Case Report

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Applying Donath–Landsteiner test for the diagnosis of paroxysmal cold hemoglobinuria

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Abstract:

61-year old male patient was admitted to the hospital with clinical picture of hemolytic anemia with hemoglobinuria. Patient was suspected to have Infectious Mononucleosis (IM) with Auto Immune Hemolytic Anemia (AIHA). DAT was positive with anti-C3d specificity. Donath Landsteiner (DL) test was positive; confirming Paroxysmal Cold Hemoglobinuria (PCH). The final diagnosis was IM with PCH. Patient was managed conservatively and discharged after seven days. DL test specifically detects a biphasic autoantibody (IgG type) that binds to RBCs at cold temperatures, and fixes complement on the RBC membrane. However RBCs are only lysed upon warming to 37C when complement cascade proceeds to completion.

Keywords:

Autoimmune hemolytic anemia, Donath–Landsteiner test, paroxysmal cold hemoglobinuria

Introduction

Paroxysmal cold hemoglobinuria (PCH) is classified under cold autoimmune hemolytic anemia (AIHA). The pathology is defined by complement-mediated intravascular hemolysis caused by a biphasic polyclonal IgG autoantibody. The stimulus for production of this antibody is likely a form of molecular mimicry in which microbial antigen shares structural similarity to the *P* antigen on human red blood cells (RBCs), resulting in immunogenic cross-reactivity. This acquired autoantibody causes intravascular hemolysis and can be confirmed *in vitro* by Donath–Landsteiner (DL) test. Here, we report a suspected case with signs and symptoms of AIHA that was confirmed as PCH by DL test.

Case Report

Clinical presentation

A 61-year-old male patient was admitted to the hospital with chief complaints of fever with rash all over the body and loss of appetite (for 1 week), dark-colored stools, and urine (for 3 days) and shortness of breath. The patient had anemia, leukocytosis, thrombocytopenia, hyponatremia, hypoalbuminemia, and unconjugated hyperbilirubinemia. Peripheral smear showed signs of hemolytic anemia (anisopoikilocytosis, spherocytes, and polychromasia), and lactate dehydrogenase was raised. Urine findings showed the presence of hemoglobin and bilirubin. Treating physician advised high-resolution computed tomography chest that revealed multiple lymphadenopathy with mild bilateral pleural effusion.

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Differential diagnosis and laboratory workup

Initial differential diagnosis included lymphoma and infectious mononucleosis (IM) with AIHA. Lymphoma was ruled out on excision biopsy of cervical lymph node. Meanwhile, the patient hemoglobin dropped further and bone marrow biopsy revealed lymphocytosis, and tests for anti- Epstein–Barr virus antibodies were positive. Physician suspected PCH and, therefore, patients' samples were sent to laboratory for direct antiglobulin test (DAT) and indirect antiglobulin test (IAT). While IAT was negative, DAT was positive. Additional test with monospecific antiglobulin reagent exhibited anti-C3d antibody. The thermal amplitude of autoantibodies ranged from 4°C to 22°C. Negative CD55 and CD59 markers ruled out paroxysmal nocturnal hemoglobinuria.

Confirmation of the diagnosis

In view of clinical history and positive DAT with C3d specificity, treating physician and laboratory physician discussed and decided to perform DL test. DL test was positive, confirming PCH [Figure 1]. The final diagnosis was, therefore, IM with PCH though P specificity of autoantibody could not be confirmed.

Management and follow-up

The patient was managed with antiviral (acyclovir), steroids (prednisolone), and RBC transfusion (one unit). The patient recovered completely and was discharged after 7 days on tapering doses of prednisolone. The patient is doing fine after 1-month follow-up with complete disease remission and normal laboratory parameters.

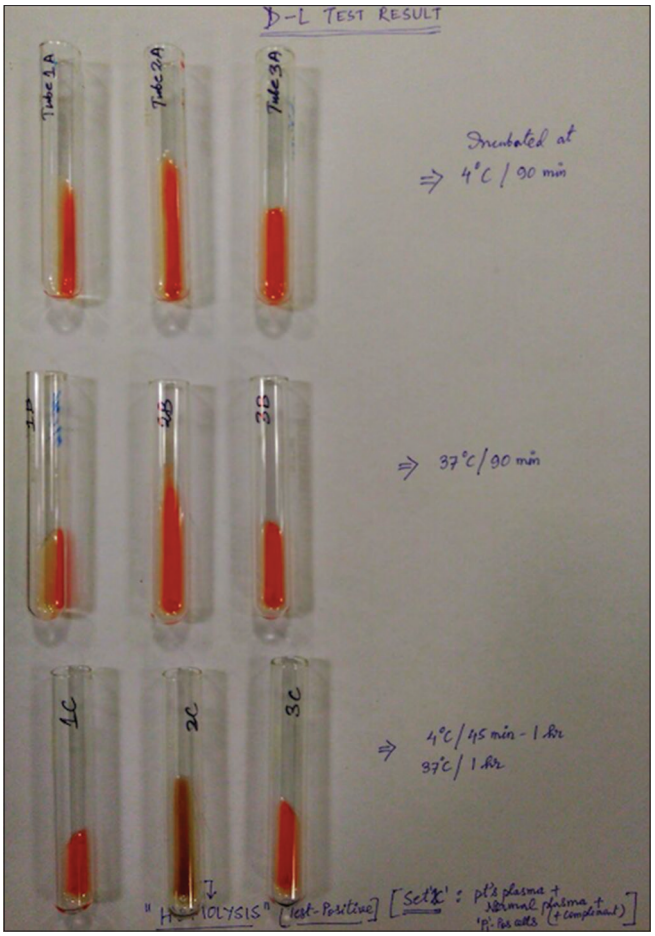

Figure 1: Three sets of three tubes as 1A-1B-1C; 2A-2B-2C; 3A-3B-3C. Set 1 and 2 had patient serum and Set 2 and 3 had fresh normal serum as source of complement. P1-positive cells were added to all nine tubes. Three sets "A," "B," and "C" were incubated at 4°C only, 37°C only, and 4°C followed by 37°C, respectively. After centrifugation, tube "2C" showed hemolysis leading to test being interpreted as positive

Discussion

Dressler first described PCH in 1854, but Donath and Landsteiner first described the pathogenic autoantibody responsible for hemolysis in 1904.[1-3] PCH is often described as a unique form of AIHA characterized by paroxysms of severe anemia and hemoglobinuria upon exposure to low temperatures, due to massive intravascular hemolysis.[4]

The autoantibody binds to RBCs at low temperatures (usually in peripheral circulation), and upon warming (when sensitized RBCs pass through central circulation), it activates complement and causes intravascular lysis of RBCs. Thus, this autoantibody has also been called "biphasic" due to different temperatures of attachment to RBC and activation of complement resulting in hemolysis.[5] This biphasic nature of antibody is proved by DL test as done in the present case.

Although there are no Indian data on the prevalence of PCH, the annual incidence of PCH in Europe is estimated as 0.4 cases per 100,000 people. European data show a prevalence ranging from 1.6% to 40% of AIHA cases.[6] The disease has preponderance for male gender and affects young children more than the elderly.[7-9] The disease usually presents 2–3 weeks' postinfection (viral or bacterial) with upper respiratory tract and gastrointestinal symptoms along with PCH.[6,10,11] In the present case, the patient was an elderly male with a recent history of viral infection followed by the onset of signs and symptoms of hemolysis.

Acute PCH cases are mostly transient and self-limited and resolve spontaneously within days and weeks after onset. Management is largely supportive and symptomatic and is directed toward treating anemia and prevention of complication due to intravascular hemolysis.[6,8] Similarly, in the present case, the patient was successfully managed with medical interventions only, was discharged after 1 week of supportive treatment, and continues to do well at 1 month of follow-up.

DAT in AIHA can be specific for IgG ± C3d or only C3d. PCH and cold agglutinin disease (CAD) are two subtypes of AIHA that present with only C3d specificity.[4,5] These two can be further differentiated on the basis of thermal amplitude and DL test. PCH has positive DL test due to biphasic IgG-type autoantibody whereas CAD usually presents with IgM-type agglutinating antibody that has thermal amplitude ≥30°C and titers >1000 at 4°C.[4,5] Positive DL test in this patient confirmed the suspicion of PCH. However, to avoid preanalytic error in DL testing, the blood sample was kept at 37°C until serum was separated from cells in laboratory to prevent adsorption of anti-P antibodies onto autologous RBCs at low temperatures. Determination of the *P* specificity of the autoantibody could not be performed in this patient due to lack of appropriate reagent red cells. The thermal amplitude of antibodies was <30°C and titers were 256, thus favoring the diagnosis of PCH.

Conclusion

DL test is a specific and key test for establishing the diagnosis of PCH. Coordination between treating physician and laboratory physician is important for the diagnosis and management of such patients.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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