International Journal of Research in Medical Sciences
Raturi M et al. Int J Res Med Sci. 2016 Jan;4(1):339-342
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20160055

Case Report

Laboratory work-up and its translational significance in cold agglutinin syndrome

Manish Raturi, Shamee Shastry*

Department of Immunohematology & Blood Transfusion , Kasturba Medical College, Manipal University, Manipal-, Karnataka, India

Received: 28 November 2015
Accepted: 18 December 2015

***Correspondence:**
Dr. Shamee Shastry,
E-mail: shameegirish@gmail.com

ABSTRACT

Autoimmune hemolytic anemia (AIHA) is characterized by the presence of agglutinins directed against autologous erythrocytes causing their reduced survival. Approximately 20% of AIHA are associated with cold-reactive antibodies. About half of patients are termed idiopathic without any underlying causes. Secondary cases are associated with underlying diseases or with certain drugs. We report herein a case of idiopathic cold autoimmune hemolytic anemia in a 65 year old lady without any underlying cause who presented with grouping discrepancy and responded well to treatment. Blood transfusion was completely avoided by keeping her warm and other conservative management including hematinic. At the time of discharge from the hospital her haematocrit reached 24%. Patient now is being followed as an outpatient and she continues to work daily albeit acro-cyanosis occurs sometimes on exposure to cold water. To our knowledge a careful communication between the transfusion services and clinicians can avoid unnecessary blood transfusions in such cases especially in clinically responding patients.

Keywords: Cold autoimmune haemolytic anaemia, Acro-cyanosis, Hematinic

INTRODUCTION

Immune haemolytic anaemias present with reduced survival of erythrocytes because of the deposition of immunoglobulin or complement on the red cell membrane. They are often grouped according to the presence of autoantibodies, alloantibodies or drug associated antibodies. The autoimmune haemolytic anaemia (AIHA) is characterized by the presence of agglutinins directed against autologous erythrocytes due to an altered immune response causing their reduced survival. AIHA has traditionally been classified based on the temperature at which there is maximum activity of this auto agglutinins.[1]

The incidence of AIHA is estimated at 10-30 cases per one million populations.[2] Approximately 20% of AIHA are associated with cold-reactive antibodies.[3] Cold auto-agglutinins are usually demonstrable optimally at 4°C and if benign their titre will be typically below 64. Occasionally, the antibody has increased thermal amplitude and will agglutinate cells at room temperature (20-24°C). These are usually IgM immunoglobulins and therefore can quiet efficiently activate complement mediated hemolysis in the patients. The clinical effects of cold agglutinins occur as a result of temperature dependent auto-agglutination in the peripheries and associated haemolysis. The degree to which these processes occur is dependent upon the titre and thermal range of the antibody.[4] The cold agglutinins may be directed against many antigens present on the erythrocyte

membrane however, most common specificity lies with Ii or Pr system.[5] We report herein a case of idiopathic cold AIHA in a 65 year old lady with no underlying cause or lymphoma who presented with acral cyanosis on exposure to cold and her management during the hospital stay.

CASE REPORT

A 65 year old Indian female (known case of diabetes mellitus type II, hypertension and dysthymia) presented to our hospital with bluish discoloration (acral cyanosis with aggravation of symptoms on exposure to cold) along with pruritus of fingers. She came with the signs of icterus, pallor and hepatomegaly. Twenty days prior to her admission she had experienced low grade fever with rhinorrhoea, cough and difficulty in breathing suggestive of respiratory tract infection. She was multiparous with three surviving children and no previous history of transfusion. Fever was associated with cough with whitish, non-blood stained sputum. There was accompanying burning micturition at that time. Cough dyspnoea and burning micturition subsided on taking appropriate treatment but tiredness and weakness persisted. Clinical laboratory investigations of blood specimen showed Haemoglobin (Hb): 5.8 g/dL, Haematocrit (HCT): 16.7%, Reticulocyte count was 8.55 %, immature reticulocyte fraction 0.57, mean reticulocyte volume 116.2 fL, Total bilirubin : 0.7mg/dL, All RBC indices were raised, lactic acid dehydrogenase (LDH) 1833 IU/L. Urine analysis demonstrated sugar & protein as negative, absence of urine casts or crystals and RBCs. A direct anti-globulin test was positive (Grade 4+) for anti-C3d and weakly positive (Grade w+) for IgG. Peripheral blood smear showed RBC agglutination and normocytic normochromic anaemia with polychromasia and occasional spherocytosis; Leukocytes: 10.4 X 10^9/L; Platelets were mildly increased in number 467 X 10^9/L. In microscopic observation from bone marrow aspiration there was gross clumping however, sections revealed increased erythropoiesis with no evidence of any infiltrative process. Bone biopsy was however, inadequate for interpretation. Her serum protein electrophoresis revealed raised alpha-1 and gamma globulins.

Immune-haematological work up

Both EDTA and plain samples were asked and they showed tinge of haemolysis with visible spontaneous agglutinates (Figure 1). Her blood grouping by CTT (conventional tube technique) showed discrepancy, where forward grouping suggested to be AB Rh D positive while the reverse grouping showed varying grades of agglutination with in-house prepared A1, B and O pooled cells (Table 1). The forward grouping was repeated by washing cells with warm saline six times whereas the reverse test tubes were incubated for 30 minutes at 4°C and 37°C each (Table 2).

Table 1: Initial blood grouping showed discrepancy

Reagent	Grade of reaction
Anti- A	1+
Anti- B	4+
Anti- D	3+
Auto Control	3+
A1 cells	3+
B cells	1+
O cells	2+
Interpretation*	? Cold Ab

Interpretation*: Type IV discrepancy (Forward AB Positive; Reverse pan-agglutination).

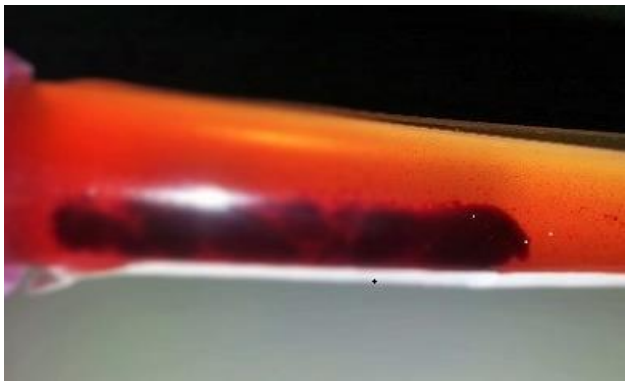

Figure 1: Visible cold agglutinates present in the laboratory EDTA sample of patient.

Table 2: Repeat reverse typing at different temperature range.

Temperature	A1 cells	B cells	O cells	Final interpretation*
4°C	3+	1+	2+	? Cold Ab
37°C	Hemolysis (H) $^+$	0	0	Group B

Final interpretation*****: Blood grouping was confirmed to be as B Rh D positive.

Further immuno-haematological tests

Patient's EDTA sample showed visible agglutinates while performing direct anti-globulin test (DAT). In order to resolve the same we asked for freshly drawn sample in a pre-warmed syringe, immediately kept the sample at 37°C incubator and washed around 1mL of packed RBCs six times with pre-warmed saline as per the manufacturer's instructions. The direct coombs test this time showed (4+ grade) along with control (6% albumin) as weak positive (w+). To disperse these agglutinates further we treated RBCs with 0.01M DTT and repeated the DAT. The direct coombs test performed this time

showed (4+ grade) along with the control (6% albumin) as negative. Therefore an interpretation of truly positive DAT could be made. On mono-specific typing of direct anti-globulin test, patient's erythrocytes were found coated with C3d (4+) and IgG (w+). To diagnose autoantibodies, screening antibody test with commercially available Diamed Gel cards (Biorad) showed pan-agglutination (Table 3). The Donath-Landsteiner test performed was negative.

Table 3: Antibody screen results for the patient.

Screening cell	I panel	II panel	III panel	Auto control
Agglutination* (Grades)	3+	3+	3+	3+

*Agglutination is read from 0, which is a negative result, to 4+, which is the strongest possible agglutination.

Thermal Amplitude Test

Although the IgM involved in CAS has higher affinity for the RBC membrane antigens at 4°C, the presence of haemolysis suggests that it is binding in vivo at physiologic core body temperature. The thermal amplitude test was used to check the titre of the antibody. Serial dilutions of the serum then were incubated at various temperatures of 4°C, 22°C and 37°C respectively, with both adult and umbilical cord O RBCs. A set of auto control was also put along with adult as well as umbilical cord RBCs. The choice of these reagent RBCs was based on the fact that CAS is due almost invariably to an autoantibody to the I antigen, which is present on adult RBCs. Less commonly, the antibody recognizes the i antigen, a similar but less complex carbohydrate antigen found on foetal cells. During the test the resulting autoantibody was found to be of auto anti-I specificity (Table 4 and 5).

Table 4: Thermal amplitude test results for patient.

Temperature	Adult OI cells	Auto-control	Cord Oi cells
4°C	4+	4+	0
22°C	3+	3+	0
37°C at saline phase	1+	1+	0

Table 5: Titre in patient using saline agglutination technique.

Parameters	4°C	22°C	37°C
Versus Adult OI red cell	1:1028(1+)	1:128	1:1
Versus autologous red cell	1:512 (1+)	1:64	1:1
Versus Cord OI red cell	0	0	0

DISCUSSION

At the turn of the 20th century, Landsteiner first described blood agglutination at cold temperatures. Subsequently, Clough and Richter in 1918 found a pathologic association of cold agglutination with RBC breakdown and its occurrence with respiratory infections.[6] Cold auto-agglutinins are commonly found in human sera, mostly IgM antibodies with a narrow thermal range and low titre (<64) of activity making them clinically insignificant or benign in nature.[2] Their specificity will be mostly directed against carbohydrate antigens, most commonly Ii antigen.[7] They can cause in-vitro agglutination of anti-coagulated blood at room temperature visible to naked eye. Biochemical similarity between various carbohydrate antigens contributes to the development of complex antibodies that shows specificity to co presenting antigens. Clinical significance of pathogenic cold agglutinins is mostly restricted to cold agglutinin syndrome and rarely to haemolytic reactions. They routinely express high titre activity at 4oc (> 1000) and a high thermal amplitude. In 90% of patients, cold agglutinin disease is mediated by an IgM molecule, which has a molecular weight of nearly one million Daltons (1000 kDa). With this size, the molecule can span the intercellular distance between red cells. Agglutination occurs at 4°C in the micro-titre well without the use of any anti-globulin antisera, thus the term "cold agglutination."

In this case report patient was found to be suffering from CAS with Anti-I cold agglutinin specificity. Albeit the samples (both EDTA and plain) came to us for pre-transfusion testing, they showed haemolysis warranting further workup. It was at this stage only that cold agglutinins were apparent to us in the EDTA sample mimicking like a clot. Literature suggests that anyone who has observed just one such similar case previously, the diagnosis of CAS immediately comes to the mind. Clues to the diagnosis of cold agglutinin disease included the presence of cold induced acro-cyanosis and Raynaud phenomenon. The in vitro phenomenon of agglutination results in arte-factual changes such that automated particle counters records a false increase in the mean corpuscular volume to levels as high. Similar findings were seen in this case. In a retrospective study from a single institution reported on 58 patients, the direct anti-globulin test revealed C3 in 74% of patients, C3 + IgG in 22.4%, and IgG alone in only 3.4%.[8] Similarly this case also showed DAT as C3 (4+) along with IgG (weak positive).

Similar to our case Swiecicki and co-workers reported a patient who had a cold antibody that had both a high titer (greater than 4096 at 4°C) and a wide thermal range (4 to 37°C) but no haemolytic complications despite the persistence of a positive DAT and cold auto-agglutinin.[6] The wide thermal amplitude rather than the titre is more critical in explaining the clinical significance of an antibody. Thermal amplitude test was performed on a

fresh pre-warmed blood sample which demonstrated reactivity with adult OI cells at all the temperature ranges (4°C to 37°C) albeit it showed, lesser reactivity at 37°C. Titration studies at 4°C and room temperature showed titres of 1028 and 128 respectively with adult OI cells. Reduction of the strength of reaction with pre-warming of the sample at 37°C distinctively pointed towards the absence of a clinically significant antibody.

Treatment of CAS is dependent on its aetiology and severity. For some it may need moving to warmer temperatures and /or climate. Therefore avoidance of cold becomes primary treatment modality. Folic acid supplementation and other haematinics are also recommended for such cases.[2] In this case the patients' clinical course was quite stable, although she had mild exacerbations particularly once during an episode of upper respiratory infection. Although the role of steroids is profound in warm AIHA they are rarely helpful in managing CAS also.[9] In our case patient responded well to steroids treatment and blood transfusion was avoided completely. Blood warmers are advantageous in the management of patients having cold agglutinins however; opinions vary considerably on their routine usage.[10]

Patient was managed conservatively by keeping her warm (using blankets). Her initial haematocrit had reached a low 14% but then gradually improved during the course of her hospital management. At the time of discharge from the hospital her haematocrit reached 24% and her general condition improved. Currently patient is being followed as an outpatient and she continues to work daily albeit the complaints of bluish discoloration of fingers and toes occur sometimes on exposure to cold water.

CONCLUSION

Usually cold agglutinins are benign however, the differences between benign and pathogenic cold agglutinins have not been completely characterized, and further studies will provide insights as to what makes an autoantibody pathogenic. To our knowledge a careful communication between the transfusion services and clinicians can avoid unnecessary blood transfusions in such cases especially in clinically responding patients.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

REFERENCES

1. Khosroshahi BN, Mohammad J, Hossein V, Ahmadi A, Keivan S, Mahdi K. Cold Autoimmune Hemolytic Anemia due to High-grade non-Hodgkin's B cell Lymphoma with Weak Response to Rituximab and Chemotherapy Regimens. Int J Hematol Oncol Stem Cell Res. 2015;9(3):157-60.
2. Gehrs BC, Friedberg RC. Autoimmune hemolytic anemia. American Journal of Hematology. 2002: 258-71.
3. Petz L, Garratty G. Immune Hemolytic Anemias: Second Edition. 2nd ed. Immune Hemolytic Anemias. Philadelphia. 2004:1-591.
4. McNicholl FP. Clinical syndromes associated with cold agglutinins. Transfus Sci. 2000;22(1-2):125-33.
5. Roelcke D. Cold agglutination. Transfus Med Rev. 1989;3(2):140-66.
6. Swiecicki PL, Hegerova LT, Gertz MA. Cold agglutinin disease. Blood. 2013:1114-21.
7. Marcus DM. A review of the immunogenic and immuno modulatory properties of glycosphingolipids. Mol Immunol. 1984;21(11):1083-91.
8. Gertz MA. Cold hemolytic syndrome. Hematol/ Educ Progr Am Soc Hematol. 2006;19-23.
9. Silberstein LE, Berkman EM, Schreiber AD. Cold hemagglutinin disease associated with IgG cold-reactive antibody. Ann Intern Med. 1987;106(2):238-42.
10. Donham JA, Denning V. Cold agglutinin syndrome: Nursing management. Hear Lung J Acute Crit Care. 1985;14(1):59-67.

Cite this article as: Raturi M, Shastry S. Laboratory work-up and its translational significance in cold agglutinin syndrome. Int J Res Med Sci 2016;4:339-42.