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Interferences of Primary Cold Agglutinin with Pre-Transfusion Testing and Automated Peripheral Blood Cell Counting – A Case Report.

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Abstract

Cold autoimmune haemolytic anaemia (AIHA) can be attributed to various factors, including primary cold agglutinin disease (CAD). Cold AIHA in CAD is typically due to the presence of autoantibodies against I. The majority of antibodies found in cold AIHA are of the IgM subtype, which exhibits optimal binding to red blood cells (RBCs) at 0°C to 4°C. The presentation of the primary CAD is variable. Certain patients presented with severe haemolytic anaemia, while others incidentally experienced discrepancies in the pretransfusion testing and interference in full blood count testing due to the presence of cold agglutinin. Here, we present a case of CAD secondary to clonal B lymphoproliferative disease (LPD). A 54-year-old male presented with a fever and had melaena. During blood collection for diagnostic purposes, it was seen that the blood had a tendency to clot readily. Additionally, his blood group showed discrepancies in the forward and reverse groupings. In this particular instance, we highlighted the strategy of minimising and resolving these interferences to facilitate the diagnosis.

Keywords
Cold Agglutinin Disease, CAD, Autoimmune Haemolytic Anaemia, Haemolysis, Discrepancy

Introduction

The classification of autoimmune haemolytic anaemia (AIHA) into warm, cold and mixed autoantibody types is based on the thermal amplitude of the implicated autoantibodies. Approximately 75% of AIHA are

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caused by warm type autoantibody (WAIHA) which mostly are of IgG subtype, that optimally bind at 37^{0}C. The sensitized RBCs are phagocytized in the reticuloendothelial system (RES) depending on the type of the protein that coats the erythrocytes, either IgG, C3b or both that leads to extravascular haemolysis [1]. Most antibodies in cold AIHA are IgM subtype that optimally binds to RBCs at 0 - 4^{0}C. IgM is effectively activating the complement system due to its pentameric structure. IgM activation on the surface of the RBC results in C3 cleavage and deposition of C3b on the erythrocyte surface. C3b binds to the complement receptors on the surface of the phagocytes leading to erythrocyte destruction in the liver. C3b is converted to C3d on the surviving erythrocytes leading to a positive direct antiglobulin test, a key diagnostic feature of Cold Agglutinin AIHA(CA-AIHA) [1-4].

Case Report

A 54-year-old male presented with fever and melaena for four days. No history of vomiting, haematemesis or abdominal pain was reported. He was haemodynamically stable, and his physical examination revealed pallor with no bruises, lymphadenopathy or hepatosplenomegaly. The full blood count (FBC) analysis was performed using Sysmex XN-1000 (Kobe, Japan). Selected parameters of the FBC analysis results of the peripheral blood samples done at room temperature and after prewarming at 37^{0}C are depicted in Table 1. Red cell morphology examination showed marked agglutination, which obviously reduced in the prewarmed blood sample, as shown in Figures 1A and 1B. The direct Coombs test with complement (C3d) monospecific was positive. Lactate dehydrogenase (LDH) was elevated at 1077 IU/L. No evidence of monoclonal antibody in the serum was found. Cold agglutinin titre was 1:2048 with anti-I specificity. Cold agglutinin titre test is done by incubating the patient's serum with washed red cells of adult and cord blood group O at 4^{0}C, room temperature and 37^{0}C to look for antibody specificity (Anti-I or Anti-i) and the titration of antibody.

Table 1: Parameters of the peripheral blood sample analysis at room temperature and after pre-warming at 37^{0}C

Parameters	Room temperature	Pre-warming at 37^{0}C	Reference range
Haemoglobin (g/L)	94	116	135 – 176
Haematocrit (%)	14.3	32.4	39.8 – 52.2
RBC count (10^6/mm^3)	1.38	3.55	4.4 – 6.2
MCV (fL)	103.6	91.3	80.8 – 99.7
MCH (pg)	68.1	31.8	26.6 – 33.8
MCHC (g/L)	657	349	315 – 363
PLT count (10^3/mm^3)	119	296	150 - 450
MPV (fL)	10.2	10.6	9.1 – 12.6
Reticulocyte count (%)	4		1 - 2
WBC (10^3/µL)	4.92	5.77	4.0 - 10.0
Neutrophil (10^3/µL)	1.81	2.24	2.0 - 7.0
Lymphocyte (10^3/µL)	2.49	2.78	1.0 - 3.0
Monocyte (10^3/µL)	0.48	0.61	0.2 – 1.0
Eosinophil (10^3/µL)	0.12	0.12	0.02 – 0.5
Basophil (10^3/µL)	0.02	0.02	0.02 – 0.1

MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, MCV: Mean corpuscular volume, MPV: Mean platelet volume, PLT: Platelet, RBC: Red blood cell. WBC: White blood cell

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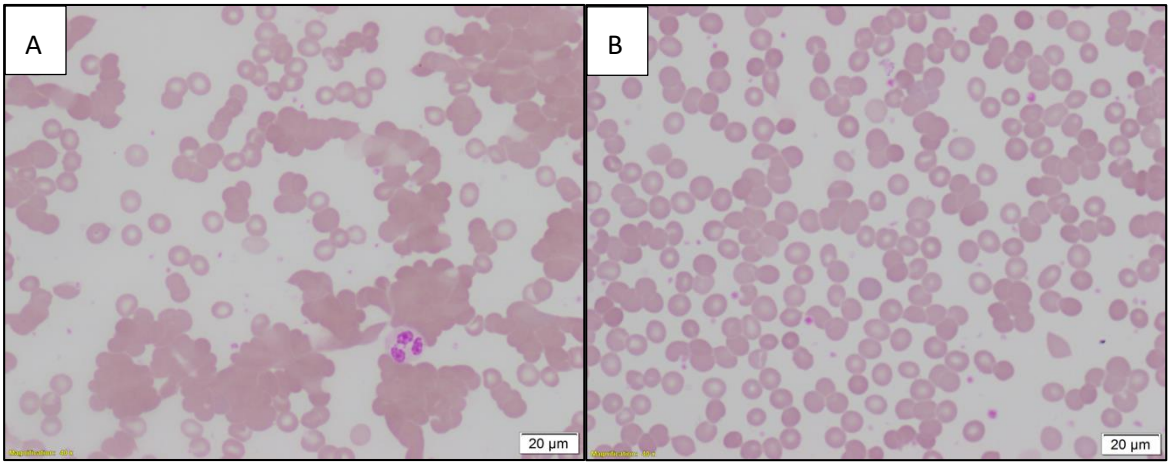

Figure 1: Red cell morphology examination showed marked red blood cell agglutination (A)(40x magnification), which obviously reduced after prewarming the blood sample (B)(40x magnification).

A discrepancy was identified between the results of the forward (anti-B; agglutination reaction grade was 4+) and reverse blood grouping (no reaction with A_1 cells). The repeated blood grouping with increased serum volume in the reverse grouping procedure by adding two additional drops of serum showed 1+ agglutination reaction grade with A1 reagent red blood cells. The weak reaction is probably due to hypogammaglobulinemia secondary to the underlying disease. The blood group is concluded as B, Rh(D) positive. The test for antibody screening was negative.

Serology for *M. pneumoniae*, Epstein-Barr virus, viral hepatitis and HIV were negative. Screening for connective tissue disease was also negative. Bone marrow aspirate and trephine biopsy were done and showed involvement with LPD. The abnormal lymphoid cells in the bone marrow showed immunopositivity with the CD20, CD79a, BCL2, CD5 and PAX5 (Figure 2) and were negative for cyclin D1 and SOX11. The cytogenetic study showed a normal 46 XY karyotype. Accordingly, the diagnosis of LPD-associated CAD was established. He was treated with rituximab and prednisolone and completed 12 cycles of rituximab. Treatment was successful based on clinical assessment, and the latest full blood count showed WBC 7.01 $x10^3/\mu L$, RBC $4.96x10^6/mm^3$, Hb 152 g/L, MCV 92.0 fl, MCH 30.6 pg, platelet $196x10^3/mm^3$.

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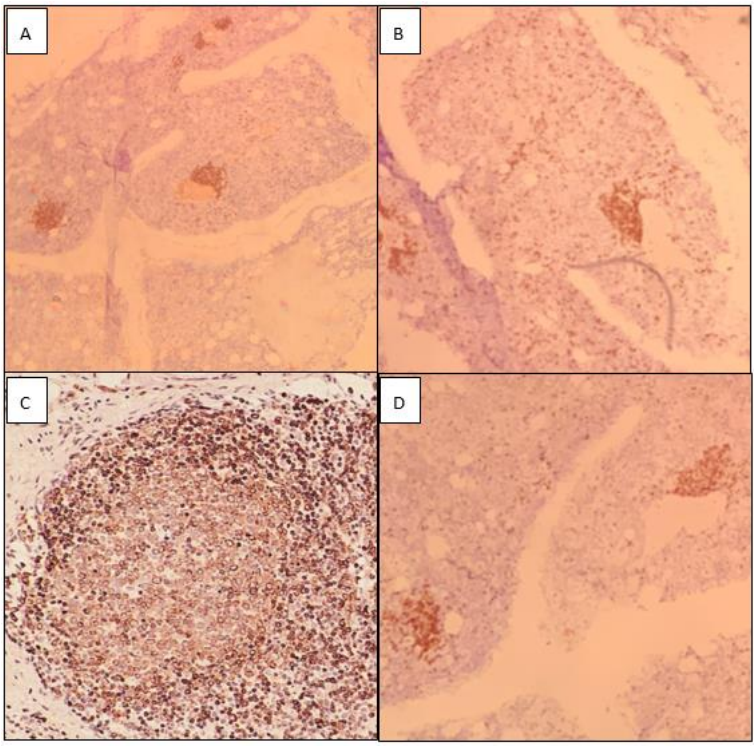

Figure 2: Immunocytochemistry analysis of the bone marrow biopsy showing infiltration by clonal B lymphoid cells that were positive for CD20 (A), CD79a (B), BCL2 (C), CD5 and PAX5 (D). Pictures taken under 10x magnification power for A, B and D, while C was taken under 20x magnification power.

Discussion

CA-AIHA accounts for 25% of AIHA and can be subclassified into primary cold agglutinin disease (CAD) and secondary cold agglutinin syndrome (CAS) [2]. Primary CAD is a rare (1: 1000000) disease characterized by the premature destruction of RBC (haemolysis) [2,4]. Around 90% of patients with CAD had detectable serum kappa light chain monoclonal antibodies or clonal bone marrow lymphoid cells due to underlying lymphoproliferative disease (LPD). In a few cases, occult lymphoma is a cause of CAD, as the lymphoma diagnosis may become apparent long after the original CAD presentation [5]. Primary CAD includes low-grade LPD or unclassified B-cell lymphoproliferation in the bone marrow [6]. Investigating underlying diseases as a cause of CAD is required for proper long-term disease control [7-9].

Primary CAD should be differentiated from secondary CAS, which is usually associated with an autoimmune condition, infections (*Mycoplasma pneumoniae*, Epstein-Barr virus and HIV) or associated with clonal B-cell disorders [2,4]. In the study by Randen et al., clonal B cells were found in most CAD patients [9]. Primary CAD is defined by chronic haemolysis, a significant CA titre > 64 at 4^0C with positive DAT. At the same time, Primary CA-associated LPD has specific bone marrow histopathologic features compared to Lymphoplasmacytic Lymphoma, marginal zone lymphoma and other lymphoma entities [9].

Monoclonal antibodies are seen in primary CAD or secondary to lymphoproliferative diseases. Polyclonal antibodies are detected in post-infectious CAD as a complication of mycoplasma, Epstein-Barr virus, or cytomegalovirus infections [4]. Based on a study in 1995 by Michaux *et al*., trisomy 3 is consistent with

chromosome change in CAD, usually preceding LPD [10]. However, this patient had a normal 46 XY karyotype.

Difficulties in diagnosing CAS are possibly misdiagnosed due to the lack of diagnostic criteria and specific molecular or immunohistochemical technologies to support the diagnosis beyond pathological appearances, which include gross and microscopic appearances [4].

The reason for the finding of higher RBC count in the pre-warming at 37^0C, as compared to room temperature, can be attributed to the fact that the agglutinated RBCs are mistaken as leukocytes, leading to spuriously lower RBC count and unreliable other RBC parameters. In addition, the agglutinated RBCs are counted as one RBC, which also contributed to the low RBC count. RBCs and platelets were measured by a haematology analyser using the principle of hydrodynamically focussed impedance. This is usually affected by the cell size alteration, such as in the case of red cell agglutination. The finding of higher MCV in the prewarmed sample also agreed with the result reported by another study [11-13].

A type 2 ABO discrepancy (missing antibody) was detected during pretransfusion ABO grouping at room temperature. Repeat reverse grouping at room temperature with increased serum-to-cell ratio demonstrated the presence of 1+ reaction at A1 cells with no agglutination at B cells. Increasing the serum-to-cell ratio, will improve the sensitivity of the antiglobulin and facilitate the antigen-antibody reaction [14]. In another case report submitted by Lodi et al., the blood group of a 48-year-old male patient was not determined due to cold agglutinins, and the patient died from complications due to the emergency transfusion of O Rh-positive [9]. This report shows the need to confirm the patient's blood group before any blood transfusion. Any discrepancy or uncertainty needs to be solved before the transfusion.

Conclusion

Primary cold agglutinin disease associated with B LPD is one of differential diagnoses in patients with cold AIHA. Without awareness of the room-temperature interference with pretransfusion testing procedures, life-threatening as well as delaying proper management occurs. Thorough investigation is essential to identify the underlying cause and for the appropriate management planning of CAD.

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Conflict of Interest Disclosure

We declare that we have no conflict of interest.

References

1. Berentsen S, Tjønnfjord GE. Diagnosis and treatment of cold agglutinin mediated autoimmune hemolytic anemia. *Blood reviews*. 2012/5// 2012;26(3):107-115. doi:10.1016/J.BLRE.2012.01.002
2. Berentsen S. Role of Complement in Autoimmune Hemolytic Anemia. *Transfusion Medicine and Hemotherapy*. 2015/12// 2015;42(5):303-303. doi:10.1159/000438964
3. Berentsen S. How I manage cold agglutinin disease. *British journal of haematology*. 2011/5// 2011;153(3):309-317. doi:10.1111/J.1365-2141.2011.08643.X
4. Swiecicki PL, Hegerova LT, Gertz MA. Cold agglutinin disease. *Blood*. 2013/8// 2013;122(7):1114-1121. doi:10.1182/BLOOD-2013-02-474437

5. Berentsen S, Ulvestad E, Langholm R, et al. Primary chronic cold agglutinin disease: a population based clinical study of 86 patients. *Haematologica*. 2006;91(4):460-466.
6. Jäger U, Barcellini W, Broome CM, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: Recommendations from the First International Consensus Meeting. *Blood Reviews*. 2020/5// 2020;41:100648-100648. doi:10.1016/J.BLRE.2019.100648
7. Genty I, Michel M, Hermine O, Schaeffer A, Godeau B, Rochant H. [Characteristics of autoimmune hemolytic anemia in adults: retrospective analysis of 83 cases]. *La Revue de Medecine Interne*. 2002/11// 2002;23(11):901-909. doi:10.1016/S0248-8663(02)00688-4
8. Ercan Ş, Çalişkan M, Koptur E. 70-year old female patient with mismatch between hematocrit and hemoglobin values: the effects of cold agglutinin on complete blood count. *Biochemia Medica*. 2014/10// 2014;24(3):391-395. doi:10.11613/BM.2014.042
9. Randen U, Trøen G, Tierens A, et al. Primary cold agglutinin-associated lymphoproliferative disease: a B-cell lymphoma of the bone marrow distinct from lymphoplasmacytic lymphoma. *Haematologica*. 2014/3// 2014;99(3):497-504. doi:10.3324/HAEMATOL.2013.091702
10. Michaux L, Dierlamm J, Wlodarska I, et al. Trisomy 3 is a consistent chromosome change in malignant lymphoproliferative disorders preceded by cold agglutinin disease. *British journal of haematology*. 1995;91(2):421-424. doi:10.1111/J.1365-2141.1995.TB05315.X
11. Yasar NE, Ozgenc A, Bolayirli IM, Adiguzel M, Konukoglu D. Unexpected laboratory results in cold agglutinin disease. *International Journal of Medical Biochemistry*. 2018;1(1):40-43. doi:10.14744/ijmb.2017.09797
12. Rim JH, Chang MH, Oh J, Gee HY, Kim J-H, Yoo J. Effects of cold agglutinin on the accuracy of complete blood count results and optimal sample pretreatment protocols for eliminating such effects. *Annals of laboratory medicine*. 2018;38(4):371-374.
13. Wahed A, Dasgupta A. Complete Blood Count and Peripheral Smear Examination. *Hematology and Coagulation, Elsevier*. 2015:1-14.
14. Reverberi R, Reverberi L. Factors affecting the antigen-antibody reaction. *Blood Transfus*. Nov 2007;5(4):227-40. doi:10.2450/2007.0047-07