USE OF CELL-FREE NUCLEIC ACIDS IN MATERNAL BLOOD FOR NON-INVASIVE PRENATAL DIAGNOSIS

A. Bustamante-Aragones
Department of Genetics. Fundacion Jimenez Diaz, Madrid, Spain

Several noninvasive tests, such as ultrasound and serum screening, may be performed on both mothers and fetuses in order to monitor pregnancy outcome. Conventional prenatal diagnosis (PD) is currently available to women whose pregnancies are at risk for genetic disorders in the fetus. However, sample collection for PD is done by invasive procedures which carry a risk of miscarriage of around 1%. Owing to this, the last decade has seen great developments in noninvasive prenatal diagnosis (NIPD).

NIPD is currently defined as the study of fetal genetic material circulating in maternal peripheral blood. NIPD was initially based on the separation and study of fetal cells in maternal blood. However the substantial progress in the field was made when circulating cell-free fetal DNA (ccffDNA) in maternal blood was discovered. The existence of cell-free DNA in the blood was firstly described in the late 1940s in cancer patients. Based on this finding and the fact that placental tissue is also an invasive element, Lo's group hypothesized that fetal DNA could be found circulating in maternal blood. Hence, in 1997 they demonstrated the existence of fetal DNA in maternal blood by means of detection of Y-specific chromosome sequences in maternal plasma in male-bearing pregnancies. It opened new lines of research in alternative technologies that may facilitate safe diagnosis.

At present, it is widely accepted that apoptosis of trophoblastic cells is the primary source of ccffDNA, which explains the high turnover of circulating DNA and its rapid disappearance after delivery. Although detectable early in pregnancy (from the 5th week) and increasing in percentage throughout gestation, ccffDNA represents only a small fraction (less than 20%) of the total DNA in a pregnant woman's circulation (the other 80% being cell-free maternal DNA).

Clinical applications:

Applications derived from the study of ccffDNA in maternal blood can be divided into two categories: 1) high genetic-risk families, including sex determination in cases of at risk of X-linked diseases and detection of specific paternally inherited single-gene disorders; 2) routine antenatal care in all pregnancies, including aneuploidy screening, particularly trisomy 21 (Down syndrome), and determination of Rhesus factor status in RhD-negative women.

The coexistence of maternal DNA in the sample restricts NIPD to the study of paternal tracts that are not present in the maternal genome are thus certain to be of fetal origin. However, recent technical advances are also opening these studies to application in detecting mutations of a maternal origin.

At present the only two tests based on the analysis of ccffDNA in maternal blood that have been translated into clinical practice are fetal sex assessment and fetal RhD determination. Fetal sex determination in maternal plasma provides an initial screening to determine the need for subsequent invasive testing in cases at risk of X-linked disorders. This new prenatal approach has brought about an approximately 50% reduction in the need for invasive testing in case of sex-linked diseases.

The other clinical test, fetal RhD determination, is performed in pregnancies of RhD(-) women in which Hemolytic disease of the newborn (HDN) may occur in case of an RhD(+) fetus. Prenatal determination of fetal RhD condition (positive or negative) limits the need for anti-D treatment to only those pregnancies with RhD(+) fetuses. Incorporation of this prenatal test in maternal blood leads to better monitoring of pregnancies at risk for HDN, cost reductions for health-care systems, and the elimination of unnecessary exposure to blood products that can cause infection.

While diagnosis of some dominant diseases (e.g.,Huntington´s disease, achondroplasia or myotonic dystrophy), and some recessive diseases (e.g., cystic fibrosis, retinal dystrophies or hemoglobinopathies), have been described, widespread use of ccffDNA for detecting monogenic diseases and aneuploidies is technically challenging and is still under research.

For the detection of fetal aneuploidies, different strategies have been tested including recent technologies such as microarrays or massive sequencing. Diagnosis of trisomies 21, 13, or 18 have been reported in the literature, although to date, no consensus exists as to the best way to detect fetal aneuploidy based on fetal nucleic acids from the maternal circulation. At present the prohibitive costs associated with some of these technologies are an impediment for their clinical use.

Although the majority of work in NIPD is focused on ccffDNA, circulating cell-free fetal RNA (ccffRNA) from genes expressed in the placenta has also been reported in maternal blood. Detection of trisomy 21 and some pregnancy complications have been reported by using ccffRNA.

The introduction of NIPD into clinical practice raises numerous ethical, social, and legal implications, due to the ease with which the test can be performed. Best-practice guidelines are currently being designed in order to standardize protocols and increase test quality.