Serological and molecular analyses of a patient with anti-G and anti-D due to alloimmunisation during her pregnancy

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Dear Sir,

G antigen (Rh12) is expressed on red blood cells that carry D and/or C antigen[1-3]. Serine is the critical amino acid for G antigen expression. Anti-G can cause haemolytic disease of newborn, which is rare, but responsible for neonatal morbidity and mortality. However, Anti-G antibody is difficult to distinguish from anti-D in combination with anti-C. Accurate identification of these antibodies is important in the clinical management of pregnancies. Here, we describe a 76-year old, gravida-3, para-3, female patient with AB RhD negative D− C− E− c+ e+, who was positive for antibodies that are routinely screened because of a potential need for transfusion. Her husband's and son's blood group were B RhD positive D+ C+ E+ c+ e+ and B RhD positive D+ C+ E− c+ e+, respectively.

The antibodies in the patient's serum were identified by an 11-cell screening panel using a gel column method in a DiaMed-ID LISS/Coombs system (Cressier, Switzerland) (Table I). There were 4+ reactions with all D+ and/or C+ red blood cells. The serological reactivity implied that the red blood cell antibodies resembled IgG anti-D and anti-C. However, anti-G appears serologically identical to anti-D and -C. Therefore, differential adsorption-elution studies were performed to define the identification of the antibodies in the woman's serum. Her serum was adsorbed with Ccdee cells; the adsorbed sera were tested against selected red blood cells (O type, ccDEE, ccDEe, and ccDee) using the indirect antiglobulin method. There were agglutination reactions of 2+ or 4+ with all D+ red blood cells, indicating that anti-D were present in the serum. In another test, the woman's serum was adsorbed with ccDEE cells, then the adsorbed sera were tested against O type Ccdee. No reactivity was seen with Ccdee red blood cells, which excluded the presence of anti-C. The elution from the adsorbed ccDEE cells reacted with D+ and/or C+ red blood cells. These results indicate that anti-G was present in the elution. The woman's antibodies were, therefore, anti-D and anti-G, but not anti-C.

RHD and *RHCE* genotyping were performed by polymerase chain reaction (PCR with sequence-specific primers (SSP) and PCR restriction fragment length polymorphism (RFLP). Briefly, multiplex PCR-SSP was used to detect the *RHD*-specific polymorphisms located in *RHD* intron 4 and *RHD* exon 7. Samples negative

Table I - Results of the patient's serum with panel of reagent RBCs for identification of blood group antibodies by microcolumn method.

Cell	Rh						Kell						Duffy		Kidd		Lewis		P	MNS				Luth		Xg	Results
	D	*C*	*E*	*c*	*e*	*Cʷ*	*K*	*k*	*Kp^a*	*Kp^b*	*Js^a*	*Js^b*	*Fy^a*	*Fy^b*	*Jk^a*	*Jk^b*	*Le^a*	*Le^b*	*P1*	*M*	*N*	*S*	*s*	*Lu^a*	*Lu^b*	*Xg^a*	*AHG (1:8) dilution*
1	+	+	0	0	+	+	0	+	0	+	nd	nd	+	0	0	+	0	0	+	+	+	0	+	0	+	+	4+
2	+	+	0	0	+	0	+	+	0	+	nd	nd	0	+	+	0	0	+	+	+	0	0	+	0	+	+	4+
3	+	0	+	+	0	0	0	+	0	+	nd	nd	0	+	+	0	0	+	+	0	+	0	+	0	+	+	4+
4	0	+	0	+	+	0	0	+	0	+	nd	nd	0	+	+	+	0	+	0	0	+	+	+	0	+	nd	4+
5	0	0	+	+	+	0	0	+	0	+	nd	nd	+	+	0	+	0	+	+	+	0	+	0	0	+	+	0
6	0	0	0	+	+	0	+	+	0	+	nd	nd	+	0	0	+	+	0	+	+	+	0	+	0	+	+	0
7	0	0	0	+	+	0	0	+	0	+	nd	nd	0	+	+	0	0	+	+	+	0	+	0	0	+	0	0
8	+	0	0	+	+	0	0	+	0	+	0	nd	0	0	+	0	0	0	+	0	+	0	+	0	+	0	4+
9	0	0	0	+	+	0	0	+	0	+	nd	nd	0	+	0	+	+	0	+	+	+	+	+	0	+	+	0
10	0	0	0	+	+	0	0	+	+	+	nd	nd	+	0	+	0	0	+	+	+	+	+	0	0	+	+	0
11	0	0	0	+	+	0	0	+	0	+	nd	nd	+	0	+	+	0	0	0	0	+	0	+	+	+	+	0

RBCs: red blood cells; 0: no agglutination; nd: the specific antigen can not be determined; AHG: anti-human globulin.

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for intron 4 and exon 7 were further tested for the *RHD* promoter region and *RHD* exon 10[4]. PCR-SSP was also used to detect *RHCE*-specific polymorphisms located in the *RHCE* gene[4]. PCR-RFLP was performed to determine the *RHD* zygosity. Primers and thermocycling conditions were identical to those described previously[4,5]. PCR amplifications for *RHD* zygosity were digested by *PstI* at 37 °C for 3 hours and fragments were resolved on a 1% agarose gel. *RH* genotyping results indicated that the patient, her husband and son have *cde/cde*, *CDe/cDE*, and *CDe/cde* genotypes, respectively, which are all consistent with the phenotypes.

These results prompted us to review the patient's medical records. Her first baby, born 50 years ago, was well and did not present any signs of haemolysis or icterus. Two years after giving birth to her first child, our patient gave birth to a male baby, delivered by Caesarean section. Since Rh was not tested in most hospital transfusion services in the 1960s, and about 99.7% individuals were RhD positive in China, the patient was probably transfused with two units of RhD-positive whole blood after surgery. A slightly delayed haemolytic transfusion reaction occurred in the patient 1 week after transfusion and her baby died. The woman gave birth to her third child 4 years later after her first delivery. The baby died in the week following birth because of severe haemolytic disease of newborn. Although the medical records did not indicate haemolytic disease of newborn in second baby we presume that this was the cause of death, given the slightly delayed haemolytic reaction that occurred in the patient in the week after transfusion. In retrospect, we think that the anti-D+G alloantibodies appeared after the first delivery and contributed to the deaths of the woman's second and third babies.

In summary, this D− C− E− c+ e+ woman had IgG anti-D+G due to alloimmunisation during pregnancy. Testing for anti-G should be performed whenever anti-D and anti-C are identified. Antenatal antibody screening, Rh immunoglobulin prophylaxis and foetal treatment might have saved her babies' lives.

The Authors declare no conflicts of interest.

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