

Clinical Utility of Non-Invasive Prenatal Screening from Maternal Blood

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Fetal chromosomal abnormalities, especially trisomy 21 (Down syndrome) are frequent so that their prenatal detection represents one of the main goals of maternal-fetal medicine.

Abnormalities of the number and structure of chromosomes frequently appear in humans, appearing for: about 10% of gametes, for normal and fertile persons, 3% of 10 week fetuses and 2% of those of 15-16 weeks of gestation, 50-60% of early miscarriages (15-25% of all pregnancies), 10% of all stillborns (1% of all pregnancies), 0,7-1% of all live-borns (>1:120), 2% of all pregnancies for women over 35 in the moment of the conception (1).

The conventional methods for prenatal diagnosis – amniocentesis, biopsy from chorionic villus – present a risk of miscarriage of 0.03% and respectively 0.08% (2). The correlation between the maternal age, the size of the nuchal translucency and the values of the serological markers from the maternal serum - free β HCG and PAPP-A determine a detection rate for Down syndrome of ~90% with a rate of false positive results of 5% (3).

The fetal genetic material can be found in the maternal circulation giving the possibility of diagnosing the genetic disorders of the fetus. The intact fetal cells can be identified in the maternal blood but do not represent an useful

source of fetal genetic material because they are very rare and can persist for years from a previous pregnancy. In comparison, the cell-free fetal nucleic acids do not contain cell membrane (cdDNA and cfRNA, exist in larger quantities in the maternal blood are found only during a current pregnancy, presenting a larger potential for prenatal genetically screening of the fetus (4).

The analysis of the cell-free fetal DNA from the maternal blood represents an efficient screening method for detecting genetic fetal disorders. The concept became possible after discovering (in 1997) the fact that a large percentage (10%) of the fragments of cell-free DNA (~ 150 kb each) in the maternal plasma has fetal origins (5).

The first clinical application of this discovery has been the non-invasive of the fetal Rh from the maternal blood. DNA can be detected from the maternal blood starting with the 9 week of gestation. The concentration of cell-free DNA modestly increases with ~0.1%/week until week 21 and increases rapidly with 1%/week until the due date. The fetal DNA represents approximately 10-15% of the cell-free DNA in the maternal born at the end of the first trimester and the beginning of the second gestation trimester, but can go up to 50% close to the due date (6-8).

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The cfDNA concentration is influenced by many factors, the most important one being the maternal weight. The cell-free maternal DNA decreases with the increase of the maternal weight and can be insufficient for testing the obese patients. The clearance rate of the fetal cfDNA in the maternal blood stream is very fast (few hours from the birth) (9).

An important challenge in the analysis of cell-free DNA (cf DNA) in the maternal plasma is the fact that this is mixed with a larger quantity of free-cell maternal DNA. The separation of the 2 sources of DNA is not always possible. The first generation tests based on cf DNA was focused on detecting the trisomies 13,18,21 and of the sex chromosomes (10).

The analysis of the monogenic disorders is not yet possible but platform have been developed that can detect the microdeletion /micro-duplications syndromes. The main source of free-cell DNA in the maternal plasma is represented by the apoptosis of the trophoblast cells. Another possible source for cfDNA is the apoptosis of the fetal erythroblasts that generate cfDNA that crosses the placenta and enters into the maternal blood stream (6).

The clinical studies performed until this day for the non-invasive prenatal screening have used three methods (11-13).

Massive parallel sequencing (MPSS)

These methods are used for the analysis by sequencing millions of cfDNA fragments from the maternal plasma (both of fetal and maternal origin). Taking in consideration the fact that the sequence of the entire human genome is known, the origin of each fragment can be determined and the quantity of DNA fragment from each chromosome can be quantified. In trisomic pregnancies the number of molecules deriving from the supplementary copy of a chromosome, as percentage of the total number of sequenced fragments, is larger than in diploid pregnancies (14).

Chromosome-selective sequencing (CSS)

While the MPSS analyzes fragments from all the chromosomes, through CSS a selective analysis is performed targeted to specific areas of chromosomes 13,18, 21, X and Y (11,12).

Moreover, CSS determines the polymorphisms of other chromosomes, with the goal of assessing the fetal fraction. The minimum

amount of fetal DNA necessary for this kind of testing is of 3-4%.

Single nucleotide polymorphisms

SNP represent variations of the DNA structure that contribute to the individual variability. The principle of the method is that the fetus has different SNPs from the mother. Using the multiplex PCR methods (a variant of PCR) ~ 20 000 polymorphic loci are simultaneously analyzed on chromosome 13,18, 21, X and Y levels (11,12).

Practical aspects of the cfDNA testing in the maternal blood

Before testing genetic counseling, informing the patients is necessary on the limits of this method. The prenatal non-invasive testing does not represent a diagnosis test, despite the high sensitivity and specificity. Is important to remember the fact that the test does not replace the prenatal diagnosis methods (chromosomal analysis from the biopsy of chorionic villus or amniotic fluid), but it decreases the number of useless invasive methods (15).

The notable advantages are represented by the minimum gestational age for the test to be performed (9 weeks), high accuracy (>99.9%) and by the fact that it does not represent any danger for the fetus. The high efficiency has been proved for single pregnancies. As a disadvantage – 1-5% of the singleton pregnancies have no result after the first sampling (7).

The performances of the non-invasive prenatal testing for the screening for Aneuploidies performed and studied through a Meta-Analysis on 18 studies, with ~13000 singleton pregnancies) (16) highlighted the following data:

The non-invasive fetal testing for twin pregnancies is complex due to the fact that the 2 fetuses can be monozygotic (genetically identical) or dizygotic (meaning that one of the fetuses can be aneuploid). An ultrasound scan can detect the chorionicity but not the zygosity. For the monozygotic twin pregnancies the testing is performed similar to the way is performed for singleton pregnancies. For the dizygotic pregnancies each fetus participates with different cdDNA quantities in the maternal blood stream.

The aneuploid fetus can participate with a DNA quantity of <4% leading to false-negative results, due to the fact the euploid fetus can participate with a satisfying DNA quantity.

Aneuploidy	DR	FPR
Tris 21	99.0% (95%CI=98.2-99.6) (95%CI= 0.03-0.14)	67.2+/-13.3 1299 (38.1)
Tris 18	96.8% (95%CI=94.5-98.4)	0.15% (95%CI=0.08-0.25)
Tris 13	92.1%(95%CI=85.9-96.7) 0.20%(95%CI=0.04-0.46)	874 (25.6) 1200 (35.2)
Monosomy X	88.6%(95%CI=83.0-93.1) 887 (7.6)	0.12% (95%CI=0.05-0.24) 293 (8.6)
Other aneuploidies of sex chromosomes	93.8%(95%CI=85.9-98.7) 0.12%(95%CI=0.02-0.28) 6312 (54.1)	1146 (33.6) 1577 (46.3) 1819 (53.4)

Taking in consideration these facts, the use of the chromosome-selective sequencing is recommended as to correctly determine the fetal fraction for twin pregnancies (17).

Currently, the non-invasive prenatal testing is chosen by many pregnant women instead of invasive diagnosis methods. For these patients is essential to benefit from pre-testing genetical counseling as to understand the limitations of such technology that represents a screening test. Moreover, there are few data on the usefulness of such test for pregnant women with a low risk of fetal chromosomal abnormalities (18-20).

Approximately 5% of the tests do not comply with the minimal analysis criteria. Recent studies have revealed that this group for which

a result cannot be obtained have a high risk of aneuploidy so a new sampling and/or a diagnosis test is recommended (20). □

CONCLUSION

The cfDNA testing from the maternal blood will have a more important part in the maternal-fetal medicine, becoming more popular both for medical specialists and for pregnant women. It is important to remember that this is a screening test, not replacing the diagnosis tests so that the therapy decisions must not be made exclusively according to the result of such test. Moreover, it does not detect other many chromosomal fetal abnormalities and it does not give information on all genetic disorders. For the population with a low risk, the relation cost-efficiency is not favorable to cfDNA-based testing, the classic screening tests remaining the first intention.

Approximately 3% of the non-invasive prenatal tests based on free-cell DNA from the maternal blood do not have a result, some of the pregnancies being with aneuploid fetuses, this remaining an important disadvantage.

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