Editorial Commentary

Non-invasive fetal *RHD* genotyping

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From analysis of a standard blood sample from a pregnant woman, it is possible to predict fetal blood groups using non-invasive prenatal testing of cell-free fetal DNA (cffDNA) (1). Knowledge of fetal blood groups is valuable for identifying incompatibility cases where the fetus has inherited a paternal gene or gene variation determining an antigen which the pregnant woman does not have herself and thus is unknown to the maternal immune system. During a pregnancy, the maternal immune system may produce antibodies against the antigen. In the form of IgG, the maternal antibodies can be transferred across the placenta into the fetal blood circulation where the antibodies can facilitate the destruction of fetal red blood cells, leading to hemolytic disease of the fetus and newborn (HDFN) (2).

For RhD negative pregnant women, fetal *RHD* genotyping can help clinicians in the management of D immunized women, securing diagnosis and timely treatment. For non-immunized RhD negative pregnant women, Rh prophylaxis is used to hinder the women in becoming immunized (2). Fetal *RHD* genotyping can guide the targeted use of Rh prophylaxis, so that only those RhD negative pregnant women who carry an RhD positive fetus are administered antenatal prophylaxis (3). This strategy adopts a rational use of often limited anti-D immunoglobulin and avoids unnecessary treatment of women carrying an RhD negative fetus (4). It is an important objective to avoid unnecessary treatment of pregnant women, thus avoiding unnecessary exposure of anti-D and the potential risk of infection, although highly theoretical, associated with the exposure to a blood-derived product.

Since the first fetal *RHD* genotyping assays were introduced around 20 years ago, several countries worldwide have implemented a service for D immunized women (5,6), and in several European countries, fetal *RHD* genotyping is now implemented as an antenatal screening assay to guide targeted prophylaxis on a national basis (7).

cffDNA is present in the maternal blood in very low concentrations, especially in early pregnancy (3). Consequently, reliable fetal *RHD* genotyping requires careful attention to each step of the method to ensure high assay sensitivity. In addition, the high polymorphism of the Rh blood group system requires careful attention in the selection of the *RHD* exons to be tested and the interpretation of the results.

In this issue, Londero *et al*. present a validation of an assay for fetal *RHD* genotyping intended for guiding targeted prophylaxis (8). Londero *et al*. use a well-validated *RHD* exon combination to amplify the fetal *RHD*. With samples from 133 pregnant RhD negative women, of which more than half were taken from the first trimester, they demonstrate 100% concordance between their assay-based prediction of the fetal RhD type and the newborn RhD type, assessed after birth by standard cord blood serology.

In addition to various validation steps, they also investigate the performance on frozen cffDNA which is shown to perform equally as well as testing the DNA directly after DNA extraction. Having the possibility to

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freeze extracted cffDNA and test it later provides valuable flexibility in a clinical setting.

They also compare manual and automated DNA extraction, implementing automated DNA extraction, which offers higher reproducibility, less hands-on time and is known to limit the risk of contamination and human error (3).

Londero *et al*. found one result which suggested a potential fetal *RHD* variant, but a correct prediction was made. It is always valuable to investigate such cases to get an understanding of the variants that exist in the population and how they may affect the outcome of the assay. However, in the routine clinical handling of cases with inconclusive results, prophylaxis can simply be offered without the need of deeper investigation into the type of variant.

Noninvasive prenatal testing of cffDNA for fetal blood grouping has become an important tool assisting in the prevention of HDFN. Fetal *RHD* genotyping represent one of the first successful clinical applications of cffDNA, and its performance in guiding targeted prophylaxis has been documented extensively in the literature, underlining its capacity for clinical use (7). The study from Londero *et al*. adds further evidence to the reliability of fetal *RHD* genotyping.

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