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

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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 trimestrer. 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, ant-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

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

If fœtus negative

No specific antenatal monitoring

If fœtus positive

Anti-RH1 D<5IU/ml Anti-KEL1 T<32	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≥32	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 or Kit QIAamp MinElute®Virus

Plasma volume 800 µl
Elution volume 70 µl

Plasma volume 500 µl
Elution volume 40 µl

Amplification
ABI 7300 or *LightCycler®*

DNA volume 5µl
PCR volume 10µl

Fœtal ***RHD*** genotype are done using Free DANA fetal kit RHD® CEIVD from *Jacques Boy*. In addition *RHD* exon 6 PCR in duplicate is used if patient carry *Dpsi* allele

All negative results are confirmed on a second sample

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 *ABO to* determine the maternal DNA quantity
-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

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

Results

over six years

Variant / Test result	*Rhd deletion*	*Rhd DPsi*	*Rhd (C)ce^s*	*Other silent*	Total
Fetus +	957	65	25	0	1047
Fetus – confirmed +	1	0	0	0	1
Fetus – non confirmed	26	4	0	0	30
Fetus undetermined	13	7	1	20	41
Fetus – confirmed -	194	9	0	0	203
Total	1191	85	26	20	1322

Sensibility : 98,4%
Specificity : 95,3%
VPN : 100 %

-42% of patients have a dosage ≥ 5IU/ml
-56% of the 1st sample are between 11-18GW

20% of pregnancies are compatible for anti-RH1 allo-immunised women

Fetus - non confirmed	21
Fetus + confirmed	85
Fetus + non confirmed	32
Fetus undetermined	12
Fetus - confirmed	122
Total	272

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

-78% of patients have a titration ≥ 32
-62% of the 1st sample are between 12-18GW

47% of pregnancies are compatible for anti-KEL1 allo-immunised women

over one years

	RHc
Fetus +	55
Fetus - confirmed	13
Total	68

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

-24% of patients have a dosage ≥ 500UCHP or a titration ≥ 4.
-49% of the 1st sample are between 12 and 18 GW

20% of pregnancies are compatible for anti-RH4 allo-immunised women

	RHE
Fetus +	15
Fetus - confirmed	11
Total	26

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

-15% of patients have a dosage ≥ 700UCHP or a titration≥ 8.
-54% of the 1st sample are between 12 and 18 GW

Specific antenatal monitoring

42% 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.