep vein thrombosis and was diagnosed with AIHA. However, on flow cytometric red cell analysis, the authors found that they possessed PNH-like defects. Their patient was treated with steroids, antibiotics and an anti-thrombotic agent and made a good clinical recovery. Interestingly, they noted that the clinical recovery corresponded with improvement in the flow cytometry anomalies \[6\].

Figure 1: Blood film appearances showing polychromasia (large bluish red cells) and spherocytes (small round red cells with no central pallor).
PNH has been historically managed with blood transfusions and iron and folate supplementation. Anticoagulation needs to be considered due to the thromboembolic risk, which is the principal cause of death in patients with PNH. If the diagnosis of PNH had been missed in our patient, it could have resulted in serious and even life threatening consequences from thromboembolic complications. Eculizumab, a humanized monoclonal antibody against terminal protein C5, inhibits complement activation and thus intravascular haemolysis. It has been shown to reduce intravascular haemolysis, stabilize haemoglobin, reduce transfusion dependence and improve quality of life in patients with PNH [5]. In the UK patients diagnosed with PNH are managed via the PNH National service. Poor outcomes are linked with the AA-PNH, older age and poor performance status [2].

This case highlights the possibility of a dual pathology in cases of haemolytic anaemia and the need to broaden investigations where clinical signs and symptoms diverge from a unifying diagnosis.

CONFLICT OF INTEREST STATEMENT
None declared.

CONSENT
Written consent not required as patient is deceased.

FUNDING
Nil (not required).

ETHICS
Verbal consent from family. Formal ethical approval not required.

GUARANTOR
Dr U. Srilangalingam (usrilangalingam@nhs.net).

Acknowledgements
We thank the patient for allowing us to write and publish this report.

REFERENCES
1. Takeda J, Miyata T, Kawagoe K, Iida Y, Endo Y, Fujita T et al. Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria. Cell. 1993;73:703–11.
2. Socie G, Schrezenmeier H, Muus P, Lisukov I, Roth A, Kulasekararaj A et al. Changing prognosis in Paroxysmal Nocturnal Haemoglobinuria disease subcategories; an analysis of International PNH Registry. Intern Med J. 2016.
3. Chen G, Zeng W, Maciejewski JP, Kcyvanfar K, Billings EM, Young NS. Differential gene expression in hematopoietic progenitors from paroxysmal nocturnal hemoglobinuria patients reveals an apoptosis/immuneresponse in ‘normal’ phenotype cells. Leukemia. 2005;19:862–8.
4. Ruiz-Delgado GJ, Vazquez-Garza E, Mendez-Ramirez N, Gomez-Almaguer D. Abnormalities in the expression of CD55 and CD59 surface molecules on peripheral blood cells are not specific to paroxysmal nocturnal hemoglobinuria. Hematology. 2009;14:33–7.
5. Hillmen P, Young NS, Schubert J, Brodsky RA, Socie G, Muus P et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. The New England journal of medicine. 2006;355:1233–43.
6. zuPańska B, Bogdanik I, Fabijanska-Mitek J, Pyl H. Autoimmune haemolytic anaemia with a paroxysmal nocturnal haemoglobinuria-like defect. European Journal of Haematology 2009;62:346–9.