Non invasive prenatal fetal blood group genotyping in the monitoring of allo-immunized anti-KEL1 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 caracterised 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-RH1 immunoglobulin has resulted in a major reduction in the incidence of anti-RH1 immunization in pregnancy, the maternal anti-RH1 allo-immunization is the most common cause of feto-maternal red blood cells incompatibility resulting in HDFN. Many laboratories worldwide provide non invasive fetal RHD genotyping as a routine service to help the practitioners to greatly improve the accuracy follow-up in anti-RH1 allo-immunized pregnant women .

The aim of this presentation is the evaluation of non invasive prenatal fetal genotyping to guide the follow-up of anti-KEL1 allo-immunized pregnant women. This antibody is involved in severe antenatal hemolytic disease.

Methods

Monitoring of allo-immunized anti-KEL1 pregnant women in France

positive antibody screen at first trimester of pregnancy

Antibody Identification = Anti-KEL1

Non invasive fetal *KEL1* genotyping from maternal blood from 13 GW

Antibody Titration

If fetus *KEL1* negative for 2 samples

No specific antenatal monitoring
Antibody screen every six weeks

If fetus *KEL1* positive for 2 samples

Anti-KEL1<1/32

Specific antenatal monitoring
- Normal ultrasound
- Monthly anti-KEL1 titration from 18 GW

Anti-KEL1≥1/32

Specific antenatal monitoring
-Weekly ultrasound and fetal middle cerebral peak systolic velocity of blood flow (MCAPSV)
-Monthly anti-KEL1 titration from 18 GW

Non invasive fetal *KEL1* genotyping

1-Blood samples of patient at least 13 GW

3 x 5 ml of blood
collected on EDTA and received before 48h

↓ *Centrifugation*

6 x 1 ml plasma
(- 20° C)

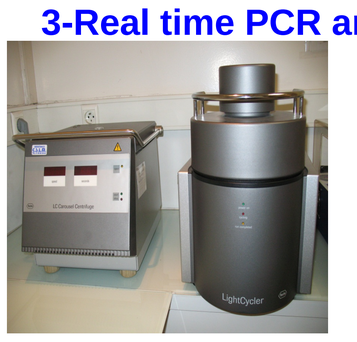
2-Manual extractions

(QIAGEN)

Kit QIAamp MinElute®Virus Vaccum Kit

Plasma volume 800 µl
Elution volume 70 µl

3-Real time PCR amplifications



-PCR-SSP in triplicate
to identify *KEL1* fetal allele
-Amplification of *ABO* determine the maternal DNA quantity
-Amplification of a DNA tracer (maize)

LightCycler®

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

Results over three years

124 allo-immunized anti-KEL1 women had non invasive fetal *KEL1* genotyping

-More than 78 % of the allo-immunized anti-KEL1 pregnant women genotyped had an antibody with a titer higher than 1/32.
-More than 62% of blood samples received were collected between 11 and 18 GW

	KEL1
Fetus - non confirmed	12
Fetus + confirmed	47
Fetus + non confirmed	13
Fetus undetermined	5
Fetus - confirmed	47
Total	124

→ **Specific antenatal monitoring**

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

For 38% of the allo-immunized women, the pregnancy was compatible.

Conclusion

Non invasive *KEL1* 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 monthly an anti-KEL1 titration and weekly a search for signs of fetal anemia (MCAPSV).