A case of severe foetal anaemia due to anti-M isoimmunisation salvaged by intrauterine transfusions

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Introduction

Haemolytic disease of the foetus and newborn (HDFN) is caused by transplacental passage of maternal IgG antibodies directed against an antigen of paternal origin present on the foetal red blood cells. IgG antibodies coat foetal antigens and cause decreased red blood cell survival leading to fetal anaemia. The antibodies causing severe forms of HDFN are anti-D, anti-c and anti-K1. However, more than 50 different red-cell antigens have been reported to be associated with HDFN and no prophylactic immune globulins are available to prevent the formation of these antibodies[1]. Anti-RhD was once the major aetiology of HDFN, but with routine antenatal administration of anti-D to all vulnerable women, maternal allo-immunisation to other red cell antigens as a cause of HDFN is becoming a greater concern.

Anti-M is typically a naturally occurring immunoglobulin M (IgM) antibody, optimally reactive at 4 C and considered clinically insignificant. This saline agglutinin was first identified by Wolff and Johnsson in 1933[2]. Anti-M rarely causes agglutination of red cells at 37 C or in the antiglobulin phase of testing. Rarely, the alloanti-M can be of IgG type or a combination of both IgG and IgM. These have a potential to cause haemolytic transfusion reactions and HDFN but have been very rarely implicated as the cause of severe HDFN requiring intrauterine transfusion (IUT). The MN determinants are carried on the transmembrane protein glycophorin A, are fully developed on foetal red cells, and can be detected as early as 9 weeks' gestation[3].

This case report describes a woman with a history of six intrauterine deaths, all between 11 to 32 weeks of gestation. Her seventh affected pregnancy was salvaged by intrauterine administration of packed red blood cell transfusions at 29 weeks of gestation, after she was found to be allo-immunised against M antigen.

Case report

A 32-year old female presented in the Department of Maternal and Reproductive Health of our Institute for an antenatal check-up at the 20th week of pregnancy. She gave a history of six previous pregnancies from the same husband, all of which had culminated in intrauterine foetal deaths between 11 to 32 weeks of gestation. She gave no history of transfusions in the past and had not received anti-D immunoprophylaxis in any of the previous pregnancies. On laboratory investigations, her thyroid profile was normal, tests for anti-phospholipid antibody were negative and she had no history suggestive of any autoimmune disease. A blood sample was sent to the immunohaematolgy laboratory for blood grouping and antibody screening as part of a routine protocol for antenatal cases.

On work-up, her blood group was found to be B Rh(D) positive using a gel card technique (DiaClon, Bio-Rad Laboratories, DiaMed GmbH, Switzerland). For antibody screening, the indirect antiglobulin test (IAT) was performed using a three-cell panel (ID-DiaCell, Bio-Rad Laboratories, DiaMed GmbH) and showed a pattern of reaction. The antibody was identification studies were done by IAT using an 11-cell panel (ID-DiaPanel, Bio-Rad Laboratories, DiaMed GmbH) and the antibody identified was anti-M. Subsequently, the patient's serum was tested in the IAT phase with three sets of M antigen-negative O group red cells, three sets of M antigen-positive O group red cells and one set of papain-treated M antigen-positive O group red cells using a gel card technique. The serum reacted with all M antigen-positive red cells but did not react with any of the M antigen-negative red cells or the papain-treated cells, thus confirming that the antibody was anti-M. The ABO blood group of the woman's husband's was also determined and was found to be B Rh(D) positive. The red cells of both the patient and her husband were phenotyped for other clinically significant red cell antigens using commercially available antisera (DiaClon): the results are shown in Table I. The patient's red cells were negative for M antigen (M−N+) whereas those of her husband were homozygous positive (M+N−).

Table I - Red cell phenotype of the patient and her husband.

	D	C	c	E	e	K	k	Jk^a	Jk^b	Fya	Fyb	M	N	S	S
Patient	+	+	+	−	+	−	+	+	−	−	+	−	+	+	−
Husband	+	+	−	+	+	−	+	−	+	+	+	+	−	+	+

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The anti-M titre, evaluated using homozygous M+ cells, was 32. To determine the immunoglobulin class of the antibody, the serum was treated with dithiothreitol (DTT) and then the IAT was performed. The reaction persisted after DTT treatment, suggesting the presence of an IgG component. Subclass analysis performed using gel cards (Diamed DAT IgG1/IgG3, Switzerland) after adsorption of antibody on red cells homozygous for M antigen (M+N−), showed a 3+ reaction for IgG1 and 2+ for IgG3.

Percutaneous umbilical blood sampling was planned at 21 weeks of gestation because the foetus showed features of hydrops on ultrasound examination. Middle cerebral artery-peak systolic velocity (MCA-PSV) was >1.59 MOM, which is quite suggestive of HDFN. A foetal blood sample obtained through cordocentesis confirmed the diagnosis of HDFN, as the foetal haemoglobin and haematocrit values were 5.9 g/dL and 17.7%, respectively.

A cord blood sample was sent to our laboratory for immunohaematological workup of HDFN. Blood grouping was performed using the conventional tube technique after washing the red cells six times. The blood group of the foetus was B RhD positive. A direct antiglobulin test (DAT) performed with a gel card as well as with the conventional tube technique using polyspecific anti-human globulin (DiaMed, Switzerland) was negative. Cord blood was typed using commercially available antisera as M+N− and thus a provisional diagnosis of anti-M HDFN was made.

The consultant obstetrician was informed and a management strategy was planned for this patient, which consisted of intrauterine transfusion. Group O Rh D negative packed red blood cell units, which were collected within 7 days, were selected and typed for M antigen. A unit negative for M antigen was cross-matched with maternal serum. The unit was leucofiltered (Imugard III RC, Terumo BCT Japan, Tokyo, Japan) and its haematocrit was adjusted to near 80%. The packed red blood cell unit was irradiated before being issued. Three intrauterine transfusions were administered through the intravascular route at 28, 32 and 35 weeks and the foetus was monitored weekly by means of the MCA-PSV. The patient delivered a male baby weighing 2.4 kg at 36 weeks of gestation by lower segment Caesarean section. The transfused red cells had been successful in raising the foetal haemoglobin concentration to 12.1 g/dL and haematocrit to 37% at the time of birth. The neonate had mild jaundice at birth and so received phototherapy, but exchange transfusion was not required. On follow-up for over 12 months, the baby did not show any haematological abnormality or developmental delay.

Discussion

Antenatal screening for the detection of antibodies that have been implicated in HDFN is usually limited to anti-D, although maternal alloimmunisation to non-RhD antigens continues to contribute to perinatal morbidity and mortality. In a large prospective cohort study on the epidemiology of alloantibodies other than anti-D in pregnancy, conducted in the Netherlands, Koelewijn *et al.* found a 1:80 prevalence of positive antibody screens and a 1:300 prevalence of clinically relevant alloantibodies other than anti-D, primarily of the specificities anti-E, -K, and -c, as well as a 1:500 prevalence of pregnancies with alloantibodies other than anti-D at risk of HDFN (father antigen-positive)[4]. There are currently no clear treatment guidelines for non-RhD HDFN, and involved pregnancies are frequently managed in a manner similar to Rh-D incompatible pregnancies. HDFN of varying degrees of severity has been reported in association with anti-M which may require treatment with intrauterine blood transfusion and exchange transfusions or may even lead to intrauterine death. As described in the literature, in antenatal screening for antibodies, anti-M ranges from the second commonest non-Rh antibody after anti-Kell[5] to a rare finding[6]. Here we report a case of anti-M immunisation and severe foetal anaemia in a pregnant woman who had had six prior intrauterine deaths of undiagnosed cause. We identified anti-M by a thorough serological work-up of the maternal serum in the seventh pregnancy and successfully treated the foetal anaemia by providing M antigen-negative packed red blood cells for repeated intravascular transfusions of the foetus *in utero*.

Very few cases of severe HDFN associated with anti-M have been reported in the literature. Most of the reported cases of anti-M associated with foetal anaemia, describe intrauterine foetal death between 10 to 35 weeks of gestation[7]; our patient's obstetric history is compatible with this. The severity of HDFN is not directly correlated with the anti-M titre, which seems to be an unreliable predictor, because the antibody titre varies depending on the technique used to determine it, the incubation temperature, suspension media and whether the reagent red cells used have homozygous or heterozygous expression of M antigen.

The prevalence of anti-M in pregnant women with a positive antibody screen is 10-14%, but the incidence of HDFN related to this antibody is reported to be extremely low[8,9]. De Young-Owens *et al.* from Ohio State University reported 26 years of experience of pregnancies with anti-M[9]. The study comprised 90 women with 115 pregnancies: anti-M accounted for 10% of all positive antibody screens but there were no cases of HDFN, mild or severe. Anti-M antibodies are naturally occurring IgM but 50-80% of subjects with this antibody additionally have an IgG component[10], which has the potential to cause HDFN. In the present case too, the patient's serum retained reactivity against

M+ cells even after DTT treatment which destroys the IgM component. Among very few cases of HDFN due to anti-M the first case was reported in 1959, in which a high titre anti-M resulted in foetal death and a severely affected child at 35 weeks, in a twin pregnancy[11].

DAT analyses in the foetus and the newborn in our case were negative. Previously reported cases[12,13] have offered two hypotheses in explanation of a negative DAT in anti-M-mediated haemolysis. One is very rapid intravascular haemolysis and the other is destruction of erythroid progenitors rather than mature erythrocytes, just as with cases affected by anti-K and anti-Ge.

Intrauterine death due to anti-M was reported as early as 1965[14]. Thus, recognition and treatment of these cases is crucial. Lin *et al.* described a case of anti-M alloimmunisation as the cause of foetal haemolytic anaemia and intrauterine foetal death[15]. Intensive treatment with plasmapheresis was give in this case from 17 weeks of gestation but foetal erythroblastosis progressed. Intravascular transfusion was unsuccessful, so intrauterine intraperitoneal and intracardiac transfusions were given. Intravenous immunoglobulin (1 g/kg/day) was also administered, but the foetus died eventually. The patient reported here was treated with three successful episodes of intravascular intrauterine transfusions with an increase in foetal haematocrit after each episode.

Another possible therapeutic modality during pregnancy is plasmapheresis. Furukawa and co-workers[16] reported the case of a woman with multiple intrauterine deaths due to anti-M, who was successfully managed by antenatal plasma exchange and delivered a live child. Another treatment option is immunoglobulin injection into the foetal abdominal cavity (IFAC). Matsuda *et al.*[17] described a case of severe foetal anaemia associated with maternal anti-M antibody which was treated by direct injection of pooled human immunoglobulin into the foetal abdominal cavity.

In view of the uncommon occurrence of HDFN due to anti-M, a thorough history of previous pregnancies and careful serological work-up accompanied by assessment of foetal well-being by ultrasound and MCA-PSV should direct the invasive procedures such as cordocentesis. Foetal blood samples only confirm the foetal M type and the severity of foetal anaemia. Obstetricians and paediatricians must be aware of the fact that confirmed M antigen-negative red cells are required for intrauterine or exchange transfusion.

Keywords: haemolytic disease of the foetus and newborn (HDFN), anti-M, intra-uterine transfusion.

The Authors declare no conflicts of interest.

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