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The Presence of the Anti-D Alloantibody in the Plasma of a Pregnant Woman Initially Typed as RhD-Positive

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ABSTRACT

Background: Although the general population can be classified as rhesus D (RhD)-positive or -negative, there are individuals whose RhD status is determined to be a D variant, i.e., the weak D, partial D or DEL phenotype. Through this case presentation we want to draw attention to the possibility of incorrect RhD serologic interpretation, which can lead to the omission of anti-D administration.

Methods: We present the case of a 27-year-old pregnant woman, initially serologically typed as RhD-positive, in the 31st week of gestation, who was diagnosed with the anti-D alloantibody in her second pregnancy.

Main findings: In the 34th week of gestation, the anti-D titer was 1:1 (a score of 7), in the 35th week it was 1:2 (a score of 18), in the 37th week it was 1:2 (a score of 15) and on the delivery day (39th week) it was 1:2 (a score of 16). Anti-D was eluted from the red blood cells of the newborn without requiring therapy. The RhD typing of the mother was conducted and determined to be partial D category IV type 4.

Principal conclusion: Partial D category IV type 4 can still cause a discrepancy between serologic typing and genotyping, increasing the incidence of anti-D alloimmunization.

Key words: RhD, anti-D immunoglobulin prophylaxis, pregnancy.

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INTRODUCTION

The anti-D alloantibody from the rhesus (Rh) blood group system is clinically significant, as it can cause a hemolytic transfusion reaction and hemolytic disease of the fetus and newborn (HDFN). Although most people can be classified as RhD-positive or -negative, there are some individuals whose RhD status is determined to be a D variant, i.e., a weak or partial D (1). It is estimated that 0.6% to 1.0% of Caucasian women have serologic weak RhD in their red blood cells. Eighty percent of them have weak D type 1, 2 or 3, which can be considered as D-positive (2). The most common partial D variant among Caucasians is DNB (3). As it is highly polymorphic, the RhD gene results in a large number of RhD variants (4). Since 1946, more than 200 D variants have been identified that can be differentiated by molecular genotyping. In transfusion practice, the most important task is to differentiate weak D types 1, 2 and 3 because these variants can be considered RhD-positive in transfusion treatment. This allows for saving RhD-negative products and reducing immunoglobulin prophylaxis administration (1). Different RhD alleles encode aberrant RhD proteins, which leads to raising the possibility of different serologic reactions and to anti-D alloimmunization (5). The D variant should be considered in cases of significant discrepancies in the strength of reactions performed with different anti-D reagents, or between actual and prior test results, or when anti-D is detected in an individual serologically typed as RhDpositive (6). All these discrepancies are still a problem in transfusion practice and can increase the risk of alloimmunization (4). Therefore, in the case of a discrepant RhD status, patients should be treated as D-negative until a final determination is undertaken (6). D category VI (DVI) is clinically the most relevant variant, as it is the one most commonly involved in anti-D immunization and HDFN (7). In order to prevent HDFN caused by RhD maternal-fetal incompatibility, administration of the anti-D immunoglobulin is

indicated (8). On the other hand, to prevent giving unnecessary prophylaxis, it is now possible to perform fetal Rh genotyping based on the mother's plasma (9).

However, we want to emphasize that there are still cases of serologically RhD-positive persons who can develop anti-D. Through this case presentation, we want to draw attention to the possibility of incorrect RhD serologic interpretation, which can lead to the omission of anti-D administration when it is required, increasing the incidence of anti-D alloimmunization and HDFN.

MATERIALS AND METHODS

The ABO blood group and K antigen were determined using a DG Gel ABO/Rh (2D) + Kell card (Diagnostics Grifols, S.A. Passeig Fluvial, 24 – 08150 Parets del Vallès, Spain). The rhesus phenotype was identified with a DG Gel Rh Pheno card (Diagnostics Grifols, S.A. Passeig Fluvial, 24 – 08150 Parets del Vallès, Spain).

An indirect antiglobulin test (IAT) and direct antiglobulin test (DAT) were performed with the DG Gel Coombs card (Diagnostics Grifols, S.A. Passeig Fluvial, 24 - 08150 Parets del Vallès, Spain). The identification of detected irregular antibodies was carried out using gel technology with a 15-red-blood-cell panel, Identisera Diana Extend/Identisera Diana Extend P (Diagnostics Grifols, S.A. Passeig Fluvial, 24 - 08150 Parets del Vallès, Spain) and 11-red-blood-cell panel, Panocell-10 (Immucor, INC. Norcross, GA 30071, USA). Elution was performed with an acid elution kit Gamma ELU-KIT ® II (Immucor, Inc. Norcross, GA 30071, USA).

RhD antigen typing was performed using anti-D clones: NovaClone™ anti-D Immunoglobulin G (IgG) + Immunoglobulin M (IgM) Monoclonal Blends IgM D175-2, IgG D415-1E4 (Immucor Gamma, Dartmouth, NS, Canada), MONO Gnost® MG reagent, IgM RUM-1, IgG MS-26 (BioGnost, Zagreb, Hrvatska), anti-D DG Gel ABO/Rh(2D) (P3X61, MS-201) (Diagnostics Grifols, S.A. Passeig



Fluvial, 24 – 08150 Parets del Vallès, Spain), DG Gel Confirm P, monoclonal anti-D, IgM antibodies of human origin, clone MS-201 (Diagnostics Grifols, S.A. Passeig Fluvial, 24 – 08150 Parets del Vallès, Spain) and DG Gel Confirm monoclonal anti-D (clones P3X290, P3X35, P3X61 and P3X21223 B10) (Diagnostics Grifols, S.A. Passeig Fluvial, 24 – 08150 Parets del Vallès, Spain). Anti-D titration was performed with the tube technique.

Deoxyribonucleic acid (DNA) was isolated with the Ready DNA Isolation Spin Kit (inno-train Diagnostik, Germany), and molecular typing was conducted by a polymerase chain reactionsequence specific primer (PCR-SSP) method with fluorometric reading of the signal in the FluoVista device (inno-train) using a commercial RBC-FluoGene CDE kit (innotrain).

CASE PRESENTATION

We present the case of a 27-year-old pregnant woman in the 31st week of gestation who was diagnosed with a positive IAT in her second pregnancy. She was blood group B RhD-positive, with the Rh phenotype Ccee, and K antigen-negative. Two years ago, when her first pregnancy was considered, we could not detect any irregular antibodies in the plasma, and the IAT was negative. She has never been transfused. Alloantibodies in the IgG class against the RhD antigen were identified in her second pregnancy using gel technology with commercially made cell panels.

Table 1. Reaction of the woman's red blood cells with different clones of anti-D

Anti-D serum	Reaction	Technique
NovaClone™; IgM D175-2; IgG D415- 1E4	4+	Plate
MONO Gnost® MG reagent IgM RUM-1, IgG MS-26	4+	Tube
anti-D DG Gel ABO/Rh(2D) (P3x61; MS-201)	4+	Micro card

DG Gel Confirm P (monoclonal anti-D,		Micro
IgM antibodies of	4+	card
human origin, clone		caru
MS-201)		
DG Gel Confirm		
monoclonal anti-D		
(mixture of IgG and		Micro
IgM antibodies of	4+	card
human origin, clones		card
P3x290, P3x35, P3x61		
and P3x21223 B10)		

The anti-D titer was unmeasurable by tube technique and the DAT was negative. The blood group of the baby's father was A RhD-positive, with the Rh phenotype Ccee, and K antigen-negative. Because anti-D alloantibodies were identified in the plasma of the woman, who was initially determined as RhD-positive, we performed a serologic RhD typing with different clones of anti-D immunoglobulins (Table 1).

As we obtained various results, we performed a crossmatch by IAT with a gel micro card between the woman's plasma and donors' red blood cells (RhD-positive and RhD-negative), as well as with cord-blood red blood cells that were B RhD-negative (Table 2).

Table 2. Crossmatch results between the woman's plasma and the red blood cells of blood donors and cord blood

Red blood cells	Crossmatch with the woman's plasma	
Blood donor B	Negative reaction	
RhD-negative		
Blood donor B	Positive reaction	
RhD-positive		
Cord blood B RhD-	Negative reaction	
negative		

Anti-Landsteiner-Wiener (LW) antibodies were excluded by negative crossmatch results between the woman's plasma and cord blood. Furthermore, RhD genotyping was performed and the partial D category IV type 4 was determined. The anti-D titer was monitored until delivery. In week 34 of gestation the anti-



D titer was 1:1 (a score of 7), in week 35 it was 1:2 (a score of 18), in week 37 it was 1:2 (a score of 15) and on the delivery day (week 39) it was 1:2 (a score of 16). The newborn's blood group was B RhD-positive, with the Rh phenotype Ccee, and K-negative. The DAT was positive (DAT IgG-positive). Thus, an anti-D was eluted from the neonatal red blood cells. The laboratory findings for the newborn on the first day of life were: leukocytes 20.6x109/L; red blood cells 5.04x1012/L; hemoglobin 188 g/L; hematocrit 0.534; platelet count 157x109/L; total serum bilirubin level 49.8 µmol/L; direct bilirubin 7.98 µmol/L. On the second day, the total serum bilirubin level increased to 96.28 μmol/L and the direct level to 8.48 μmol/L, and on the third day to 157.18 µmol/L and 9.38 µmol/L, respectively. The newborn did not receive any therapy.

DISCUSSION

Here we reported anti-D in a pregnant woman initially typed as RhD-positive, but with the molecular partial DIV type 4. Anti-D was detected in her second pregnancy. We want to emphasize that in her first pregnancy she did not receive anti-D immunoglobulin prophylaxis because she was initially serologically typed as RhD-positive.

are limited data about anti-D immunization in D variants. A study from Ohio reported 0.47% of anti-D immunization in women initially typed as RhD-positive or weakly positive (10). DVI, with the phenotype frequency 1:6214 in Germany, is clinically still the most important D variant in Europeans because of the capacity of alloimmunization (11). Therefore, D-typing strategies in several European countries protect carriers of D category VI (DVI) from anti-D immunization, but not others (1). It is important to emphasize that one of the clinically most important partial D variants is D category IV (DIV).

DIV type 4 is a hybrid RHCE gene, and the key changes from the standard allele are (7:1048-7:1061). The nucleotide changes relative to "standard RhD" are c.(1048 G>C;1053 C>T;

1057 GGA>TGG; 1060 GC>AA), while the changes at the amino acid level are D350H, G353W, A354N (12-13).

There are no literature data on the molecular basis of D variants in the Bosnian and Herzegovinian population, but there are data from Croatia, which is geographically close to Bosnia and Herzegovina. Using molecular testing for the samples with discrepant results from RhD serologic testing in two Croatian centers, Zagreb and Split, the most common weak D type was weak D type 3, followed by type 1, type 14, type 2, type 11/RhD (M295I) and types 4.2 and 15. In Zagreb (representing the population of central Croatia) the most prevalent type was weak D type 3 and in Split (corresponding to the population of the Mediterranean part of Croatia) the most prevalent type was weak D type 1. In Croatia, only one sample (0.6%) was typed as RhD weak 4.2, which is rarely present in Europe but common in Africa. It was found in the Mediterranean part of Croatia, which is involved in international maritime traffic (14). In the obstetric population of Split (the Mediterranean part of Croatia) during the period 1993-2012, three cases of anti-D alloimmunization in pregnant women initially not typed as RhD-negative were recognized. Anti-D occurred in 0.9% (184 samples) of women at risk. Out of 184 women with the anti-D alloantibody, 181 cases occurred in women serologically typed as D-negative but three cases were partial D carriers (DVa n = 2, DNB n = 1), initially typed as RhD-positive. These cases were recognized as D variants only following immunization after pregnancy. No cases of anti-D formation in women with weak D initially identified as RhD-positive were reported (15). There are also data from Croatia that the most common D variant in primiparous individuals is D Va, with a prevalence of 0.08% (16).

As mentioned above, the distribution of D variants varies among different counties, regions and populations. In addition, their recognition depends on the choice of D typing reagents used.



In general, there are many data demonstrating the importance of identifying weak D types in persons who are initially determined to be RhD-negative. There are not many which indicate the possibility of the presence of the weak or partial D type in persons who are initially identified as RhD-positive, which could be important, especially in the obstetric population. The range of clones of anti-D serums in serologic testing is crucial.

CONCLUSION

In this paper we reported a positive reaction in serologic D typing by a woman who was partial D category IV type 4.

The administration of an anti-D immunoglobulin prophylaxis to RhD-negative women highly reduces the incidence of anti-D alloimmunization and HDFN. Therefore, it is very important to recognize D variants, especially in the obstetric population. This case presentation reminds us how important it is to perform an investigation of D weak and partial D variants in Bosnia and Herzegovina and choose an anti-D serum based on the results.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTIONS

ITD: conceived and designed the study, collected the data, analyzed the data, interpreted the results, prepared the tables, drafted the manuscript, edited and revised the manuscript and approved the final version of the manuscript; APM: collected the data, analyzed the data, interpreted the results and drafted the manuscript; SK: collected the data, analyzed the data and interpreted the results; JK: collected the data, analyzed the data and interpreted the results; JA: edited and revised the manuscript and approved the final version of the manuscript.

ETHICAL BACKGROUND

Institutional review board statement: The study was conducted according to the guidelines of the Declaration of Helsinki.

Informed consent statement: Written informed consent was obtained.

Data availability statement: We deny any restrictions on the availability of data, materials and associated protocols. Derived data supporting the findings of this study are available from the corresponding author on request.

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