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Original article

Relevance and costs of *RHD* genotyping in women with a weak D phenotype

Intérêts et coûts du génotypage du gène RHD chez les femmes enceintes présentant un affaiblissement antigénique RH1

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Abstract

Objectives. – For pregnant women, the serologic test results of D antigen will determine the frequency of RBC antibody detection as well as the indication for RhIG prophylaxis. *RHD* genotyping is the only method that may provide clear guidance on prophylaxis for women with a weak D phenotype. This analysis evaluated the economical implications of using *RHD* genotyping to guide RhIG prophylaxis among pregnant women with a serological weak D phenotype.

Methods. – We compared the costs of 2 strategies in a cohort of 273 women with weak D phenotype. In the first strategy, we did not perform genotyping and all women with weak D phenotypes were treated as if they were D–, thus considered to be a risk of RhD alloimmunization. These women all received the prophylactic follow up. In the second strategy, *RHD* genotyping was performed on all women with a serologic weak D phenotype. Then, the follow-up will be determined by phenotype deduced from genotype.

Results. – On the studied cohort, the additional expense occurred by genotyping is 26,536 €. *RHD* Genotyping has highlighted 162 weak D Type 1, 2, 3, that could safely be managed as D+ and 111 partial D to consider as D–. By comparing the 2 strategies, the savings generated by genotyping the patients of our cohort are € 12,046 for the follow up of one pregnancy. Knowing that in France, a woman has on average 2 pregnancies and that the genotyping is carried out only once, the savings generated for the following pregnancies would be € 38,581.

Conclusions. – Performing *RHD* genotyping for pregnant women with a weak D phenotype enables to clearly identify weak D type 1, 2 or 3 from the other variants at risk of alloimmunization. This analysis generates savings in terms of follow-up schedule of pregnant women and RhIG prophylaxis. It also allows saving of D– products for patient with a weak D type 1, 2 or 3 in case of a transfusion need.

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Keywords: *RHD* genotyping; RhIG prophylaxis; Weak D phenotype; Weak D; Partial D

Résumé

Objectif. – Chez la femme enceinte, le statut RH : 1 ou RH : –1 détermine le calendrier de suivi immuno-hématologique ainsi que l'indication d'une prophylaxie anti-D. Seul le génotypage permet de déterminer ce statut en cas d'affaiblissement antigénique RH1. L'objectif de notre travail est d'évaluer l'intérêt et l'impact financier du génotypage *RHD* des patientes présentant un antigène RH1 affaibli dans le cadre du suivi immuno-hématologique obstétrical.

Méthodes. – Nous avons comparé les coûts de 2 stratégies sur une cohorte de 273 patientes. Dans la première stratégie sans génotypage *RHD* : toutes les patientes avec affaiblissement RH1 en sérologie sont considérées comme RH : –1 et bénéficient du suivi complet incluant RAI supplémentaires, génotypage fœtal et injection d'IgG anti-D. Dans la 2^e stratégie, le génotypage *RHD* (facturé 97,2 €) est réalisé chez toutes les patientes présentant un affaiblissement RH1, puis le suivi est réalisé en fonction du statut RH1 ou RH–1 déduit du génotype.

Résultats. – Sur la cohorte étudiée, le surcoût généré par le génotypage *RHD* est de 26 536 €. Il a permis d'identifier 162 D faible type 1, 2 ou 3 à considérer RH : 1 et 111 autres variants à considérer comme RH : –1. En comparant les 2 stratégies, l'économie générée grâce au génotypage réalisé sur les patientes de notre cohorte est de 12 046 € pour le suivi d'une grossesse. Sachant qu'en France, une femme a en moyenne 2 grossesses et que le génotypage est réalisé une seule fois, l'économie générée pour les grossesses suivantes serait de 38 581 € par grossesse.

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Conclusion. – La réalisation du génotypage *RHD* chez les femmes enceintes présentant un affaiblissement RH1 permet de d'identifier clairement les D faibles 1, 2 et 3 des autres variants à risque d'allo-immunisation. Cette analyse n'entraîne pas de surcoût et génère même des économies significatives en terme de suivi immuno-hématologique et de prophylaxie anti-D, puisque les D faibles type 1, 2 ou 3 identifiés sont considérés comme D+. De plus, elle permet l'économie de CGR D négatif chez ces patientes D faibles en cas de besoin transfusionnel.

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Mots clés : Génotypage du gène *RHD* ; Prophylaxie anti-D ; Phénotype D affaibli ; D faible ; D partiel

1. Introduction

Among the compulsory tests for pregnant women is ABO-D typing at the start of pregnancy. D antigen phenotype determines the appointment schedule for immuno-hematological monitoring of the patient. However, test laboratories are confronted with a “weak D antigen” in around 0.2 to 1% of typing tests [1]. In fact, the RH blood group system is the most polymorphous of the blood group systems. Therefore there is a considerable number of variants of the *RHD* gene, leading to D antigens known as “weak variants” and “partial variants”, at the origin of a weak D phenotype [2]. *RHD* gene genotyping is used to explore serological reactions ambiguities of the D antigen and to specifically identify the incriminated antigen [3]. *RHD* genotyping is used to identify weak variants (type 1, 2, or 3): the most common in the European population. Weak D type 1, 2 or 3 are not likely to be alloimmunized, so they could be safety managed as D+ in the obstetrical and transfusion context. Other than these 3 weak variants, *RHD* genotyping can also be used to screen other weak variants or partial variants, at risk of anti-D alloimmunization, and therefore, to be considered as D– in the transfusion and obstetrical context. In this case, the patients should receive RhIG prophylaxis and immuno-hematological monitoring to avoid anti-D alloimmunization likely to compromise their obstetrical future [3,4].

Currently, ABO-D and RHK type tests are performed twice in D+ pregnant women as well as 2 RBC antibody detection tests throughout the pregnancy: one antibody detection test in the 3rd month and one in the 9th month (pre-transfusion antibody detection test) [5]. For D– women, the pregnancy monitoring timeline defined by the order of 1992 [6] includes two ABO-D and RHK type and four RBC antibody detection tests: one before the end of the 3rd month, one in the 6th, 8th and 9th month. According to the 2005 recommendations by the National College of French Gynecologists and Obstetricians [7] these D– patients should also have a routine injection of RhIG at 28 weeks amenorrhea, to prevent fetal-maternal anti-D alloimmunization. However, it is recommended to also carry out, in this context, fetal *RHD* genotyping on maternal blood (test listed since May 2017) [8], to only provide women pregnant with an D+ child with RhIG prophylaxis [8,9]. As the incidence of the *RHD* gene is 0.6 in the French population, D– women with D– fetus represent 40% of the cases [10,11]. Also, in situations with high risk of bleeding, D– women should receive “targeted prophylaxis”, in combination, when appropriate, with a Kleihauer-Betke test to detect the presence of fetal erythrocytes. Finally, after giving birth,

D– women should receive RhIG prophylaxis if the newborn is D+. In that case the dose will be adapted according to the Kleihauer-Betke test result [7].

The objective of our work is to evaluate the relevance and financial impact of *RHD* gene genotyping in women with weak D antigen as part of obstetrical immuno-hematological monitoring.

2. Methods

Over a 18-month period, we compared the costs of weak D antigen exploration by *RHD* genotyping in women of child-bearing age with a weak D phenotype, to the costs incurred by a precautionary D– result. Antigen weakness is detected by serologic ambiguity defined by a reaction during the filtration technique of $\leq 2+$, or a mixed field in a non-transfused patient, and/or a discrepancies in the results between two techniques used. However, there is a lack of consensus on the exact definition of ambiguity and on the exploration methods [1].

The costs of monitoring D+ and D– patients were calculated, including RBC antibody detection test, *RHD* fetal genotyping on maternal blood and RhIG prophylaxis in D– women.

In our study, due to the rareness of *RHD* variant gene, we considered women with a weak D phenotype as heterozygous: they present a variant *RHD* allele and a delete *RHD* gene in *trans*. This means two consequences for the study:

- the realization of a fetal *RHD* genotype on these women will amplify the mother's *RHD* gene and results for the fetus will be uninterpretable. The laboratory will then conservatively consider the fetus as D+;
- statistically, these women will be twice as likely to have a D– child (50% chance to transmit a weak *RHD* allele, 50% chance a delete *RHD* allele). Knowing that a D– women has 40% chances to have a D– fetus [10,11], these patients will have 20% chances to have a D– fetus.

These costs are shown in Table 1. These costs do not include ABOD group and RHK phenotype, tests carried out regardless of D status.

We defined 2 strategies (Table 3) in order to identify the savings that could be made by identification of a weak D type 1, 2 and 3 by *RHD* genotyping. The first strategy without *RHD* genotyping: all women with a serological weak D phenotype were treated as D– and thus considered to be at risk of RhD alloimmunization. In the 2nd strategy, *RHD* genotyping is carried out in women with a weak D antigen. Those found by genotyping

Table 1
Determination of follow-up costs according to the D status of the mother and the newborn.

Testing during pregnancy	Cotation in B ^a or price	Status D+/D– of mother and newborn		
		Cost 1	Cost 2	Cost 3
		Mother D+	Mother with a weak D considered as D– so with a fetal RHD genotyping ininterpretable (performed once time)	
		Newborn D+ (find at the birth)	Newborn D– (find at the birth)	
Antibody screen: 3rd month	B39	€ 10.53	€ 10.53	€ 10.53
Fetal RHD genotyping if mother is D– or considered as D–	B260		€ 70.2	€ 70.2
Antibody screen: 6th month	B39		€ 10.53	€ 10.53
Antibody screen: 8th month	B39		€ 10.53	€ 10.53
Antibody screen 9th month	B39	€ 10.53	€ 10.53	€ 10.53
RhIG 300 µg at 28th week of amenorrhea if mother is D– and fetus D+ or indetermined	83.78 €		€ 83.78	€ 83.78
Kleihauer test if newborn is D+ at the birth	B70		€ 18.9	
RhIG 200 µg at the delivery if newborn is D+ at the birth	60.36 €		€ 60.36	
Total costs		€ 21.06	€ 275.36	€ 196.10

^a Price of B = 0.27 €.

to have alleles encoding a weak D type 1,2 or 3 phenotype were managed as D+ (cost1 Table 1). Women with other variants are considered to be D– [3] (cost 2 and 3 Table 1).

The *RHD* genotyping tests were carried out at the Marseilles-Baille site, using the IMMUCOR[®] RHD BeadChip Bioarray. The method uses eMAP[®] (Elongation mediated Multiplexed Analysis of Polymorphisms) technology capable of studying 35 polymorphisms and of identifying over 65 variants. The cost of the RHD BeadChip kit per patient is around 80 Euros (not including DNA extraction). The non-listed test is invoiced at the price of € 97.20 by the EFS (HN360).

3. Results

Over the 18-month period, 273 patients of childbearing age (age 15 to 50) with a weak D antigen, were tested by *RHD* genotyping. These patients come from 4 EFS regions: Rhône-Alpes Auvergne, Pyrénées-Méditerranée, Alpes-Méditerranée, and Réunion Island. The list of the variants identified and the related RHCE phenotypes can be found in Table 2.

In the strategy without *RHD* genotyping, all women with a weak D antigen are considered D– and receive comprehensive monitoring, including additional antibody detection test, RHD fetal genotyping and RhIG injection. In theory, 60% of the women D– have a D+ fetus (incidence of the *RHD* gene: 0.6 in the French population) and 40% a D– fetus. In this strategy, women with a weak D are twice less likely to have a child found D– at birth (we considered these women as heterozygous genotype: *RHD* variant/delete *RHD* gene) so they have 20% chance to have a fetus D–, then 80% chance to have a fetus D+.

The strategy without genotyping costs € 70,814 in our cohort (Table 3).

In the strategy with *RHD* genotyping, all the women with a weak D phenotype have a *RHD* genotyping.

The cost overrun generated by *RHD* genotyping test is € 26 536 (273 × € 97.20). This strategy identified 162 weak D type 1, 2 or 3 (i.e. 59% of the cohort) to be considered D+, for whom savings were made in terms of antibody detection test, RhIG prophylaxis and fetal genotyping. Genotyping also identified 111 other variants to be considered D–. With regard to immuno-hematological monitoring of these patients (D+, D– with D+ child, D– with D– child), this strategy costs € 58,768 (Table 3). In total, the savings made by using *RHD* genotyping amount to € 12,046 for our cohort during pregnancy monitoring (costs of strategy without RHD genotyping minus cost of strategy with RHD genotyping for weak D).

Bearing in mind that in France, a women has 2 pregnancies on average [12] and that *RHD* genotyping is only carried out once [13] we determined the theoretical costs of a 2nd pregnancy on the scale of our cohort. This strategy, in which the *RHD* genotyping result is already known, would therefore cost only € 32,233 (cost of the strategy with *RHD* genotyping € 58,768: minus costs of *RHD* genotyping test: € 105,746 + € 10,789), therefore savings of € 38,581 for a 2nd pregnancy (cost strategy without *RHD* genotyping minus € 32,233) (Table 3).

4. Discussion

Prevention of anti-D alloimmunization by using RhIG significantly reduces the number of hemolytic diseases of the newborn [14] thus the need to establish the D status of pregnant women with certainty. This is not always the case since laboratories may come across a weak D antigen, which is considered D– as a precautionary measure, without any additional tests. This

Table 2
Distribution of the main variants detected.

D variant phenotype	RHD variant (ISBT nomenclature)	Rh C/c and E/e phenotype	Number of patients
To consider as D+			
Weak D type 1	<i>RHD*01W.1</i>	Ccee CCee ccee	81 1 3
Weak D type 2	<i>RHD*01W.2</i>	ccEe	61
Weak D type 3	<i>RHD*01W.3</i>	Ccee CCee	15 1
To consider as D–			
Weak D type 5	<i>RHD*01W.05</i>	ccEe	3
Weak D type 4.0 or 4.3	<i>RHD*09.03.01 or RHD*09.05</i>	Ccee ccee	6 27
DAR	<i>RHD*09.01</i>	Ccee ccee	9 43
DAU4 or DV type 5	<i>RHD*10.04 or RHD*05.05</i>	ccee	4
DAU5 or DV type 1	<i>RHD*10.05 or RHD*05.01</i>	ccee	5
DIIIb	<i>RHD*03.02</i>	ccEe	1
DFR1 or DFR3	<i>RHD*17.01 or RHD*17.03</i>	Ccee	1
Weak D type 18	<i>RHD*01.18</i>	Ccee	2
Weak D type 25	<i>RHD*01.25</i>	Ccee	1
Weak D type 29	<i>RHD*01.29</i>	ccee	1
Weak D type 51	<i>RHD*01.51</i>	Ccee	1
Weak D type 61	<i>RHD*01.61</i>	Ccee	1
Other		Ccee ccee	2 4
Total			273

Table 3
Cost comparison of the two strategies.

Strategy without <i>RHD</i> genotyping		
273 women with a weak D phenotype considered as D–		
80% of cost 2: 218 women	218 × cost 2	€ 60,028.5
20% of cost 3: 55 women	55 × cost 3	€ 10,785.5
Total costs		€ 70,814
Strategy with <i>RHD</i> genotyping for weak D		
273 women with a weak D phenotype		
162 women with <i>RHD</i> weak type 1, 2, 3 considered as D+ after genotyping		
	162 × genotyping costs (€ 97.2) ^a	€ 15,746
	162 × cost 1	€ 3412
111 women with other variant considered as D– after genotyping		
	111 × genotyping cost ^a	€ 10,789
80% of cost 2: 89 women	89 × cost 2	€ 24,507
20% of cost 3: 22 women	22 × cost 3	€ 4314
Total costs		€ 58,768

^a Cost only applicable for the first pregnancy.

means these D– women are considered to be at risk of alloimmunization and eligible to receive immunoprophylaxis in excess. Our analysis compared the costs of 2 strategies: the first without *RHD* genotyping: all women with a weak D phenotype are considered as D–, the second strategy: with *RHD* genotyping for weak D phenotype, which highlights weak variants of type 1, 2 or 3 which could be safely managed as D+. These patients to be considered as D+ do not therefore require additional prophylaxis or RBC antibody detection test or fetal genotyping. Even if adding *RHD* genotyping to exploration into serologic ambiguity generates an additional cost of 97.20 € per pregnant woman, it reduces the cost of further blood tests and RhIG injections. In our cohort of 273 patients, € 12,046 savings are made for one pregnancy. Also, we calculated the costs of monitoring D– women on the basis of the minimum required tests, without taking account of the additional costs incurred by targeted RhIG injections and Kleihauer-Betke tests or flow cytometry carried out in certain high-risk situations [7]. These tests make taking care of D– women even more expensive. In addition to saving costs, reducing RhIG prophylaxis removes an ethical problem of using a product of human origin in limited quantities for patients who do not require it.

In addition, the strategy without genotyping cannot identify women with a weak D type 1, 2 or 3. Nevertheless, women with a weak D phenotype considered as D– would benefit from fetal *RHD* genotyping. The presence of *RHD* gene exons from the mother will make the test not readable and in these cases, the fetus will be conservatively considered a D+ and women would benefit from prophylaxis.

Therefore, the calculated cost savings of € 12,046 for our cohort is minimized, especially as the savings were calculated on the basis of a single pregnancy.

Bearing in mind that in France a woman has 2 pregnancies on average [12], and that *RHD* genotyping is only carried out once, this strategy is all the more economic, the more pregnancies a patient has.

These results are consistent with the cost studies conducted in other countries [15].

Also, our analysis demonstrates the benefit of genotyping weak type 1, 2 or 3 variants and of avoiding RhIG injections, a product of human origin, when not necessary. Finally, we did not include the impact on D– red blood cell-sparing in the event a transfusion is required in these patients eligible to receive D+ transfusion.

There is a lack of consensus on the definition of weak D antigen, and especially on the intensity threshold to be reached to allow genotyping. Conventionally, the threshold is set at 2+, however, certain partial variants at risk of alloimmunization show an intensity of $\geq 2+$ [16,17], whereas it is important to identify them to prevent alloimmunization likely to compromise the obstetrical future of the patients. In this case, the alert point for genotyping in the aim of detecting variants at risk of alloimmunization is a discrepancy between 2 techniques, or clearer weakening with the anti-CDE reagent if it is available [18].

In the absence of an appeal point, patients with partial D presenting positive reaction, unambiguous reactions during phenotyping, and who are at risk of anti-D alloimmunization and require D– monitoring, are not detected. Identifying these patients with D+ phenotype but partial D antigen, would involve *RHD* genotyping in all D+ women [15].

Basing the decision to carry out *RHD* genotyping or not on the RHCE phenotype result or on the patient's geographical origin is delicate. In effect, even if most weak D types 1, 2 or 3 have a Ccee or ccEe phenotype, a high number of variants to be considered D– in our cohort (10%, 27 patients) also have these phenotypes. Geographical origin, which could also be an indicator, is sometimes complicated and not always reliable, due to the diversity of the populations.

Although *RHD* genotyping is available, this test and its price remain non-listed.

This study has demonstrated the relevance of *RHD* genotyping in the management of prevention of anti-D alloimmunization

in pregnant women with a weak D. This genotyping generate savings, especially when women have two or more pregnancies.

Disclosure of interest

The authors declare that they have no competing interest.

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