Risk assessment for RH-1 pregnant women when a negative non-invasive fetal *RHD* genotyping result is not confirmed

Da Silva N.R. (1), Deray M. (1), Huguet-Jacquot S. (1), Toly-Ndour C. (1), Maisonneuve E. (2), Oudin O. (1), Saulet P. (1), Cortey A. (3), Carbonne B (4), J.M. Jouannic (2), Brossard Y. (1) and A. Mailloux (1)

(1)Unité Fonctionnelle d'expertise en Immuno-Hémobiologie Périnatel, Centre National de Référence en Hémobiologie Périnatale (CNRHP), Pôle de Biologie Médicale et Pathologie, Hôpital St Antoine, GHU Est Parisien, AP-HP, Paris, France.

(2) Département de Gynecologie- Obstétrique et de Médecine fœtale, Pôle Périnatalité, Hôpital Trousseau, GHU Est Parisien, AP-HP, Paris, France.

(3)Unité fonctionnelle clinique (soins des incompatibilités foeto-maternelles et ictère néonatal), Centre National de Référence en Hémobiologie Périnatale (CNRHP), Service de Medecine Fœtale, Pôle Périnatalité, Hôpital Trousseau, pôle périnatalité, GHU Est Parisien, AP-HP, Paris, France.

(4)Département de Gynécologie Obstétrique, Centre Hospitalier Princesse Grace, Monaco, Monaco.

Background

Feto-maternal blood group incompatibility is common and may result in hemolytic disease of the fetus and newborn (HDFN). This disease is characterised by anemia and hyperbilirubinemia which may lead to fetal hydrops, kernicterus or death. Three antibodies are associated with severe fetal disease: anti-RH1 (D), anti-RH4 (c) and anti-KEL1 (Kell). Although the widespread use of anti-D immunoglobulins has resulted in a major reduction in the incidence of RhD immunisation in pregnancy, the maternal anti-RH1 allo-immunisation is the most common cause of feto-maternal red blood cells incompatibility resulting in HDFN. Many laboratories wordwide provide non-invasive fetal *RHD* genotyping as a routine service to help the practitioners to greatly improve the accuracy follow-up in pregnant women RH:-1 allo-immunised or to restrict the use of anti-D immunoglobulins only to RH:-1 women bearing a *RHD* positif fetus.

The aim of this presentation is to evaluate the risk assessment for RH:-1 pregnant women when a negative non-invasive foetal *RHD* genotyping result is not confirmed when the presence of free fetal DNA is not tested.

Methods

Non invasive fetal *RHD* genotyping

Blood collected on EDTA and **received before** 72h

↓ ***Centrifugation***

6 x 1 ml plasma (<- 20° C)

Extraction

EasyMag Biomerieux

Plasma volume 800 µl
Elution volume 70 µl

or

Kit QIAamp MinElute®Virus

Plasma volume 500 µl
Elution volume 40 µl

Amplification

ABI 7300

or

LightCycler®

DNA volume 5µl
PCR volume 10µl

Non-invasive fetal *RHD* genotyping were done using Free DNA fetal kit RHD® CEIVD from *Jacques Boy* which allows amplification of *RHD* exon 10, 7 and 5 from peripheral maternal blood. Furthermore, for RH:-1 pregnant women carrying non-functional *RHDpsi* allele, amplification of *RHD* exon 6 was used.

The 1st blood samples are between 11-34GW

Non-invasive fetal *RHD* genotyping results from 1477 RH:-1 pregnant women were compared with or without confirming negative fetal *RHD* results on a second sample. Fetal genotype results were compared with the fetal *RHD* genotype determined on amniotic cell or the phenotype of the red blood cells of the babies at birth.

Results over six years

All negative results are confirmed on a second sample

Sensibility : 98,4%
Specificity : 95,4%
VPP : 99,6%
VPN : 100 %

	Fœtus RH1	Fœtus RH:-1	Total
Test +	1015 991 mothers with *deletion* 5 mothers with *(C)ce*^{s} 19 mothers with *Dpsi*	4 1 fetus with *Dsilent* 3 fetus with *Dpsi*	1019
Test – confirmed	**0**	427 422 mothers with *deletion* 5 mothers with *Dpsi*	427
Test undetermined	19 7 mothers with *deletion* 2 mothers with *Dpsi* 7 mother with *Dsilent* 3 mothers with *Dpartial*	12 2 mothers with *deletion* 2 mothers with *Dpsi* 3 mother with *Dsilent* 1 mothers with *(C)ce*^{s} 1 fetus with *Dpsi* 3 fetus with *(C)ce*^{s}	31
	1034	443	1477

All negative results are NOT confirmed on a second sample

Sensibility : 98,3%
Specificity : 95,3%
VPP : 99,6%
VPN : 98,5%

	Fœtus RH1	Fœtus RH:-1	Total
Test +	1010 986 mothers with deletion 5 mothers with *(C)ce*^{s} 19 mothers with *Dpsi*	4 1 fetus with *Dsilent* 3 fetus with *Dpsi*	1019
Test – confirmed	**5***	427 422 mothers with *deletion* 5 mothers with *Dpsi*	427
Test undetermined	19 7 mothers with *deletion* 2 mothers with *Dpsi* 7 mother with *Dsilent* 3 mothers with *Dpartial*	12 2 mothers with *deletion* 2 mothers with *Dpsi* 3 mother with *Dsilent* 1 mothers with (C)ce^{s} 1 fetus with *Dpsi* 3 fetus with(C)ce^{s}	31
	1034	443	1477

* Blood samples between 19-25 GW

Conclusion

When negative tests are not checked, the negative predictive value of non-invasive fetal *RHD* genotyping is only 98%. This rick is may be allowed for non anti-RH1 allo-immunised women, but it is not acceptable for anti-RH1 allo-immunised women.