An interesting case of atypical venous thrombosis with low red blood cells

Giuseppe Chiariello, Raimondo Cavallaro, Giuseppe Covetti, Maria Carla Attilia Pisano, Antonella Schettini, Tiziana D’Aniello, Rossella Nappo, Emilio Aliberti, Arcangelo Iannuzzi

Department of Medicine and Medical Specialties, Section of Internal Medicine 2, Antonio Cardarelli Hospital, Naples, Italy

Correspondence: Arcangelo Iannuzzi, Department of Medicine and Medical Specialties, Antonio Cardarelli Hospital, Via A. Cardarelli 9, 80131 Naples, Italy.
Tel.: +39.081.7472121 - Fax: +39.081.7472121. E-mail: arcangelo.iannuzzi@aocardarelli.it

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Abstract

Sex: F Age: 65 years. Recent abdominal colic. Petechial-like manifestations with painful discolored skin lesions (suggestive of dermal veins thromboses) on the chest and abdomen. Blood levels of glucose, electrolytes, amylase, lipase, total protein, renal-function tests, the prothrombin time, the international normalized ratio, the partial-thromboplastin time and albumin were normal. Laboratory testing revealed hemolytic anemia: Red cells 2.94 x10^6/UL; Haemoglobin 8.9 g/dl; Lactate dehydrogenase 950 IU/L. Computed tomography (CT) of the abdomen and pelvis performed after the administration of intravenous contrast material, revealed a central filling defect in the hepatic veins and their branches that was compatible with acute thrombosis of the hepatic veins and their branches. It was suspected paroxysmal nocturnal haemoglobinuria for which flow cytometric analysis was requested.

Case Report

Sex: F Born in Naples. Age: 65 years. Diagnosis on admission: Suspected Vasculitis.

History: Admitted to Emergency Department with cutaneous rash and abdominal pain. Patient came to ward from short-stay observation unit. She reported recent abdominal colic, and taking a prescribed antibiotic from which she said that had developed an allergic skin reaction. Previous seizures for which she is now taking Levetiracetam 500mg oral once nightly. Awake and collaborative, eupnoic at rest. No chest pain. Blood Pressure 125/70 mmHg, Heart Rate 88 bpm, SpO₂ 93% in air. Apyrexic. Vesicular murmur heard on auscultation of the chest. Abdomen soft and diffusely tender with no rigidity or guarding. No ankle swelling. Evidence of recent petechial-like manifestations with painful discolored skin lesions (suggestive of dermal veins thromboses) on the chest and abdomen.

Blood tests: Urea 42.0 mg/dl; Creatinine 0.76 mg/dl; Total bilirubin 0.45 mg/dl; Direct bilirubin 0.1 mg/dl; Indirect bilirubin 0.3 mg/dl; Lactate dehydrogenase 950 IU/L; Ferritin 893 ng/ml; Red cells 2.94 x10^6/UL; Haemoglobin 8.9 g/dl; White cells 9.05 x10^3/UL;
Platelets 221 x10^3/UL; Haptoglobin 0.08 g/L; ANA-Titre: Present 1:160; ANA-pattern: Speckled; Anti dsDNA Ab 3.0 (Negative); ENA negative; Indirect antiglobulin test negative; Direct Coombs test negative.

US Abdomen: Liver, pancreas and spleen free from focal lesions. Biliary tract not dilated. Gall bladder with mostly dense biliary contents. No signs of urinary stasis. Urinary bladder empty. Diffuse distension of the colon with faecal impaction. No evidence of collections in the peritoneal cavity.

CT Thorax with contrast: No focal changes of lung parenchyma. No focal changes of pleural lining. No mediastinal or peri-bronchial lymphadenopathy. Regular opacification of the pulmonary trunk and main branches of the pulmonary artery. Trachea and bronchi patent. No evidence of pleural effusion. Nodular hyperplasia of the thyroid with expansion into the mediastinum.

CT Abdomen and Pelvis with contrast: No evidence of peritoneal effusion. Liver within normal limits, homogenous density of parenchyma with no evidence of focal lesions. Biliary vessels not dilated. Portal vein and splenic vein patent. The presence of non-occluding thrombotic-like defects in the hepatic veins and their branches is noted. Patent superior vena cava and inferior vena cava. Spleen, pancreas and adrenal glands within normal limits of size and volume with homogenous density. Kidneys in situ, within normal limits of size and volume, with regular enhancement post- contrast. Renal pelvis not dilated. Bilateral renal cysts (25mm). Bladder empty. No evidence of lymphadenopathy in the coeliac, lumbar-aortic, iliac, inguinal and obturator nodes. Retroperitoneal vessels follow regular course and are of normal size with normal opacification of lumen.

The presence of venous thrombosis in abnormal sites (hepatic and dermal veins) in a patient with no hepatic pathology, together with abdominal pain and intravascular haemolysis (increased LDH, negative Coombs anaemia, reduced haptoglobin), even in the absence of evident haemoglobinuria, has led us to suspect paroxysmal nocturnal haemoglobinuria (PNH)
Discharge diagnosis: Paroxysmal nocturnal haemoglobinuria complicated by hepatic vein thrombosis. Nodular hyperplasia of the thyroid with mediastinal expansion in euthyroid patient.

Discussion

PNH is a condition of clonal origin from multipotent haematopoietic stem cells characterised by anaemia, bone marrow failure and thrombophilia due to an acquired mutation of the PIG-A gene (Xp22.1), in which there is a reduced synthesis of the glycosylphosphatidylinositol (GPI) anchor on the haematopoietic stem cell membrane. According to the escape theory, the PIGA gene undergoes a series of somatic mutations in haematopoietic stem cells, in a two-step process, which determines clonal expansion. The PIGA gene produces a glycosyl transferase protein which is an essential component of the biosynthetic pathway that produces GPI. Following somatic mutation of the PIGA gene a protein is formed with partial or complete loss of enzymatic function, resulting in an almost complete absence of GPI-anchored proteins. Among the cellular membrane constituents that are GPI-anchored are the CD55 complement inhibitory proteins [decay accelerating factor (DAF)] and CD59 [membrane inhibitor of reactive lysis (MIRL)]. Deficiency of these two proteins results in complement mediated intravascular haemolysis. Although the loss of GPI-anchored proteins affects all cells derived from hematopoietic stem cells (erythrocytes, granulocytes, monocytes, lymphocytes, and platelets), only CD55 and CD59 deficiency contributes clinically to the pathophysiology of PNH with complement-mediated intravascular haemolysis. The relationship between deficits in CD55 and CD59 and other manifestations of PNH, such as medullary aplasia and thrombophilia, is not well known. Prevalence is not well known due to little available data but the prevalence of patients with PNH clones is estimated to be 15 per million, whereas the incidence is 1 in a million in the total population. There is
no difference in incidence between the sexes and the median age of diagnosis is just over thirty years, even if there are documented cases in children, adolescents and older adults\(^5\). The pathophysiology of intravascular haemolysis in PNH is due to the fact that normal red blood cells are protected from the attack of complement by GPI-anchored proteins CD55 and CD59, while in PNH red blood cells are not protected by these anchored GPI proteins and are lysed by complement, giving rise to haemoglobinaemia and anaemia. Intravascular haemolysis also leads to a rise in levels of LDH, indirect bilirubin and reticulocytes and a reduction in levels of haptoglobin. As it develops intravascular haemolysis favours the development of thrombotic phenomena. Haemoglobinaemia favours the depletion of nitric oxide (NO), presenting as abdominal pain, dysphagia, sexual dysfunction and pulmonary hypertension. Haemoglobinuria can result in renal damage, both as tubular toxicity and interstitial nephritis with subsequent acute and/or chronic renal insufficiency. Psycho-somatic implications are also important in this condition, with chronic fatigue, apathy, malaise, and perception of a poor quality of life in the majority of patients suffering from this disease. The clinical manifestations of this disease are varied, with some patients developing a pathology predominantly characterised by complement mediated haemolysis while others predominantly display signs of medullary aplasia with modest signs of intravascular haemolysis. Clinical heterogeneity, although not completely understood, could be due to the interrelations between PNH and immune-mediated medullary aplasia, taking into account that PNH is an acquired, non-malignant, clonal haematopoietic stem cell disease. Understanding the pathophysiology which that is unique both to the haemolytic action of the complement and to the immune mediated medullary aplasia provides the basis for the correct management of the disease. The two main aspects which characterise PNH and distinguish it from other haemolytic anaemias are primarily the involvement of haematopoietic stem cells, not only of the red blood cell, and also, unlike other intrinsic anomalies of erythrocytes, PNH is an acquired and non-hereditary disease. Early diagnosis can avoid clinical complications
possibly created by the typical diagnostic delay that accompanies patients who have rare diseases, improving management and quality of life for patients\textsuperscript{6}.

For rare diseases, exploring and intercepting cases before they are overtly apparent is a challenge, with the aim being to provide precise and accurate care as soon as possible\textsuperscript{7}. Presently the gold standard for obtaining a definitive diagnosis of PNH is flow cytometry with antigens that bind to the GPI protein. The diagnosis of PNH is straightforward when flow cytometric analysis of the peripheral blood reveals a population of glycosyl phosphatidylinositol anchor protein-deficient cells\textsuperscript{8}. Until a few years ago the only curative treatment option for PNH was allogenic transplant, which was burdened with a high morbidity and mortality\textsuperscript{9,10}. Symptomatic palliative treatment was confined to non-targeted therapy with blood transfusions, corticosteroids, oral anticoagulants or heparin in the case of thrombotic events. Currently, however, we have specific monoclonal antibodies (eculizumab) that binds with high affinity to C5, so that the terminal complement activity is blocked while the proximal complement functions remain intact. In the TRIUMPH study eculizumab led to a complete reduction in the number of transfusions received and a reduction of LDH to normal concentrations, with a net improvement in the relative score of asthenia\textsuperscript{11}. This drug also results in an 85% reduction of thrombotic events and an improvement in renal function with reduced damage in patients with renal damage caused by haemolysis. In 2003 eculizumab was designated an orphan drug in Europe, in 2007 was inserted onto List 648 in Italy for the treatment of patients with PNH and in 2008 a decree regarding reimbursement and sales price (high) in Italy (AIFA register) was published. Side effects are rarely clinically significant (nasopharyngitis, urinary tract infections, upper airways infections, dizziness, headache, nausea, diarrhoea).
Conclusions: The 5 key questions about PNH

Is PNH a neoplastic disease?

No, PNH is a disease of clonal cells in which mutation of the PIGA gene is used as a marker of clonality, but it is not a neoplastic disease like chronic myeloid leukaemia or acute myeloid leukaemia in which clone cells mutate and expand indefinitely until specific therapy is not started. Also in PNH expressivity of the clone changes from case to case: in some cases PNH is hardly appreciable at flow cytometric analysis (as in the subclinical form), sometimes it is fairly well expressed (as in the form that accompanies medullary aplasia), in other cases it is widely represented, being expressed in most hematopoietic stem cells (classical form of PNH); in no case does PNH invade other organs and tissues outside the bone marrow.

Does flow cytometric analysis only reveal mutated clones on red blood cells?

No, it is detectable in all cells derived from haematopoietic stem cells (erythrocytes, granulocytes, monocytes, lymphocytes and platelets): an accurate diagnosis requires that flow cytometry is performed not only on red blood cells (many are destroyed by intravascular haemolysis and others are potentially coming from blood transfusions) but also on polymorphonuclear cells, in particular granulocytes and monocytes, which are not lysed by the Membrane Attack Complex (MAC). Today flow cytometry is the gold standard for the diagnosis and monitoring of PNH and is the most sensitive method to reveal cells that lack GPI anchoring proteins. Modern flow cytometers are currently capable of analysing at least 6 fluorescent parameters or antigens on a routine basis. This increase in the level of sophistication and complexity means that small populations of abnormal cells can be easily discovered, although in some circumstances the clinical significance of results is not fully understood. Although CD55 and CD59 are widely used to find abnormal granulocyte clones, there is data which suggests that these reagents are not as valid as other antibodies and antigens for the diagnosis of PNH. Recent data suggests that one of the best available agents
to study antigens bound to GPI on leukocytes is fluorescent aerolisine or FLAER\textsuperscript{13}. This is an inactive variant conjugated to the fluorochrome of the aerosolised protein which is bound specifically to GPI anchors and will therefore be absent from granulocytes and monocytes deficient in GPI anchors (PNH cells). FLAER has significant advantages as a reagent in the diagnosis of PNH since, unlike antibodies against the GPI-linked antigens normally studied, its binding is less affected by the maturity of the cell. Also, it can be used in multi-colour combinations with monoclonal antibodies directed to both GPI-linked and non-GPI-linked antigens in the detection of EPN clones\textsuperscript{14}. For this reason this method was used in the case.

**Why does the mutation of the PIGA gene cause PNH?**

A normally functioning PIGA gene codes for the synthesis of an enzyme (acetyl glucosamine transferase) which allows the glycation of inositol phosphate (uridine diphosphate N-acetylglucosamine + phosphatidyl inositol → N-acetylglucosaminyl phosphatidyl inositol); this is a fundamental initial enzymatic stage in allowing the construction of GPI-anchored proteins. Among the particularly important proteins which bind to GPI, and inhibit haemolytic action via alternative pathway of complement, are DAF (decay accelerating factor, also called CD55) and MIRL (membrane inhibitor active reactive lysis, also called CD59). While the reason for intravascular haemolysis is clear in PNH, the pathophysiology related to the occurrence of thrombotic events, another typical characteristic of PNH, is not yet completely understood even though several possible explanations have been proposed\textsuperscript{15}.

**Which complement pathways provoke intravascular haemolysis in PNH?**

Chronic intravascular haemolysis is caused by the alternative pathway of complement, a component of innate immunity and an evolved mechanism to protect the host from pathological organisms. This is different to the classical pathway of complement which forms part of acquired immunity and requires a reaction with an antigen to activate; the alternative...
pathway is always active. The alternative pathway of the complement cascade needs two fundamental components in order to function: amplification of C3 and C5 convertases and the activation of the MAC. Safeguarding systems are in place to protect the host from continuous attack by the alternative pathway. In the case of red cells, protection against cell lysis mediated by the alternative pathway comes mainly thanks to two proteins: CD55 and CD59, which act on different levels of the complement cascade. CD55 regulates the formation and activity of the C3 and C5 convertases, whereas CD59 blocks the formation of MAC, which is composed of C5b, C6, C7, C8 and many molecules of C9. The absence of CD55 and CD59 on the red blood cell membrane represents the distinctive element of clonal mutation in PNH and explains the pathophysiology of complement mediated haemolysis, and also justifies why Coombs testing is negative.

**Why is eculizumab effective in treating classical PNH?**

Eculizumab is a humanised monoclonal antibody which binds to a factor C5 of complement, inhibiting the activation of C5 into C5b by C5 convertase in the alternative pathway of complement and blocking the formation of the MAC and therefore intravascular haemolysis. Treatment with eculizumab therefore reduces the need for blood transfusions, improves anaemia and greatly improves quality of life in patients by reducing the symptomatology associated with chronic haemolysis (malaise, tiredness, fatigue); with treatment bilirubin, haptoglobin and LDH return to normal. Eculizumab does not cure the disease in the sense that red blood cells are still deficient in GP1-anchored proteins and defective cells continue to be produced even if they are not lysed by complement. From a patho-physiologic point of view, the drug should not have an effect on thrombotic complications of the disease and these patients need to continue heparin or oral anticoagulation if they have had a thrombotic event, but there is some evidence from clinical studies that eculizumab reduces incidence of thrombotic events in these patients, particularly in the Budd-Chiari syndrome. Finally,
because patients who have a deficit in C5 are more at risk of meningitis, patients in therapy with eculizumab have to be prophylactically vaccinated against meningitis B, C and Y.

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Table 1. When to test for PNH.

| 1) Intravascular haemolysis (haemoglobinuria)                                      |
|-----------------------------------------------------------------------------------|
| 2) Unexplained haemolysis + (see next column)                                      |
|   | Hyposideraemia                                                                      |
|   | Abdominal pain                                                                       |
|   | Atypical thrombosis in situ                                                        |
|   | Granulocytopaenia / Thrombocytopaenia                                               |
| 3) Acquired haemolytic anaemia with negative Coombs, in absence of schistocytes and|
|   | in absence of infective disease.                                                    |
| 4) Atypical thromboses, see next column (especially if accompanied by haemolytic   |
|   | anaemia or cytopaenia)                                                               |
|   | Hepatic veins (Budd-Chiari)                                                         |
|   | Portal vein and splenic vein (in absence of cirrhosis)                               |
|   | Cerebral veins                                                                      |
|   | Dermal cutaneous veins                                                               |
| 5) Marrow aplasia (aplastic or hypoplastic)                                         |

Figure 1. Flow cytometric analysis.