A rare case of multiple alloantibody detection in a woman of childbearing age

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ABSTRACT

Anti-G antibodies in alloimmunized woman.
The Rhesus G antigen is expressed on C+ and D+ erythrocytes. As a consequence, anti-G antibodies can mimic the presence of anti-C plus anti-D specificity on Indirect Antiglobulin Test . It is not possible to discriminate between the three antibodies in routine testing because anti-G can only be highlighted with the adsorption-elution test. During pregnancy, it is important to identify anti-G antibodies because their presence has been associated with hemolytic disease of the fetus and newborn (HDFN). It is also of fundamental importance to identify the antibodies really present because when anti-D is absent rhesus prophylaxis must be administered. The present case report describes the findings in a 44-year-old woman, who was group A Rh negative (phenotype ccdee), weak D negative; antibodies screening by Indirect Antigloblin Test (IAT) was positive. Antibody identification by IAT revealed anti-C, anti-D and anti-Jka antibodies. Due to the concomitant presence of anti-C and anti-D, the test for anti-G antibody was carried out by using adsorption-elution test. The results showed that in the patient's blood anti-G, anti-C, anti-D and anti-Jka antibodies were present. This case report illustrates the usefulness of using manual methods together with the automated methods in the Immunohematology Laboratory. In fact, the simultaneous presence of anti-C and anti-D must always induce to check for the presence of anti-G which can be identified only with adsorption-elution methods.

Keywords: *indirect antigloblin test, hemolytic disease of the newborn*

CASE PRESENTATION

This case report is about a 44-year-old woman, currently not pregnant, with a previous pregnancy that led to the birth of a child with health problems, who came to our observation in the Immunohematology Laboratory of the Policlinic in Bari. There was no significant medical history, no past history of blood transfusion or transplantation. The patient was undergoing medically assisted fertilization for a further pregnancy.

For this reason, she has been subjected to several immunohematological tests that were performed using Capture solid phase technology (NEO Iris, Immucor) and DG Gel technology (Erytra, Grifols).

The patient's blood group was A Rh negative (phenotype ccdee), weak D negative (NEO Iris, Immucor). Monospecific Direct Antiglubulin Test (DAT, anti-IgG and anti-C3d) was negative (Erytra, Grifols). Antibody Screening by Indirect Antigloblin Test (IAT), using three-cell antigen panel (Screen-Cyte 0,8%, Grifols), was positive.

An antibody identification by IAT using an extended 15-cell panel (Identisera Diana Extend, Grifols) with ID-cards at 37 °C was then performed. The pattern of antibody reactivity was suggestive of anti-C, anti-D and anti-Jka antibodies. This identification was repeated using ID-cards at 4 °C and showing the same result: the pattern was suggestive of anti-C, anti-D and anti-Jka antibodies. A titration test gave the following results: anti-D/C 1:64, anti-Jka 1:2 by both NEO Iris and Grifols.

The extended red cell antigen phenotype, performed by using Capture methods (NEO Iris, Immucor), was: K-, Fya+Fyb+, Jka-Jkb+, M+N+, Kpa-Kpb+, P1-, Lua+Lub+, Lea-Leb+.

Further investigations included analysis of the husband's blood and the use of second-level methods (adsorption-elution tests) on the patient's samples.

The husband's blood group was O Rh positive, phenotype CcDee. Extended red cell antigen phenotype was: K-, Fya+Fyb+, Jka+Jkb-, M+N-, Kpa-Kpb+, P1+, Lua-Lub+, Lea-Leb+ (NEO Iris, Immucor).

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These findings were consistent with the presence of the antibodies detected in the serum of the patient: the husband was strongly positive for C, D and Jka antigens whereas the patient was negative.

Because of the simultaneous presence of anti-C and anti-D, a research for the presence of anti-G antibody was carried out performing adsorption-elution tests.

Second level methods were used to identify the different types of antibodies in patient's plasma.

In particular, adsorption-elution test allows to remove the antibodies from the serum or plasma by using red cells with the corresponding antigens (Adsorption) and to elute the antibodies from red blood cells (Elution). In this particular case, the adsorption step was performed using O group red blood cells, with a specific phenotype useful for our purposes, sorted out from our blood bank. Specifically, the plasma + red cells (ccDJka-) at 1:1 ratio were incubated for 30 minutes at 37 °C. Subsequently, red cells were eluted using rapid acid elution (Elu-kit II, Immucor) to reveal any attached antibodies. The antibody identification was performed by solid-phase IAT using a commercial panel for identification (Capture-R Ready-ID, Extend I and II, Immucor) and a gel card system using an extended 11-cell panel (Data-Cyte, Grifols): the results were suggestive for the presence of anti-G antibodies. At the same time, a second adsorption was performed using the eluate + red cells (CcdJka-). Also in this case, the results (after elution and antibody identification) were suggestive for the presence of anti-G antibodies.

In summary, the second level manual methods identified anti-G antibodies (Figure 1) and confirmed the presence of anti-C, anti-D and anti-Jka antibodies (Table 1) as already indicated by the routine testing using automated analyzer (Erytra, Grifols).

DISCUSSION

The "Rhesus G antigen" was first described in 1958 by Allen et al (1). It originates by Serine on position 103 of the RH polypeptide and is encoded by RHD gene and by the RHCE*Ce (C allele of the RHCE gene) (2). The G antigen (RH12) is a member of the Rh system of blood group antigens. It is expressed on erythrocytes bearing the C and/or D antigen. Furthermore, erythrocytes that do not carry either of these two antigens, typically do not express the G antigen. Nevertheless, rare cases of erythrocytes with rGr (cGe/ce) phenotype and G antigen but not D and C antigens (due to RHD-RHCE gene conversion) have been reported (3,4).

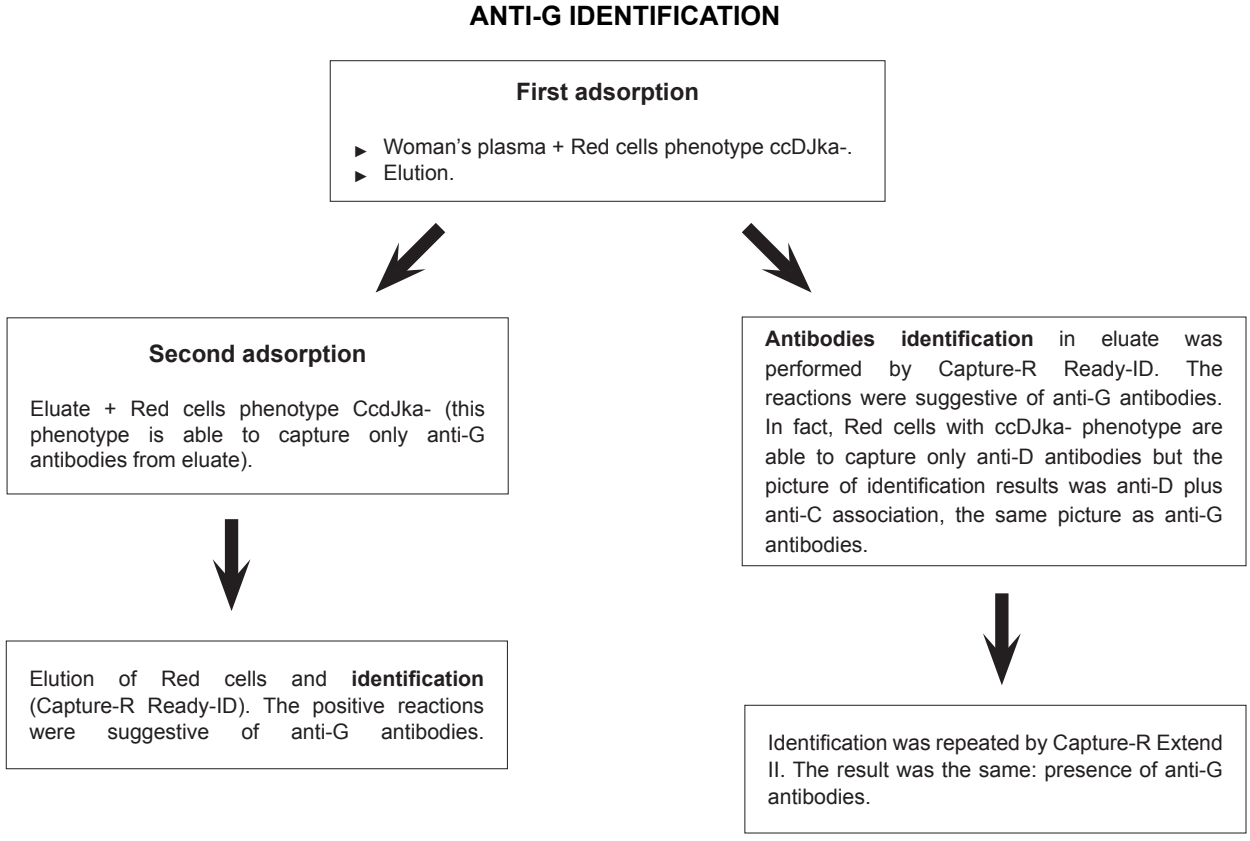

Figure 1
Flow chart of sequential adsorption elution tests used to remove anti-G antibodies from the plasma. Identification was performed by Capture-R Ready-ID and Capture-R Extend II (Immucor) for confirmation. The results indicate the presence of anti-G antibodies.

Table 1

Detection of anti-C, anti-D and anti-Jka by using adsorption elution methods.

ANTI-C ANTIBODIES

Antibodies identification was performed by three consecutive adsorptions for exhaustion anti-D, anti-G and anti-Jka:

- First adsorption: woman's plasma + Red cells phenotype ccDJka+;
- Two following adsorptions: adsorbate + Red cells phenotype ccDJka+;
- Antibodies identification in adsorbate was performed with 11-cell panel, Data-Cyte (Grifols). The reactions were suggestive of anti-C antibodies.

ANTI-D ANTIBODIES

Antibodies identification was performed by four consecutive adsorptions for exhaustion anti-C, anti-G and anti-Jka:

- First adsorption: woman's plasma + Red cells phenotype CcdJka+;
- Three consecutive adsorptions: adsorbate + Red cells phenotype CcdJka+;
- Antibodies identification in adsorbate was performed with 11-cell panel (Data-Cyte, Grifols), plus Cell 2 of 15-cell panel (Identisera, Grifols) to have an extra positive well for anti-C identification. The reactions were suggestive of anti-D antibodies.

ANTI-Jka ANTIBODIES

Antibodies identification was performed by following steps:
- Adsorption: woman's plasma + Red cells phenotype ccdJka+;
- Elution;
- Antibodies identification in eluate was performed with Capture-R Extend I (Immucor). The reactions were suggestive of anti-Jka antibodies.

Anti-G is an antibody found in individuals with phenotype rr (ccdee) who lack the C and D antigens and have been exposed to these antigens because of transfusion, pregnancy or transplantation. It appears as a blend of anti D plus anti C antibodies on IAT (since it is adsorbed by both C-D+ and C+D- erythrocytes), so it cannot be serologically differentiated (5). Furthermore, anti-D, anti-C and anti-G antibodies can be found alone or in various combinations.

Differentiating anti-G from the other two antibodies is not important for transfusion practice since D-C- erythrocytes will be always chosen for transfusion. Furthermore, serological cross-match will detect if a rare D-C-G+ unit has been selected for transfusion. On the opposite, it is important in obstetrics when pregnant women express anti-G and not anti-D: they can still develop anti-D so they require rhesus prophylaxis. Moreover, anti-G can cause mild and, rarely, severe hemolytic disease of the fetus and newborn (HDFN) (6,7).

The interest of the present case is due to the rare presence of four antibodies at the same time in a alloimmunized woman. In particular, the results indicate that patient's antibody profile is anti-C+anti-D+anti-G+anti-Jka. Palfi et al. (8) evaluated the frequency of these antibody combinations in the sera from alloimmunized women. The frequency of D+G, D+C and D+C+G antibodies occurred in 25.9%, 11.1% and 48.1% of the examined women, respectively; overall, anti-G was detected in 24/27 samples (88.9%) (8). Anti-Jka is associated with mild anemia in pregnancy although rare cases of severe anemia have been reported (9,10). Anti-C and anti-D as well as anti-G antibodies can lead to HDFN with different degrees of severity (11). Unfortunately, in routine testing, anti-G mimics the reactivity pattern of anti-C and anti-D antibodies so it is not possible to discriminate between the three patterns when using automated analyzers. On the other hand, in women of childbearing age or pregnant, it is crucial to find out which antibodies are really present because when anti-D is absent, rhesus prophylaxis must be administered. It is possible to discriminate between anti C, anti-D and anti-G only using manual methods as the adsorption-elution test, as the present case demonstrates.

The importance of having available second level manual methods along with automated methods in the Immunohematology Laboratory is thus confirmed.

We can conclude that when anti-C + anti-D are detected during pregnancy or in alloimmunized women, it is appropriate to look for the presence of anti-G, considering that it may cause HDNF. Moreover, the differentiation of anti-D, anti-C and anti-G is necessary and useful in deciding whether, in case of pregnancy, RhD prophylaxis should be administered.

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CONFLICT OF INTEREST

None.

REFERENCES

1. Allen FH Jr, Tippett P A. A new Rh blood type which reveals the-Rh antigen G. Vox Sang 1958;3:321-30.
2. Faas BH, Beckers EA, Simsek S, et al. Involvement of Ser103 of the Rh polypeptides in G epitope formation.

Transfusion 1996;36:506-11.
3. Lirochon J, Doinel C, Rouger P, et al. Biochemical identification and characterization of the G antigen in the human blood group Rh. Immunology 1988;64:337-40.
4. Jernman R, Stefanovic V, Korhonen A et al. Case report: Severe hemolytic disease of the fetus and newborn due to anti-C+G. Immunohematology 2015;31:123-7.
5. Soumya D, Shamee S, Murugesan M, et al. What is it really? Anti-G or Anti-D plus Anti-C: Clinical Significance in Antenatal Mothers. Indian J Hematol Blood Transfus 2017;33:259-63.
6. Hadley A G, Poole G D, Poole J et al. Haemolytic disease of the newborn due to anti-G. Vox Sang 1996;71:108-12.
7. Chen J, Liu F. A case of mild HDFN caused by anti-C, anti-D, and anti-G: diagnostic strategy and clinical significance of distinguishing anti-G from anti-D and anti-C. Transfusion Apher Sci 2020;59:102602
8. M Palfi M, Gunnarsson C. The frequency of anti-C + anti-G in the absence of anti-D in alloimmunized pregnancies. Transfus Med 2001;11:207
9. Mittal K, Sood T. Clinical significance of rare maternal anti-Jka antibody. Indian J Hematol Blood Transfus 2016;32:497-9.
10. Baek EJ, Park S C, Kwon Y H et al. Hemolytic disease of newborn due to anti-Jka and the duration of antibody persistence. J Pediatr Child Health 2013;49:E101-2.
11. Rai P, Sharma G, Singh D et al. Approach to a Pregnant Woman with Anti D + Anti C Reactivity Pattern: A Diagnostic Conundrum. J Clin Diagn Res 2017;11:ED06-8.