J Bras Patol Med Lab. 2018 Aug; 54(4): 249-252.

10.5935/1676-2444.20180043

Interference of cold agglutinin autoantibodies in erythrogram interpretation: a case report and literature review

Interferências das crioaglutininas na interpretação do eritrograma: relato de caso com revisão da literatura

Bruno Miguel B. Costa; Maria C. Vellés; Maria Mariana F. B. Viana; Catarina Isabel M. Rebelo

Centro Hospitalar do Tâmega e Sousa E.P.E., Serviço de Patologia Clínica, Porto, Portugal.

ABSTRACT

The erythrogram is one of the components of the blood count that includes red blood cell (RBC) quantification and evaluation. A correct interpretation and validation of the results obtained in an erythrogram require experience and critical awareness of health professionals. It is imperative to evaluate the interference of physiological variables, collection procedures, manipulation of samples and endogenous variables (such as the presence of cold agglutinin autoantibodies), since these may falsify the results obtained. Cold agglutinin autoantibodies are predominantly immunoglobulin type M (IgM), which cause agglutination of RBC at temperatures below 37°C, and may appear in cases of autoimmune hemolytic anemia and atypical pneumonia, among other pathologies. The presence of erythrocyte agglutination interferes with RBC and reticulocyte counts, determination of the globular volume and the blood count indices. A set of laboratorial procedures may be performed in order to eliminate the interference of these agglutinins in the results of the erythrogram. If these procedures do not correct the values obtained, the only result of the erythrogram that can be validated is hemoglobin, since the remaining results are falsified due to the presence of cold agglutinin autoantibodies.

Key words: erythrogram; cold agglutinin autoantibodies; interferences.

INTRODUCTION

The blood count is currently one of the most frequently prescribed laboratory tests and is frequently used as an aid in screening, diagnosis and monitoring the many different pathologies(1). This laboratory test is divided into erythrogram, leukogram and thrombogram, including quantification and morphological evaluation of red blood cells (RBC)(1, 2). The erythrogram includes RBC quantification and evaluation, as well as RBC, hemoglobin (HGB) and hematocrit (HCT) count and RBC indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MHC), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW or RDW-CV or RCDW and RDW-SD)(1-3). For a correct interpretation and validation of the results obtained in an erythrogram it is imperative to take into account that physiological variables, collection procedures, manipulation of samples and endogenous variables are able to falsify the results obtained(4, 5). One of the endogenous variables that may compromise the results is the presence of cold agglutinins, first described by Landsteine in 1903(5). These autoantibodies, predominantly immunoglobulin class M (IgM), may appear in some patients with autoimmune hemolytic anemia and atypical pneumonia, among other pathologies(6, 7). Cold agglutinins cause erythrocytes agglutination at temperatures below 37°C, interfering with RBC and reticulocytes counts, and the determination of globular volume and RBC indices(7, 8).

First submission on 06/12/18; last submission on 06/12/18; accepted for publication on 06/19/18; published on 08/20/18

CASE REPORT

An 85-year-old female patient diagnosed with autoimmune hemolytic anemia (cold agglutinins) underwent routine analysis. A peripheral venous blood sample was collected in an anticoagulant tube (EDTAK3, 3 ml, VACUETTE®, Premium, Greiner Bio-one, Austria) and then processed on the XN-2000 autoanalyzer (Sysmex Corporation, Kobe, Japan). Four different procedures to analyze the sample were performed in order to evaluate the interference of the cold agglutinin autoantibodies in the erythrogram results:

- procedure I – sample analysis without any pretreatment. The sample followed the normal laboratory processing circuit being collected and transported to the laboratory for analysis. This transport occurred at room temperature (below 37°C);
- procedure II – sample was incubated in an laboratory oven at 37°C for 30 minutes;
- procedure III – sample was incubated in water bath at 37°C for 30 minutes;
- procedure IV – sample was centrifuged at 3500 rpm for 10 minutes. Plasma volume replacement (in this case 1.5 ml) by equal volume of saline 0.85%. Sample homogenization and incubation in water bath at 37°C for 30 minutes.

The results obtained for the four procedures, referring to the different parameters that compose the erythrogram, are shown in **Table**.

TABLE – Results of the erythrogram for procedures I, II, III and IV

Results	Erythrogram (units)							
	RBC (10^6/ul)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW-SD (fl)	RDW-CV (%)
Procedure I	0.19	10	2	105.3	526.3	500	52.4	28.9
Procedure II	1.49	9.6	16.9	113.4	64.4	56.8	n.d.	n.d.
Procedure III	1.97	9.7	21.6	109.6	49.2	44.9	n.d.	n.d.
Procedure IV	3.19	9.9	29.6	92.8	31	33.4	56.3	16.8

RBC: red blood cell; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-SD: distribution range of erythrocytes measured as standard deviation; RDW-CV: distribution range of erythrocytes measured as coefficient of variation; n.d.: not determined.

DISCUSSION

Cold agglutinins at low titers (1:32) may be present in children and adults without causing any problem or pathology, but at high titers (greater than 1:1000) may cause hemolysis[9]. These predominantly IgM autoantibodies may be monoclonal or polyclonal, each one having a different etiology and prognosis[10].

Hemolytic anemia caused by cold agglutinin autoantibodies may be of primary origin (association of hemolytic anemia with Raynaud's phenomenon) or of secondary origin, in response to an infectious process [e.g., *Mycoplasma pneumoniae*, infectious mononucleosis, cytomegalovirus, human immunodeficiency virus (HIV)], as well as in neoplasms (lymphomas, chronic lymphocytic leukemia) or induction by certain drugs[11].

Although these antibodies react better at 4°C, pathological cold agglutinin autoantibodies are more reactive at 28°C-31°C[12-14]. RBC agglutination is reversible with increasing temperature occurring both *in vitro* and *in vivo*[12, 13]. In the present case, the agglutination *in vitro* due to a decrease in temperature was first detected macroscopically by the presence of small clusters of RBC along the wall of the collection tube with anticoagulant and, subsequently, microscopically by the observation of RBC clusters in the peripheral blood smear.

The presence of cold agglutinin autoantibodies may cause serious problems in the laboratory diagnosis in the determination of blood groups, due to the interference of hemolysis in some biochemical parameters and the interference in erythrogram results due to erythrocytes aggregation[8].

The rule of the three principles is a valuable tool for beginning the interpretation of erythrogram results. According to this rule, the hematocrit result is obtained by multiplying the hemoglobin value by three, and the hemoglobin result can be obtained by multiplying the number of RBC by three[11]. However, this rule has a counterpart, since it can only be used in patients with normal values[12]. The presence of results that do not comply with the formulas presented should refer to the search for significant changes in the RBC indices, as well as the observation of the erythrocyte morphology in the peripheral blood smear[1].

The RBC indices (MCV, MCH and MCHC) are a valuable tool for the analysis and interpretation of the erythrogram, amongst these indices, we should pay particular attention to MCHC, since this parameter is not only an indicator of color but also an indicator of problems with the sample (MCHC normal value is 30 to 36 g/dl)[1, 11]. The presence of cold agglutinin autoantibodies is one of the most frequent cases of a false positive result for MCHC[12, 14, 15].

In the case presented, an 85-year-old female patient diagnosed with autoimmune hemolytic anemia (cold antibodies), we verified in procedure I, that the rule of three was violated, since the presence of RBC aggregates is counted by the autoanalyzer as a single RBC, it resulted in low RBC counts and therefore decreased HCT. In this case, the HGB assay was not inferred as the only real parameter to be validated, obtaining physiologically impossible values of MCH and MCHC. The sample warming at 37°C (procedure II) aimed at reducing the inference of the cold agglutinin autoantibodies and, although the results improved considerably, the HGB would be the only result to be validated. The inclusion of the procedure III appeared in the attempt to improve the results obtained, since the warming in water bath allows a uniform heating of the sample, thus improving the performance of procedure II. In spite of a significant improvement of the results, the inclusion of the procedure IV was fundamental to obtain results within the reference values. Plasma replacement by saline allowed the removal of some of the cold agglutinin autoantibodies, and obtaining results within the reference values allowed their validation.

CONCLUSION

The interference of cold agglutinin autoantibodies in the erythrogram results can be solved through a set of laboratory procedures. However, it is not always possible to solve this problem, so the laboratory will have to rely on the professionals' experience so that false results are not validated, in which cases only the hemoglobin result may be validate. This procedure must always be complemented with visualization of a peripheral blood smear and its description in order to guide the clinician for an assertive diagnosis.

RESUMO

O eritrograma é um dos componentes do hemograma que inclui a quantificação e a avaliação eritrocitária. Uma correta interpretação e validação dos resultados obtidos em um eritrograma requer experiência e sentido crítico dos profissionais de saúde. Torna-se imperativo avaliar a interferência de variáveis fisiológicas e de colheita, a manipulação das amostras e as variáveis endógenas (como a presença de crioaglutininas), uma vez que estas podem falsear os resultados obtidos. As crioaglutininas são autoanticorpos predominantemente do tipo imunoglobulina da classe M (IgM), as quais provocam aglutinação dos eritrócitos a temperaturas inferiores a 37°C, podendo aparecer em casos de anemia hemolítica autoimune e pneumonias atípicas, entre outras patologias. A presença de aglutinação eritrocitária interfere na contagem de eritrócitos, reticulócitos, determinação do volume globular e dos índices hematimétricos. Laboratorialmente, existe um conjunto de procedimentos que podem ser executados de modo a eliminar a interferência dessas aglutininas nos resultados do eritrograma. Caso esses procedimentos não corrijam os valores obtidos, o único resultado do eritrograma que poderá ser validado é o da hemoglobina, visto que os resultados restantes estão falseados devido à presença de crioaglutininas.

Unitermos: *eritrograma; crioaglutininas; interferências.*

REFERENCES

1. Failace R. Hemograma: manual de interpretação. 5 ed. Porto Alegre: Artmed; 2009. p. 21-30.

2. Lewis S, Bain B, Bates I. Hematologia prática de Dacie e Lewis. 9 ed. Porto Alegre: Artmed; 2006. p. 30-48.

3. Hoffmann LP, Polletti C, Roehrig KS, et al. Avaliação dos índices hematimétricos emitidos pelos contadores hematológicos Pentra 120 Range e Sysmex XT-2000i. Rev Bras Análises Clínicas. 2007; 39(1): 25-8.

4. Plebani M. Errors in clinical laboratories or errors in laboratory medicine? Clin Chem Lab Med. 2006; 44(6): 750-9.

5. Narayanan S. The preanalytic phase. An important component of laboratory medicine. Am J Clin Pathol. 2000; 113(3): 429-52.

6. Swiecicki PL, Hegerova LT, Gertz MA. Cold agglutinin disease. Blood. 2013; 122(7): 1114-21.

7. Gupta V. Assessment of red blood cell parameters and peripheral smear at different temperatures in case of cold agglutination disease. Ann Med Health Sci Res. 2014; 4(1): S25-8.

8. Stamminger G, Beier L. Use of the XE-2100 in a patient with cold auto-immune hmolytic anemia. Sysmex J In. 2000; 10(1): 3-12.

9. Axelson JA, LoBuglio AF. Immune hemolytic anemia. Med Clin North Am. 1980; 64: 597-606.

10. Gallo JQ, Picado MM, Taylor CG. Síndrome aglutininas frías. Acta Méd Costarric. 2004; 46 (4): 204-7.

11. Silva PH, Alves HB, Comar SR, Hennberg R, Merlin JC, Stinghen ST. Hematologia laboratorial: teoria e procedimentos. Porto Alegre: Artmed; 2015. p. 193.

12. Walters J. O poder dos índices hematimétricos. Desmistificando as regras de 3. Seminário em Português da Sysmex; 2011.

13. Rosenfeld R. Hemograma. J Bras Patol Med Lab. 2012: 48(4): 255-8.

14. Kalyani R, Thej MJ, Thomas AK, Raveesha A. Chronic cold agglutinin disease: a case report with review of literature. JCDR. 2012; 6(3): 480-2.

15. 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.

CORRESPONDING AUTHOR

Bruno Miguel Barbosa da Costa

Centro Hospitalar do Tâmega e Sousa, E.P.E.; Serviço de Patologia Clínica; Avenida do Hospital Padre Américo, 210; 4564-007; Guilhufe-Penafiel, Portugal; e-mail: BrunCosta1@gmail.com.