

A 75-Year-Old Man with a History of a Positive Ham Test

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INTRODUCTION

A 33 year-old man was first admitted in November 1976 with suspicion of acute hepatitis. He was feeling well until a week prior to admission when he developed symptoms of an upper respiratory infection along with anorexia, fatigue, abdominal cramping, and dark urine. His relevant social history was positive for alcohol and cigarette use. Physical exam revealed no cardiac or pulmonary abnormalities. His liver was tender to palpation but not enlarged. He was discharged with the diagnosis of viral hepatitis and was lost to follow-up.

He was admitted in October of 1977 for evaluation of jaundice, which was initially presumed to be a complication of chronic alcohol use. He reported right upper quadrant pain unrelated to food intake or position, anorexia and fatigue and one episode of brown urine. He denied recent infection, hematochezia, nausea, or vomiting. The patient denied injection drug use or known exposure to anyone with viral hepatitis. Physical exam revealed no abnormalities except for right upper quadrant tenderness. Chest x-ray was without evidence of granulomatous disease. His lab work was consistent with a hemolytic process including a total bilirubin of 2.5mg/dL (normal range, 0.1-1.2mg/dL). He also had an elevated lactate dehydrogenase, ranging from 334 to 560 U/L (normal range, 105-333 U/L). A bone marrow biopsy revealed no abnormalities. He then underwent liver biopsy which was reported as consistent with "early sarcoidosis."

The patient was lost to follow-up again for many years thereafter and returned to clinic in January of 1999 for a routine health maintenance visit. At the time, he was feeling well overall. He did, however, report being diagnosed with paroxysmal nocturnal hemoglobinuria (PNH) at a different health facility in 1985 based on a positive Ham test. He had no subjective complaints at the time the Ham test was performed, but was noted to have had mild pancytopenia. He was asked to follow up every six months to monitor his PNH. He was again lost to follow up for three years during which time he experienced two exacerbations of his PNH. The first was in June 2000 for which he was hospitalized and required blood transfusions. The second exacerbation was

in December 2001, precipitated by a flu-like syndrome. During both episodes he was treated successfully with prednisone for five weeks. He returned to clinic yearly and was stable without hematuria, dizziness, or fatigue. Complete blood counts were drawn at least annually and these were reportedly normal.

There have been no additional episodes of hematuria. He has not had any episodes of thrombosis or required any blood transfusion. Due to the relatively indolent nature of the patient's disease, the diagnosis of PNH based on the Ham test was questioned and a confirmatory flow cytometry of leukocyte and erythrocyte CD55/CD59 expression was performed in 2017. GPI anchor deficient cells were detected in 19.99% of granulocytes, 45.63% of monocytes and 82.95% of erythrocytes, consistent with a diagnosis of PNH. The patient is not on treatment and is being followed closely by the Hematology clinic.

DISCUSSION

PNH is an acquired hematopoietic stem cell disorder caused by a somatic PIG-A gene mutation.¹ Cells containing this mutation are unable to synthesize cell surface glycosyl phosphatidylinositol (GPI) anchors. When GPI is absent, cells cannot effectively secure the complement regulatory proteins delay accelerating factor (DAF or CD55) or membrane inhibitor of reactive lysis (MIRL or CD59).² The protein MIRL prevents the formation of the membrane attack complex on cell surfaces, while DAF inhibits C3 convertase activity.³ Without GPI, the amplification of the complement system goes unchecked. Red and white blood cells as well as platelets become susceptible to complement destruction, specifically by C3b and C4b.⁴ Resulting cell lysis leads to the classic PNH symptoms from hemolysis and, ultimately, thrombosis and bone marrow destruction. (Figure 1)

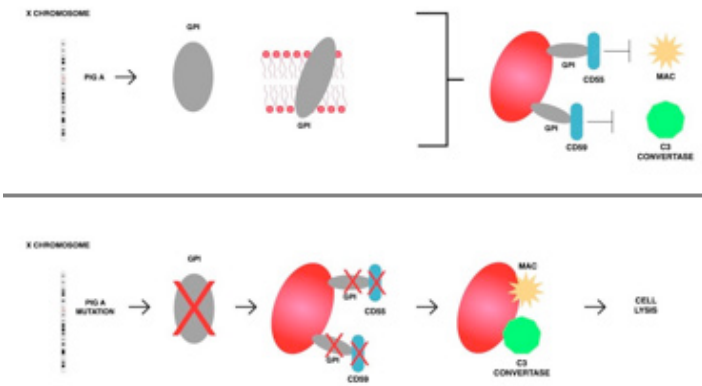


Figure 1. See text for explanation.

PNH is a rare disorder; although its incidence is not truly known, it is estimated to be between 0.1-0.2/100,000/yr.⁴ It is primarily diagnosed in young adulthood with the median age of onset being in the fourth decade.^{5, 6} The median survival after diagnosis is ten years with supportive therapy, but survival is highly variable and dependent upon the extent of hemolysis, thrombosis and bone marrow failure. Long term survival as well as frequency of complications may be based on clone size population, which could explain the long asymptomatic periods seen in our patient.⁷ This disorder may occur in higher frequency among Asian populations who are more likely to present in an atypical fashion, such as with marrow aplasia. Caucasian populations are more likely to present with the classic symptoms of thrombosis and hemoglobinuria.⁸

Clinical presentations can vary widely. More than 90% of patients with PNH exhibit hemoglobinuria at some point in their disease.⁹ Patients may also complain of weakness, dizziness, and fatigue. Thrombosis is seen in 29-44% of cases, resulting from endothelial dysfunction caused by nitric oxide depletion by free hemoglobin.^{4, 11} Manifestations of PNH resulting from NO depletion include erectile dysfunction, esophageal spasm, dysphagia, chest pain, and abdominal pain.^{12, 4, 9} The higher the PNH clone population in a given patient, the higher the risk for thrombosis.^{7, 10, 11} For reasons that remain unclear, the most frequent site of thromboses are in atypical locations such as the hepatic vein or superior mesenteric vein; arterial thrombosis is rare.^{12, 4, 13} Multiple studies have demonstrated the relationship between PNH clone size, risk for thrombosis and overall prognosis. For example, a 10% increase in clone size results in the odds ratio for thrombosis is estimated to be 1.64.⁵

PNH is also associated with bone marrow failure.¹² The leading hypothesis for this failure suggests that the PNH clone has a survival advantage over normal hematopoietic cells during

autoimmune T cell attack, as is also seen in aplastic anemia. The member D subset of natural killer cells target bone marrow stem cells for destruction through interaction with binding proteins linked through GPI. PNH cells lack this anchor; therefore, they cannot be targeted and are spared in these autoimmune processes. There is a documented relationship between age of PNH onset and likelihood of marrow failure, with increased bone marrow failure found in those with younger age of onset.²

Historically, the diagnosis of PNH was established by a positive Ham test, developed in the 1930s when Dr. Thomas Ham administered ammonium chloride to his patients and found that a lower blood pH induced hemolysis and hemoglobinuria.⁹ Erythrocytes with increased sensitivity to complement, as seen in PNH, will lyse when mixed with acidified control serum. In the Ham test, a patient's erythrocytes are mixed with ABO compatible serum. A portion of this serum is acidified; another portion of serum is heated to inactive complement. A positive Ham test is lysis in the acidified serum, and no lysis in the heated serum.¹

Better testing has been developed in the intervening eighty years: Flow cytometry has been found to be more specific, sensitive and quantitative in establishing a definitive diagnosis of PNH. These tests can measure PNH cell frequency at levels as low as 0.01%, whereas the Ham test required at least 10% lysis for a positive result.^{14, 1} With flow cytometry, multiple cell lines should be measured when assessing the degree of stem cell abnormality. Granulocyte and erythrocyte measurements give the most accurate assessment of stem cell condition.

Lymphocytes are not optimal to measure as their expression of GPI varies and they have a longer circulation life. Monocyte analysis, while performed, can be somewhat challenging due to smaller numbers of these circulating cells.^{1, 15} The proportion of abnormal granulocytes is often found to be higher than abnormal red cells, due to the fact that the half-life of abnormal red cells is reduced in comparison to granulocytes. The difference in survival of these two cell lines is an important consideration, as the amount of deficient granulocytes more accurately represents the number of abnormal cells delivered from the bone marrow to the peripheral circulation.^{14, 15} As indicated earlier a smaller clone population may be indicative of fewer PNH complications and a more benign disease course.¹⁰

Aplastic anemia, a disease in which the bone marrow loses the ability to produce all three cell lines, due to an auto-immune phenomenon, shares several pathophysiologic features with PNH. Distinguishing these processes can be complicated because patients presenting with aplastic anemia can often show features of PNH and many with PNH show some level of marrow failure. Over fifty percent of patients at presentation with pancytopenia have expanded PNH cell populations detected by flow cytometry. The link between PNH and aplastic anemia is believed to be related to PNH clone cells' ability to escape the immune destruction seen in aplastic anemia.¹⁶

The management of PNH was transformed with the development

of eculizumab, a monoclonal antibody that targets the terminal complement component C5 and prevents its conversion to C5a and C5b.^{3, 15} Prior to this treatment, patients were essentially managed with supportive measures such as transfusions, B12, folate, iron replacement, and growth factor support. Eculizumab was studied in a randomized, double-blinded placebo controlled phase 3 trial in 87 transfusion dependent PNH patients.³ Treatment began with a 600mg infusion weekly for the first four weeks. During the fifth week patients were administered a 900mg dose and subsequently a 900mg dose every two weeks. Hemoglobin levels stabilized in 48% of drug treated patients compared to none of the control patients. Eculizumab patients required significantly less transfusions than control patients and there was a significant reduction in lactate dehydrogenase levels in patients treated with eculizumab compared to the control patients, reflecting a reduction in hemolysis.^{3, 17}

Adverse events reported most commonly were pyrexia in 9% of patients and viral infections in 6% of patients; there were no reported cumulative toxicity effects. However, patients receiving eculizumab have up to a 2,000 times greater risk for meningococcal infection as compared to normal hosts. In the randomized trial establishing the efficacy of eculizumab, two cases were reported of meningococcal sepsis, both patients survived with appropriate antibiotic treatment. The CDC recommends administering meningococcal vaccine against serogroups A, C, W, Y, and B for patients before receiving eculizumab.¹⁸

As evidenced by the clinical history of our patient and the discussion above, PNH is a disease with a widely variable presentation and course. New diagnostic techniques allow us to accurately assess clone size and use monoclonal antibody therapy targeting terminal complement to more effectively treat the disease and improve quality of life. This case study highlights the need to include this relatively rare disease in one's differential diagnosis of intravascular hemolysis.

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