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(CASE REPORT)

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Panagglutination of red blood cells complicating blood typing: A Malagasy case report

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Abstract

Introduction: Blood grouping appears to be simple to perform and interpret. Recommendations have been established to ensure the accuracy of the results. However, there are cases that create difficulties and may distort the result. We report a case of blood grouping problem with panagglutination of red blood cells found in the laboratory of University Hospital Center (UHC) Andrainjato Fianarantsoa Madagascar.

Case description: A 63-year-old woman of Asian origin was hospitalized in the Internal Medicine Department for fever and physical asthenia. She had a family history of beta-thalassemia minor. The blood count showed pancytopenia (regenerative anemia at 31 g/L, neutropenia at 1.7 G/L, thrombocytopenia at 34 G/L), with a probable presence of cold agglutinin (MCHC = 400 g/L). The blood grouping by globular test allowed to find a positive rhesus group AB, but the serum test was in favor of group A. The repeat of the haemogram and the blood grouping after incubation of the sample for one hour at +37° C made it possible to correct the MCHC and to objectify a positive blood group A rhesus respectively. The latter was confirmed by the pre-transfusion compatibility test with the tested isogroup blood and the transfusions performed were without incident.

Conclusion: Panagglutination of red blood cells, probably related to the presence of cold agglutinin in our case, represents one of the sources of blood grouping errors that should be avoided by the application of strict blood grouping rules.

Keywords: Blood grouping; Cold agglutinin; Compatibility; Panagglutination

1. Introduction

Hemovigilance is a very important issue in blood transfusion. Apart from infectious risks, the control of immunological risks is essential [1]. Blood grouping is one of the basic tests for the biological qualification of blood donations. It can be defined as a classification based on the presence or absence of antigenic substances on the surface of red blood cells and/or serum antibodies [2]. It is a specific test that represents, together with other tests, a mandatory pre-transfusion evaluation. Blood grouping is a safety test on which the life of the transfused patient depends. Therefore, it must be performed with the utmost rigor [3].

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Although this test seems to be easy to perform and to interpret, there may be discrepancies in the results, which may lead to difficulties in interpretation. Through this article, we report a case of a problem related to blood group determination encountered in the laboratory of the University Hospital Center Andrainjato Fianarantsoa Madagascar. Our main objective is to report this unusual case, and secondly to remind the modalities of grouping for the awareness of each practitioner.

2. Description of the case

This is a case of a 63-year-old woman, college supervisor, from the Asian continent, referred to the internal medicine department for physical asthenia and dyspnea evolving for 2 weeks before her admission, associated with a fever of +38° C for several days. In her personal history, she was hypertensive under treatment, complicated by cardiopathy and stage II hypertensive retinopathy. A notion of metrorrhagia of great abundance, of undocumented cause, occurred 12 years ago. There was a history of beta-thalassemia minor in her sister.

On physical examination, the patient was apyretic (+37°2 C), with peripheral oxygen saturation assessed at 95% on room air, blood pressure at 100/60 mmHg. She had a poorly tolerated clinical anemia syndrome (mucocutaneous pallor, tachycardia at 105 bpm, dyspnea at 25 cpm). The lymph nodes are free, no hepatosplenomegaly. The rest of the clinical examination showed nothing special.

The results of the biological assessments are shown in the following table (Table 1).

Table 1 Results of initial biological tests

Tests performed		**Results**	***Reference value***
Hemogram	Red blood cells	1,69	*3,50 – 5,50 T/L*
	Hemoglobin level	31	*110 – 160 g/l*
	Hematocrit	0,11	*0,40 – 0,55*
	MCV	106	*80 – 95 fl*
	MCH	42	*27 – 31 pg*
	MCHC	400	*320 – 360 g/l*
	Leukocytes	2,5	*4,0 – 10,0 G/L*
	Neutrophils polynuclear	68% (1,7)	*2,0 – 7,5 G/L*
	Eosinophils polynuclear	1% (0,0)	*<0,4 G/L*
	Basophilic polynuclear	0% (0,0)	*<0,2 G/L*
	Lymphocytes	25% (0,6)	*1 – 4 G/L*
	Monocytes	6% (0,2)	*0,1 – 1,0 G/L*
	Platelets	34	*150 – 450 G/L*
Reticulocyte count		192,8	*20 – 120 G/L*
ESR (at first hour)		84	*4 – 10 mm*
C-reactive protein (CRP)		2,1	*<6 mg/l*
ASAT		62	*<45 UI/L*
ALAT		16	*<41 UI/L*
Total bilirubin		125	*<21 µmol/l*
Direct bilirubin		6,5	*<5 µmol/l*
Creatinine		93	*45 – 105 µmol/l*

Lactate dehydrogenase		756	*225 – 450 UI/L*
Blood ionogram	Chlorine	98	*96 – 108 mmol/l*
	Potassium	3,7	*3,5 – 5,0 mmol/l*
	Sodium	138	*135 – 145 mmol/l*
Sars-Cov-2 antigen test		Negative	
Direct Coombs test		Positive	

MCV: Mean Corpuscular Volume; MCH : Mean Corpuscular Hemoglobin; MCHC : Mean Corpuscular Hemoglobin Concentration; ESR : Erythrocyte sedimentation rate; ASAT : Aspartate amino-transferase; ALAT : Alanine amino-transferase

Table 2 First blood grouping performed

Blood grouping						
Beth-Vincent test				**Simonin test**		
Anti-A	**Anti-B**	**Anti-AB**	**Anti-D**	**RBCT-O**	**RBCT-A**	**RBCT-B**
+++	+++	+++	+++	-	-	+++
Interpretation : AB Rhesus Positive				Interpretation: A		

RBCT: Red Blood Cells-Test

In total, there was severe pancytopenia, including a very regenerative macrocytic anemia associated with a significant increase in the MCHC to 400 g/L. Biological signs of hemolysis (anemia + predominantly free hyperbilirubinemia + elevated LDH with positive direct Coombs test) were demonstrated. For blood grouping, a discrepancy in results between the Beth-Vincent blood test and the Simonin serum test was noted (Table 2).

In this situation, the suspicion of the presence of cold agglutinin prompted us to incubate the whole blood sample in a water bath at +37° C for one hour. A repeat blood count immediately afterwards showed normalization of the CCMH level. The blood grouping was also redone and the result was as follows (Table 3).

Table 3 Blood grouping after sample incubation

Blood typing after sample incubation						
Beth-Vincent test				**Simonin test**		
Anti-A	**Anti-B**	**Anti-AB**	**Anti-D**	**HT-O**	**HT-A**	**HT-B**
+++	-	+++	+++	-	-	+++
Interpretation : A Rhesus Positive				Interpretation: A		

Rhesus-positive blood group A was retained as the final result. The pre-transfusion compatibility test between the patient's plasma and the red blood cells of the group A rhesus positive blood bag was correct. Thus, the transfusion of 4 bags of whole blood and one bag of fresh frozen isorhesus grouped plasma was uneventful.

3. Discussion

In Madagascar, the national blood transfusion policy aims to strengthen the competence and performance of each hospital in terms of hemovigilance. The creation of regional blood transfusion centers (CRTS) with appropriate infrastructures is one of the achievements in this direction [4]. The Andrainjato University Hospital laboratory works in close collaboration with the Haute Matsiatra Fianarantsoa CRTS. As part of its remit, blood grouping is an important activity. According to the recommendations, it must be systematically carried out by two different technicians, with two different batches of reagents and two different techniques including the Simonin serum test and the Beth-Vincent blood test [2,5]. In order to issue a definitive blood group card, in addition to the conditions mentioned above, a second sample will be necessary at a distance from the first.

The problem encountered in blood grouping is a rare phenomenon, but can have serious consequences if not detected. Firstly, it can falsify the result; and secondly, it can delay the transfusion act in case of emergency. The frequency of this incident varies according to the studies. According to Bhallil O et al in Rabat in 2015, the analysis of 700 blood groupings performed shows the presence of interpretation difficulties in 3.14% of cases [5]. A much lower rate was found by a multicenter study performed by Ferrera-Tourenc et al at the ten laboratories of the Alpes-Méditerranée in France, with a figure of 0.04% [6]. To our knowledge, no data on the frequency of difficult grouping has been reported in Madagascar to date.

According to the literature, the main difficulties encountered in blood grouping are the double population visualized as a carpet of agglutinates on a background of free red blood cells, as well as the discrepancy between the two tests, either by lack of agglutination or by excess agglutination [7]. In a Moroccan study, 59% of problematic groupings showed a double population pattern, all of which are of transfusion origin [5]. Indeed, this situation could be multifactorial: recent transfusion (less than 3 months), congenital origin (dizygotic twins), post hematopoietic stem cell transplant and hematological malignancies (transient), ... [8,9].

Regarding the discrepancy between serum and blood tests, the presence of irregular antibodies is one of the possible causes. In our case, the presence of cold agglutinins caused a polyagglutinability of the red blood cells in the globular test, leading to the wrong assumption of a group AB. Cold agglutinins are anti-erythrocyte antibodies that agglutinate red blood cells at temperatures below +37° C. Their agglutination is optimal at +4° C and can be observed up to +20 or +25° C, this phenomenon being reversible after warming [10]. Among cold agglutinins, we can distinguish alloantibodies (anti-M, anti-P1, anti-Lea) which are natural irregular antibodies rarely responsible for transfusion accidents; and autoantibodies which, in vitro, can be responsible for spontaneous agglutination of sensitized red blood cells, visible on the blood smear and sometimes also macroscopically on the anticoagulated blood tube [11].

In vivo, agglutination may occur in superficial vessels of the extremities where the temperature may drop to +28 or +31° C. It causes signs of accrocyanosis and may lead to necrosis of the extremities if ischemia is prolonged [12]. In the deep circulation, these cold agglutinins can activate the complement system which can lead to intravascular hemolysis. Thus, the pathogenicity of cold agglutinins is more related to their thermal amplitude than to their concentration.

In our case, the hemolysis workup was positive, in favor of the dreadful complications of these cold agglutinins. A few cases have been reported in the literature, including a case of mixed autoimmune hemolytic anemia with cold agglutinins induced by an immunological checkpoint inhibitor [13]. Chandesris et al found that the titer and thermal amplitude correlated with the hemolytic activity of cold agglutinin in 52 patients. Secondary causes were prevalent (77.6%) with mainly autoimmune diseases (n = 19), lymphoid hemopathies (n = 11) and infections (n = 10) [14].

Faced with the problem of blood group determination, some perspectives can be considered. In the case of inconsistent reactions or any other anomaly observed, the use of internal quality controls can ensure the quality of the reagents used. In addition, different controls can be performed: the autologous control, which consists of putting the subject's plasma and his or her own red blood cells together to detect autoantibodies, and the allo control, which is performed by putting test red blood cells "O" together with the subject's plasma to detect alloantibodies. In case of difficulty with blood grouping, it is systematically repeated using the tube technique, which consists of preparing a saline suspension with the red blood cells in physiological serum at a 5% dilution. Some interpretation difficulties can be solved by washing the red blood cells in saline solution 3 to 4 times in order to repeat the blood test, or by heating the serum in a water bath at +37° C to repeat the plasma test.

4. Conclusion

The difficulty of blood grouping by panagglutination of red blood cells due to the presence of cold agglutinins described in this case reminds us of the need to apply all the modalities required in the practice of this examination. The importance of knowing the alternatives to the problems that may occur helps to avoid a delay in the transfusion act as well as the mismanagement of the labile blood product stock by abusive use of O-negative cells or AB-negative plasma.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Zmouli N, Seghier F. Haemovigilance. Clinical and Biological Transfusion. 1 Nov 2014, 21(4):256-7.

[2] Gallet A, Bazin A, Mauny J, Malherbe M, Dupuis M. Blood grouping, redundancies and relevance. Clinical and Biological Transfusion. 1 Nov 2021, 28(4, Supplement):S82-3.

[3] Zmouli N, Seghier F. Transfusion safety. Clinical and Biological Transfusion. 1 Nov 2014, 21(4):268.

[4] Stivionade. Blood transfusion in Madagascar: progress for the future [Internet]. All about transfusion. [cited 27 March 2023].

[5] Bhallil O, Benseffaj N, Ouadghiri S, Drissi Bourhanbour A, Essakalli M. Blood grouping: difficulties of interpretation. Journal of Medical Biology. 2015, 3(12):280-5.

[6] Ferrera-Tourenc V, Gouvitsos J, Chabrières C. Incidence of ABO mismatches detected in EFS Alpes-Méditerranée: analysis of causes over eight years and at regional level. Clinical and Biological Transfusion. 2012, 19:280-310.

[7] Khan MN, Khan TA, Ahmed Z. Discrepancy in ABO blood grouping. J Coll Physicians Surg Pak. 2013, 23(8):590-2.

[8] Chiaroni J, Lauroua P, Roubinet F. Decision support in immuno-haematology: Interpretation of ABO blood grouping and its difficulties. Clinical and biological transfusion. 2000, 7(1):84-95.

[9] Mannessier L, Chiaroni J, Roubinet F. The difficulties of ABO blood grouping. Hematology Review. 2002, 8(5):370-5.

[10] Judd WJ. How I manage cold agglutinins? Transfusion. 2006, 46:324-6.

[11] Jamal O, Sekkach Y, Messaoudi N, Doghmi K. Clinical and aetiological profile of cold agglutinin syndrome. The Journal of Internal Medicine. 1 déc 2021, 42:A308.

[12] Nakagawa H, Kato C, Miyata Y. Necrosis due to cold agglutinin disease. CMAJ. 27 juin 2022, 194(25):E889-90.

[13] Bisiou S, Witkowski P, Palassin P, Castille E, Jauzelon B, Marine LR, and al. Immunological checkpoint inhibitor-induced mixed autoimmune haemolytic anaemia with cold agglutinins: a case report and review of the literature. The Journal of Internal Medicine. 1 June 2022, 43:A198.

[14] Chandesris MO, Schleinitz N, Ferrera V, Bernit E, Mazodier K, Gayet S, and al. Cold agglutinins, circumstances of discovery in adults and significance in clinical practice: retrospective analysis of 58 patients. The Journal of Internal Medicine. 1 Dec 2004, 25(12):856-65.