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Cold Agglutinin Disease; A Laboratory Challenge

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Introduction: Autoimmune haemolytic anemia (AIHA) is a complex process characterized by an immune reaction against red blood cell self-antigens. The analysis of specimens, drawn from patients with cold auto-immune hemolytic anemia is a difficult problem for automated hematology analyzer. This paper was written to alert technologists and pathologists to the presence of cold agglutinins and its effect on laboratory tests.

Case Presentation: A 72-year-old female presented to the Shafa laboratory for hematology profile evaluation. CBC indices showed invalid findings with the Sysmex automated hematology analyzer. Checking the laboratory process showed precipitation residue sticking to the sides of the tube. After warming the tubes, results become valid and the problem attributed to cold agglutinin disease. In this situation, aggregation of RBCs, which occurs at $t < 30°C$, causes invalid findings meanwhile working with automated hematology analyzer.

Conclusions: Knowledge of this phenomenon can help prevent wasting too much time and make an early and accurate diagnosis.

Keywords: Cold Agglutinin Disease; Hemolytic Anemia; Sysmex Autoanalyzer

1. Introduction

Autoimmune haemolytic anemia (AIHA) is a complex process characterized by an immune reaction against red blood cell self-antigens. AIHA is classified into warm and cold reactive antibody types (1). Cold agglutinin disease (CAD) has traditionally been classified into a primary or idiopathic type not associated with lymphoma or other diseases and a secondary type accompanied by malignant disease, most often lymphoma (2). The term "cold" is primarily derived from the immune biology of CAD, not from clinical features of patients. The autoantibodies responsible for hemagglutination at low temperatures, cold agglutinins (CA), bind to erythrocyte carbohydrate antigens at a temperature optimum of 0 - 4°C. Binding of CA causes agglutination of erythrocytes and the antigen-antibody complex induces complement-mediated hemolysis. CA may be found in the sera of healthy subjects as well as patients with AIHA of the cold reactive types or primary CAD (3). Essential clinical manifestations of primary CAD are hemolytic anemia and cold-induced circulatory symptoms (4). The analysis of specimens, drawn from patients with cold autoimmune hemolytic anemia is a difficult problem for automated hematology analyzer. Therefore, the first suspicion of CAD comes from failed attempts of hematology laboratory to obtain a meaningful RBC count and other indices. This paper was written to alert technologists and pathologists to the presence of cold agglutinins and its effect on laboratory tests.

2. Case Presentation

A 72-year-old female presented in Shafa laboratory with a history of lethargy and weakness. Blood was taken for CBC evaluation and analyzed by automated heamatology analyzer (Sysmex kx-21 N). After taking the hematology results, total bilirubin and lactate dehydrogenase enzyme measured by autoanalyzer Hitachi 917 and protein electrophoresis were performed on a sample of blood serum. In addition, titration of cold agglutinin was estimated to make a definite diagnosis. Moreover, mono test was performed to rule out causative infection.

Aggregation of RBCs, which occurs mainly at temperatures lower than 30°C, causes invalid findings when working with automated hematology analyzer (Sysmex KX 21 N). On the other hand, few values change erroneously such as RBC count, HCT, MCV and MCHC, which two latter indices show an increase. In fact, proportion between 3 fold of Hb and HCT did not correlate and this makes mismatching of CBC results. In this condition, tubes were checked and precipitation residue found sticking to the sides of the tube. To rule out a

problem, the technologist was instructed to redraw a new sample. Another CBC test was run, but the results remained unchanged. Suspecting cold agglutinins, the tube warmed at 37°C for 20 minutes and after the second run, the results become valid. However, there were large clumps of red cells on the slide before warming the tubes, which should be differentiated from rouleaux formation. RBC count decreased due to doublet erythrocytes being counted as a single cell, thus resulting in a falsely high MCV. Hematocrit lowered as the volume of doublets is slightly less than two cells. The platelet count was normal. Furthermore, mild leukocytosis and anemia (Hb = 8.2 g/dL) were present. Although in biochemistry profile, total bilirubin and LDH enzyme activity were increased, protein electrophoresis seemed normal. Approximately, cold agglutinin titer measured 1:2048. Infection or overt malignancy was ruled out as the result of mono test was negative.

Moreover, bone marrow aspiration and trephine biopsy performed and the result revealed mild erythroid hyperplasia and slight increase in lymphocyte population, while progenitor morphology and number of platelets were normal. These findings are sufficient to exclude the case for secondary chronic CAD. The case was referred for treatment with Zytux© for a while and the results were satisfactory.

3. Discussion

All patients with CAD have hemolysis, but rare patients are not anemic, because the hemolysis is fully compensated. Approximately 90% of patients experienced cold-induced acrocyanosis and/or Reynaud phenomena (5). In most cases, immune-mediated hemolysis occurs extravascularly and associated with IgG antibodies on the surface of red cells. In rare syndromes, IgG antibodies cause direct intravascular hemolysis, such as paroxysmal cold hemoglobinuria. Besides, there are rare cases with extravascular hemolytic syndromes caused by IgM polyclonal or monoclonal antibodies. On the other hand, it was demonstrated that red cell agglutination occurs at 3°C, so-called cold antibodies. The term "cold agglutinin" is misleading, because it implies that the disease has a relation with cold exposure. In fact, the term is derived from the immunology of cold agglutinin disease. Because cold agglutinin disease has a strong association with several lymphoproliferative disorders and IgM monoclonal gammopathies, its management differs significantly from that associated with warm autoimmune hemolytic anemia. In addition, hemolytic anemia associated with monoclonal IgM proteins is more serious; it is chronic and sustained, because the IgM monoclonal protein persists indefinitely. The source of IgM monoclonal protein is a population of cells typically found in the bone marrow, often in sufficient number to allow a firm diagnosis of non-Hodgkin lymphoma or Waldenström's macroglobulinemia (3, 6). Monoclonal IgM κ in serum (or rarely IgG, IgA or λ phenotype) and cellular κ/λ ratio > 3.5 (or rarely < 0.9) in B lymphocyte population and clonal lymphoproliferative bone marrow disorder confirmed by immunohistochemistry but not required for diagnosis (5). In primary chronic CAD, bone marrow biopsies show absence of plasmacytoid cells, presence of plasma cells predominantly outside the nodular lymphoid infiltrates. IGHV4-34 restriction and absence of MYD88 L265P mutation strongly suggested that cold agglutinin-associated lymphoproliferative disease is a distinct entity different from lymphoplasmacytic lymphoma (7).

Cold agglutinin antibodies are found in the serum of approximately 55% of patients with primary atypical pneumonia, a respiratory disease caused by Mycoplasma pneumonia.

Cold agglutinins may also be produced by other diseases including liver disease, chronic sepsis, acquired hemolytic anemia, leishmaniasis and black water fever. Most of these diseases have symptoms easily distinguished from those of primary atypical pneumonia. The prevalence of primary CAD was 16 cases per million inhabitants. The incidence rate was 1 per million annually. It is typically an affliction of older adults with a peak incidence at around 70 years of age and both sexes are affected with a slight female predominance. Primary CAD represents a spectrum of clonal lymphoproliferative bone marrow disorders, in most cases with morphological signs of lymphoma (8). In CAD patients, nearly all cold agglutinins are of the IgM type and mostly directed against the I antigen on RBC membranes. The mechanisms of red-cell agglutination and subsequent destruction have been elucidated in detail. Essential clinical manifestations of primary CAD are hemolytic anemia and cold-induced circulatory symptoms. Exact estimates of the severity of anemia and the frequency of cold-induced symptoms have not been provided until the recent years (3).

The first suspicion of CAD comes from failed attempts of haematology laboratory to obtain a meaningful RBC count and indices. Cold agglutinins in high titer tend to give spurious macrocytosis and low red cell counts with impossibly high MCHCs. Warming the blood or the diluents eliminates this problem (9). A patient with high titer antibodies can pose extremely difficult serologic problems for the blood bank laboratories. Often incompatible units are released due to the residual agglutination from the cold auto-antibody (10). Therefore, definite and early diagnosis is highly rewarded.

Not all patients require pharmacological therapy, but treatment is more needed than previously thought. Corticosteroids should not be used to treat primary CAD. The most efficient treatment to date is fudarabine (fludarabine phosphate or Fludara) and rituximab in combination (5). Some researchers show that TNT 003, a mouse monoclonal antibody targeting complements protein C1S, prevents induction of in vitro hemolysis by cold ag-

glutinins (CA). If successfully transferred into the clinical setting by further studies, these findings may result in a novel therapeutic principle for a frequently difficult problem (11). As in most autoimmune cytopenias, low prevalence of CAD makes it difficult to design and conduct randomized trials (12). Resolving blood typing discrepancies in presence of cold agglutinins with warm washed (37°C saline) or 2 ME treated RBCs and reverse ABO tests at 37°C (control with group O RBCs) or with autoadsorbed or group O adsorbed serum-plasma seem to be a good suggestion in treatment (13).

CAD is not an indolent disease and this paper was written to alert technologists and pathologists to the presence of cold agglutinins and its effect on laboratory tests. Knowledge of this phenomenon can help prevent wasting too much time and make an early and accurate diagnosis. The recent treatment modalities using Rituximab and Fludaribine have shown good results, thus emphasizing the need for an early diagnosis of CAD.

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Authors' Contributions

Diagnosis of the case: Abbas Haratyan. Diagnosis of the case and writing the manuscript: Zahra Nikousefat. Drafting of the manuscript: Moosa Javdani and Ali Jalili. Revising the manuscript: Mohammad Hashemnia.

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