Cleveland Clinic
Laboratories

Noninvasive Prenatal Screening White Paper

By: Lana Bandoim

Background

During pregnancy, a woman's blood contains cell-free DNA (cfDNA) from the fetus. The amount of cfDNA increases during each trimester, but noninvasive testing that uses cell-free DNA is possible starting from week 10 of the first trimester. A cfDNA analysis can reveal the risk of the fetus having trisomies and other aneuploidies, along with determining the sex.[5]

The American College of Obstetricians and Gynecologists (ACOG) recommends noninvasive prenatal screening for pregnant women who have an increased risk of fetal aneuploidy.[1] Guidelines from the American College of Medical Genetics and Genomics (ACMG) for noninvasive prenatal screening using cell-free DNA (NIPS) also indicate healthcare providers should offer patients genetic counseling along with information about the test and guide them through the process.[2] Both amniocentesis and chorionic villus sampling (CVS) may increase the risk of miscarriage and other complications, so healthcare providers may want to start with a screening test such as NIPS and evaluate the risk for chromosome abnormalities before offering diagnostic testing. [3]

Compared to nuchal translucency testing or maternal serum screening, NIPS tends to have a higher detection rate.[6] Noninvasive prenatal screening using cell-free DNA has also shown significantly lower false-positive rates in clinical studies compared to standard screening tests, which can decrease the need for more invasive procedures.[7] Moreover, NIPS poses no physical risk to the mother or fetus.

Clinical Indications

Healthcare providers may recommend the NIPS test to pregnant women with certain risk factors. This includes women who are 35 or older and who have a fetal ultrasound that indicates a risk for aneuploidy. Other risk factors for women are having a family history of aneuploidy, having a previous child with trisomies or other aneuploidies and being a known carrier of balanced translocations or chromosome abnormalities.[1] NIPS testing is routinely carried out during the first trimester and may begin after 10 weeks of gestation.

The NIPS test is suitable for singleton pregnancies and twin pregnancies[4] but not triplet pregnancies. It is also appropriate for IVF pregnancies or self- and non-self egg donor pregnancies. Gestational carriers may also have this test.

Methodology

NIPS is a noninvasive laboratory test that can screen for an abnormal number of chromosomes in fetal cells and can also determine the sex of a fetus. The NIPS test relies on a maternal blood draw from a vein to conduct a cell-free DNA (cfDNA) analysis. The test requires two 10-mL cell-free DNA blood collection tubes. The specimen must be whole blood.

The circulating cell-free fetal DNA is processed from the maternal blood for DNA sequencing. DNA analysis relies on the Roche Ariosa cFS system and microarray technology. It uses the Digital ANalysis of Selected Regions (DANSR)[5] to amplify the cfDNA samples and targets chromosomes 4, 13, 18, 21, X and Y. Since this system does not require whole genome sequencing, it saves time while improving efficiency. Only chromosomes 21, 18, 13, X and Y are compared to chromosome 4 to determine aneuploidy.

The test analyzes single nucleotide polymorphisms (SNPs) and reports the fetal fraction.[8] SNPs tend to be the most common genetic variations with each one signifying a specific nucleotide. They are used to distinguish between the fetal and maternal DNA.

The FORTE (Fetal-fraction Optimized Risk of Trisomy Evaluation) algorithm further evaluates the fetal fraction and risk factors for aneuploidy. [6] This proprietary technology can include multiple factors during analysis such as maternal age, gestational age and fetal fraction in the probability score. The inclusion of these additional risk factors creates a weighted assessment and a better distinction between high- and low-probability results.[10]

Results are available three to seven days after the lab gets the blood sample. Then, the healthcare provider receives the results and has the responsibility to contact the patient and follow-up with her.

Mutations Detected

The NIPS test screens for trisomy 21 (Down syndrome), trisomy 13 (Patau syndrome) and trisomy 18 (Edwards syndrome) to determine if the fetus is at high or low risk of having these abnormalities. It also checks the risk of having common X and Y chromosome aneuploidies such as Klinefelter syndrome and Turner syndrome.

Other Detections

The same NIPS test can also determine fetal sex. If Y chromosome is detected, it indicates a male fetus. The lack of Y chromosome suggests a female fetus.

Test Advantages

The main advantage of the NIPS test is that it uses a higher fetal fraction of 4 percent, which produces a greater confidence in results. Using a higher fetal fraction increases test sensitivity and reduces the potential for false-positives or false-negatives.

Another advantage of NIPS is it that offers a quicker turnaround time for results. Other genetic sequencing tests require seven to 10 days, but NIPS only needs three to seven days to deliver results from the time the lab receives the blood sample. This may be more appealing to both healthcare providers and patients who are often eager to receive test results faster.

Test Limitations

It is important to note that NIPS is a screening test and not a diagnostic test, so positive results require additional testing and diagnostic confirmation. NIPS cannot determine with 100 percent accuracy if a fetus has a chromosomal abnormality. A positive result is a not a diagnosis of a fetal aneuploidy. Likewise, a negative result is a not a guarantee of fetal health or a normal pregnancy.

An insufficient blood volume or late receipt of a blood sample can also affect testing. Samples that are not in two 10-mL cell-free DNA blood collection tubes cannot be tested. In addition, the maternal blood must be drawn within seven days of being sent to the lab, and older specimens will be rejected.

The maternal blood sample cannot be drawn before 10 weeks of gestation because there will not be enough fetal cell-free DNA for analysis.

A low fetal fraction in the maternal blood sample can lead to results being indeterminate. This means that there is not enough fetal DNA for a complete analysis. A low fetal fraction may also produce a false negative.

NIPS can screen for trisomy 21, 13 and 18, but it cannot show results for other trisomies. It cannot detect single-gene disorders or other genetic conditions like triploidy.

NIPS is an indirect method to check a fetus' chromosomes since it relies on cell-free DNA. This type of DNA comes from the placenta, so it is possible for it to be different from the actual DNA in a fetus.

It is also possible for a mother's chromosomal abnormalities to be confused with the fetal DNA during NIPS to create a false positive. In addition, vanishing twin syndrome may affect results because the DNA can persist in maternal blood.[8]

Other test limitations include NIPS being less effective for women who are obese or carrying multiples such as triplets. This test is also not recommended for women who have bone marrow transplants.

The false-positive rates for fetal cfDNA analysis are 0.09 percent for trisomy 21, 0.13 percent for trisomy 18, 0.13 percent for trisomy 13, 0.23 percent for monosomy X and 0.14 percent for other sex chromosome aneuploidies.[9] These rates are for singleton pregnancies and can vary for twins or other multiples.

Test Results and Interpretation

Test results are either positive or negative. Positive results show a high probability of fetal aneuploidy that is greater than 99/100 or 99 percent. The recommendation for positive results is genetic counseling and diagnostic testing. Negative results show a low probability of less than 1/10,000 or 0.01 percent of fetal aneuploidy. The recommendation for negative results is genetic counseling if clinically indicated. A negative test result does not guarantee that a fetus does not have chromosome abnormalities.

The positive predictive value (PPV) reveals the probability that a fetus who receives a positive test result will actually have the condition. The PPV for NIPS is 98 percent for trisomy 21, 92 percent for trisomy 18 and 69 percent for trisomy 13.[6]

In some cases, results are indeterminate because of insufficient data such as the amount of cell-free fetal DNA being too low in the maternal blood sample for analysis. Healthcare providers will want to counsel patients and offer to repeat the test at a later date or do a different screening procedure.

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