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Discovery

Review and report on HAM test

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General Note
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ABSTRACT

The Ham's test is done to diagnose paroxysmal nocturnal hemoglobinuria (PNH). The test checks whether red blood cells become more fragile when they are placed in mild acid. It can also be used to diagnose another rare disorder called congenital dyserythropoietic anaemia. Paroxysmal nocturnal hemoglobinuria (PNH), is an rare acquired stem cell disorder associated with periodic hemolytic events. This benign clonal disease is caused by abnormalities of the Xlinked phosphatidylinositol glycan class A (PIGA) gene and is associated with cytopenias and thrombosis. Variety of laboratory abnormalities observed in PNH includes bone marrow hyperplasia, hypoplasia, hematologic cytopenias, microcytosis macrocytosis, decreased leukocyte alkaline phosphatase (LAP), hemoglobinuria, hemosiderinuria, as well as associated iron deficiency. The laboratory tests include the sucrose hemolysis test for screening and Ham's acid hemolysis test for confirmation.

Keyword: Paroxysmal nocturnal hemoglobinuria, Thrombosis, Cytopenias, bone marrow, hemosiderinuria, Hemolysis.

1. INTRODUCTION

In December 1937, Thomas H. Ham's case study detailed his new procedure for diagnosis of PNH in three patients. Now referred to as the "Ham test," it was adopted by the medical community and was the first diagnostic test for PNH. Paroxysmal nocturnal emoglobinuria (PNH) is a rare, acquired, life-threatening disease of the blood. The disease is characterized by destruction of red blood cells (hemolytic anemia), blood clots (thrombosis), impaired bone marrow function, and a 3 to 5% risk of developing leukemia. A disease caused by a defect in the glycosylphosphatidylinositol (GPI) anchor, due to an acquired abnormality in the PIG-A gene. This leads to partial or complete absence of all GPI-linked proteins, particularly CD59 (membrane inhibitor of reactive lysis) and CD55 (decay accelerating factor), resulting in an increased sensitivity of the affected cells to the action of complement.

The first description of paroxysmal hemoglobinuria was by the German physician Paul Strubing in 1882. A more detailed description was made by Dr. Ettore Marchiafava and Dr. Alessio Nazari in 1911 with further elaborations by Marchiafava in 1928, and Dr. Ferdinando Micheli in 1931. The Dutch physician Enneking coined the term "paroxysmal nocturnal hemoglobinuria" in 1928. Paroxysmal nocturnal hemoglobinuria affects both sexes equally, and can occur at any age, although it is most often diagnosed in young adulthood. The clinical manifestations of PNH are primarily related to abnormalities in the hematopoietic system, including hemolytic anemia, a hypercoagulable state, and diminished hematopoiesis. PNH affects only 1-2 persons per million of the

population and is a disease of young adults (median age of diagnosis 35-40 years of age) with occasional cases diagnosed in childhood or adolescence. PNH is closely related to aplastic anemia. In fact, up to 30% of newly diagnosed cases of PNH evolve from aplastic anemia. Similarly, the risk developing PNH after treatment for aplastic anemia with immunosuppressive therapy (anti-thymocyte globulin and cyclosporine) is approximately 20 to 30%. The median survival after diagnosis is 10 years; however, some patients can survive for decades with only minor symptoms.

The premature destruction of red blood cells seen in paroxysmal nocturnal hemoglobinuria is caused by a component of the immune system called complement. Complement consists of a group of proteins that work together to destroy foreign invaders such as bacteria and viruses. To protect the individual's own cells from being destroyed, this process is tightly controlled by complement-regulating proteins. Complement-regulating proteins normally protect red blood cells from destruction by complement. In people with paroxysmal nocturnal hemoglobinuria, however, abnormal red blood cells are missing two important complement-regulating proteins that need the GPI anchor protein to attach them to the cell membrane. These red blood cells are prematurely destroyed, leading to hemolytic anemia. In the past, the treatment of PNH had been largely empirical. As with most medical disorders, therapy is largely given to ameliorate the symptoms or complications rather than the cause of the disease. However, with improved understanding of the underlying pathogenesis, more rational therapies are emerging.

2. SIGNS AND SYMPTOMS

People with paroxysmal nocturnal hemoglobinuria have sudden, recurring episodes of symptoms (paroxysmal symptoms), which may be triggered by stresses on the body, such as infections or physical exertion. During these episodes, red blood cells are prematurely destroyed (hemolysis). Affected individuals may pass dark-colored urine due to the presence of hemoglobin, the oxygen-carrying protein in blood. The abnormal presence of hemoglobin in the urine is called hemoglobinuria. In many, but not all cases, hemoglobinuria is most noticeable in the morning, upon passing urine that has accumulated in the bladder during the night (nocturnal). The premature destruction of red blood cells results in a deficiency of these cells in the blood (hemolytic anemia), which can cause signs and symptoms such as fatigue, weakness, abnormally pale skin (pallor), shortness of breath, and an increased heart rate. People with paroxysmal nocturnal hemoglobinuria may also be prone to infections due to a deficiency of white blood cells.

3. DIAGNOSIS

The genetic defect responsible for causing PNH has recently been identified. Knowledge of the genetic defect will allow researchers to study the disease in a manner that was not previously possible, and may give insight for developing more effective therapies. PNH is caused when mutations of the PIG-A gene occur in a bone marrow stem cell. Stem cells give rise to all the mature blood elements including red blood cells (RBC) which carry oxygen to our tissues, white blood cells (WBC) which fight infection, and platelets (PLT) which are involved in forming blood clots. Therefore, the affected stem cell passes the PIG-A mutation to all cells derived from the abnormal stem cell. Cells harboring PIG-A mutations are deficient in a class of proteins called, GPI-anchored proteins. Certain GPI-anchored proteins protect red blood cells from destruction; others are involved in blood clotting, while others are involved in fighting infection. Therefore, the majority of the disease manifestations i.e., hemolytic anemia, thrombosis, and infection result from a deficiency of these GPI-anchored proteins.

If your doctor suspects PNH, he may order a variety of blood tests to confirm the diagnosis. The sucrose hemolysis (sugar water) test and Ham test are available at almost all institutions, but can be falsely negative if you have received recent red blood cell transfusions. Over the past several years flow cytometry has become the gold standard for making the diagnosis. Like the sucrose hemolysis and Ham tests, this is a relatively easy blood test, but the result is not affected by blood transfusions. Unfortunately, not all laboratories perform this test.

In general, the diagnosis of PNH should be considered in a patient with any of the following manifestations:

- Evidence of acquired hemolysis, specifically with a negative direct antiglobulin (Coombs) test of the red cells.
- Evidence of intravascular hemolysis (eg, hemoglobinemia, hemoglobinuria, hemosiderinuria, elevation of plasma lactate dehydrogenase levels, reduction in plasma haptoglobin levels).
- Granulocytopenia and/or thrombocytopenia in the presence of an elevation in the reticulocyte count or evidence of intravascular hemolysis.
- Venous thrombosis, particularly of the abdominal or cerebral veins (eg, Budd-Chiari syndrome, mesenteric or portal vein thrombosis, thrombosis of cerebral or dermal veins) and signs of intravascular hemolysis.
- Aplastic anemia.
- Myelodysplastic syndrome, refractory anemia variant.
- Episodic dysphagia or abdominal pain with evidence for intravascular hemolysis.

4. HAM'S TEST

Also known as a acidification Test, Ham's test is used to gauge any increase in the fragility of red blood cells when they are placed in mild acid. A positive test can confirm the diagnosis of PNH, short for paroxysmal nocturnal hemoblobinuria, which is a condition where an abnormal cell surface leads to the premature destruction of the cells. The condition is primarily caused by a defect in the formation of a red cell surface protein anchor - known as GP1. This defect will then contribute to the other surface proteins being unable to remain tethered to the cell surface resulting in enhanced sensitivity to complement mediated cell destruction. The condition is not restricted to any particular age and is known to affect both sexes in equal proportion. The condition is characterized by the low count to red blood cells, white blood cells and platelets as well as a reddish or brownish tinge to the urine as a result of the release of hemoglobin into the circulation and urine after the breakdown of red blood cells.

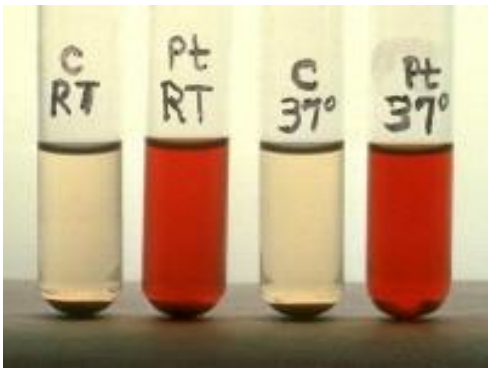

Figure 1
Ham's Test

4.1. Procedure

As with most blood tests, the Acidification test will require a sample of blood be drawn from the arm of the patient (venipuncture) after the puncture site has been sufficiently cleansed with an anti septic and an elastic band is placed a little higher up the arm to restrict blood flow to the vein. This will cause the vein to swell with blood - making it more prominent and easy to draw a sample from. After the blood has been drawn with the help of a needle, the puncture site will be covered to prevent any extra loss of blood. Some patients may experience fainting or dizziness just after the drawing of blood while others may merely notice a throbbing around the puncture wound.

4.2. Inference

If the test result is positive, means it will confirm the diagnosis of PNH. (Shows lysis of Red cells in acidified serum samples with patients cell (not with normal cells). A negative test is normal.

Abnormal results may be due to:

- Paroxysmal nocturnal hemoglobinuria
- Congenital dyserythropoietic anemia

5. COMMENTS

PNH is a beguiling disease that is caused by somatically mutable "hot spots" within the X-linked PIG-A gene. This non-neoplastic, clonal, stem cell disorder is best defined by FCM, which has recently superseded Ham's test, which for many decades was the dominant diagnostic test for PNH.

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