Original Article

# Clinical and serological characterization of cold agglutinin syndrome in a Tertiary Care Hospital in Eastern India

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**Abstract**

**Background and Aim:** Cold agglutinin syndrome (CAS) primary or secondary represents approximately 16-32% of autoimmune hemolytic anemia cases. Most patients present with mild, chronic hemolytic anemia with exacerbation of the condition in the cold environment. Red cell transfusions are only indicated when there is a life-threatening anemia causing crisis. We studied the clinical and serological characterization of CAS with the aim that the information gained from this study would help in proper diagnosis and management of these patients. **Materials and Methods:** The prospective study included nine patients who were admitted with severe anemia. Detailed work-up were conducted to establish the diagnosis, severity of *in vivo* hemolysis and transfusion management. **Results:** All patients presented with pallor, weakness, fatigue and painful fingers and toes with exacerbation of symptoms in winter months. Secondary CAS was observed in three patients suffering from malignant lymphoma. Red cells of all patients were coated with complements (C3) more specifically C3d. In one patient suffering from malignant lymphoma, the cold autoagglutinin titer was as high as 4096. Autoantibody in seven patients was specific to "I" antigen and one to "i" antigen. **Conclusions:** We conclude that detailed clinical and serological characterization is needed to diagnose and manage CAS. Whereas avoidance of cold exposure is the primary therapy, but no critical patient should be denied blood transfusion due to serological complications. All transfusion services should follow the correct protocol to maximize blood safety in CAS.

**Key words:**

Alloadsorption, autoimmune hemolytic anemia, blood transfusion, cold agglutinin syndrome, cold agglutinin, cold autoantibody

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## Introduction

Autoimmune hemolytic anemia (AIHA) is generally classified according to the characteristic temperature reactivity of the red cell autoantibody. While warm autoantibodies react most strongly near 37°C and exhibit decreased affinity at lower temperature, cold autoantibodies bind to red cells most strongly near 4°C with little affinity at physiologic temperature. Occasionally, patients have a combination of warm and cold autoantibodies. Cold agglutinin syndrome (CAS) represents approximately 16-32% of AIHA cases.[1,2] Primary or idiopathic CAS generally affects older adults with a slight female preponderance.[3] Infection and lymphoproliferative disorders are the predominant causes of secondary cases. The typical case of an infectious etiology involves mycoplasmal pneumonia or infectious mononucleosis in an adolescent or young adult. Other infectious agents include various virus and bacteria.[4,5] While infectious etiologies may produce transient CAS, lymphoproliferative disorders typically produce a more chronic course.[6]

Patient with primary or secondary cold AIHA have a mild, chronic hemolytic anemia producing pallor and fatigue, however there is an exacerbation of the condition in a cold environment. Episodes of acute hemolysis with hemoglobinemia and hemoglobinuria are more common in the winter months. Patients also present with acrocyanosis or experience Raynaud's phenomenon during exacerbations.[7-9] Patients with CAS have more homogenous direct antiglobulin test (DAT) results than with warm AIHA. Since the pathophysiology of CAS typically involves immunoglobulin M (IgM) autoantibodies and complements, patients almost exclusively have positive DAT with anti-C3 and polyspecific reagents and a negative result with anti-IgG.[4] Cold autoantibodies react more strongly at 0-4°C than at higher temperatures. These antibodies can be detected in most healthy individuals with the majority being benign. Pathological cold autoantibodies are characterized by wide thermal amplitude and high titer with thermal amplitude as the better predictor of hemolysis.[10] Primary CAS and CAS secondary to lymphoproliferative disorders usually exhibit higher titer than CAS secondary to infection.[4] Cold autoantibodies commonly show specificity against the "Ii" blood group system, with approximately 90% directed against the "I" antigen and most of the remaining ones directed against the "i" antigen.[11]

Avoidance of cold exposure is the primary therapy in CAS. For secondary CAS treating the underlying disease is the main stay of treatment. In cases with severe hemolysis, immunosuppression and plasmapheresis may be beneficial. Red cell transfusions are only indicated when there is a life-threatening anemia causing crisis.[12]

Ours being a Tertiary Care Hospital with an established Hematology Department, we encounter patients of cold AIHA regularly. Therefore, we planned to conduct a study on the clinical and serological characterization of CAS with the aim that the information gained from this study would help in diagnosis and management of these patients.

## Materials and Methods

The study was conducted from July 2011 to December 2013 which included nine patients who were admitted with severe anemia, all having history of recent blood transfusions elsewhere and required urgent blood transfusion again. Requisition for packed red blood cells (PRBC) with blood samples for blood grouping and crossmatching were received. Characteristically all anticoagulated samples received in the blood bank were autoagglutinated. A group IV blood group discrepancy was observed in all samples. Samples were maintained at 37°C and red cells adequately washed with warm saline. Warm red cell suspensions were used to confirm the forward ABO blood grouping, typing and DAT result. Discrepancy in reverse grouping was resolved using prewarmed serum as well as autoadsorbed serum. For all samples blood grouping was confirmed using the conventional test tube method. DAT and crossmatching using prewarmed serum were performed by Gel technology (BIO-RAD DiaMed, Cressier s/Morat, Switzerland). Samples showing positive DAT with polyspecific antihuman globulin (AHG) were further tested with gel card containing monospecific AHG (anti-IgG, anti-IgM, anti-IgA, anti-C3c and anti-C3d). In all techniques performed agglutination reactions were graded as 4+, 3+, 2+, 1+, weak and negative and documented accordingly. Specificity of cold agglutinins was determined as described before using pooled adult group 'O' red cells and group 'O' cord red cells.[13] To exclude paroxysmal cold hemoglobinuria (PCH) the Donath-Landsteiner (DL) screening test was performed following the AABB protocol.[13] All serum samples were subjected to a new cold alloadsorption technique that was developed in-house. As standard ZZAP technique is used only for cold autoadsorption and applies only for patients with no history of recent transfusion therefore the in-house cold alloadsorption technique has been developed and performed in lieu of the standard ZZAP autoadsorption technique.[13]

### Papain low ionic strength solution (LISS) cold alloadsorption (in-house developed technique)

Since all patients had recent history of blood transfusion, group 'O' allogenic red cells of phenotype R1R1 (with K–, Fya–, Jka+), R2R2 (with K–, Fya+, Jka+) and rr (with K–, Fya–, Jka–) were selected for cold alloadsorption.[13] Equal volumes of commercially available 1% papain and washed packed allogenic group "O" red cells of each phenotype were mixed adequately and incubated at 37°C for 10 min in separate test tubes. Then equal volumes of patient's serum, commercially available LISS (BIO-RAD DiaMed) and each washed papainized cells were mixed properly, incubated at 4°C for 45 min keeping the three tubes in horizontal position to ensure maximum contact of serum and LISS with red cells. The mixture was centrifuged for 5 min at 1000 g and the absorbed serum-LISS mixture was then harvested for further analysis.

Since the harvest contained equal volumes of serum and LISS therefore to rule out dilutional effect a parallel indirect (IAT) with only serum was performed. No dilutional effect was observed as the strength of IAT in both was identical.

### Validation of the technique

1. Harvested serum was mixed with pooled allogenic group "O" red cells and incubated at 4°C for 45 min. Presence of agglutination indicated adsorption failure or incomplete adsorption and serum deserved further adsorption.
2. Harvested serum was mixed with pooled allogenic group "O" red cells and incubated at 4°C for 45 min. Absence of agglutination indicated complete adsorption.
3. If the adsorption was complete then an IAT was performed using the adsorbed serum and commercial cell panel (BIO-RAD DiaMed, Cressiers/Morat, Switzerland). If the result was negative then it indicated absence of alloantibody in the serum. A positive result indicated the presence of single or multiple alloantibodies in the patient serum.
4. Further test for antibody identification was performed using a commercial 11 cell panel (BIO-RAD DiaMed, Cressier s/ Morat, Switzerland).
5. A total of 13 samples containing cold autoantibodies were subjected for the validation study.

## Results

Seven of the nine patients under study were beyond 50 years of age with a male preponderance. All patients presented with pallor, weakness, fatigue and painful fingers and toes with exacerbation of symptoms in winter months. While patients had acrocyanosis and numbness of the extremities, one patient experienced episodes of hemoglobinuria with a negative DL test. Three old patients who presented with a history of mild continuous fever, weight loss, adenopathy and splenomegaly were later diagnosed with malignant lymphoma through computed tomography scans and biopsy. In the remaining patients, no underlying illness could be identified. None of the patients presented with pulmonary or cardiac signs. The process flow of the clinical and serological diagnosis of CAS has been depicted in Figure 1. Figure 2, a box plot analysis describes the indicator values of *in vivo* hemolysis in our patients. The mean hemoglobin, reticulocyte count, serum bilirubin and serum lactate dehydrogenase were 6.8 g/dl, 6.3%, 3.2 mg/dl and 911.8 IU/ml respectively. For all samples, peripheral blood smears revealed clumps of RBC and polychromasia. Table 1 shows the detailed serological characterization of the cold autoagglutininins found in the patients. Red cells of all patients were coated with complements (C3) more specifically C3d. In one patient suffering from malignant lymphoma, the cold autoagglutinin titer was as high as 4096. Although the antibodies reacted strongly at 4-22°C, but thermal range of reactivity also extended to 32°C. Autoantibody in seven patients was specific to "I" antigen and one patient specific to "i"antigen. In a young patient with severe anemia antibody specificity was predicted as "anti-Pr." In another patient, Anti-E was found as an underlying alloantibody.

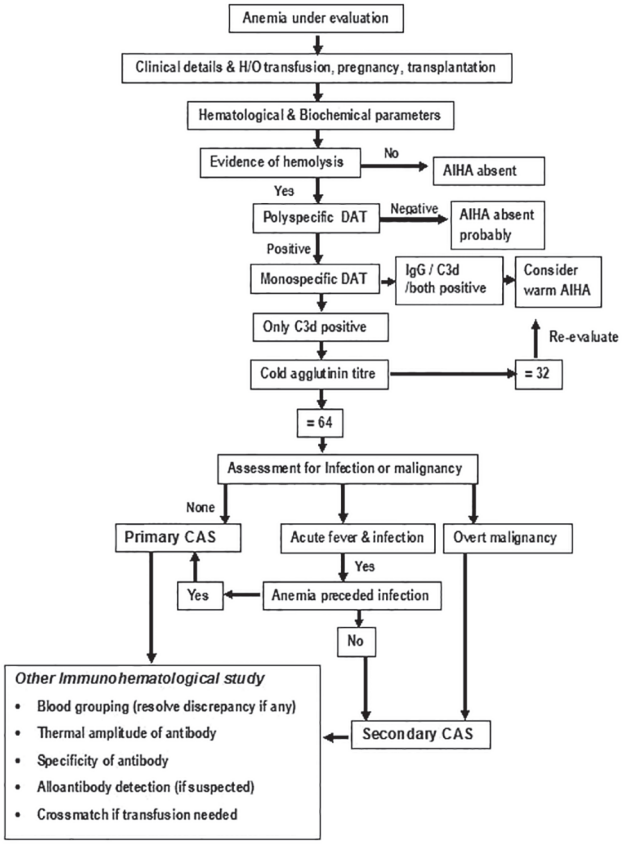


**Figure 1:** Clinical and serological diagnosis of cold agglutinin syndrome

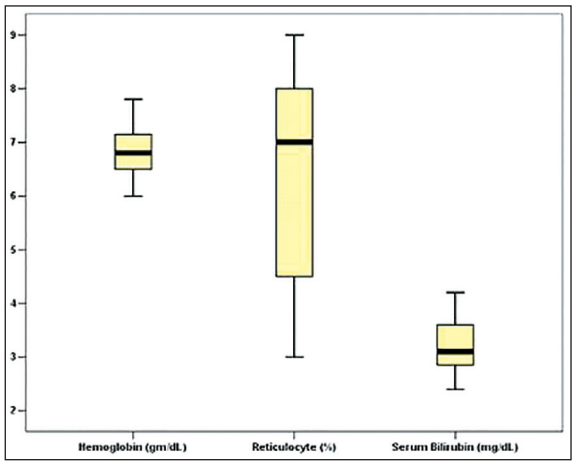


**Figure 2:** Hematological and biochemical details of cold agglutinin syndrome patients (*N* = 9)

## Discussion

In CAS episodes of acute hemolysis with hemoglobinemia and hemoglobinuria are more common in winter months. Only few patients of CAS suffer from life threatening intravascular lysis.[14] Ours being a Tertiary Care Hospital all patients under study presented with severe *in vivo* hemolysis and were referred from other hospitals. All patients had symptoms of anemia and admitted with deranged values of hemolytic parameters. Most of these patients presented in the winter months with two patients who were recently exposed to extreme cold in the hills of north eastern India. As described by Gehr *et al.* PCH is characterized by an acute attack of high fever, chills, back and/or leg pain and abdominal cramping.[4] Other symptoms include headache, nausea, vomiting, diarrhea and typically hemoglobinuria. None of our patients presented with the typical symptoms of PCH except an old patient who visited the emergency with episodes of passing cola color urine. The DL screening test was negative in all our patients. Malignant lymphoma as the underlying cause of CAS was confirmed in three patients. These patients had a long history of anemia and received several blood transfusions elsewhere.

Underlying alloantibody (Anti-E) was detected in one of these multi-transfused patients. A validated new in-house cold alloadsorption technique was developed and performed to detect this alloantibody. Antigen 'E' negative PRBC was transfused to the patient who had hemoglobin of 6.8 g/dl.

A detailed working protocol has been developed for the detailed characterization of CAS [Figure 1]. This protocol enabled the correct diagnosis of the anemic patients with regards to their autoantibody type, subtype, titer, specificity and thermal amplitude. These further helped in the transfusion and pharmacological management of the patients.

Cold agglutinins require cold environments to bind red cells. Subsequent to binding red cells, IgM autoantibodies fix C1 and initiate the classical complement cascade. If the complement cascade progresses to the membrane attack complex intravascular hemolysis results. Otherwise, bound C3 leads to extravascular hemolysis.[4] We observed that red cells of all the patients under study were coated with only complements (C3c or C3d or both). Cold agglutinins in all our patients were pathological with a high titer and wide thermal amplitude and majority of these were specific to "I" antigen [Table 1]. Isolated "i" specificity was observed in an old patient suffering from Hodgkin's lymphoma. As discussed by other authors cold agglutinin specific to "i" antigen is a rare phenomenon and is usually encountered in CAS with underlying lymphoproliferative disorders or infectious mononucleosis.[15] In one patient of idiopathic CAS the cold agglutinin reacted equally strongly with both cord and adult red cells, and the agglutination disappeared with papain pretreatment. This finding further suggested anti-*Pr* specificity as discussed earlier by Roelcke in 1989.[9] Other reported specificities included *Gd, Sa, Lud, Fl, Vo, M, N, D* and *P* the determination of which were beyond our scope.[16]

In CAS red cell transfusions are only indicated when there is a life-threatening anemia causing crisis. As most cold autoantibodies are directed against "I" antigen, and "I" antigen negative donor units are extremely rare so red cell transfusion may potentiate hemolysis.[4,14,17] All patients in the present study required red cell transfusion. Since 'I' antigen negative donor units are extremely rare therefore to reduce transfusion related hemolysis firstly, we used in-line blood warmer and secondly kept all patients optimally warm. For the patient who developed underlying alloantibody-E, PRBC units lacking 'E' antigen were issued for transfusion. All patients were advised to avoid cold exposure, keep themselves warm and those with secondary CAS underwent appropriate management under the department of hematology.

We conclude that detailed clinical and serological characterization is needed to diagnose and manage CAS. Whereas avoidance of cold

**Table 1: Immunohematological details of CAS patients (*n* = 9)**

| Age (years) | Sex | Hb. (g/dL) | DAT (poly) | DAT (mono) | Titre (4°C)/ score | Thermal amplitude | | | | Specificity | Allo-antibody |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 4°C | 22°C | 32°C | 37°C | | |
| 64 | Male | 6.8 | 3+ | C3d (3+) | 2048/125 | 4+ | 4+ | 2+ | Negative | I | Anti-E |
| 27 | Male | 7.2 | 3+ | C3d (3+) | 512/101 | 4+ | 3+ | 1+ | Negative | I | None |
| 40 | Male | 6.4 | 3+ | C3c (1+) C3d (3+) | 1024/113 | 4+ | 3+ | 1+ | Negative | I | None |
| 65 | Male | 7.8 | 3+ | C3d (3+) | 512/99 | 4+ | 2+ | w+ | Negative | i | None |
| 78 | Female | 6.6 | 3+ | C3d (2+) | 1024/107 | 4+ | 4+ | 1+ | Negative | I | None |
| 54 | Male | 5.4 | 4+ | C3c (2+) C3d (4+) | 4096/139 | 4+ | 3+ | 2+ | Negative | I | None |
| 67 | Male | 7.1 | 3+ | C3d (2+) | 512/101 | 4+ | 2+ | 1+ | Negative | I | None |
| 70 | Male | 6.9 | 3+ | C3c (2+) C3d (3+) | 2048/113 | 4+ | 3+ | 2+ | Negative | ? Pr | None |
| 58 | Female | 6.5 | 4+ | C3d (3+) | 1024/107 | 4+ | 3+ | 1+ | Negative | I | None |

Hb = Hemoglobin, DAT = Direct antiglobulin test, CAS = Cold agglutinin syndrome

exposure is the primary therapy, but no critical patient should be denied blood transfusion due to serological complications. All transfusion services should follow the correct protocol to maximize blood safety in CAS.

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