

# Cold Agglutinin Disease: A Transfusion Perspective!

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## ABSTRACT

Cold agglutinin disease is a type of auto immune hemolytic anemia characterized by cold agglutinins which were first described by Landsteiner in 1903. The implicated autoantibodies are IgM type which bind to red blood cells (I, i, H antigen) at lower or colder temperatures leading to hemolysis. The hemolytic potential of such antibodies is usually determined by their titer, specificity and thermal amplitude. In this case, 31 year old female presented with fever, severe pallor and jaundice in a cold winter night. The samples were sent to Department of Transfusion Medicine to provide compatible blood units for transfusion in view of severe anemia. Visible agglutinates in anticoagulated sample and discrepant results in immunohematology laboratory were the first observation to suspect cold agglutinins. The resolution of same was done with simple pre-warming technique using conventional tube test. High titers correlated with severity of symptoms due to hemolysis. The compatible blood units obtained were transfused with special precautions and transfusion was uneventful. This case highlights the importance of simple step of maintaining blood sample of patient of cold antibodies at 37°C and using conventional tube technique for immunohematological tests.

**Key words:** Cold agglutinin, pre-warming technique, conventional tube technique, incompatible crossmatch, autoantibody titer

## INTRODUCTION

Auto immune hemolytic anemia (AIHA) is categorized by clinical presentation and characteristics of implicated autoantibodies. Cold agglutinin disease (CAD) is a type of cold auto immune hemolytic anemia, not so common

as warm AIHA, with reported incidence of 7.7%. [1] CAD is usually characterized by IgM autoantibodies reacting at colder temperatures but sometimes may have broader thermal amplitude. [2] Because of such properties, anticoagulated sample shows autoagglutination of red blood cells and presents as erroneous laboratory results in hematological and immunohematological tests. This poses a great challenge to determine correct blood type of patient as well as selecting and providing a compatible blood unit for transfusion. Here, we report a case of idiopathic cold agglutinin disease presenting with discrepant results in immunohematology laboratory and resolution of same with prewarming technique and conventional tube test.

## CASE REPORT

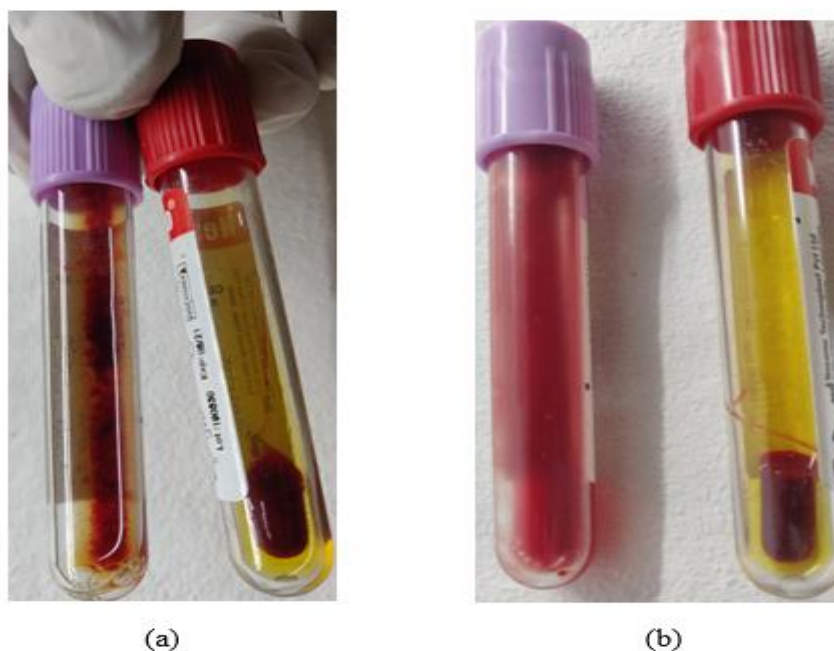
A 31-year-old female, presented to Emergency department on a cold winter night, with complaints of yellowish discoloration of skin and sclera for 3 days, fever for past 1 week and ongoing weakness and fatigue for past 1 month. The history also revealed that for past 4-5 years, she has been developing weakness, lethargy and diminished routine activities during each winter season. Causes of fever due to infections such as malaria, typhoid, mycoplasma pneumoniae and other bacterial, viral or parasitic ones were ruled out. Causes of anemia due to nutritional deficiency or non-immune causes of hemolytic anemia were also ruled out. Patient's samples were sent for hematological and biochemical analysis (Table 1) and in view of severe anemia

(Hemoglobin-3.3 g/dl), 2 units of packed red blood cells (PRBC) were requested from Department of Transfusion Medicine. The

EDTA sample so received for patient was found to have visible agglutinates (Figure 1).

**Table 1: Hematological and biochemical laboratory parameters of patient on admission**

INVESTIGATION	OBSERVED VALUES	UNITS	BIOLOGICAL REFERENCE INTERVAL
Hemoglobin	3.3	g/dl	11.50-15.00
Hematocrit	3.1	%	36-46
MCV	88.01	fL	80.00- 100.00
MCH	103.8	Pg	27.00- 32.00
MCHC	117.9	g/dl	32- 35
RDW-CV	16.69	%	11.6- 14.0
Platelet count	325.3	Thou/mm <sup>3</sup>	150-450.00
Bilirubin, total	5.04	mg/dl	0.30- 1.20
Bilirubin, direct	0.74	mg/dl	< 0.20
LDH	4139.0	U/L	140- 280



**Figure 1: (a) EDTA vial showing visible autoagglutinates at room temperature (b) Autoagglutinates in EDTA vial disappeared after warming at 37°C**

Blood grouping and compatibility testing as performed by column agglutination technique (CAT) revealed ABO blood group discrepancy (Type 3/4). The cell grouping showed the blood group to be A RhD positive while the serum grouping had agglutination in all the columns (Table 2). Two units of PRBCs were crossmatched and both were found to be incompatible at immediate spin as well as anti- human globulin phase (AHG phase) (Table 2). The blood grouping was repeated by conventional tube technique (CTT) also and showed similar results. Direct antiglobulin test (DAT) and indirect antiglobulin test (IAT) were found to be positive by both the technologies (CAT =4+, CTT = 2+).

**Table 2: Blood grouping and compatibility results with initial sample at room temperature (CAT and CTT)**

Anti-A	Anti-B	Anti-D	Autocontrol	A1 Cells	B Cells	O Cells	Anti- A1 lectin
4+	0	4+	2+	4+	4+	4+	4+

Blood group: ? A Rh D positive

DAT, IAT, Autocontrol: Positive (CAT 4+, CTT 2+)

Compatibility test: Incompatible at Immediate spin & AHG phase (CAT 4+, CTT 4+)

Saline replacement technique was used to rule out Type 3 discrepancy but it could not be resolved. Repeat samples (both plain & EDTA) were asked with a caution to use pre-

warmed syringes and vacutainers and to be transported immediately after drawing, which were further stored at 37<sup>0</sup>C. Later, serum and saline warmed at 37<sup>0</sup>C were used to repeat blood group testing and washing red cells for preparation of red cell suspension respectively. All tests were repeated using CTT (Table 3).

**Table 3: Blood grouping and compatibility results with repeat sample warmed at 37<sup>0</sup>C (CTT)**

Anti-A	Anti-B	Anti-D	Autocontrol	A1 Cells	B Cells	O Cells	Anti- A1 lectin
4+	0	4+	2+	0	4+	0	4+

Blood group: A RhD Positive

DAT, Autocontrol: Positive (CAT 4+, CTT 2+)

IAT: CAT 4+, CTT negative

Compatibility test: Compatible at Immediate spin & AHG phase by CTT only

DAT was strongly positive with polyspecific AHG (negative for monospecific IgG and positive for C3) prompting for consideration of cold (IgM) antibodies. The antibody screen was pan reactive with a positive autocontrol indicating presence of autoantibodies. The titration of autoantibody was performed (CTT) using patient serum. Serial double dilutions of patient's serum were prepared in saline, ranging from 1 in 2 to 1 in 4096. Two drops of each dilution were mixed with one drop of 3-5% suspension of pooled group O adult (I) red cells and kept for incubation at 4<sup>0</sup>C for 1 hour. Tubes were centrifuged at end of one hour and examined for macroscopic agglutination. Results were recorded and the titre of cold autoantibody was reported as 1024 (Titers above 64 are considered significant).<sup>[3]</sup> To determine the specificity of cold agglutinins, three similar sets of dilutions were prepared to be tested with group O adult (I) red cells, group O cord (i) red cells and autologous red cells. All tubes were incubated and results were recorded. The interpretation of results confirmed anti-I specificity as there was no reaction with group O cord (i) red cells. Compatibility testing was repeated using warm serum and two group specific compatible units in AHG phase were obtained using CTT. The units were transfused to patient under close monitoring with special precautions- avoidance of cold exposure, covering patient with blankets, maintaining a warmer temperature in the room. The transfusion was reported to be uneventful. The post transfusion hemoglobin was 6.0g/dl. Patient was

simultaneously also given broad spectrum antibiotics and supportive care for suspected underlying condition. Patient was also started on steroids which were tapered slowly. Patient's condition improved significantly (Hb- 8.2 g/dl) and she was discharged in stable condition and was advised to visit out-patient department for further follow-up.

## DISCUSSION

Typical age of presentation of CAD is middle age to elderly with younger age presenting with idiopathic CAD as compared to secondary which follows viral infections or malignancy or any such condition.<sup>[1]</sup> Characteristic clinical manifestations include symptoms of chronic anemia such as fatigue, dyspnoea on exertion, weakness; dark urine, acrocyanosis, pallor, jaundice and in some cases hepatosplenomegaly or lymphadenopathy may also be present. Clinical features vary from patient to patient with dependence on thermal amplitude of cold agglutinins and titer.<sup>[1,2]</sup> Our patient presented in winter season with pallor, jaundice, fatigue and weakness and was a young female without any preceding illness. Severity of anemia gets augmented in winters as temperature of peripheral circulation falls and patient may present with acute hemolytic episode as was noted in this case (Hb= 3.3g/dl; LDH = 4139 U/L). Some patients may present with acrocyanosis which has to be differentiated from Raynaud's phenomenon. Cutaneous necrosis is an unusual presentation reported in few patients.<sup>[4]</sup>

The characteristic laboratory finding includes auto agglutination of anticoagulated blood samples as blood cools to room temperature which is reversed by warming to 37°C. In this case, autoagglutination of anticoagulated blood sample which was later reversed by warming to 37°C, was the first observation to raise suspicion of CAD. The agglutination of red cells leads to unexpected cell counts and peripheral blood smear shows agglutinates. This creates discrepancy in blood grouping and other immunohematological test procedures too. The presence of cold autoantibodies necessitates further work-up so as to differentiate from clinically non-significant benign cold antibodies. Cases with erroneous results for patient's complete blood count and blood type occurring due to cold antibodies, have been reported in literature. [5,6] Yasar et al reported a case of unexpected complete blood counts along with haptoglobin and glycosylated haemoglobin. [7] Kalyani et al also reported that first suspicion of CAD is inability to measure red cell counts and other indices; and suggested to warm the sample at 37°C. [8]

In another report by Lodi et al, a case of 48- year old male patient whose blood group could not be determined and patient died after emergency transfusion of universal O Rh positive PRBC as titers of autoantibody were very high with anti-IH specificity. [9] Mohanty et al reported a patient of CAD secondary to SLE where a blood group discrepancy was reported with high titers of cold agglutinin and patient was transfused the least incompatible PRBC and transfusion was uneventful. [10] In a case reported by Kaur et al, a patient posted for cardiopulmonary bypass graft surgery had incidental detection of cold agglutinin (titer of 128) with normal hematological profile and no evidence of hemolysis. [11]

In present case, blood group discrepancy was resolved, titers and specificity of cold agglutinin were determined and high titers correlated with

severity of symptoms due to hemolysis. Patient was given compatible transfusion under close monitoring with special precautions. This highlights the importance of simple step of maintaining blood sample of patient of cold antibodies at 37°C to perform laboratory tests early as most of the cases present with severe anemia and require blood in emergency. Supportive care plays an important role in management of these patients such as avoidance of cold exposure, maintaining the temperature of surroundings. CAD has better prognosis as compared to other AIHA.

## CONCLUSION

Cold agglutinins can cause erroneous results in hematological and immunohematological tests leading to unnecessarily delay in patient management. Often this is the first observation to suspect cold agglutinins. Special precautions while drawing blood samples, maintaining blood sample at 37°C to perform laboratory tests and using conventional tube technique are key factors in providing accurate results and avoiding unnecessary delay in patient management.

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