Abstract

In 1997, Prof. Dennis Lo discovered the presence of cell-free fetal DNA (cffDNA) in the maternal plasma and serum of pregnant women. This finding started the development of new non-invasive prenatal diagnosis methods, which are currently in the forefront of the advanced care of mother and fetus. Non-invasive genetic tests based on the detection of paternally inherited alleles, including determination of fetal sex in cases at risk of X-linked disorders or congenital adrenal hyperplasia and *RHD* or *RHCE* genotyping in alloimunized pregnancies, were quickly introduced into routine practice.

This thesis focuses on the basic characteristics of cffDNA and fetal cells in maternal circulation and its usage for non-invasive prenatal diagnosis, especially in cases of placental insufficiency related complications, e.g. preeclampsia and IUGR. This severe disorder is characterized by placental dysfunction with an abnormal invasion of trophoblasts and a defect in the transformation of maternal spiral arteries, leading to placental ischemia followed by increased apoptosis of trophoblast associated with an elevated concentration of cell-free nucleic acids in maternal circulation. Until recently, cffDNA quantification studies were mostly done using amplification of *SRY* or *DYS-14* genes localized on chromosome Y, and were thus restricted to pregnancies with a male fetus. For this reason, researchers were interested in finding a universal fetal marker that would enable the detection and quantification of fetal DNA in maternal circulation in all pregnant women regardless of fetal sex. Recent studies have suggested the possibility of using a difference in the methylation of the promoter region of the *RASSF1A* tumor suppressor gene between the mother and fetus.

In our study, we tested the feasibility of this universal fetal marker and quantified fetal (*SRY*, *DYS-14*, hypermethylated *RASSF1A*) and total (*GLO* gene, *RASSF1A*) cffDNA in preeclampsia with or without IUGR during the onset of placental insufficiency. In addition to a retrospective study, we prospectively screened women at risk of placental insufficiency throughout gestation and tried to find out if quantification of cell-free nucleic acids in maternal circulation could be useful as a non-invasive screening method for the prediction of later onset severe pregnancy complications associated with placental insufficiency.

Increased levels of cell-free DNA were detected in pregnancies with preeclampsia with or without IUGR relative to controls (*RASSF1A*, $p < 0.001$; *SRY*, $p = 0.009$; *GLO*, $p < 0.001$). *RASSF1A*, *SRY*, and *GLO* achieved 93%, 94% and 92% accuracy for differentiation between a normal pregnancy and preeclampsia with or without IUGR. Among 14 patients at risk, 8 pregnancies developed placental insufficiency related complications. Concerning all three markers, an elevation of cell-free DNA was demonstrated in 5 out of 8 patients at the earliest 26 weeks before the onset of symptoms. These data indicate that cell-free DNA concentrations can be significantly elevated in the plasma of patients who later developed

placental insufficiency related complications. However, this is highly individual, and not a rule for all cases, and probably depends on the actual occurrence of excessive placental trophoblast apoptosis.