Use of non invasive prenatal fetal blood group genotyping in the monitoring of allo-immunized pregnant women: experience of the French National Center For Perinatal Hemobiology (CNRHP)

Da Silva N.R. (1), Huguet-Jacquot S. (1), H. Delaby (1), Toly-Ndour C. (1), Oudin O. (1), Saulet P. (1), Mordie M. (1), Disdier S. (1), Cortey A. (2), 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)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.

Background

The French "Centre National de Référence en Hémobiologie Périnatale" (CNRHP) is dedicated to biological and clinical diagnosis and treatment of feto-maternal red blood cells incompatibilities. This disease is common and may result in hemolytic disease of the fetus and newborn (HDFN), 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). High concentration of anti-RH3 (E) can too led to HDFN during the third pregnancy trimester. Since the discovery of free fetal DNA into peripheral maternal blood, non-invasive prenatal determination of fetal *RHD* genotype on maternal blood is used in the management of pregnancies of RH:-1 (D negative) women. CNRHP provide non invasive fetal genotyping as a routine service to help the practitioners to improve the accuracy follow-up in pregnant woman anti-RH1, anti-KEL1, anti-RH4 and anti-RH3 allo-immunised.

The aim of this presentation is the review of non-invasive fetal genotypes used in the CNRHP in determing of feto-maternal RH1, KEL1, RH4 or RH3 incompatibility status in order to spare a specific antenatal monitoring.

Methods

Monitoring of allo-immunised anti-RH1 or anti-KEL1 or anti-RH4 or anti-RH3 pregnant women in France

positive antibody screened at first trimester of pregnancy

Antibody Identification
Anti-RH1, anti-KEL1, anti-RH4 or anti-RH3

Non invasive fetal genotyping from maternal blood from at first trismester of pregnancy 12GW/13GW/13GW/13GW

If fœtus negative

No specific antenatal monitoring

If fœtus positive

Antibody Titration(T) and/or Dosage (D)

Anti-RH1 D<1µg/ml Anti-KEL1 T<16	Anti-RH4 D<500 UCHP/ml Anti-RH3 D<700 UCHP/ml

Specific antenatal monitoring
-Normal ultrasonography
-Antibody titration and/or dosage
every 2 weeks, anti-RH1, RH4 dosage and titration from 18 GW
monthly, anti-KEL1 titration from 18 GW
every 4 weeks, anti-RH3 dosage and/or titration from 18GW

Anti-RH1 D≥1µg/ml Anti-KEL1 T≥16	Anti RH4 D≥ 500 UCHP/ml Anti-RH3 D≥ 700 UCHP/ml

Specific antenatal monitoring
-Weekly ultrasonography and velocimetry Dopler
-Antibody titration and/or dosage
every 2 weeks, anti-RH1, RH4 or RH3 dosage and titration from 18 GW
monthly, anti-KEL1 titration from 18 GW

UCHP=CNRHP concentration unit determined with standard anti-D

Non invasive fetal *RHD* or *KEL1* or *RHc* or *RHE* genotyping

Blood collected on EDTA and **received before 72h/48h/72h/72h**

Centrifugation

6 x 1 ml plasma (<- 20° C)

Extraction
EasyMag Biomerieux

Plasma volume 800 µl
Elution volume 70 µl

Fœtal ***RHD*** genotype are done using Free DNA fetal kit RHD® CEIVD from *Jacques Boy (amplification RHD exon5,7,10)*. In addition *RHD* exon 6 PCR in duplicate is used if patient carry *Dps*i allele

All negative results are confirmed on a second sample

Amplification
ABI 7300

or

VIA7

DNA volume 5µl
PCR volume 10µl

Fœtal ***KEL1*** genotype is done using:
-PCR-SSP in triplicate to identifie *KEL1* fetal allele
-Amplification of *ABO to* determine the maternal DNA quantity
-Amplification of a DNA tracer to validate extraction step

All results are conclued on two extractions and amplifications, and confirmed on a second sample

Extractions
Kit QIAamp MinElute®Virus

Plasma volume 500 µl
Elution volume 40 µl

Amplification
ABI 7300

DNA volume 5µl
PCR volume 10µl

Fœtal ***RHc*** **or** ***RHE*** genotype is done using:
-PCR-SSP in triplicate to identifie *RHc* or *RHE* fetal allele (*Finning et al , transfusion 2007*)
-Amplification of a DNA tracer (maize) to validate extraction step

All negative results are conclued on two extractions and amplification and confirmed on a second sample

Results over two years

Variant / Test result	*Rhd deletion*	*Rhd DPsi*	*Rhd (C)ce^s*	*Other*	Total
Fetus +	274	22	11	0	307
Fetus – non confirmed	27	1	0	0	28
Fetus undetermined	13	0	1	18	32
Fetus – confirmed -	60	4	0	0	64
Total	374	27	12	18	431

Sensibility : 97,3%
Specificity : 88,1%
VPN : 99,6%

-32% of patients have a dosage ≥ 1µg/ml
-17% of patients have a dosage unknown

-59,5% of the 1st sample are between 11-18GW

15% of pregnancies are compatible for **anti-RH1** allo-immunised women

Fetus - non confirmed	21
Fetus + confirmed	35
Fetus + non confirmed	16
Fetus undetermined	5
Fetus - confirmed	51
Total	128

Sensibility : 96%
Specificity : 69,2%
VPN : 100 %

-47% of patients have a titration ≥ 16
-24,3% of patients have a titration unknown
-63% of the 1st sample are between 12-18GW

40% of pregnancies are compatible for **anti-KEL1** allo-immunised women

Fetus - non confirmed	10
Fetus +	149
Fetus undetermined	1
Fetus - confirmed	30
Total	190

Sensibility : 100%
Specificity : 100%
VPN : 100 %

-8% of patients have a dosage ≥ 500UCHP or a titration ≥ 4.
-15% of patients have a dosage or a titration unknown
-39,6% of the 1st sample are between 12 and 18 GW

15,8% of pregnancies are compatible for **anti-RH4** allo-immunised women

Fetus - non confirmed	16
Fetus +	66
Fetus undetermined	1
Fetus - confirmed	47
Total	130

Sensibility : 100%
Specificity : 100%
VPN : 100 %

-10,8% of patients have a dosage ≥ 700UCHP or a titration≥ 8.
-15% of patients have a dosage or a titration unknown
-36% of the 1st sample are between 12 and 18 GW

36,2 % of pregnancies are compatible for **anti-RH3** allo-immunised women

Conclusion

Non invasive fetal genotyping is a powerful tool to diagnose a feto-maternal red blood cells incompatibility and allows to legitimize a costly and heavy specific antenatal monitoring only to pregnant women carrying incompatible fetus.