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Impact of non-invasive fetal *RhD* genotyping on management costs of rhesus-D negative patients: results of a French pilot study

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ABSTRACT

Objectives: Fetal rhesus D (*RhD*) status determination using circulating cell-free fetal DNA from maternal plasma or serum is now recognized in Europe as a reliable and useful tool. A few countries are presently using this test in their management policy of rhesus D negative patients. The objective of this study is to evaluate the impact of this test on the costs of managing RhD-negative pregnant women, whether or not they are allo-immunized.

Study design: A prospective follow-up of rhesus D negative women during their pregnancy was performed in three French obstetric departments. Non-invasive fetal RhD genotyping was performed in the first trimester and pregnancies were followed The costs of all procedures (biological tests and medication) associated with patient management in relation to their RhD-negative status were calculated according to different management options.

Results: A comprehensive follow-up, including medical and biological monitoring, was obtained for 99 of the 101 patients included in the study. Patients were separated into two groups: the "Adverse Event" group (AE, $n = 23$) for which a potentially sensitizing event occurred and the "No Adverse Event" group (NAE, $n = 76$). Fetal RhD status was accurately determined in all cases. The mean cost per patient was estimated at 237€ (range: 115–644) with differences observed depending on the group, notably 331€ (range: 236–644) for the AE group and 208€ (range: 115–366) for the NAE group. Various cost simulations were performed according to various policies of allo-immunization antenatal prophylaxis. Variations ranged from +36.2% to +105.3%.

Conclusion: This study demonstrates that fetal RhD genotyping early during pregnancy is not an effective cost-reduction strategy whether or not antenatal prophylaxis is given. The economic issues could, however, be overcome by the fact that there is a major clinical benefit to offering the test systematically to all RhD-negative pregnant women while avoiding unnecessary testing and immunoglobulin injections.

1. Introduction

Fetal rhesus D (RhD) status determination using circulating cell-free fetal DNA from maternal plasma or serum is now well accepted by many obstetricians in Europe as a reliable and useful tool [1–4]. Management of allo-immunized pregnant women is its main and indisputable indication. Non-invasive first-trimester fetal RhD status determination permits the avoidance of unnecessary invasive procedures which are likely to further aggravate immunization due to induced feto-maternal hemorrhage.

The test may also be offered to non-sensitized RhD-negative pregnant women prior to invasive prenatal diagnostic procedures. Such strategy offers several advantages. First, it avoids unnecessary administration of anti-D immunoglobulin (a blood-derived product) in the case of an RhD-negative fetus, allowing much simpler and less stressful patient follow-up. Secondly, it allows physicians to manage women carrying an RhD-positive fetus in a single-step procedure (i.e. amniocentesis plus anti-D injection), thus eliminating the time-delay between invasive sampling and anti-D prophylaxis.

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Whether or not systematic non-invasive fetal RhD genotyping should be conducted in the third trimester for all RhD-negative pregnancies is still a matter of debate in countries where the prevalence of RhD allo-immunisation is low (0.9‰ in France). Recommendations were issued in 2005 by the French College of Obstetricians and Gynecologists regarding prevention of feto-maternal RhD allo-immunization. Obstetricians were advised to systematically administer 300 μg of anti-D immunoglobulin (Rhophylac®) at the 28th week of gestation. In addition, non-invasive fetal RhD genotyping, which is now available in some laboratories in France, has been recommended in order to develop an elective strategy for immunoprophylaxis of women carrying a RhD-positive fetus [5–7]. It is still unclear, however, how the test should be set up, and what its impact on the costs of managing RhD-negative patients might be. The question is of importance as about 170,000 pregnant women in France are targeted by these recommendations.

The aims of this prospective pilot study were to perform non-invasive fetal RhD genotyping early during pregnancy in a non-invasive manner, and prospectively to evaluate the overall costs of antenatal prophylaxis. Based on the results, the costs of potential strategies were evaluated, including routine antenatal anti-D prophylaxis (RAADP).

2. Material and methods

2.1. Patients

Overall, 101 women agreed to participate, none of whom was allo-immunized. Except for eight women (one Afro-Caribbean woman and seven from North Africa), all were of Caucasian origin. At the time of non-invasive fetal RhD genotyping, the mean gestational age was 13 ± 3 weeks, ranging from 7 ± 2 to 17 ± 1, when based on the date of the last menstrual period or first-trimester ultrasound measurements taken between 11 and 13 weeks. Local ethics committee approval was obtained for this study.

Follow-up of pregnancy for allo-immunization antenatal prophylaxis was performed in all three centers, according to the recommendations of the French College of Obstetricians and Gynecologists, which include at least anti-D antibody testing at months 1, 6, 8, and upon delivery. If a sensitizing event occurred during pregnancy (i.e. amniocentesis, abdominal trauma, or uterine bleeding), 200 μg of anti-D immunoglobulin (Rhophylac®) were given, and feto-maternal hemorrhage was evaluated using the Kleihauer-Betke test. Depending on the results, an additional immunoglobulin injection could be administrated. Since the guidelines were not yet published at the time of this study, 300 μg of anti-D immunoglobulin (Rhophylac®) were not systematically given at 28–32 weeks for RAADP. Newborn blood group was determined at birth, and anti-D immunoglobulin was administered to RhD-positive infants.

2.2. Non-invasive fetal RhD genotyping

Peripheral blood samples were collected from RhD-negative pregnant women, with no particular medical history, attending the antenatal clinic at three public teaching hospitals. All women gave informed consent. Researchers were blinded to the father's RhD status.

A total of 5 ml of blood was collected into Vacutainer SST® tubes (Becton Dickinson, Meylan, France) at admission. Immediately after clotting, serum was obtained by centrifugation for 10 min at $3000 \times g$ and sent to the laboratory within the following 2 days. Upon receipt of the samples, all sera were aliquoted and stored at −30 °C until further processing, if the assay was not performed on the same day.

The procedure has been previously described, except for some minor modifications [10]. Briefly, as tracer for DNA extraction efficiency and detection of inhibitory effect of DNA extract a low amount (250 pg) of mouse DNA (Sigma, Grenoble, France) was added to each patient's sample (1 ml of serum) immediately prior to DNA extraction. DNA was extracted by the Total Nucleic Acid LV extraction procedure on the MagNaPure Compact instrument (Roche Diagnostics) and eluted in 50 μl of elution buffer, 10 μl of which were used per polymerase chain reaction (PCR). Amplification was carried out in a LightCycler® v2.0 instrument (Roche Diagnostics, Meylan, France). PCR reactions were set up in a final volume of 20 μl using the Fast DNA Master Hybridization Probes Kit (Roche Diagnostics, Meylan, France) with 0.5 μM of each primer targeted at exon 10 of *RHD* gene, 0.25 μM of each probe (Sigma Aldrich, France), 1.25 units of uracil DNA glycosylase (UDG) (Biolabs, Saint-Quentin en Yvelines, France), and 4.75 mM of magnesium chloride. Following an initial 1-min incubation at 50 °C, a first denaturation step of 8 min at 95 °C was followed by an amplification performed for 50 cycles of denaturation (95 °C, 10 s, ramping rate 20 °C/s), annealing (56 °C, 10 s, ramping rate 20 °C/s), and extension (72 °C, 20 s, ramping rate 2 °C/s).

Each sample was treated twice for DNA extraction, and the RhD assay was performed in duplicate on each DNA extract. Results were considered definitive only when the four PCR reactions were concordant. During each run, known sera obtained from patients carrying an RhD-positive or RhD-negative fetus were used as positive and negative controls.

As the findings regarding fetal RhD status were not disclosed to the mother or the medical staff, they had no impact on the pregnancy. The results were compared with newborns' RhD serology when available.

2.3. Cost evaluation

For all patients, direct medical costs associated with patient management in relation to their RhD-negative status were calculated, all costs being reimbursed by the French National Health Service. This includes the costs of biological tests (screening for anti-D antibodies, Kleihauer-Betke testing, and blood group determination), the drug (Rhophylac®), as well as the associated medical or nursing procedures (blood sampling and anti-D immunoglobulin administration) during pregnancy and delivery (Table 1).

The cost impact of RAADP at 28–32 weeks and fetal RhD genotyping was evaluated based on the following hypotheses:

- Fetal RhD genotyping performed during the 3rd trimester in order to offer RAADP only to women carrying an RhD-positive fetus. Newborn serology was checked either systematically at birth (3TA) or only in the case that RhD genotype was determined to be negative (3TB).
- Fetal RhD genotyping performed during the 1st trimester in order to detect women not at-risk (carrying an RhD-negative fetus),

Table 1
Analysis input variables.

Product and medical act	Price €
Rhophylac® 200 μg	61.57[a]
Rhophylac® 300 μg	85.16
Irregular antibody screening	13.5
Kleihauer-Betke test	18.9
Blood group determination	20.3
Nursing procedure (veinopuncture or immunoglobulin injection)	4.5

[a] Anti-D immunoglobulin Natead® 100 μg having recently been replaced by anti-D immunoglobulin Rophylac® 200 μg in France, the total cost per patient was calculated with the latter.