Non invasive prenatal fetal blood group genotyping in the monitoring of allo-immunized anti-RH4 pregnant women: experience of the French National Center For Perinatal Hemobiology (CNRHP)

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Background

Maternal-feto blood group incompatibility is common and may result in hemolytic disease of the fetus and newborn (HDFN). This disease is caracterized by anemia and hyperbilirubinemia which may lead to fetal hydrops, kernicterus or death. Three antibodies are associated with severe fetal disease: anti-RH1, anti-RH4 and anti-KEL1. Although the widespread use of RhD immune globulin 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. 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 and KEL1 allo-immunised but this assistance are not yet provided to monitoring pregnant woman RH4 allo-immunised. Among the 300 patients/years followed by CNRHP, about 100 have severe immunisation (RH4 antibody dosage higher than 500UCHP/ml) leading to specific antenatal monitoring if partner is RH4.

The aim of this presentation is the evaluation of non invasive prenatal fetal genotyping to guide the follow-up of allo-immunised anti-RH4 pregnant women.

Methods

Monitoring of allo-immunised anti-RH4 pregnant women in France

positive antibody screen at first trismester of pregnancy

Antibody Identification = Anti-RH4

Non invasive fetal *RHc* genotyping from maternal blood from 13 GW

Antibody titration and/or dosage

If fœtus *RHc* negative for 2 samples

If fœtus *RHc* positive

No specific antenatal monitoring

Anti-RH4 titer <4 and/or <500UCHP

Anti-RH4 titer ≥4 and/or ≥500UCHP

Specific antenatal monitoring
- Normal ultrasonography
-Every two weeks, anti-RH4 titration and dosage from 20 GW

Specific antenatal monitoring
-Weekly ultrasonography and velocimetry Dopler
-Every two weeks, anti-RH4 titration and dosage from 20 GW

Non invasive fetal *RHc* genotyping

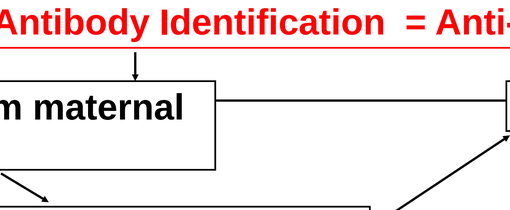
1-Blood samples of patient at least 13 GW

3 x 5 ml of blood
collected on EDTA and received before 72h

↓ *Centrifugation*

6 x 1 ml plasma
(<- 20° C)

2-Manual extractions

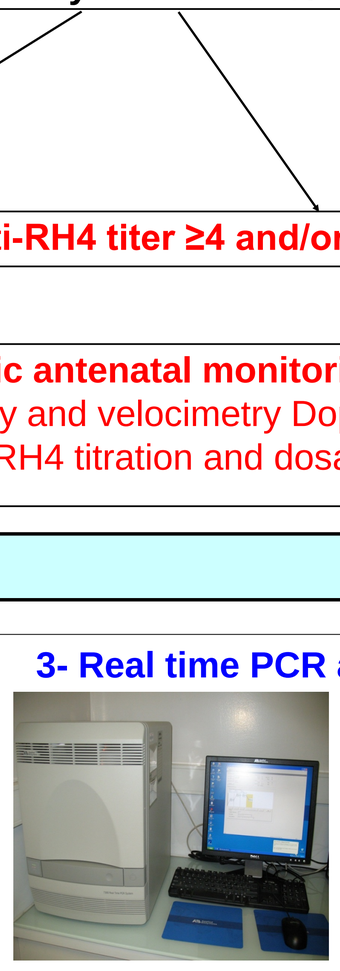


(QIAGEN)

Kit QIAamp MinElute®Virus Vaccum Kit

Plasma volume 500 µl
Elution volume 40 µl

3- Real time PCR amplifications



-PCR-ASP in triplicate
to identifie *RHc* 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

ABI7300

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

Results over one years

68 allo-immunised anti-RH4 women had non invasive fetal *RHc* genotyping

- 24% of the pregnant allo-immunised anti-RH4 women genotyped had an antibody with respectively a dosage or a titer higher than 500UCHP or ½. No dosage or titer informations were available for 2 patients.
- 49% of blood samples received were collected between 12 and 20 GW
- RH2 and RH4 father phenotype were avaliable for less than 25% of pregnancy

	RHc
Fœtus +	55
Fœtus - confirmed	13
Total	68

Specific antenatal monitoring

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

For 20% of the allo-immunised women, the pregnancy was compatible.

Conclusion

Non invasive *RHc* 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 with every two weeks an anti-RH4 dosage and titration and weekly a search for signs of fetal anemia (Velocimetry Doppler).