REVUE GENERALE

Biological diagnosis of fetal-maternal incompatibilities

Diagnostic biologique des incompatibilités fœto-maternelles

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Résumé

Introduction: L'allo-immunisation fœto-maternelle résulte d'une suite de phénomènes liés à l'introduction d'un antigène étranger dans la circulation maternelle. La gravité de l'atteinte est due à une immuno-hémolyse entraînant la survenue d'une anémie fetale et/ou d'une anémie néonatale. Le diagnostic des incompatibilités fœto-maternelle érythrocytaire est basé principalement sur l'identification d'agglutinines irrégulières et le titrage/dosage des anticorps.

Objectif: Dans la présente revue de littérature, nous résumons les connaissances actuelles sur les principales méthodes d'exploration des incompatibilités fœto-maternelle érythrocytaire. Nous présentons les avantages et inconvénients de chaque méthode afin d'améliorer nos connaissances sur le diagnostic et le suivi des incompatibilités fœto-maternelle érythrocytaire.

Méthodes: Nous avons analysé la littérature médicale et les études publiées sur la thématique des incompatibilités fœto-maternelles. Les sites consultés sont PubMed, Transfusion, Elsevier et CNHRHP. Les mots clés utilisés lors de nos recherches sont "recherche anticorps irrégulier", "dosage pondéral", "cytométrie flux", "test Kleihauer".

Conclusion: Le diagnostic des incompatibilités fœto-maternelles doit être fait pendant la grossesse. Celui-ci repose sur le respect du calendrier de surveillance des agglutinines irrégulières. De plus, la technique du génotypage des groupes sanguins du fœtus sur sang maternel a révolutionné le diagnostic des incompatibilités fœto-maternelles. Le diagnostic des incompatibilités fœto-maternelles érythrocytaires et le succès de leur prise en charge reposent sur une coopération et un dialogue multidisciplinaire entre biologistes, obstétriciens et néonatologistes.

Mots-clés : incompatibilité fœto-maternelle, anticorps, agglutinines, titrage

Abstract

Introduction: Fetal-maternal incompatibilities result from a series of phenomena related to the introduction of a foreign antigen into the maternal circulation. The severity of the attack is due to immune-haemolysis resulting in the occurrence of fetal anaemia and/or neonatal anaemia. The diagnosis of fetal-maternal incompatibilities is mainly based on the identification of irregular agglutinins and the titration/assay of antibodies.

Objective: In this literature review, we summarize the current knowledge on the main methods for exploring fetal-maternal incompatibilities. We present the advantages and disadvantages of each method in order to improve our knowledge of the diagnosis and follow-up of fetal-maternal incompatibilities.

Methods: We analysed the medical literature and published studies on the topic of fetal-maternal incompatibilities. The sites consulted are PubMed, Transfusion, Elsevier and CNHRHP. The key words used during our research are "irregular antibody research", "weight assay", "flow cytometry", and "Kleihauer test".

Conclusion: Diagnosis of fetal-maternal incompatibilities should be made during pregnancy. This is based on compliance with the monitoring schedule for irregular agglutinins. In addition, the technique of genotyping fetal blood groups on maternal blood has revolutionized the diagnosis of fetal-maternal incompatibilities. The diagnosis of fetal-maternal incompatibilities and the success of their management are based on cooperation and multidisciplinary dialogue between biologists, obstetricians and neonatologists.

Keywords: fetal-maternal incompatibilities, antibodies, agglutinins, titration

INTRODUCTION

Fetal-maternal incompatibilities result from a series of phenomena related to the introduction of a foreign antigen into the maternal circulation with the consequence of the production of irregular antibodies. Transplacental passage of maternal IgG antibodies occurs via FcγRn receptors present on the placenta from the first trimester of pregnancy. These antibodies once in the fetal circulation form antigen-antibody immune complexes on the fetal red blood cell, resulting in immune adherence and erythrophagocytosis by splenic macrophages. The severity of the attack is due to fetal and neonatal immune-haemolysis leading to the occurrence of fetal anaemia and/or neonatal anaemia. Fetal anaemia can lead, in the most severe forms, to hydrops and/or fetal death *in utero* if untreated. Neonatal anaemia can lead to hyperproduction of bilirubin with a consequent risk of jaundice which can go as far as hyperbilirubinemic encephalopathy due to fixation of unbound bilirubin in the basal ganglia if immediate and appropriate management is not provided (1- 3).

The diagnosis of fetal-maternal incompatibilities (FMI) is mainly based on the identification of irregular agglutinins and the titration/assay of antibodies. If anti-erythrocyte antibodies are present in the mother's blood, identification of the specificity is essential. If the antibody is associated with a risk of fetal and/or neonatal haemolytic disease, quantification is mandatory. Various tests of varying interest are available for the quantification of an antibody. These tests can be used to estimate the risk of haemolytic damage to the foetus and/or newborn in order to make appropriate arrangements for biological and ultrasound monitoring of the pregnancy and to anticipate the conduct to be held at birth. In the present review, we summarize the current knowledge on the main methods of investigation of FMI available to date. We present the advantages and disadvantages of each method in order to improve our knowledge on the diagnosis, monitoring and management of FMI to improve the follow-up of pregnant women and reduce the risk of fetal and neonatal mortality.

METHODS

As part of this literature review, we analysed the medical literature and studies published on the topic of fetal-maternal incompatibility. We consulted the sites of PubMed, Transfusion, Elsevier and CNHRHP. The keywords used during our research are "irregular antibody", "weight assay", "flow cytometry", "Kleihauer test", "fetal-maternal incompatibilities", "alloimmunization". We selected articles published in both French and English. In this review, we present tests for screening and identification of antibodies, determination of antibody specificity, and quantification of the amount of fetal hematite in maternal blood.

The search of irregular agglutinins

The search for anti-erythrocyte antibodies or irregular agglutinins (SIA) is the first step before any identification of irregular agglutinin (4). Compliance with the SIA schedule during pregnancy is essential. Indeed, SIA makes it possible to detect cases of FMI and promotes better management of incompatible pregnancies. This helps to prevent the risk of miscarriage and death *in utero*. In most European countries, SIA is compulsory in the first trimester of pregnancy. If the patient is RH1 negative, the SIA is repeated at the 6^{th} and 8^{th} month of pregnancy (5, 6). In Africa, very few *data* exist on the timing of SIA in pregnant women. In Mali, SIA is rarely requested during prenatal consultations (7). Immunohematological monitoring of pregnant women is limited to tests for ABO and rhesus D (8, 9).

Any positive SIA must be followed by an identification of the agglutinin found (10). It is imperative to determine the specificity of the antibody as the risk of developing haemolytic disease in the new-born depends on the specificity of this antibody. Antibody quantification should be regular throughout pregnancy as antibody levels may increase in incompatible pregnancies. The other elements of the biological monitoring in this context are to assess the likelihood of an incompatible pregnancy by phenotyping the biological father and/or performing fetal genotyping (11, 12). When maternal antibody levels are high, ultrasound monitoring of the foetus should be undertaken weekly with peak systolic velocity measurements to the mean cerebral artery (13). If the measurements are repeatedly elevated and/or if there is an effusion in the serous membranes on ultrasound, fetal blood should be drawn, followed by fetal transfusion if necessary.

There are four major groups of antibodies based on the risk of fetal and/or neonatal damage. The first group includes the three most dangerous antenatal antibodies: anti-RH1, anti-KEL1 and anti-RH4. The second group contains the anti-RH3 antibodies which can lead to a risk of haemolysis in the foetus. Antibodies of the third group have only exceptionally and at very high levels been described as possibly being responsible for the development of severe fetal anaemia. Finally, the fourth group includes antibodies whose risk is almost exclusively postnatal. Table 1 shows these antibodies together with an assessment of the risks of fetal anaemia and neonatal haemolytic disease (3, 14).

Antibodies parameters influencing haemolytic risk

The haemolytic risk depends on several biological parameters, the most important of which is the specificity of the antibody. Another important parameter is the affinity of the antibody for red blood cells. This last parameter encompasses many factors including the mode of immunization, the specificity of the antibody, genetics and the number of incompatible pregnancies.

Table 1: Main specificities of anti-erythrocyte antibodies and obstetrical risk

Blood group	Antibody specificity	Risk of fetal anaemia (hb<6g/dl preterm)	Risk of neonatal haemolytic disease
RH system	Anti-D (RH1)	Yes (frequent - from 15 SA)	Yes
	Anti-C (RH2)	No	Yes
	Anti-E (RH3)	Yes/rare	Yes
	Anti-c (RH4)	Yes (from 20SA)	Yes
	Anti-e (RH5)	Yes (exceptional)	Yes
	Anti-Cw (RH8)	Yes (exceptional)	Yes
	Anti-G (RH12)	Yes (exceptional)	Yes
	Other anti RH including « anti-public and anti-private », linked anti-antigens, …	Yes (exceptional)	Yes
Kell system	Anti-Kell (KEL1)	Yes (frequent - from 15SA)	Yes
	Anti-Kpa (KEL3)	Yes (exceptional)	Yes
	Otheranti-KEL including « anti-public »	Yes (exceptional)	Yes
MNS system	Anti-M (MNS1	Yes (rare)	Yes
	Anti-N (MNS2)	No	Rare
	Anti-S (MNS3)	Yes (exceptional)	Yes
	Anti-s (MNS4)	No	Yes
	Anti-U (MNS5)	Yes (exceptional)	Yes
	Other anti-MNS including « anti-private »	Anti-Vw (exceptional) Anti-Mur (exceptional)	Yes
FY system	Anti-Fya (FY1)	Yes (exceptional)	Yes
	Anti-Fyb (FY2)	No	Yes
	Other anti-FY including « anti-public »	No	Yes
Jk system	Anti-Jka (JK1)	Yes (exceptional)	Yes
	Anti-Jkb (JK2)	No	Yes
	Other anti-JK including « anti-public »	No	Yes
P1PK1 system	Anti-P1 (P1PK1)	No	No
Lu system	Anti-Lua (LU1) andLub (LU2)	No	No
LE system	Anti-Lea (LE1), Leb (LE2), other	No	No
Other system of blood groups	Anti-Vel	Yes (exceptional)	No
	Anti-LAN	Yes (exceptional)	No
	Anti-Jra (JR1)	Yes (rare)	No
	Anti-Dia	Yes (exceptional)	No
	Anti-Coa	Yes(exceptional	No
	Anti-GE3	No	Yes
H &I system	Anti-H, anti-HI	No	No
ABO system	Anti-A (ABO1)	No	Yes
	Anti-B (ABO2)	Yes (exceptional)	Yes
Auto antibody and auto papaïne	Auto-antibodies et auto-papaïne	No	No

The affinity of an antibody to an antigen is assessed by the titration technique using an indirect antiglobulin test putting in contact with hematies native tests with dilutions of the patient's serum (15). The antibody concentration directly influences the haemolytic power of the antibody. The weight assay makes it possible to assess this antibody concentration. However, this weight determination is generally not carried out in african laboratories. In addition to the weight assay, antibody titration is a less accurate way of estimating antibody concentration. Antibody titration can be done in laboratories provided that R1R2 red blood cells (D C+E+c+e+) are available.

The three antibodies mainly involved in severe fetal anaemia are anti-D (RH1), anti-Kell (KEL1) and anti-c (RH4) (3). Anti-D (RH1) is always present in the first trimester of pregnancy but it may be at the limit of detection. The use of SIA technique using enzyme-treated red blood cells (papain) therefore facilitates its detection. The main pitfall is to confuse it with a residual passive anti-RH1 following an injection of IgG anti-D (IgRhD). Anti-Kell (KEL1) causing severe fetal anaemia is always present in early pregnancy at very high levels above 1/64. The use of enzyme-treated red blood cells is of no value in detecting this antibody as it is present at high levels from the start. Anti-c (RH4) involved in severe fetal anaemia is also always present in the first trimester of pregnancy. It can be at the detection limit. Its detection is greatly facilitated by the use of enzyme-treated red blood cells (3).

Antibody titration

Titration allows the quantification of all anti-erythrocyte antibodies, whatever their specificity, as long as test red blood cells expressing the corresponding antigen are available. It is based on the principle of hemagglutination. The reference technique uses the indirect antiglobulin test (IAT or indirect Coombs test) in a tube (16). The serum to be studied is placed in the presence of test red blood cells possessing the target antigen. The titration technique can be performed for RH system antibodies and also for other antibodies. After incubation, the test red blood cells to which the antibodies present in the maternal serum have bound are washed and centrifuged in contact with an anti-human IgG antiglobulin. The antibody titer corresponds to the inverse of the greatest geometric dilution of the serum for which an agglutination visible to the naked eye is observed. A first direct reading, before the addition of anti-IgG, allows to see if an IgM component is present. The titer will depend in part on the antibody concentration but mainly on its affinity for the target antigen. As a result, a high-affinity antibody can have a much higher titer than a low-affinity antibody despite a much lower concentration. This accounts for the frequent discrepancies observed between the titer and the concentration of the antibody and the impossibility of establishing a close correlation between these two parameters. This technique is not standardized and there is great interlaboratory variability (17). Many variations of titration techniques can be used, including gel techniques based on techniques for finding irregular agglutinins. They generally give higher titer values (18). For monitoring pregnant women, it is recommended to carry out comparative titrations with the previous serum. In order to consider an increase in titration significant, more than one dilution difference from the previous serum is required.

Titration in liquid medium by indirect antiglobulin test

This technique remains the most used because its implementation is simple. It consists of establishing a titer by determining the maximum dilution of the sample which still induces a hemagglutination reaction visible to the naked eye. It can be used for all types of antibodies, as long as there is at least one test-cell carrying the corresponding antigen in glass tubes. It is recommended to use a mixture of at least three heterozygous expression test hematies for antigen to limit variations in titre due to non-uniform donor expression of antigen, which is sometimes observed for the MNS, FY and JK systems (10). Some laboratories use the Marsh score which assesses the affinity of antibodies more finely. It is calculated by adding the agglutination patterns observed for each dilution of the serum sample.

The higher the titer of maternal serum antibodies and the higher the Marsh score, the greater the risk of developing severe haemolytic disease of the foetus and newborn. If the serum titer differs by two or more dilutions from a previous serum, the titer increase is considered significant. Immunization is then considered to be progressing. Threshold titers, from which it is estimated that there is a risk of severe fetal anaemia, have been established. They vary according to the country and the nature of the antibodies (11, 19,20). For anti-D, the titer of 16 is commonly accepted as critical, but from one institution to another, the threshold value can vary from 8 to 32 (21). For anti-Kell, recent studies have not observed serious cases of haemolytic disease of the foetus and new-born with titers < 16 (22). For the anti-c, there is not strictly speaking a critical titer described in the literature. The threshold titers used for anti-D are generally extrapolated for anti-c. The anti-c is often unrefined and the titers found are, most of the time, low to moderate. Cases of neonatal haemolytic disease, sometimes severe, have been observed with titers of 8 and the weight assay of antibodies, from titer 4 according to a study at the CNRHP (23).

For the other antibodies, little information is available in the literature. However, no severe fetal anaemia has been described for titers ≤ 32 (24). The critical threshold

used in France is 64, with an exception for anti-E and anti-Jra. Anti-Jra (JR1) is an antibody directed against a high frequency (anti-public) antigen. Its prevalence is low but a certain number of cases of severe fetal anaemia have been described with this antibody which seems particularly dangerous from an obstetric point of view. For this antibody, the biologist recommends to the clinician a specific fetal ultrasound follow-up from the titer of 16 (25, 26). Anti-M has the particularity of often combining IgG and IgM respectively able and unable of crossing the placental barrier. Titration of the IgG fraction alone is recommended from an overall antibody titer of 32. It requires first inactivating the haemagglutinating power of the IgM by treating the serum with dithiothreitol (DTT). An overall titer of anti-M $\geq$ 64, with a titer of the IgG fraction alone $\geq$ 16 is considered at risk (14, 27). The titration of anti-A and anti-B IgG is of very controversial interest in predicting the risk of severe fetal and neonatal haemolytic disease by ABO incompatibility. It requires prior treatment of the serum with DTT to inactivate the IgMs and only titrate the IgG fraction. Anti-A and anti-B IgGs sometimes include a high proportion of IgG2 whose haemolytic power is low because this subclass of IgG is less well recognized by the Fc receptors on monocytes and activates the complement less well than the IgG1 or IgG3. Moreover, the expression of antigens A and B is not restricted to erythrocytes and is not integral in the new-born (28). For these reasons, the titer of total maternal IgG is not necessarily a good reflection of the neonatal haemolytic risk. This titration may be of interest in women whose previous child has developed severe neonatal haemolytic disease due to ABO incompatibility. Indeed, in this case, the titer of maternal IgG seems to be associated with the severity of neonatal involvement, but without a clearly established threshold value (29).

Advantages and disadvantages of liquid titration

The tube titration technique has the advantage of being quite easily achievable by many laboratories. Moreover, it remains very powerful and very informative. Indeed, it is very sensitive to the affinity of antibodies with respect to their target antigen and therefore to their haemolytic power. A very affine antibody with a low concentration may have a higher titer than an antibody with a low affinity but a high concentration. Thus, at equal concentration, an antibody with a high titer will be much more at risk of inducing severe haemolytic disease in the foetus or new-born than an antibody with a moderate titer. However, the titration of anti-erythrocyte antibodies by the indirect antiglobulin tube test is not very reproducible and the intra- and inter-laboratory results vary considerably. This is due to a discontinuous reading of the values, a source of inaccuracy, and to an end point of hemagglutination depending on many variables that are difficult to standardize (30).

Gel titration

Developed for several years on column-filtration type supports, the indications for gel titration are the same as for the tube technique. Due to the presence of potentiating molecules in the column/filtration supports, the results observed are often different from those obtained by the technique in liquid medium. Test red cells can be suspended in saline or BFI medium, a condition that also affects the test result. If the results generally seem overestimated for antibodies of the RH system, this tendency is less obvious for those directed against other blood group systems, in particular for anti-Kell whose serum titer is sometimes underestimated (30, 31). In addition, it would seem that samples with the same titer by the tube technique can have titers varying up to five dilutions apart by this technique. To date, no critical titer correlated with clinical data has been established. Laboratories that have switched to the gel titration technique often use the same threshold titers as with the other technique for anti-D and anti-C. The advantage of the gel titration technique is the possibility of automation. With automation, it can be expected that inter-operator variability will be reduced and thus the reproducibility of assay results will be improved (18). The use of antibody titration results with this new technique is likely to be cautious and may lead to a significant increase in the number of pregnancies considered to be at risk, with clinical follow-up not always necessary for patients. If more precise thresholds are then established with experience and hindsight, they may differ from those of the tube technique, which will require good communication on the part of biologists.

Other titration techniques

With automation, other titration techniques are developing: in microplates, by immunocapture techniques. Like the column/filtration techniques, they will have to be demonstrated clinically in order to be able to define critical thresholds. The laboratories using them will have a key role in advising clinicians when submitting analysis results.

Special case of anti-D microtitration

Microtitration is a technique implemented in France at the end of the 1990s following the generalization of antenatal Rh immunoprophylaxis (32). This technique is only applicable for anti-D and only quantifies low concentrations of antibodies. It can be used to ensure that anti-D is “passive” or, on the contrary, to detect the onset of weak immunization in a patient.

Anti-D titration technique

Based on the principle of haemagglutination, this technique involves the use of column/filtration type media and red blood cells of the D+C-E-c+e+ phenotype (RH:1,-2,-3,4,5) treated with papain (papainized). An

alternative with native red blood cells remains possible. The phenotype of the test red blood cells is chosen so that they are compatible with other antibodies sometimes associated with anti-D, such as anti-C or anti-E. In this way these antibodies (except for anti-G) cannot interfere with the titration. An anti-D immunoglobulin standard solution (6 ng/mL or 0.03 IU/mL if the technique uses papainized test red blood cells or 24 ng/mL or 0.12 IU/mL if it uses native test red blood cells) is diluted in a two-fold series in the six wells of the column/filtration rack. The sera are diluted in the same way, in isotonic sodium chloride solution, and the haemagglutination intensities are compared. The anti-D concentration is calculated by multiplying the reciprocal of the last reactive dilution of the sample with the concentration of the dilution of the standard with the same reaction intensity. If the concentration of anti-D found is less than or equal to that expected according to the charts established in view of the pharmacokinetics of IgRh, it is a "passive" anti-D (32). If it is higher, the presence of an anti-D immunization can be evoked. This technique has shown its interest for patients with a negative D phenotype (RH1) in the absence of information on a possible injection (anti-D immunoglobulin), and for any intensity of reaction inconsistent with the date of injection announced. It can make it possible to correct false diagnoses of "passive antibodies" or, more rarely, false diagnoses of immunization (33).

Advantages and disadvantages of anti-D microtitration

Microtitration has few disadvantages compared to its added value because it is easy to implement for any immunohematology laboratory and can be automated, at least for the dilution and distribution of serums and red blood cells. However, it requires having a standard connected to the international standard, which can be the limiting factor. It is still recommended, for any value found ≥ 24 ng/ml, to supplement the microtitration with a weight assay to obtain a more precise concentration value (23).The microtitration technique is therefore the only one that can accurately determine the absence or absence of anti-RH1.

Weight measurement

The weight assay can be performed for all the antibodies of the RH system: anti-RH1(D), anti-RH2(C), anti-RH3(E), anti-RH4(c), anti-RH5(e). The weight assay makes it possible to determine critical levels of antibodies beyond which there is a risk of severe fetal anaemia. These rates alert the clinician so that a specific monitoring of the foetus can be set up. This specific follow-up is intended to identify signs of anaemia requiring therapeutic intervention (34). However, the predictive value of the antibody assay should not be overestimated as it does not explore the ability of the antibody to activate macrophage Fc receptors and additionally there are fetal variables that can attenuate the haemolytic potential of antibodies.

The weight determination of the anti-erythrocyte antibodies by continuous flow hemagglutination

This is the reference technique for estimating the concentration of anti-D antibodies (32). It is based on the principle of hemagglutination in a continuous flow in an autoanalyzer. The weighted assay technique is quite laborious but it allows the concentration of antibodies to be established and is very useful in the management of patients with alloimmunisation against the five major antigens of the RH system (24, 35, 36).

The weighted assay technique requires a continuous flow autoanalyzer, which is becoming less available on the market. The maintenance of the equipment is essential and its use requires a certain amount of expertise for the management of leaks, breakages, clogging, etc. Furthermore, the results obtained from one type of autoanalyser to another are not completely correlated, hence the importance of always performing the assays in the same laboratory. The intermediate precision of the technique shows a fairly high coefficient of variation, between 5 and 20% depending on the studies (32). The results are still influenced by a certain number of factors including the freshness of the red blood cells, the precision of the dilutions, the incubation temperature and the environmental conditions. For RH system antibodies, the weight assay detects reactivation of immunization earlier (37). It has also been shown that the concentration of maternal anti-D antibodies is correlated with the bilirubinemia of the first hours of life in new-borns (37).Concerning anti-c antibodies, which generally have a moderate titer due to their often low initial affinity, the concentration established by weight assay allows a better assessment of the postnatal hemolytic risk (23). For anti-E, anti-e and anti-C antibodies, the weight assay associated with titration allows a more precise assessment of the risk of haemolytic disease. This technique is not applicable to the quantification of anti-Kell. Indeed, assays of these antibodies by continuous flow hemagglutination have been conclusive: problem of reproducibility, lack of correlation with clinical data.

Flow cytometry techniques

The Kleihauer test is the reference test for detecting and quantifying maternal-fetal haemorrhage (MFH). However, this test has its limitations, particularly in the presence of maternal haemoglobin pathology. In this context, only a complementary flow cytometry technique (FCT) combining fetal haemoglobin and carboninc anhydrase labelling can be used to conclude whether or not MFH is present. The FCT provides several pieces of information including the percentage of red blood cells recognised by the patient's serum antibodies and

the average number of IgG molecules bound per red blood cell (38).

ELISA techniques

It is an immuno-enzymatic assay on a solid support. The addition of an enzyme-bound antiglobulin (indirect ELISA) or other enzyme-bound antigen-specific antibody (competitive ELISA) allows, after addition of the substrate, the development of an enzymatic reaction (39). Other techniques involving additional manual steps have also been developed, such as SOL-ELISA. This method is very sensitive and highly specific for anti-D and anti-Kell assays (40). ELISA techniques have many manual steps and are quite laborious. For anti-D, the results obtained by ELISA and continuous flow hemagglutination seem to be correlated, and the coefficients of variation are similar (40). However, the ELISA techniques have the advantage, compared to the continuous flow hemagglutination technique, of being able to measure many antibody specificities and to differentiate the proportion of IgG subclasses. A multicenter study compared the results obtained for nine serums containing an anti-D by weight assay on an auto-analyser, ELISA (by competition) and flow cytometry: the concentrations found were globally well correlated and no test seemed superior to another (41).

Genotyping techniques

In proven RH1, KEL1 and RH4 alloimmunizations, knowledge of the fetal genotype makes it possible to diagnose fetal-maternal incompatibility and to justify heavy and restrictive fetal monitoring. Fetal RHD and KEL1 genotyping is a non-invasive test and has the advantage of avoiding fetal risks and aggravation of immunization associated with invasive sampling. This validated and proven technique has revolutionized the monitoring of pregnant women (42, 43). In pregnant women, it is possible to amplify genes carried by the foetus and not by the mother using a gene amplification technique based on DNA extracted from maternal plasma. This made possible non-invasive fetal RHD genotyping from the blood of RH1 negative pregnant women (44). If the test is positive, specific monitoring is warranted. The risk of a false negative genotyping could have dramatic consequences here, which justifies all means to avoid this pitfall. In this context, it seems essential to never give a definitive negative result without a systematic check on a second sample remotely from the first. In the event of a positive result, this control is not necessary (45).

Kleihauer test

Several methods can be used to identify and quantify fetal red blood cells in maternal blood with varying degrees of sensitivity, specificity and accuracy (46, 47). The Kleihauer test remains the most efficient and the most universally applicable (48). It is a cytochemical test used to distinguish, on a blood smear fixed with ethanol, red blood cells rich in fetal hemoglobin after elution of adult haemoglobin at pH= 3.2. The critical step is elution, the development of which must be monitored using a suspension consisting of fetal red blood cells mixed with adult blood. The Kleihauer test allows an etiological diagnosis of fetal/neonatal anaemia or fetal death. It also allows screening and follow-up of spontaneous FH or induced by trauma or obstetric procedures. Finally, the Kleihauer test allows dosage adjustment of anti-RH1 IgG in RH1-negative women who are pregnant or have recently given birth (49). The Kleihauer test has the disadvantage of being a manual technique, not standardized, often criticized and requiring trained and trained personnel. It also presents a difficulty in cell counting which can lead to a large interindividual variation (50).

DISCUSSION

Many quantification tests exist but there is no ideal biological test to predict the severity of haemolytic disease in the foetus and new-born due to the complexity of its pathophysiology involving both maternal parameters, placental, and foetus. Quantification tests are only informative of part of the mechanism, which explains the very great variability of the clinical pictures observed for a given result. They can only be interpreted in relation to a threshold to define the risk of severe fetal anaemia, possibly instituting clinical and ultrasound monitoring specific to pregnancy. However, the different quantification techniques are complementary. Indeed, the results of titrations and weight assays provide different information which, when combined, makes it possible to better understand the overall risk of haemolytic disease in the foetus and new-born. Titration remains the technique of choice throughout the world because of the simplicity of its execution and because it allows rapid quantification of anti-erythrocyte antibodies, whatever their specificities, if at least one informative test haematode carrying the antigen is available. It is also evolving with the arrival of automatable techniques, whether on column/filtration support or by other methods. These new techniques will nevertheless pose the problem of the new risk thresholds to be established. In the United Kingdom and France, the weight determination of anti-erythrocyte antibodies by continuous flow hemagglutination is still used. Although it completely replaces titration for anti-D and anti-c in the United Kingdom, in France, it is carried out in addition to titration in order to specify the risk of fetal and neonatal damage (2). Quantification tests remain essential for the follow-up of pregnancies complicated by anti-erythrocyte alloimmunization. They must be implemented quickly as soon as a risky antibody is identified in a

pregnant woman. Their results must be accompanied by an interpretation describing the associated fetal and neonatal risk.

CONCLUSION

The diagnosis of any fetal-maternal incompatibilities must be made during pregnancy and not urgently, in the face of fetal anaemia complications. This is based on well-conducted biological monitoring: compliance during pregnancy with the irregular agglutinin monitoring schedule, regular titrations and weighted determinations of anti-erythrocyte antibodies. In addition, many advances have improved the diagnosis of FMIs with the implementation of non-invasive diagnosis of incompatibilities, genotyping of fetal blood groups on maternal blood. Monitoring by Doppler velocimetry of the cerebral artery for fetal anaemia complications, the use of new therapies and the evolution of transfusion therapies have contributed to revolutionizing the management of FMI. Finally, the diagnosis of fetal-maternal erythrocyte incompatibilities and the success of their management rely on cooperation and multidisciplinary dialogue between biologists, obstetricians and neonatologists.

What is known about this topic?
The diagnosis of fetal-maternal incompatibilities is mainly based on the identification of irregular agglutinins and the titration/assay of antibodies.

Various tests of varying interest are available for the quantification of an antibody. These tests can be used to estimate the risk of haemolytic damage to the foetus and/or new-born.

What this study adds?
The search for irregular antibodies is an indispensable tool to detect antibodies at risk of fetal-maternal incompatibility. Antibody titration can predict the predisposition of an autoimmune haemolytic disease.

The advantages and disadvantages of liquid titration, gel titration and anti-D microtitration techniques are clearly described.

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