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Interference of cold agglutinin with RBC parameters in a non-hemolytic case

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ABSTRACT

Background: Cold agglutinin disease is a rare condition affecting 1 per million population annually. It is usually associated with hemolytic anemia. We report a case of interference of cold agglutinin with red blood cell (RBC) parameters in a non-hemolytic case.

Case: A 31-year-old male presented with complaints of a cough and fever. Routine blood investigation revealed normal hemoglobin with low RBC, packed cell volume (PCV), high mean corpuscular volume (MCH), and mean corpuscular hemoglobin concentration (MCHC). The indirect Coombs test was negative. So, interference due to cold agglutinin was suspected, which was confirmed following the disappearance of agglutinin after incubation at 37°C for 30 minutes.

Conclusion: The possibility of RBC cold agglutinin should be suspected if there is any mismatch between RBC parameters and appropriate measures should be taken for proper delivery of results.

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Abbreviations

CA, Cold agglutinin; CAD, Cold agglutinin disease; CBC, Complete blood count; K2EDTA, Dipotassium ethyelene diamine tetra acetic acid; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; PCV, Packed cell volume; RBC, Red blood cells; WBC, White blood cells.

Introduction

Cold agglutinin disease (CAD) is a type of autoimmune hemolytic anemia affecting 1 per million population annually (1,2). It accounts for 15-25% of autoimmune hemolytic anemia and is due to presence of IgM antibodies against I antigen present in red blood cells (RBC) which gets activated when the patient is exposed to cold temperature (3). The mean age at diagnosis is 68 years with male: female ratio 0.56 (4). Based on etiology, it can be primary (idiopathic) or secondary due to infection, malignancy or autoimmune condition.

Complete blood count (CBC) is one of the frequent laboratory tests which aids in screening, diagnosis and monitoring of different diseases (5). It is very imperative to provide accurate results for proper management of the patients. The presence of agglutinin, cryoglobulin, lipids, hemolysis, platelet clumps can give spurious results in CBC parameters which requires special attention (6-9). We report a case of interference of cold agglutinin (CA) which resulted in discordant red blood cell parameters without any evidence of hemolysis.

Case Report

A 31-year-old male patient presented to our medical outpatient department with complaints of a cough and low-grade fever for a month. The general condition was ill looking. Respiratory examination revealed bilateral decreased breath sound in the upper zone of lungs. Other systemic examination and vitals were normal. There was no significant past history. As a part of a routine laboratory examination, a blood sample was collected in Dipotassium Ethylenediamine tetra acetic acid (K2EDTA) vial. The sample was analyzed with an automated hematology analyzer (Sysmex XN 1000), the results are presented in Table 1. However, the rule of three was violated as RBC and packed cell volume (PCV) were significantly low compared to hemoglobin, which was normal. Similarly, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were spuriously high but white blood cells (WBC) and platelets were unaffected. The RBC histogram showed a small second population of red cells (Figure 1). The sample tube showed the visual presence of micro clumps in the room temperature. A blood smear revealed RBCs agglutination (Figure 2) and some lymphocytes with reactive changes with no features of hemolysis.

The results after incubating the sample at 37°C for 30 minutes are presented in Table 1 and there was no agglutination was found on subsequent microscopic evaluation. Reticulocyte count was normal (1.1%) and indirect Coombs's test was negative. The biochemical test showed normal total bilirubin, direct bilirubin, lactate dehydrogenase, total protein and albumin. The monospot test and sputum examination for acid fast bacilli was negative. Chest Xray revealed increased broncho vascular markings suggestive of chest infection. Furthermore, CT lung, was advised which showed mild scarring of the apical segment of the upper lung lobes likely a sequela of post infection. After treatment with azithromycin, levofloxacin and fluticasone inhaler, the patient's condition as well as the RBC parameters improved significantly.

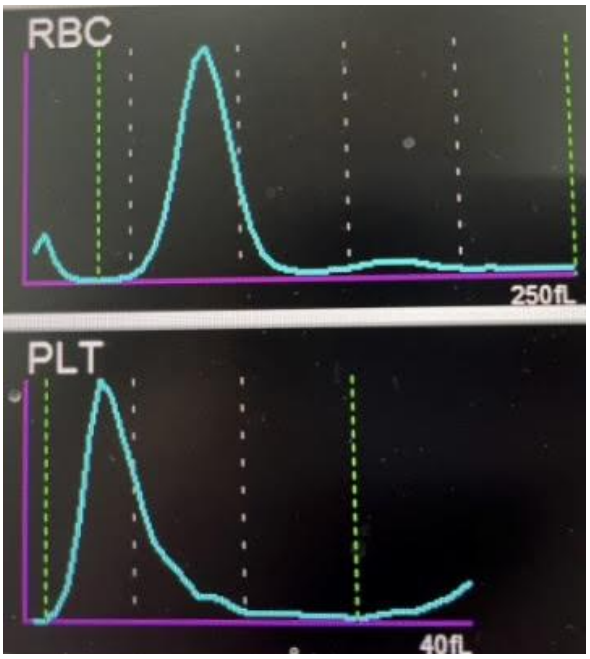

Figure 1. RBC Histogram showing a small second population (blue arrow) of agglutinated RBCs

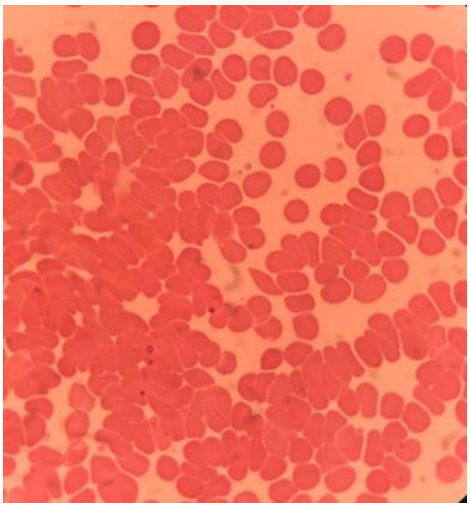

Figure 2. Blood picture showing RBC agglutination (oil immersion x 100)

Table 1. Comparative results of samples at room temperature and after incubation at 37°C.

Parameters	Room Temperature	After Incubation at 37°C	Unit
Hb	14.2	14.1	gm/dl
RBC	2.93	4.59	$\times 10^6$/ul
PCV	26.8	40.2	%
MCV	91.5	87.6	fl
MCH	48.1	30.9	pg
MCHC	52.6	35.3	gm/dl
RDW	13.1	12.9	%
TC	8.38	8.57	$\times 10^3$/ul
DC	N51 L47 M2	N50 L46 M4	%
Platelets	267	288	$\times 10^3$/ul

Hb, Hemoglobin; RBC, Red blood cell; PCV, Packed cell volume; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; RDW, Red cell distribution width; TC, Total count; DC, Differential count; N, Neutrophils; L, Lymphocytes; M, Monocytes.

Discussion

CAD is a type of autoimmune hemolytic anemia associated with agglutination of RBCs in cold temperatures (5-7). About 90% of cases of CAD are due to IgM antibodies and 10% due to IgA or IgG antibodies. CA may be present in healthy individuals but does not show activity above 4°C whereas pathological CA reacts at 28-31°C (10). The temperature at which the antibody is activated is known as thermal amplitude. The severity of the disease depends on the thermal amplitude rather than the antibody titers. The presence of CA could be either primary (idiopathic) or secondary due to lymphoproliferative disorder (lymphoma, chronic lymphocytic leukemia), infection (Mycoplasma, Epstein Bar virus, varicella) or drugs (6-8). The most common clinical features are anemia and acrocyanosis which characterized by dark purple to grayish discoloration of fingers, toes, nose on exposure to cold (11,12). Other symptoms like Raynaud's phenomena, livedo reticularis and rarely gangrene are also seen.13 However, the first suspicion of presence of CA arises due to mismatch of RBC parameters on CBC.10 CA can be monoclonal or polyclonal. The monoclonal CA are associated with hemolysis and have high titers whereas the polyclonal ones are incidental, not associated with hemolysis and have low titers (below 1:64) (3). The peculiarity of our case is the incidental detection of CA without any features of hemolysis on microscopy and biochemistry.

This could possibly be attributed to, *in-vitro* agglutination of RBC or secondarily, due to respiratory illness, similar to a study conducted by Erkus et al. (1). From a laboratory perspective, the presence of CA can interfere with blood parameters giving erroneous results (2,3,9). The principle of cell counting is based on, impedance method where cells suspended in a solution are counted on the basis of electrical impulses generated when they pass through a small aperture. Therefore, the presence of microaggregates of RBCs in CA is detected as a single cell resulting in low RBC count and hematocrit (8). This causes marked elevation of red cell indices, particularly MCHC, all of which are calculated on the basis of RBC counts. However, the value of hemoglobin is correct as it is determined after lysis of RBCs (12). So, the discordance between RBC, hematocrit and Hb along with elevated RBC indices should raise a suspicion towards the presence of CA as reported in studies conducted by various authors (1,5,6,7,9,13). Incubation at 37°C is a commonly used method to eliminate CA which was proposed by Finland et al. (9) in 1945. Likewise, agglutination can be observed visually in the wall of specimen tube, but the gold standard test is by peripheral smear examination (2,13). Additionally, the early identification of such phenomena is crucial as it can pose challenges while performing blood grouping and serological in blood bank laboratories (7,8,10).

CBC histograms and flags produced by the hematology analyzers have good potential for providing relevant information regarding many diseases. Careful analysis of these can provide a diagnostic clue to many hematological diseases like leukemia, dimorphic anemia, pseudo thrombocytopenia, malarial parasites and CA (14). The importance of histograms is further supported by our study as there was a second peak recognized due to RBC aggregated with double the normal corpuscular volume (15). Such aberrant histograms should always be checked with blood smears to avoid any conflict in diagnosis.

Conclusion

The possibility of CA should be suspected if the rule of three is not maintained between hemoglobin, RBC and PCV. As skilled laboratory consultants, we should identify the clues of RBC agglutination and take appropriate measures to obtain accurate RBC parameters which will guide correct diagnosis and management.

Declaration

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Conflicts of interest/Competing interests

The authors declare no conflict of interest.

Authors' contributions

S.M. (Sailuja Maharjan) designed the study concept and drafted the manuscript. C.T. and R.T. collected data, reviewed and revised the manuscript. S.M. (Sumnima Mainali) reviewed and revised the manuscript.

References

1. Erkus E, Kocak MZ, Aktas G, et al. A Rare Non-Hemolytic Case of Idiopathic Cold Agglutinin Disease. Clin. Lab. 2018;64:1075-8

2. Balraam KV, Masood A, Garg N, Somasundaram V. Coomb's negative cold agglutinin disease: A rare report of an incidentally detected case. Asian J Transfus Sci 2021;15:233-6.

3. Gabbard AP, Booth GS. Cold agglutinin disease. Clinical Hematology International. 2020;2(3):95-100.

4. Berentsen S, D'Sa S, Randen U, Małecka A, Vos JMI. Cold Agglutinin Disease: Improved Understanding of Pathogenesis Helps Define Targets for Therapy. Hemato. 2022; 3(4):574-594.

5. Costa BM, Velles MC, Maria C. Vellés, Viana MM, et al. Interference of cold agglutinin autoantibodies in erythrogram interpretation: a case report and literature review. J Bras Patol Med Lab. 2018 Aug; 54(4): 249-52.

6. Ercan S, Calışkan M, Koptur E. 70-year old female patient with mismatch between hematocrit and hemoglobin values: the effects of cold agglutinin on complete blood count. Biochem Med (Zagreb). 2014;24(3):391–395.

7. Nikousefat Z, Javdani M, Hashemnia M, et al. Cold agglutinin disease; a laboratory challenge. Iran Red Crescent Med J. 2015;17(10):e18954

8. Ying Q, Lv D, Fu X. et al. Resolution of serologic problems due to cold agglutinin mediated autoimmune hemolytic anemia and its transfusion decision. J Clin Lab Anal. 2021;35:e23894

9. Lin H, Feng D, Tao S, Wu J, Shen Y, Wang W. A patient with the highly suspected B cell lymphoma accompanied by the erythrocytes cold agglutination: Case report. Medicine 2023;102:25(e34076).

10. Yasar NE, Ozgenc A, Bolayirli IM, Adiguzel M, Konukoglu D. Unexpected laboratory results in cold agglutinin disease. Int J Med Biochem. 2018; 1(1): 40-3.

11. Kawai Y, Deguchi M, Mizouchi N, et al. Cold agglutinin-induced hemolytic anemia during room temperature fluid resuscitation: a case report. J Med Case Reports (2021) 15:169

12. Yordanova M. Cold agglutinins - a common clinical or laboratory problem. Clinical case. MOJ Gerontol Ger. 2020;5(2):37–40.

13. Hafid Zahid et al. Cold agglutinins revelated by abnormalities to the cell blood count: a case report. Pan African Medical Journal. 2021;38(328).

14. Buch AC, Patro N, Banikar R, et al. Analytical study of histograms and their correlation with peripheral blood smear findings. Indian Journal of Applied Reseach. 2020;10(4). 32-5.

15. Thomas A, Bhagya S, Majeed A. Clinical utility of blood cell histogram interpretation. Journal of Clinical and Diagnostic Research.2017 ;11(9):1-4

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