

Paroxysmal Cold Hemoglobinuria: Role of Hospital Transfusion Medicine and Immunohematology Department in the Diagnosis

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Abstract

Background: Paroxysmal cold hemoglobinuria (PCH), an autoimmune hemolytic anaemia caused due to polyclonal IgG anti-P autoantibody binding to red blood cell surface antigens and is characterized by hemoglobinuria, typically after exposure to cold temperatures.

Case Presentation: An 84-year-old female patient started developing breathing difficulty off and on with generalised weakness and loss of appetite. There was continuous fall in hemoglobin and continuous evidence of intravascular hemolysis since last 15 days. After ruling out many common diagnosis, Donath – Landsteiner Test was done which showed Immunoglobulin G, IgG, antibodies, appearing at 4°C and hemolysing at 37°C in the presence of complement. Peripheral smear showed anisopoikilocytosis and spherocytosis. Reticulocyte count was on the lower side (0.5%). Forward and reverse blood grouping showed no group discrepancy. Direct antiglobulin test (Direct Coombs test, DAT) was positive with the monoclonal C3 antisera and negative with the monoclonal anti-IgG. ICT, Indirect Coombs Test, was negative. Syphilis was tested negative by Treponema pallidum hemagglutination assay (TPHA).

Results: Patient was diagnosed as a case of PCH based on the test results. Conclusions: Detecting blood antibodies, auto, allo, cold or warm, is important. The role of transfusion medicine laboratory in the diagnosis of patient PCH is emphasised.

Keywords: Paroxysmal cold hemoglobinuria; Donath Landsteiner Antibody; Cold agglutinins; Direct antiglobulin test; Hemolytic Anemia

Introduction

Paroxysmal cold hemoglobinuria (PCH) or Donath-Landsteiner (DL) syndrome is an autoimmune intravascular hemolytic anaemia [1] caused by polyclonal IgG anti-P autoantibody binding to red blood cell surface antigens characterized by complement-mediated hemolysis. This condition was first described by Donath and Landsteiner 1904 [2,3].

There is classic presentation with episodic hemoglobinuria, typically following exposure to cold temperature, however hemoglobinuria may be absent and a history of cold exposure may not be present occasionally.

Case Presentation

An 84-year-old female patient reported breathing difficulty off and on with generalised weakness and loss of appetite. Patient had continuous fall in hemoglobin and continuous evidence of intravascular hemolysis since last 15 days. On investigation her laboratory reports revealed hemoglobin as 5.6 gm%, haptoglobin < 20 mg/dl, Liver function Tests LFT showed total bilirubin of 5.8 mg/dl and indirect bilirubin as 4.8 mg/dl, serum lactic acid dehydrogenase (LDH) was 680 U/L. Urine analysis was negative for red blood cells. Stool for occult blood was negative. Hemoglobin kept on falling over

the next days of follow up and was reported 4.8 gm% when the patient was transfused four units of packed red cells during the investigations. Peripheral smear showed hypochromic picture with anisopoikilocytosis and spherocytes [4]. Bone marrow aspiration revealed erythroid hyperplasia and marrow biopsy showed few scattered epithelial cells which were negative for cytokeratin markers. Gastro intestinal endoscopy and colonoscopy were normal. Thyroid profile was normal. Flow cytometry was done to rule out CLL, Chronic lymphocytic leukemia and PNH, Paroxysmal nocturnal hemoglobinuria (CD55 and CD59 markers were negative). CECT, Contrast-enhanced computed tomography study of nasal sinuses, thorax and abdomen was normal. CA-125 for ovarian cancer was negative. Serum protein electrophoresis & Hb electrophoresis was normal. G6PD level were normal. Vasculitis markers like ANA, antiDsDNA, antiGBM, p-ANCA, c-ANCA were negative. Donath – Landsteiner Test showed positive antibodies, IgG, appearing at 4°C, hemolysing the red cells in presence of complement at 37°C. Reticulocyte count was on the lower side (0.5%). Forward and reverse blood grouping showed no group discrepancy and blood group was B positive. Direct antiglobulin test (DAT) with the monoclonal anti-IgG was negative and with monoclonal C3 antisera was positive. ICT (Indirect Coombs Test) was negative. The antibodies detected did not interfere with the cross-matching test. Syphilis tested negative by TPHA. Immunohematology antisera used for blood grouping and typing were of Tulip diagnostics and those used for DCT and ICT were of Biorad make. Treatment: Oral Steroid was started at the dose of 20 mg twice daily, which maintained her Hb above 9 gm% [5]. Vitamin supplements were given and patient was encouraged to maintain

warm. Follow up in OPD after fifteen days showed hemoglobin maintained at 9 gm% and no red cells in urine but presence of the same polyclonal IgG cold autoantibody in serum.

Paroxysmal cold hemoglobinuria (PCH) is the first, and the rarest, type of autoimmune **hemolytic anemia** (AIHA) to be identified.

Etiology

Chronic PCH in adults was earlier thought to be associated with syphilis which resolved after the treatment of syphilis with appropriate antibiotics. Now most cases of PCH, both in children and adults, are acute, self-limited disorders [6,7] known to occur soon after developing upper respiratory and gastrointestinal infection symptoms. The development of paroxysmal cold hemoglobinuria within 2-3 weeks of upper respiratory or gastrointestinal symptoms has been noted.

The initial inciting event to the predisposition of D-L antibody synthesis is still unknown. Infections and neoplasms could be the underlying pathology associated with the development of D-L antibody [8].

Paroxysmal cold hemoglobinuria being of transitory nature, lack of awareness may lead to a failure in recognizing and diagnosing this uncommon syndrome.

Among the infectious agents included are measles, mumps, influenza, varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus, parvovirus B19, Coxsackie A9, Haemophilus influenzae, Mycoplasma pneumoniae and Klebsiella pneumonia [9-11]. The D-L antibody development has been reported following measles immunization. Other associations include solid organ and hematopoietic neoplasms [12].

In most cases, the P antigen must be present on the RBCs for paroxysmal cold hemoglobinuria to develop. As most people express P antigen on their erythrocytes, nearly the entire population is susceptible to reactivity by the D-L antibody.

The degree and duration of hypothermia that is required to precipitate hemolysis depends on the temperature requirement of the antibody-RBC reaction and on the concentration availability of complement.

Pathophysiology: This antibody is attached to red blood cells (RBCs) in the cold and induced hemolysis when the RBCs are warmed due to complement activity.

Paroxysmal cold hemoglobinuria results in a biphasic hemolysis in the blood, usually polyclonal, IgG. The D-L antibody, as is named after the scientists Donath and Landsteiner, is known to bind to various antigens such as I-, i-, p-, Pr-, preferably at low temperatures, on the RBC surface, but the glycosphingolipid P antigen is considered its primary target [7].

This interaction sensitizes the erythrocytes to allow further interaction with the complement system resulting in complement lysis within the vascular circulation. Intravascular hemolysis occurs preferentially at 37°C, at which temperature the antibody has dissociated yet maintains maximal complement activity.

The stimulus for production of this D-L antibody is likely a form of molecular mimicry in which a microorganism's antigen shares structural similarity to the P antigen on human RBCs, resulting in immunogenic cross-reactivity [13].

Complement-mediated injury to the RBC is an intravascular process, hemoglobinemia, hemoglobinuria, and, sometimes, renal failure may develop [14-22]. The antibody may persist for 1-8 months to several years [19].

Epidemiology

Disease prevalence

The annual incidence of paroxysmal cold hemoglobinuria is estimated as 0.4 cases per 100,000 people [10,11]. European data shows a prevalence ranging from 1.6% to 40% of autoimmune hemolytic anemia cases, high prevalence being in children [7,19,20].

Young children are the most susceptible within the general population, developing a single, brief, post viral hemolytic episode [6,21]. However, recurrent episodes have been reported [7-22].

Clinical features

Within minutes to a few hours of exposure to cold temperatures, the patient develops a combination of the following: sudden onset of back and abdominal pain, headache, leg cramps, fever, rigors, chills, acute distress, nausea, vomiting, diarrhea, and esophageal spasms. The hemoglobinuria can be severe enough to alter the urine to a dark red-brown colour, although hematuria is generally minimal or absent. Oliguria or anuria can develop upon renal dysfunction. Cold urticaria and jaundice may also occur [23]. These are the result of the release of large quantities of hemoglobin from lysed RBCs, which then act as an irritant to various tissues.

Physical signs of massive RBC hemolysis include pallor, icterus, and urticarial dermal eruption. Hepato splenomegaly can be due to an underlying lympho proliferative or other neoplastic process, and clinical examination to rule out lymphadenopathy and/or splenomegaly is obligatory.

Laboratory studies

The diagnosis of autoimmune hemolytic anemia (AIHA) is usually straightforward and made on the basis of the following laboratory findings:

- Normocytic or macrocytic anemia
- Reticulocytosis
- Low serum haptoglobin levels
- Elevated lactate dehydrogenase (LDH) level
- Increased indirect bilirubin level

A positive direct antiglobulin test with a broad-spectrum antibody against immunoglobulin and complement

Treatment and prognosis

Acute PCH tends to be transient and self-limited [1] particularly in children. Chronic PCH associated with syphilis resolves after the syphilis is treated with appropriate antibiotics. People with PCH are sometimes advised to avoid exposure to cold temperatures [2]. If anemia is severe, blood transfusion may be needed. Careful compatibility testing by the blood bank is necessary because autoantibodies may interfere with blood typing. Prednisone may be used in individuals with PCH and severe anemia.

Points in favour for our diagnosis of PCH: A sudden onset of a marked anisopoikilocytic, hypochromic anemia. Reticulocyte count low during the acute episode. Peripheral blood smear shows poikilocytosis, spherocytes and anisocytosis [4]. DL Antibody Test: Positive Monoclonal C3 antisera: showed DAT Direct antiglobulin test (Direct Coombs test, DAT) positivity Monoclonal anti-IgG DAT results negative. Antibody hemolysing the red cells at 37°C. Biochemical testing: Test results for acute hemolysis positive, including elevated LDH level, increased indirect or unconjugated bilirubin levels and low haptoglobin values.

Conclusions

People with PCH are sometimes advised to avoid exposure to cold temperatures [24]. Rapid onset of hemolytic anemia that resolves spontaneously is the classic presentation of the disease.

If anemia is severe, blood transfusion may be needed. If left undiagnosed and untreated, the patient may develop complications from intravascular hemolysis in the form of end organ failure. The Donath Landsteiner autoantibody is a biphasic hemolysin that binds to red blood cells only at low temperatures and, upon warming, induces complement activation and lysis [2,24]. In contrast to autoimmune hemolytic anemia mediated by IgM cold agglutinins, most cases of PCH are caused by non-agglutinating IgG antibodies with anti-P specificity [2,24,25]. The Donath Landsteiner antibody usually appears 1 week after the onset of illness and can persist from 1 to 3 months [25]. Donath Landsteiner testing requires coordination between the clinical and laboratory teams to ensure appropriate sample collection, delivery, and testing. To avoid pre-analytic error in Donath Landsteiner testing, it is important to maintain the blood sample at 37°C until serum is separated from cells in the laboratory. This prevents adsorption of anti-P antibodies onto autologous red blood cells at low temperatures. Careful compatibility testing is also necessary because autoantibodies may interfere with blood typing.

Detecting blood antibodies, auto, allo, cold or warm, is important. The present case illustrates the role of transfusion medicine laboratory in the diagnosis of patient PCH Paroxysmal Cold Hemoglobinuria.

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