Case Report

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Auto- or Allo-Antibody: Sometimes Difficult to Differentiate

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ABSTRACT

One of the complications of blood transfusion is the production of antibodies directed against antigens present on the erythrocyte membrane, due to genetic differences between the blood donor (BD) and the recipient. Similarly, several drugs used in daily clinical practice are implicated in the development of autoimmune hemolytic anemia. The present report presents a case where both conditions coexist.

Abbreviations: BD: Blood Donor; RBC: Red Blood Cell; IAS: Irregular Antibody Screening; PRBC: PACKED RBC; γGT: Gamma-Glutamyl Transferase; LDH: Lactic Dehydrogenase; DAT: Direct Antiglobulin Test

Introduction

Because of genetic differences, blood transfusions can lead to red blood cell (RBC) sensitization, resulting in the development of antibodies against blood group antigens (alloantibodies). Conversely, the use of antibiotics in clinical practice can also generate antibodies specifically targeting the same antigens (autoantibodies) through different immune mechanisms, making it difficult to distinguish between them in routine transfusion practice (Thornton N, et al. [1]).

Material & Methods

Case Report

A 40-year-old man with multiple traumas from a bus versus motorcycle accident, who has external fixation of a left acetabular and femoral neck fracture and extensive necrosis in the thigh without signs of inflammation and already on antibiotics (cefazolin), was referred to MS/INTO for a specialized surgical procedure. He has no history of prior transfusions; his initial blood sample showed an O RhD negative (O-) blood type and negative Irregular Antibody Screening (IAS). Two O- packed RBCs (PRBCs) were used during surgery. Twelve days later, the patient developed a fever, leukocytosis, and worsening liver and kidney function (total bilirubin=9.4 mg/dL with direct=7.6 mg/dL, γGT=826 U/L, LDH=1,116 U/L, and creatinine=1.6 mg/dL), leading to the start of meropenem and vancomycin. A culture of the necrotic lesion on the thigh was positive for Enterobacter cloacae. After five days, due to a drop in hemoglobin to 6.3 g/dL, a transfusion of PRBC O- was performed. IAS remained negative. Due to a new surgical procedure, 35 days after admission, a reserve of two PRBCs was requested from the Blood Bank, and a new blood sample was taken from the patient.

Results & Comments

A new blood sample tested positive for IAS and an autologous control. The direct antiglobulin test (DAT) was reactive with human antiglobulin anti-IgG + C3d. Adsorption-elution studies with homologous RBC (Johnson ST, et al. [2]) revealed the presence of antibodies with specificity against:

a) Three antigens of the RH blood group system (RH1, RH2, RH3) and

b) One against the KEL blood group system (KEL1) bound to the erythrocyte membrane.

Antibodies with specificity against an antigen from the MNS system (MNS3) and one from the Diego system (DI1) remained in the serum after adsorption. Considering the possibility of RHD variants arising from the mixed ancestry of the Brazilian population (Dezan ST, et al. [3]), segments of PRBC and new samples from the three BD were analyzed. One of the BD, a woman born in Rio de Janeiro and classified as Caucasian, had unknown ancestry. The other two donors (BD02 and BD03), with clearly defined ancestries, were confirmed as Afro-Brazilian (Palatnik M, et al. [4]). The erythrocyte phenotyping results for all three BD were identical in both samples (new and old), as shown in Table 1. To exclude the RHDel phenotype, adsorption-elution tests were performed using an anti-D blend reagent (monoclonal IgM + polyclonal IgG reagent from Ebram, São Paulo, SP, Brazil). Positive and negative controls were carried out in parallel. Only the positive control yielded anti-D (data not shown).

Conclusion

The hypothesis that antibodies against the RH and KEL blood group system antigens could represent alloantibodies was completely ruled out by the erythrocyte phenotypes of the three BD (Table 1). Instead, they were considered autoantibodies. Furthermore, based on the donor´s erythrocyte phenotypes, it was possible to confirm the presence of only two alloantibodies (anti-MNS1 and anti- DI1), resulting from genetic differences between the patient and one of the blood donors (Table 1). Currently, 45 days after stopping antibiotics, there is no evidence of any autoantibody attaching to the erythrocyte membrane (DAT negative).

Table 1: Erythrocyte phenotyping of the three blood donors involved in the case.

Blood Donor	Blood Group Systems									
	ABO	**RH***	**KEL**	**MNS**	**FY**	**JK**	**LE**	**P**	**LU**	**DI****
BD01	ABO:-1,-2,-3	RH:-1,-2,-3,4,5,-8	KEL:-1,2,-3,4	MNS:1,2,-3,4	FY:1,2	JK:1,2	LE:-1,-2	P:-1	LU:-1,2	DI:-1,2
BD02	ABO:-1,-2,-3	RH:-1,-2,-3,4,5,-8	KEL:-1,2,-3,4	MNS:1,2,3,4	FY:1,2	JK:1,2	LE:1,2	P:1	LU:-1,2	DI:1,2
BD03	ABO:-1,-2,-3	RH:-1,-2,-3,4,5,-8	KEL:-1,2,-3,4	MNS:1,2,-3,4	FY:-1,2	JK:-1,2	LE:-1,2	P:-1	LU:-1,2	DI:-1,2

Note: *Five anti-D reagents from different manufacturers (Diagnostic Grifols, DiaMed-Biorad, Ebram) were employed in agglutination analysis using both tube and gel column techniques.

**Antisera of human origin, frozen from patients with the appropriate antibodies. The other blood group systems were tested with reagents from Diagnostic Grifols.

Erythrocyte phenotypes are described according to the current alphanumeric nomenclature proposed by the ISBT (Daniel G, et al. [5]).

No difference between the erythrocyte phenotyping of the three donors and that of the patient regarding the RH and KEL blood group systems was observed, which would justify the development of alloantibodies. The alloantibodies detected in the patient were explained by genetic differences between him and one of the blood donors (highlighted in yellow)

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