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A Case of Polyagglutination

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Summary. A case of erythrocytic polyagglutination in a healthy blood donor is reported. After a review of current literature, the results of clinical and serologic tests which led to the diagnosis of Tn-red cells polyagglutination are presented. This is only the sixth case of Tn-activation in a healthy blood donor. The clinical and forensic significance of this rare phenomenon is discussed.

Key words: Polyagglutination – Tn-Antigen – Lectins

Zusammenfassung. Es wird über eine erythrozytäre Polyagglutination eines gesunden Blutspenders berichtet. Nach einer Revision aktueller Literatur werden Ergebnisse klinischer und serologischer Tests dargestellt, die zur Diagnose einer Polyagglutinabilität des Typs Tn führt.

Dies ist der sechste in der Literatur und mitgeteilte Fall von Tn-Polyagglutinabilität bei einem gesunden Blutspender. Über die klinische und gerichtliche Bedeutung des seltenen Phänomens wird diskutiert.

Schlüsselwörter: Polyagglutination – Tn-Antigene – Lectine

Introduction

Polyagglutination is the name given to the phenomenon by which a sample of red cells is agglutinated by all, or nearly all, human sera. This phenomenon has also been called "panagglutination". However, as Bird [1, 2] pointed out, this term is incorrect. In fact, in polyagglutination the red cells are agglutinated by all normal sera, whereas in panagglutination it is the red cells of an normal individuals which are agglutinated by a serum containing an antibody (known as anti-I) that reacts with normal red cells.

Thomsen [3] in 1927 was the first to observe a sample of human blood having lost its ABO specificity. Friedenreich [4] studied the possible cause and discovered that it was the action of bacterial or viral enzymes (neurominidase, that modified the structure of the cellular membrane.

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The I receptor (so called by Friedenreich) is able to bind the anti-I agglutinins (an autoantibody of the IGM class with a thermic optimum between 15° C and 20° C): these agglutinins are normally present in adult sera. Lind and Mac Arthur [5] proved that cord sera does not contain the antibodies that cause polyagglutination.

Actually, the activation of the I receptor is only one of the phenomena that explain polyagglutination: a careful immunohematologic study permits the identification of different types of polyagglutination, associated with typical clinical conditions.

In 1957, Moreau [6] found an individual whose red cells were agglutinated by all adult sera, but not by their anti-T antibody. The cause of the Tn type polyagglutination (the name given to this case) still is not known. Similar cases presented the following characteristics: the Tn-activation cannot be reproduced in vitro; the individuals usually present leukopenia and/or thrombo cytopenia; Tn-activation is usually permanent and may, in fact, represent a clinical syndrome [7, 8].

T-activation, on the other hand, can be reproduced in vitro with normal red cells; there is generally a high leukocyte count; the phenomenon disappears when the infective process, usually present, terminates (7, 9].

A new antigen called Cad was discovered in 1968 by Cazal et al. [10]: it was a low incidence antigen expression of a dominating gene: all the Cad-positive red cells were polyagglutinable [11].

Later on, Cazal et al. also [12] observed that in fact not all Cad-positive red cells were polyagglutinable [13].

In 1972, Bird and Wingham [14] reported on a new type of polyagglutination which they called Tk. This phenomenon has the following characteristics: it is temporary and reversible; the Tk red cells are agglutinated by Arachis hypogaea (anti-T) extract; the Polybrene test [15] was positive (whereas it was usually negative in all other cases of polyagglutination); a *Bacteroides fragilis* infection was always present [16].

The phenomenon of polyagglutination occasionally causes a problem which is not easily solved by the blood bank.

The awareness of this phenomenon is also important in the forensic laboratory, particularly in paternity testing in which all possible causes of error must be known.

For this reason, we wish to present a case of suspected polyagglutination in a blood donor registered at the Verona City Hospital Blood Bank.

Case Report

G. L., a 23-year-old male blood donor, who had already given blood (O, Ccde, D^u neg.) five times before without any problems, donated again in January 1981; anamnesis was negative, the donor was clinically healthy, and not undergoing any medical therapy. Standard tests (Hb, VES, HbsAg, VDRL) were normal. The results of ABO determination (with Groupamatic) are presented in Table 1. After 3 and 6 months the results were the same.

In January 1982, laboratory tests (Table 2) and a detailed immunohematologic screening (see infra) were carried out.

Table 1. AB0 group determination by Groupamatic in January 1981

Donor G.L.	Anti A	Anti B	Anti A+B
R.C.	+	+	+
	R.C.A	R.C.B	R.C.O
Serum	+++	+++	—

Table 2. Laboratory tests

Hb	14.7 g%
Hct	43.9%
Red cells	5,010,000/mm^3
Platelets	124,000/mm^3
Leukocytes	2,900/mm^3

Differential: neutrophils 56%, lymphocytes 35%, monocytes 7%, eosinophils 2%, not immature cells. Total bilirubinemia 2.10 mg/dl, indirect 0.45 mg/dl, reticulocyts 1.50%, MCHC 33.4%, MCH 29.3%, MCV 88 μm^3, VES 2, TAS 50, Prot C ± rheumatoid factor: negative. Hemoculture: negative. Uroculture: negative

Table 3. Determination of the surface erytrocytic antigens

System	Phenotype
Rh	Ccde
MNSs	MNs
Kell	Kell neg
Lutheran	Lu (a— b+)
Lewis	Le (a— b+)
P	(+)
Duffy	Not reaction
Kidd	J_k (a+ b+)

Materials and Methods

Erythrocyte suspension was prepared as a 5% saline suspension. A direct Coombs' test with polyvalent anti IgG and anti C_3 antisera was then carried out. The red cells were tested with 50 donor sera, 20 samples of cord sera, commercial sera from three different companies and six different lots, Arachis hypogaea extract prepared according to Bird's technique [17], commercial *Dolichos biflorus* lectin and purified anti-T antibodies according to Howard's technique [18].

At the same time, a positive test of T-activated with neuraminidase red cells was carried out according to Issitt's technique [19].

The *Salvia Schlarea, Phaseolus limensis, and Glycine soya* seeds necessary for the preparation of the other lectins were unobtainable.

The phenotypes Rh, Kell-Cellano, MNSs, Duffy, Lewis, Kidd, Lutheran, P, were then identified.

The donor's serum was tested for auto-and iso-antibodies at 4° C, 22°C and 37° C in saline, with bromelin and with Coombs' serum. A test to detect the presence of anti-T antibodies was carried out.

Saliva testing, ABO, Rh, and Lewis determination was carried out on all members of the family [20].

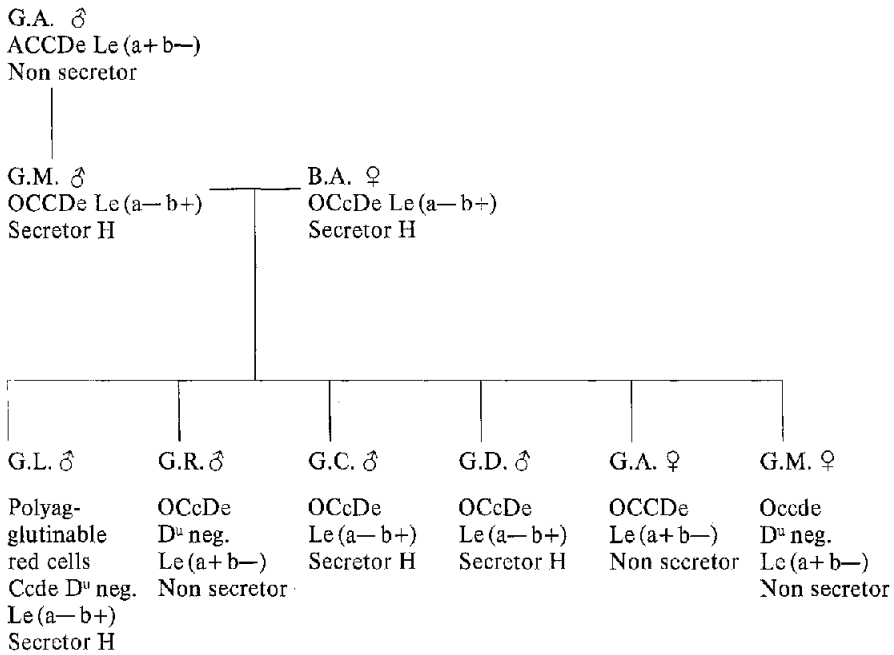

Fig. 1. The family genetic study (ABO, Rh, Lewis phenotypes, and saliva testing)

Table 4. Scheme for the recognition of polyagglutinable red cells derived from Bird [1, 2]

Red cells	Lectin			
	Arachis hypogaea	Dolichus biflorus	Salvia sclarea	Salvia hormium
T-activated	+	0	0	0
Tn-activated	0	+	+	+
Cad-polyagglutinable	0	+	0	+
Normal (Group 0)	0	0	0	0
Normal (Group A)	0	+	0	0

Serologic Results

We found a weak but certain agglutination (+ according to the AABB score) using all commercial anti-A, anti-B, and anti-AB sera; a strong agglutination (++) with all donor sera; no reaction with *Arachis hypogaea* lectin; very strong agglutination (+++) with *Dolichos biflorus* lectin; no reaction with anti-T antibodies.

The natural isoagglutinin titer was in normal range. There was no reaction with cord sera. Coombs' direct and indirect tests were negative. In the Serum we found an anti-T specific antibody (titer 1 : 32). Only substance H was present in the saliva in accordance with Lewis phenotype and the secretor status of the

members of the family (Fig. 1). Table 3 shows the determination of the surface erythrocytic antigens.

Discussion

The donor's blood group was originally O (see secretor status and the family genetic study). The last group determination was made difficult by the phenomenon of polyagglutination. The serologic results immediately excluded T and Tk types, but not Cad type; this type was excluded because it was not present in the other members of the family in accordance with Cazal's observations [11]. In the differential diagnosis it was not possible to use the lectins suggested by Bird in his schema (Table 4). Additional blood studies showed reticulocytosis, leukopenia, indirect hyperbilirubinemia, and platelets at the lower limits of the norm.

The donor was clinically healthy. Baldwin [21], with regard to this, points out that five of the 25 cases of Tn-activation which have been reported concerned healthy blood donors.

Berman [7] and Bird [22] underlined the fact that Tn-polyagglutination could exist in "pathologic" form and "non-pathologic" form. It is still not clear, however, whether anemia is due to the reduced survival of the Tn-activated red cell or to their destruction by the anti-Tn antibodies, normally present in the individual.

The use of bromelin (normally used with Groupamatic) did not eliminate the phenomenon, contrary to what other authors have reported [8, 22].

Anti-Tn antibodies were present in commercial ABO determination sera, even though they were 4 years out of date and had been stored at 4° C.

There were no problems in determining the other erythrocytic phenotypes: probably either in the preparation stage there was an absorption of anti-Tn antibodies or there was a lower titer at the beginning.

The Duffy phenotype determination proved to be very important from the forensic point of view: the commercial sera anti Fy^a and anti Fy^b with titer 1:8 did not agglutinate the donor red cells. This fact could be explained by a rare phenotype Fy (a-b-), but more probably a reduction of the sialic acid of polyagglutinable cell's surface is the cause. In 1972, Myllyle et al. [23] demonstrated this fact.

Several transfusion reactions, one of which was lethal, were caused by T- or Tn-activated red cells' transfusion [24].

The question of medical responsibility could arise from the non identification or the incorrect diagnosis of various types of erythrocytic polyagglutination.

Awareness of this phenomenon is obviously equally important during paternity testing.

This case of red cell Tn-polyagglutination in a healthy donor is only the sixth report in current literature. Its extreme rarity, together with its clinical and forensic significance were the purpose of this report.

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