ALLOIMMUNIZATION IN PREGNANCY

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1. INTRODUCTION

Alloimmunization is caused by the maternal production of antibodies to target a Fetal red blood cell (RBC) membrane antigen, which is not present in the mother (and therefore of paternal origin), in response to previous sensitisation. The red cells are destroyed by the fetal reticuloendothelial system after an antigen-antibody reaction. Fetal or neonatal anaemia secondary to haemolysis of immunological origin or haemolytic disease of the foetus and newborn (HDFN) has also been called Perinatal Haemolytic Disease (PHD) or *Erythroblastosis Fetalis*.

1.1. Blood groups involved

The antigen group mainly involved in the generation of maternal antibodies is Rhesus (Rh), specifically D, Cc and Ee. Other groups that can potentially generate maternal alloimmunization are Kell, Duffy, Kidd, etc. The main antibody involved in most cases of alloimmunization is **anti-RhD**, followed by anti-Rhc and anti-Kell. The other antigens are very infrequent causes of alloimmunization (Table 1).

TABLE 1: Antibodies causing fetal and immune anaemia (*Kenneth J Moise. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. Seminars in Fetal and Neonatal Medicine (2008), 13, 207e214*).

Antibodies associated with sever fetal involvement:

System	Ac
Rhesus	D, c
Kell	Kell (k1)

Antibodies rarely associated with severe fetal involvement:

System	Ac
Colton	Coa, Co3
Diego	ELO, Dia, Dib, Wra, Wrb
Duffy	Fya
Kell	Jsa, Jsb, k (K2), kpa, kpb, K11, K22, Ku, Ula
Kidd	Jka
MNS	Ena, Far, Hil, Hut, M, Mia, Mit, Mta, MUT, Mur, Mv, s, sD, S, S, U, Vw
Rhesus	Bea, C, Ce, Cw, Cx, ce, Dw, E, Ew, Evans, e, G, Goa7, Hr, Hro, JAL, HOFM, LOCR, Riv, Rh29, Rh32, Rh42, Rh46, STEM, Tar
Others	HJK, JFV, JONES, Kg, MAM, REIT, Rd

Antibodies not associated with severe fetal involvement:

System	Ac
Dombrock	Doa, Gya, Hy, Joa
Duffy	Fyb, Fy3
Gerbich	Ge2, Ge3, Ge4, Lsa
Kidd	Jkb, Jk3
Scianna	Sc2
Others	Vel, Lan, Ata, Jra

1.2. Causes of alloimmunization

Alloimmunization is always caused by a previous sensitisation episode:

- Fetomaternal haemorrhage during delivery or pre-partum (it is considered that alloimmunization can occur in 17% of RhD (-) pregnant women with a RhD (+) foetus in the absence of anti-D immune globulin administration).
- Transfusion of blood and blood products.
- Exchange of needles or blood-contaminated products (drug addicts).
- Organ transplants.

2. PREVENTION OF MATERNAL ALLOIMMUNIZATION ANTI-RhD

2.1. Prevention of a sensitisation event:

There are considered sensitising events:

- Delivery or caesarean section of an RhD (+) foetus
- Miscarriage or threatened miscarriage
- Ectopic pregnancy
- Molar pregnancy
- Invasive techniques (chorionic biopsy, amniocentesis, cordocentesis, cerclage, fetoscopy, etc.)
- Intrauterine fetal demise
- External cephalic version
- Maternal abdominal trauma
- Antepartum haemorrhage

In non-sensitised RhD pregnant women:

- An intramuscular dose of 300 µg of anti-D immune globulin should be administered within the first 72 hours of the sensitisation process.

- If not administered in the first 72 hours, it is still useful up to 4 weeks.
- This dose can protect fetomaternal haemorrhages up to 30 mL.
- The protective effect lasts at least 3 weeks.

In episodes of severe fetomaternal haemorrhage (intrauterine fetal demise, placental abruption, manual removal of placenta, maternal trauma, non-filarial fetal anaemia), the dose should be adjusted according to the degree of fetomaternal haemorrhage estimated by Kleihauer test, HbF determination or flow cytometry. Twenty µg of anti-D immune globulin per mL of fetomaternal haemorrhage should be administered.

2.2. Routine antepartum prevention

In the 28th week of gestation, a dose of 300 µg of anti-D immune globulin will be administered to all RhD (-) pregnant women regardless of the RhD of the partner. It will not be necessary if the fetal genotype has been studied in maternal blood or amniotic fluid and the result is RhD (-).

3. FOLLOW-UP OF THE NON ALLOIMMUNIZED PREGNANT WOMAN

3.1. All pregnant patients should have their blood group, RhD and irregular antibodies (indirect Coombs) determined. In case of presence of irregular antibodies, the antibody should be determined and titred if it is RhD.

3.2. In RhD negative pregnant women, irregular antibodies should be determined every trimester (at 10, 24 and 34-36 weeks), but never in the period of one month after the administration of anti-D immune globulin.

4. MATERNAL ALLOIMMUNIZATION

The patient who presents positive titres of irregular antibodies, regardless of their titre, will be considered as alloimmunized, although not all of them will require monitoring in a specialised unit.
The protocol described has been validated in anti-RhD alloimmunization. In case of proven anti-Kell or anti-Rhc alloimmunization, the definitions and attitude are the same, except of the antibody titration, which is usually not performed.

4.1. Low risk patients:

Low titres (<1:16) of irregular antibodies are not associated with fetal or neonatal haemolytic disease.

4.2. High risk patients:

- High titres (≥ 1:16) of Anti-RhD irregular antibodies.
- Presence of a history of high risk, regardless of irregular antibodies:
    - Fetal demise due to alloimmunization.
    - Fetal hydrops in previous pregnancies.
    - History of severe fetal/neonatal anaemia (intrauterine transfusion or exchange transfusion)
- Anti-Kell alloimmunization (any antibody titre will be considered high risk).
- Anti-Rhc alloimmunization (any antibody titre will be considered high risk).

5. BEHAVIOUR IN THE ALLOIMMUNIZED PREGNANT WOMAN

5.1 Low risk: gestation will be monitored in the general obstetric consultation.

- Fetal RhD genotyping: in maternal blood this is reliable from the second trimester onwards. In case of amniocentesis for other reasons (avoiding transplacental passage), it can be determined in amniotic fluid. Fetal Rhc or Kell genotyping in maternal blood is not currently available in our setting.
- Paternal RhD phenotype: useful for counselling in future pregnancies: if it is heterozygous there is a 50% risk of RhD (+) foetus, if it is homozygous the risk is 100%. If RhD (-) there is no possibility of a RhD (+) foetus. Kell (+) parents should be considered heterozygous.
- Serial irregular antibody tests at 10, 17, 24, 24, 29, 34 weeks (if titre is >/= 16 it becomes high risk).
- Evaluate serial middle cerebral artery Doppler studies (monthly, bimonthly).
- Usual obstetric behaviour.

5.2 High risk:

Gestation will be monitored in the specialised Fetal Haematology Office. The main objective of the monitoring is to identify the development of moderate or severe fetal anaemia as early as possible.

5.2.1. Fetal genotyping: in anti-RhD alloimmunization, if the foetus is RhD (-) it does not make sense to continue strict monitoring, so the RhD fetal genotype will be requested in maternal blood from the

second trimester onwards. In case of amniocentesis for other reasons (avoiding transplacental passage), it can be determined in amniotic fluid. Fetal RcC or Kell genotyping in maternal blood is not currently available in our country. The study of the paternal RhD phenotype will be useful only in counselling for future pregnancies.

5.2.2. Irregular antibody titre:

These have a primarily diagnostic role in anti-RhD alloimmunization. Once they have passed the critical level (1:16), subsequent titres will not be used for clinical decisions. In anti-Kell alloimmunization it is not necessary to perform titration, as there is no correlation with the degree of fetal anaemia.

5.2.3. Doppler and Ultrasound:

Doppler study is the main method of fetal control and monitoring in alloimmunized pregnant women classified as "high risk" according to this protocol.

An ultrasound will be performed **weekly** (from 16 weeks), determining:

- Middle Cerebral Artery Peak Systolic Velocity (MCA-PSV)
- Presence of any signs of hydrops or established hydrops
- Amount of Amniotic Fluid

Once the foetus has an elevated MCA-PSV or shows hydropic signs, it is considered that there is a high Probability of moderate or severe anaemia and that the foetus should undergo cordocentesis for possible eventual intrauterine transfusion. The AFI (Amniotic Fluid Index) is an indicative sign (it can be used for accurate control), but it does not determine the clinical behaviour.

Methodology of Middle Cerebral Artery Systolic Velocity (MCA-PSV) measurement:

- The foetus must be at rest and the mother in apnoea.
- Axial section of the fetal skull at the sphenoid level.
- View of the vascular polygon of Willis by colour Doppler.
- The image must be magnified, so that the closest MCA to the transducer occupies more than 50% of the screen. The MCA must be visualised over its entire length.
- The cursor should be located near the origin of the MCA and the insolation angle should be as close to 0 degrees as possible. The velocity corrector should not be used due to the angle of insolation.
- The flow velocity waveforms (between 5-10) should be similar and the highest systolic peak should be measured.

- Avoid compressing the fetal skull as this may affect velocities.
- The MCA-PSV is expressed in centimetres per second (cm/s) and is translated into multiples of the median (MoM) according to Mari et al (see Table 2). It can also be expressed in standard deviations (SD).
- It is recommended to repeat the measurement three times and use the average for the final calculation.
- When the MCA-PSV value is > 1.5 MoM, it is recommended to confirm it 24 hours later.

TABLE 2: Values of MCA-PSV corresponding to 1.5 MoM for each gestational week in the non-invasive diagnosis of fetal anemia (*Mari. Ultrasound Obstet Gynecol 2005;25:323-30*).

	MCA-PSV (cm/s)	
GA (weeks)	Median	1.5 MoM
14	19.3	28.9
15	20.2	30.3
16	21.1	31.7
17	22.1	33.2
18	23.2	34.8
19	24.3	36.5
20	25.5	38.2
21	26.7	40.0
22	27.9	41.9
23	29.3	43.9
24	30.7	46.0
25	32.1	48.2
26	33.6	50.4
27	35.2	52.8
28	36.9	55.4
29	38.7	58.0
30	40.5	60.7
31	42.4	63.6
32	44.4	66.6
33	46.5	69.8
34	48.7	73.1
35	51.1	76.6
36	53.5	80.2
37	56.0	84.0
38	58.7	88.0
39	61.5	92.2
40	64.4	96.6

5.2.4. Cordocentesis:

It is indicated up to 35.6 weeks, when moderate or severe fetal anaemia is suspected in order to perform a fetal Red blood cell (RBC) count:

- MCA-PSV > 1.5 MoM
- Hydropic foetus

5.2.5 Intrauterine transfusion:

Intrauterine transfusion is indicated if the fetal haemoglobin is below -4 SD for gestational age (moderate anaemia) (Table 3).

TABLE 3: Fetal Hb concentration corresponding to the mean for each gestational week and to – 4 SD, which is the cutoff value for moderate fetal anaemia, above which the intrauterine transfusion will be indicated (*Scheier et al., Prediction of fetal anemia in rhesus disease by measurement of fetal middle cerebral artery peak systolic velocity. Ultrasound Obstet Gynecol 2004;23:432-6*)

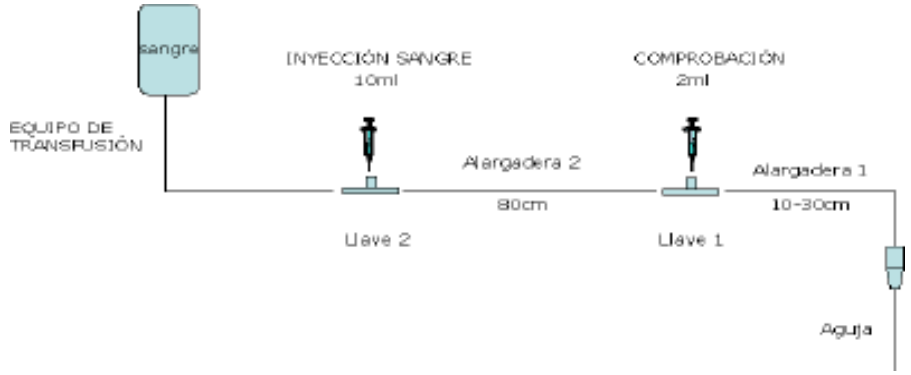
Gestational week	Mean Hb (g/dL)	Mild anemia - 2 SD (g/dL)	Moderate anemia - 4 SD (g/dL)	Severe anemia - 6 SD (g/dL)
	11.0	9.1	7.2	5.3
		9.3	7.4	5.5
	11.3	9.4	7.5	5.6
		9.6	7.7	5.8
	11.6	9.7	7.8	5.9
		9.9	8.0	6.1
	11.9	10.0	8.2	6.2
		10.2	8.3	6.4
	12.2	10.3	8.5	6.5
		10.5	8.6	6.7
	12.5	10.6	8.8	6.8
		10.8	8.9	7.0
	12.8	10.9	9.1	7.1
		11.1	9.2	7.3
	13.1	11.2	9.4	7.4
		11.4	9.5	7.6
	13.5	11.6	9.7	7.8
		11.8	9.9	8.0
	13.8	11.9	10.0	8.1
		12.1	10.2	8.3
	14.1	12.2	10.3	8.4
		12.4	10.5	8.6
	14.4	12.5	10.7	8.7

Consider transfusion in mild anaemia (Hb < -2 SD or haematocrit < 30%).

Methodology of intrauterine transfusion:

- Informed consent
- Blood is requested from the Blood Bank the day before and maternal blood is collected for compatibility testing. The blood must be group O-negative, compatible with any antibody present in the mother's serum, from a CMV seronegative donor, removal of leukocytes by filtration, irradiated and concentrated to a haematocrit between 75-85%.

- Fetal lung maturation if gestation is > 24 weeks (betamethasone 12 mg/24 h IM (intramuscular), 2 doses).
- Previous ultrasound with the assessment of the intrahepatic trajectory of the umbilical vein and placental insertion of the cord, estimation of fetal weight and cervical length.
- Nifedipine (Adalat) 20 mg (2 tablets) via oral or sublingual route if cervix < 15 mm.
- Routine antibiotic (cefazolin 2 g ev in 100 mL serum) in peripheral vein access.
- Prior to cordocentesis, the blood infusion pathway is prepared. The outlet port of the blood bag is connected distally to a 3-way stopcock, with an extension that connects to the puncture needle. The middle port of the stopcock will be connected to a 10 mL syringe that will alternately serve to aspirate the blood from the bag and to infuse it into the fetal bloodstream.



- Fetal immobilisation for fetal intramuscular puncture (norcuronium 0.1 mg/kg fetal weight + fentanyl 10 mcg/kg fetal weight).
- Cordocentesis will be performed trying to access the umbilical vein and, if feasible, the intrahepatic tract (lower risk of bradycardia and haemorrhage). It should be taken into account that in an anterior placenta, transplacental puncture facilitates the procedure but might worsen the degree of alloimmunisation.
- Fetal RBC count on the first mL of fetal blood in EDTA, making sure it is not diluted in amniotic fluid.
- Determination of Fetal blood type and direct Coombs test in the second mL of fetal blood.
- Intrauterine transfusion is indicated if fetal Hb is below -4 SD for gestational age (moderate anaemia) (Table 2). Transfusion should be considered in mild anaemia (Hb < -2 SD or Hto < 30%).

- The amount of blood to be transfused is calculated from the expected fetal blood volume, based on the gestational age and multiplied by a factor determined by the haematocrit of the foetus and the donor.
- Blood transfusion under continuous ultrasound control, in which the entire needle trajectory should be viewed in the ultrasound plane. The entry of blood into the fetal circulatory system appear as bubbles on the ultrasound. During periods when blood is not infused, the fetal heart rate should be monitored if it is not visible in the ultrasound infusion plane.
- In case of severe anaemia with fetal hydrops, it must be considered to transfuse less amount than the required to avoid hemodynamic decompensation and cardiac overloading due to hypervolemia, and then complete the transfusion within the following week.
- Final haematocrit check, after infusing 2 mL of saline in the direction of the needle.
- Post-procedural NST (non-stress test) from 26 weeks.
- Post-procedure resting for 24 h.
- Ultrasound control at 24 h.

5.2.6 Follow-up of the transfused foetus and indication for new transfusions.

- Weekly ultrasound/ Doppler assessment.
- MCA-PSV control and weekly ultrasound scanning.
- Evaluate the performance of NST at 28 weeks and beyond.
- Indication for new cordocentesis (foetuses < 36 s): in general, the drop in fetal haematocrit is 1% daily after the first transfusion but varies markedly in the subsequent ones, therefore the decision for a new transfusion will be made based on a combination of:
    - MCA-PSV: after a first transfusion, the same cut-off (1.5 MoM) is used.
    - Estimated daily reduction in fetal haematocrit by 0.9% after 2 transfusions and 0.6% after 3 transfusions.

5.2.7 Induction of labour in a high-risk alloimmunized pregnant woman.

a) Indication

- Transfused foetuses: 36-38 weeks: the timing of termination will take into account the transfusion interval (based on previous transfusion behaviour) and evidence of fetal well-being.

- Non-transfused foetuses:

- Generally, at 38 weeks
- If there is a risk of moderate or severe anaemia (MCA-PSV > 1.5 MoM or hydrops) from 36 weeks (in previous gestational ages: cordocentesis and possible transfusion).

b) Delivery route

- Suspected moderate or mild anaemia (with or without previous transfusion), there is no contraindication for vaginal delivery, but it will be taken into account:

- obstetric conditions.
- it requires surveillance with continuous monitoring and fetal biochemical assessment.

- Suspected severe fetal anaemia (persistent non-reactive NST or sinusoidal pattern or presence of hydrops): caesarean section is considered the delivery route of choice.

SPECIAL SITUATIONS:

5.2.8. History of fetal hydrops or fetal death before 20 weeks

These are highly complex cases due to the early onset of fetal anaemia, when intrauterine transfusion procedures are more difficult and riskier. All cases will be individualised but the following aspects will be taken into account:

- Fetal genotyping.
- The possibility of plasmapheresis and immunotherapy will be assessed on an individual basis. In week 10, two plasmaphereses of 2000 mL every other day, followed by gamma globulins at a dose of 0.4 grams per kilogram of weight/day x 5 days. If necessary, plasmapheresis and gamma globulins will be repeated in three weeks.
- Follow-up with MCA-PSV from week 16 of gestation.
- Cordocentesis and eventual transfusion in case of altered MCA-PSV (higher risk if before 20 weeks).

5.2.9. Gestation > 35 weeks with recent diagnosis

Late diagnosis after 35 weeks, particularly in case of an adverse previous clinical history, MCA-PSV loses specificity. Amniocentesis for amniotic fluid spectrophotometry is no longer performed, so clinical ultrasound assessment of the risk of hydrops fetalis may be helpful in indicating cordocentesis or even induction of labour.

6. NON ANTI-RhD ALLOIMMUNIZATION

The management or anti-Kell (K1) and anti-Rhc alloimmunizations is the same as in anti-RhD alloimmunization, regardless of antibody titre, and therefore are always considered high risk for fetal anaemia.

Anti-RhE alloimmunizations have an intermediate risk of fetal anaemia and therefore a monthly ultrasound control and MCA-PSV would be recommended.

Alloimmunizations by other antibodies are at low risk of fetal anaemia, although it is advisable to consult with a Fetal Medicine specialist to assess the risk.