Coomb’s negative cold agglutinin disease: A rare report of an incidentally detected case

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Abstract:
Cold agglutinin disease (CAD) is a rare type of autoimmune hemolytic anemia which usually results due to production of immunoglobulin M-type autoantibody against the I/i and H antigens on red blood cell membrane. They can be idiopathic or due to underlying lymphoproliferative disorders or atypical infections. It can have a varied presentation ranging from being incidentally detected to being totally transfusion dependent for a longer or shorter duration. Several factors play a role in determining the ability of cold agglutinins in inducing hemolysis such as antibody concentration and temperature. Here, we present a 54-year-old patient, a known case of chronic obstructive pulmonary disease who was admitted to our hospital in the winter months as a case of alcohol withdrawal syndrome. During the course of the stay, the patient developed respiratory insufficiency and went into Type II respiratory failure and hematological investigations revealed features of CAD.

Keywords:
Anemia, cold antibody, hemolytic, respiratory failure

Introduction
Agglutination of blood was not so recent as far as human knowledge goes and it was first described by the great Austrian biologist Karl Landsteiner in the early 20th century. Moreover, it was Clough and Richter in the year 1918 who identified the pathological connection of cold agglutinins with hemolysis and its incidence with pneumonias. However, it took nearly half a century after the discovery of agglutinins by Landsteiner to finally lay claim to the terminology cold agglutinin disease (CAD).

CAD is a rare type of autoimmune hemolytic anemia (AIHA) caused by cold-type immunoglobulin (Ig) M autoantibodies which exhibit greater titer and red blood cell (RBC)-binding activity as the temperature decreases and approaches 0°C. The cold agglutinin antibodies are specific for the I/i and H proteins found on the membrane of RBCs, and they are usually of IgM type in character, albeit less frequently can also be of IgG or IgA type also.

Case Report
A 54-year-old male, chronic alcoholic by nature, with antecedent comorbidities in the form of chronic obstructive pulmonary disease (COPD) presented to the casualty department of our hospital on a cold winter morning in a delirious state and was admitted to the acute medical ward as a case of alcohol withdrawal syndrome.

A day after his admission, he deteriorated clinically and developed high-grade fever with continuous cough, hypotension, and hypoxemia. Arterial blood gas analysis revealed severe respiratory acidosis, and he was immediately shifted to the intensive care unit with a diagnosis of sepsis with Type II respiratory failure.

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Blood was sampled for workup which revealed neutrophilic leukocytosis along with an interesting pattern of findings in the RBC indices. There was anemia which was out of proportion to the RBC count and indices. His hemoglobin (Hb) was 9.2 g/dl while his total RBC count given the counter was 1.6 million/mm.\[3\] The mean corpuscular volume (MCV) was 106 fl, and the mean corpuscular hemoglobin concentration (MCHC) was 55.2 g/dl.

Peripheral blood smear was evaluated which showed bandemia (shift to left in the neutrophil series) along with the presence of toxic granulation corroborating with the clinical input of sepsis. Apart from this, the striking features noted were large clumps of RBCs throughout the smear along with increase in the polychromatophilic cells and nucleated RBCs [Figure 1a-c]. A separate smear was subjected to supravital stain also which revealed a high reticulocyte count to the tune of 12% [Figure 1d].

Biochemical tests were ordered on the patient’s serum sample. The serum was slightly pinkish in color indicating hemoglobinemia. Lactate dehydrogenase levels were raised, and unconjugated bilirubin was mildly raised (2.3 mg/dl).

An interesting finding was that the blood showed agglutination even on naked eye inside the vacutainer, which on incubating at 37°C disappeared and reappeared on refrigerating [Figure 2a and b], which was confirmed microscopically also [Figure 2c and d]. Later on, Coomb’s test was undertaken on the patient’s sample which was negative surprisingly [Figure 2e]. Autoagglutination was carried out using the patient’s washed RBCs and allowing it to react with the patient’s serum at 4°C which gave a positive reaction [Figure 2f].

As a protocol of investigations, chest X-ray showed bilateral pulmonary interstitial infiltrates suggestive of atypical pneumonia. However, the causative agent could not be isolated due to constraints of the hospital. The patient was diagnosed to be a case of CAD along with atypical pneumonia, alcohol withdrawal syndrome, and COPD. He was treated on the lines of his pathologies and discharged home with advice to not expose himself to extremes of cold temperature.

**Discussion**

CAD is an extremely rare form of AIHA with an incidence in the west of 1 in million. It falls under the spectrum of immune hemolytic anemia consisting broadly of CAD and paroxysmal cold hemoglobinuria (PCH). CAD is associated with IgM-type autoantibodies directed against the RBC I antigen, whereas PCH is due to IgG-type antibody commonly referred to as the Donath–Landsteiner antibody.\[7\]

The IgM antibodies guilty for hemolysis can be differentiated by the naturally occurring IgM antibodies by their titer and thermal amplitude. The naturally occurring IgM antibodies, unlike the ones responsible for CAD, show their activity usually at 4°C and are found at a titre of less than 1:64 with hardly any recognisable activity at higher temperatures.\[4,8\] On the other hand, the pathological agglutinins usually have titers more than 1:512 and react over a wide range of temperature.\[4,8\]

Based on the etiology, they can be divided into either primary or secondary. The primary CAD or idiopathic CAD are usually seen in the elderly individuals with a slight female preponderance, whereas the secondary CAD is due to the presence of an underlying disorder which can be infections such as *Mycoplasma pneumoniae*, *Legionella*, infectious mononucleosis, malaria, HIV, hepatitis B, and hepatitis C or malignancies like commonly mature B-cell hematolymphoid malignancies, namely chronic lymphocytic leukemia/small lymphocytic lymphoma, Waldenstrom macroglobulinemia, or multiple myeloma. They can sometimes be associated with autoimmune disorders as well as in posttransplant recipient patients.

In our patient, he was a known case of COPD who was suffering from Type II respiratory failure and sepsis most likely due to atypical pneumonia as brought by the radiological findings of thorax. Following this, hematological investigations revealed anemia with spurious RBC parameters. The findings in our case was high MCV, discrepancy between Hb and total RBC count leading to spuriously low RBC count, and consequently, a very high MCHC value. This happens because RBCs clump together and form agglutinates and microaggregates which lead to them not being

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**Figure 1:** (a and b) Clumping of red blood cells (arrow) seen in low power (×10 objective – Leishman-Giemsa stain), (c) Nucleated red blood cells (arrowhead) seen along with the red blood cell agglutinates (×10 objective – Leishman-Giemsa stain), (d) Reticulocytosis seen (×100 oil immersion – supravital stain)
counted as RBC by the automated counters, thus causing a false low RBC count consequently leading to altered hematocrit and RBC indices also. MCV is raised because of aberrant increase in the volume of RBC because of microscopic agglutination and also due to reticulocytosis. MCHC is also raised because of these aggregates only.

Any analyzer measures RBC, Hb, and hematocrit directly and the other indices are derived from these parameters indirectly. Falsely low RBC count can be explained by the fact that RBC agglutinates become so large in size that analyzers either identify as a single white blood cell (WBC) or single RBC or they are totally excluded from the count. As a result of this, other RBC parameters deviate from normalcy. High MCHC is usually a good indicator of analysis or sample error such as RBC agglutination, hemolysis, or lipemia. In some cases, agglutination can be observed macroscopically also, but the gold standard is to confirm by a smear evaluation. These fanatical results can be corrected by simple ways which overcome the agglutination, i.e., by warming the sample back to 37°C either by collecting the blood in a prewarmed syringe and quickly feeding that into an analyzer and making a smear out of that or by incubating the collected sample in a water bath at 37°C and observing for the changes in the complete blood count parameters.

The WBC and Hb values are usually unaffected because the principles by which they are counted by the analyzers are different. However, platelets are prone to autoagglutination and there may be false numerical values of platelets in some cases. Once a case of CAD is diagnosed, we need to evaluate it further by doing a Coomb’s test, serum concentrations of immunoglobulins and complements, and additional battery of tests to get to the cause of CAD if they are secondary to some underlying pathology.

In our case, we did run Coomb’s test on the patient’s sample which surprisingly turned out to be negative. However, the autoagglutination carried out at room temperature and refrigeration was positive as when compared to the same sample incubated at 37°C. The cause of negative Coomb’s can be manifold. It can be due to technical fallacies of inadequate cell washing or delay in addition of reagent or it may be due to severe hemolysis where coated RBCs are cleared rapidly, or in rare cases, they may be due to IgA-type autoantibodies which may not be picked up in the routine tests. However, we do not know the exact reason behind negative Coomb’s test in our case, as in our case the patient recovered well in a few days and was discharged. Additional tests were not carried out because of cost factors considering the patient’s socioeconomic strata.

The importance of diagnosing CAD lies in the fact that the patient needs to be explained carefully for prevention of attacks in the future by counseling the individual to wear appropriate clothing and avoiding cold exposure. Transfusions should be avoided strictly in these conditions unless there is a life-threatening event. The most important indication of transfusion is maintenance of pregnancy. If possible, washed and warm RBCs should be transfused at a slow rate initially with the help of in-line warmer.
Cross-matching in these situations has to be performed only at 37°C.

Recognition of CAD is important as they may be subtle in clinical presentation and may be missed initially if overlooked while interpreting the counter values. The suspicion should arise if the “rule of three” is not maintained between the RBC count, Hb, and hematocrit values. Any wariness should prompt one to go for alternative methods of collection like prewarming of syringe followed by immediate analysis of report or by observing the reversal of agglutination after incubating the collected sample. The disease carries an excellent prognosis, especially if it is secondary to any underlying disorder except for HIV infection associated CAD. However, one must be aware of its dreadful complications such as severe hemolysis, renal shutdown, or gangrene of fingers or toes.

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Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) 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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Conflicts of interest
There are no conflicts of interest.

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