

PREVENTION OF RhD ALLOIMMUNIZATION IN RhD NEGATIVE WOMEN

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Background. Despite the introduction of anti-D prophylaxis into clinical practice, RhD alloimmunization still presents a problem to date. The actual incidence of RhD alloimmunization in pregnant women remains unknown in most countries. Anti-D immunoglobulin is administered to RhD negative women at a fixed dose and in much greater amounts than is actually necessary. On the other hand, it is not possible to diagnose cases where greater doses are needed. To optimize the prevention of RhD alloimmunization in RhD negative women, it is important to diagnose conditions that lead to fetomaternal hemorrhage (FMH), precisely determine the volume and subsequently administer the appropriate dose of anti-D immunoglobulin. The possibility to accurately detect FMH and precisely determine its volume would enable more effective and less costly prevention of RhD alloimmunization. Anti-D immunoglobulin could be administered only in indicated cases and only in doses essentially necessary for prevention of RhD alloimmunization.

Methods and results. The Cochrane and UpToDate databases of systematic reviews, as well as national guidelines, were reviewed.

Conclusions. Due to the medical significance and indispensable economic costs associated with prevention of RhD alloimmunization, it would be appropriate to establish exact methodical guidelines. The text itself should be limited to a list of potentially sensitising events during which anti-D immunoglobulin should be administered to RhD negative women if anti-D antibodies are not already present. Following each potentially sensitising event, the minimal dose of anti-D immunoglobulin necessary for prevention of RhD alloimmunization should be determined. After 20 weeks of gestation, the volume of FMH should also be determined to specify the necessary dose of anti-D immunoglobulin.

MATERNAL RhD ALLOIMMUNIZATION

Each person who lacks the red blood cell antigen and is exposed to it will create an antibody. During penetration of RhD positive fetal erythrocytes into the circulation of an RhD negative mother, the immune system may be stimulated and trigger the creation of antibodies by "alloimmunization". The same immune reaction may also occur during transfusion of antigen-incompatible erythrocytes.

Anti-D antibodies may cause a severe form of haemolytic disease of the fetus and newborn (HDFN - Haemolytic disease of the fetus and newborn). Because the RhD antigen is very potent, even parenteral administration of only 0.1 ml of RhD positive erythrocytes to RhD negative individuals will stimulate the production of antibodies. The most common cause of RhD alloimmunization is hemorrhage, during which fetal erythrocytes enter the mother's circulation.

Most cases of RhD alloimmunization however may theoretically be avoided by prophylactic administration of anti-D immunoglobulin in the necessary dose after every potentially sensitising event (Table 1).

THE INCIDENCE OF RhD INCOMPATIBLE PREGNANCIES

The incidence of RhD incompatibility varies according to race and ethnic background. Approximately 15% of the Caucasian population is RhD negative. In most other populations however, the incidence of an RhD negative phenotype is significantly lower, in African-Americans the incidence is 5-8%, in Asians and Native Americans 1-2%. In the Caucasian population, an RhD negative woman has an 85% probability that her partner will be RhD positive, in 60% heterozygous and in 40% homozygous at locus *RHD*. In approximately 10% of all pregnancies, the situation arises where an RhD negative mother will have an RhD positive child and approximately 60% of RhD negative women will have an RhD positive child in their first pregnancy.

In the Czech Republic, if prevention of RhD alloimmunization was not performed in RhD negative women following potentially sensitising events, approximately

ABBREVIATIONS

RhD	- rhesus D phenotype (RhD phenotype)
<i>RHD</i>	- rhesus D genotype (<i>RHD</i> genotype)
FMH	- fetomaternal hemorrhage
HDFN	- haemolytic disease of the fetus and newborn

2000 women would annually become alloimmunized. Although during the last three decades the introduction of anti-D prophylaxis has led to a decrease in incidence, it remains a problem even today. In the USA in the year 2001, the incidence of RhD alloimmunization was 6.7 per 1000 liveborn infants¹.

Such data are not available in the Czech Republic. However, if similar results are assumed, there are about 670 RhD alloimmunized pregnant women annually. If two-thirds of these women will have an RhD positive fetus, we may assume that there will be about 447 at-risk fetuses annually.

PREVENTION OF RhD ALLOIMMUNIZATION IN RhD NEGATIVE WOMEN

At the beginning of every pregnancy, the woman's ABO + RhD blood types are determined and screening for irregular anti-erythrocyte antibodies (from herein referred to only as "anti-erythrocyte antibodies") is performed. In the absence of anti-erythrocyte antibodies at the beginning of pregnancy, a follow-up antibody screening is performed at 28 weeks of gestation in all pregnant women (RhD negative and RhD positive). In RhD negative women it is performed before antenatal prophylaxis of RhD alloimmunization, and in RhD positive women it is performed due to the possible development of other than anti-D antibodies causing severe haemolytic disease of the newborn.

If the woman's blood type is RhD⁺ positive "weak rhesus positive" (approx. 1% of those RhD positive), prevention of RhD alloimmunization is generally not indicated.

In cases of weak RhD phenotype "weak D" (formerly labeled D^u), there is quantitative weakening of D antigen expression. All D epitopes are weakly expressed, but individuals do not form anti-D antibodies during contact with erythrocytes with normal D expression.

In contrast, partial RhD phenotypes „partial D, variant D“ are RhD positive phenotypes in which some epitopes of the RhD antigen are not expressed. During contact with RhD positive erythrocytes, individuals with partial D phenotype may form antibodies against D epitopes, which are lacking on their erythrocyte surface.

It is necessary to distinguish quantitative weakening of the RhD antigen where it is unnecessary to perform prevention of RhD alloimmunization and qualitative variants of the RhD antigen where prevention is indicated. Individual consultation with the laboratory is necessary! The effectiveness of screening for anti-erythrocyte antibodies at 28 weeks of gestation has not been confirmed but is performed in a number of countries (Europe, USA, Canada, Australia).

The incidence of antepartal RhD alloimmunization is 1–2%; however, in 90% of cases it occurs after 28 weeks of gestation^{9–10}. The incidence of RhD alloimmunization before 28 weeks is therefore 0.1–0.2%. Follow-up examination of anti-erythrocyte antibodies in all RhD negative women at 28 weeks of gestation could then enable

Table 1. Events following which anti-D immunoglobulin should be given to all RhD negative women with no anti-D antibodies^{2–7,19–23}. Dosage and timing of anti-D immunoglobulin administration^{6–7,22–23}.

Delivery of an RhD positive infant*

Abortion

- therapeutic termination of pregnancy
- spontaneous abortion followed by instrumentation
- spontaneous complete or incomplete abortion after 12 weeks' gestation
- threatened abortion before 12 weeks when bleeding is heavy or repeated or is associated with abdominal pain; in particular, if these events occur as gestation approaches 12 weeks
- threatened abortion after 12 weeks when bleeding continues intermittently, anti-D immunoglobulin should be given at approximately 6-week intervals, and the volume of fetomaternal hemorrhage should be assessed

Invasive prenatal diagnosis

- chorionic villus sampling
- amniocentesis
- cordocentesis

Other intrauterine procedures

- evacuation of the uterus because of mola hydatiforma
- multifetal reduction
- fetal therapy (insertion of shunts etc.)

Antepartum hemorrhage

when bleeding continues intermittently, anti-D immunoglobulin should be given at approximately 6-week intervals, and the volume of fetomaternal hemorrhage should be assessed

External version of the fetus

Abdominal trauma

Ectopic pregnancy

Intrauterine fetal death

Stillbirth

Dose:

before 20 weeks gestation	50 µg (250 IU)
after 20 weeks gestation**	100 µg (500 IU)

Timing:

as soon as possible, but no later than **72 hours** after the event.

* also if the RhD type of the infant has not been determined or is in doubt

** in conjunction with a test to assess the volume of any fetomaternal hemorrhage

diagnosis of 10–20 cases of RhD alloimmunization per 100 000 deliveries annually. The developed alloimmunization of the mother however does not place the fetus at risk of severe haemolytic disease in the current pregnancy. In such cases, it would therefore not be necessary to administer anti-D immunoglobulin.

Before screening for anti-erythrocyte antibodies in the mother's serum, it is always necessary to specifically inquire whether the woman had already been administered immunoglobulin anti-D (from herein referred to only as "IgG anti-D") during this pregnancy. If so, it is necessary to include this information on the examination requisition slip, because persisting levels of IgG anti-D could falsely lead to suspicion of maternal RhD alloimmunization. Similarly, if during the follow-up screening presence of anti-D antibodies is confirmed, it is best to once more inquire whether IgG anti-D was administered during this pregnancy before a diagnosis of RhD alloimmunization of the mother is established.

The half-life of the administered IgG anti-D is approximately 24 days. In 15–20% of patients who were administered IgG anti-D at 28 weeks of gestation, it is nonetheless still possible to detect a low titre of anti-D (usually 2 or 4) at the time of labour².

RhD negative women where the presence of anti-D antibodies in the serum was not confirmed, are administered a dose of 125 µg IgG anti-D intramuscularly at 28 and 34 weeks of gestation. IgG anti-D may also be administered as a single dose of 250 µg at 28 weeks of gestation.

This procedure may lead to an 80% decrease in the incidence of antepartal RhD alloimmunization (from 1% to 0,2%)⁷.

In the 1st trimester, women who are RhD negative are administered 50 µg IgG anti-D after spontaneous miscarriage followed by evacuation of the uterus, induced abortion (therapeutic termination of pregnancy), evacuation of the uterus because of mola hydatiforma, chorionic villus sampling or after operation for ectopic pregnancy.

Alloimmunization by the RhD antigen may already be detected at 6 weeks of gestation⁴.

The risk of RhD alloimmunization after spontaneous miscarriage is 1.5–2%, after induced abortion 4–5% (ref.²), after chorionic villus sampling 14% (ref.¹¹).

The total volume of fetal blood at 12 weeks of gestation = 3ml (FMH 1.5ml), sufficient dose of IgG anti-D = 30 µg. Therefore up until 12 weeks of gestation, a sufficient dose of IgG anti-D for prevention of D alloimmunization is 50 µg.

In the 2nd and 3rd trimester, after induced abortion (therapeutic termination of pregnancy), amniocentesis, cordocentesis, or after other invasive prenatal diagnostic or therapeutic procedures, after antepartum hemorrhaging, intrauterine fetal death, attempt at external cephalic version of a breech presentation, after abdominal trauma or in situations where there is a potential risk of sensitization of the mother by RhD antigens of the fetus, a dose

of 50 µg of IgG anti-D is administered to RhD negative mothers until 20 weeks of gestation, after 20 weeks of gestation 100 µg of IgG anti-D are administered. After 20 weeks of gestation, the volume of fetomaternal hemorrhage (FMH) should also be determined to specify dosing.

The risk of RhD alloimmunization after amniocentesis is 2–5% (ref.³).

In cases of continued or repeated hemorrhaging after 12 weeks of gestation, 100 µg IgG anti-D are repeatedly administered in 6-week intervals, and during each episode of hemorrhaging the volume of fetomaternal hemorrhage (FMH) should be determined to specify the dose of IgG anti-D necessary for prevention of RhD alloimmunization of the mother^{2–5}.

For RhD negative women, after delivery of an RhD positive child, if the presence of anti-D antibodies was not detected in the serum, a 50–100 µg dose of IgG anti-D must be applied intramuscularly and the volume of FMH should be determined to specify the dose of IgG anti-D necessary to prevent RhD alloimmunization of the mother. If the FMH volume is not determined, a dose of 200–300 µg is administered. The effectiveness of administering a standard dose of over 100 µg to all women has not been demonstrated^{6,22}. This dose should be administered immediately following delivery, no later than 72 hours after delivery.

A 10 µg dose of IgG anti-D administered intramuscularly should cover 0.5 ml of fetal RhD positive erythrocytes or 1ml of whole blood. This means that 125 µg (250 µg) of IgG anti-D should prevent alloimmunization after fetomaternal hemorrhage of 12.5 ml (25ml) of whole fetal blood.

In approximately 1.5% of deliveries is fetomaternal hemorrhage more than 5 ml, in only 1% of deliveries does fetomaternal hemorrhage surpass 12.5 ml and only 0.5% of deliveries have fetomaternal hemorrhage greater than 25 ml^{12–15}. However, in nearly 50% of all cases no risk factor is present^{14–16}.

A number of countries therefore recommend assessing the volume of FMH after delivery to specify the dose of IgG anti-D necessary to prevent RhD alloimmunization of the mother (Australia, Canada, USA, Great Britain, France, Ireland).

There is a greater risk of fetal erythrocytes entering maternal circulation in deliveries by caesarean section¹⁷, stillborn deliveries¹⁵, traumatic vaginal deliveries, deliveries with multiple births, deliveries with signs of premature separation of the placenta, deliveries with pathology in the third stage of labour, etc.

The goal of further studies should be establishing optimal doses of IgG anti-D. The effectiveness of immediate administration of lower doses of IgG anti-D in combination with screening of the volume of fetomaternal hemorrhage and subsequent supplementation of IgG anti-D in case of necessity should be compared with the effectiveness of administering a single larger dose of IgG anti-D to everyone.

If the amount of fetal erythrocytes which entered the maternal circulation is quantitatively determined, administration of 10 µg IgG anti-D per 0.5 ml of fetal erythrocytes or 1 ml of whole blood is indicated⁶⁻⁷.

In cases where prevention of RhD alloimmunization is not performed within 72 hours of a potentially sensitising event, it is still sensible to administer IgG anti-D within 13 days, and in special cases, administration is still recommended up to a maximum interval of 28 days postpartum⁸.

It is necessary to issue a confirming document to women who received IgG anti-D, which precisely describes the amount and form of administration. IgG anti-D should not be administered to women where the presence of anti-D antibodies was confirmed in their blood serum. Exceptions are cases of persisting levels of antenatally administered IgG anti-D. If there is not absolute certainty regarding the origin of anti-D antibodies in the mother's serum, prevention of RhD alloimmunization should be performed. IgG anti-D should also be administered in cases where the RhD status of the child is unknown.

DETERMINING THE VOLUME OF FETOMATERNAL HEMORRHAGE (FMH)

The amount of fetal erythrocytes leaked into the maternal circulation (FMH) is accurately assessed using flow cytometry. After labour, a blood sample from the mother may be drawn no sooner than 1 hour after labour and a sample of 0.5–1.0 ml of venous blood is collected into a test tube containing an anticoagulative substance (EDTA, Heparin).

It is common practise to always examine the RhD status of a child born to an RhD negative woman after labour. Assessing the volume of fetomaternal hemorrhage (FMH) is then indicated in cases, where the child is RhD positive as it allows specification of the dose of IgG anti-D needed for the prevention of RhD alloimmunization of the mother. IgG anti-D is however always administered in a minimally 100 µg dose immediately after detecting a positive RhD status of the child.

Optimal and economically most effective would be to apply a 250 µg dose of anti-D antibody in the 28th week of gestation to RhD-negative women if no anti-D antibodies were detected in their serum. After the birth of an RhD positive child, the volume of fetal erythrocytes which entered maternal circulation should be assessed and only in indicated cases should a dose of IgG anti-D be administered postpartum (Australia)⁴.

The volume of fetomaternal hemorrhage (FMH) should also be determined in all RhD negative women where the presence of anti-D antibodies was not determined, in cases of potentially sensitising events (Table 1) after 20 weeks of gestation^{5-7,20-23}.

The Kleihauer-Betke acid-elution test (UK) or the Rosette test (USA) may be used to roughly determine the volume of fetomaternal hemorrhage.

ESTABLISHING *RHD* GENOTYPE OF THE FETUS

At the beginning of pregnancy of an RhD negative woman, it is possible to establish the *RHD* genotype of the fetus from free fetal DNA circulating in maternal peripheral blood. If the fetus is RhD negative, it is unnecessary to administer IgG anti-D at 28 weeks gestation or perform RhD alloimmunization prevention in cases of potentially sensitising events (Table 1)²⁴⁻²⁷.

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