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Noninvasive Prenatal Genetic Screening Using Cell-free DNA

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Cell-free DNA (cfDNA) analysis, based on maternal blood sampling, was initially validated as a clinical prenatal screen for pregnancies at high risk for trisomy 21 (**Figure**). cfDNA screening has since been approved to determine fetal sex and screen for fetal aneuploidy, including trisomies 13, 18, and 21, in high-risk and average-risk pregnancies.[1]

How It Works

cfDNA screening (also referred to as noninvasive prenatal screening or noninvasive prenatal testing), relies on the presence of small fragments of fetal DNA, typically smaller than 150 base pairs, that freely cross the placenta and enter the maternal bloodstream beginning early in the first trimester. Once isolated, cfDNA can be identified as being of fetal origin by analyzing patterns of single-nucleotide polymorphisms (SNPs) that are unique for the fetus' genome and can be distinguished from the unique SNP patterns characteristic of maternal DNA that, in turn, is extracted from maternal leukocytes. Aneuploidy can be detected by cfDNA analysis because there is increased cfDNA from an overrepresented chromosome or a less than expected amount of cfDNA derived from an underrepresented chromosome.

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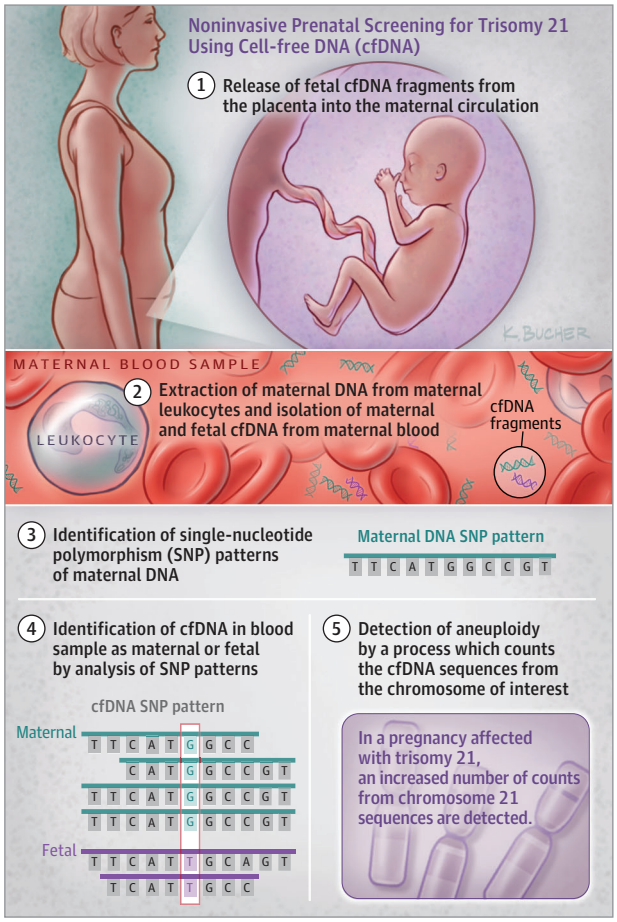
Next-generation sequencing of cfDNA is used to assess fetal aneuploidy. The genomic DNA sequence is then reconstructed from the fragments using computer algorithms. The 3 general approaches to cfDNA screening use next-generation sequencing technology. The first is shotgun genome-wide sequencing, which generates short sequences from across the genome. The generated sequences are aligned to a reference chromosome and counted. A second approach is targeted sequencing of the cfDNA that is based on next-generation sequencing, which amplifies selected chromosomal loci on the chromosomes of interest (eg, 13, 18, 21), followed by sequencing and counting. The third method amplifies and analyzes SNPs in a single nucleotide at a specific DNA locus. The maternal SNP pattern on chromosomes of interest can be determined from maternal leukocyte DNA. Computer algorithms use the known maternal SNP pattern to calculate ratios of fetal to maternal DNA. This analysis is more efficient if paternal DNA is available. The SNP-based approach is better at detecting triploidy and uniparental disomy, a condition in which 2 chromosomes are inherited from one parent and none from the other.

Although efficiency varies by laboratory, meta-analysis suggests that the overall detection rates of cfDNA-based screening are 91.3% (trisomy 13), 94.9% (trisomy 18) and 98.6% (trisomy 21). The performance of cfDNA-based screening for detection of sex chromosome aneuploidies is less robust. For example, studies suggest that the detection rate and false-positive rate for 45,X is 90.3%, and the detection rate for 47,XXY, 47,XYY and 47, XXX is 93%.[2]

Clinical Considerations

The detection rate for trisomy 21, using cfDNA screening, is often advertised as 99%. The detection rate for other genetic conditions varies widely. Clinicians and patients should know that despite a 99% detection rate, cfDNA analysis is a screening test and is not considered diagnostic because of the potential for false-positive results and because cfDNA is of placental and not truly of fetal origin. Diagnoses of these abnormalities requires sampling and analysis of fetal tissue by chorionic villous sampling or amniocentesis.

Figure. Noninvasive Prenatal Screening for Trisomy 21 Using cfDNA



Most commercial cfDNA screens assess for trisomy 13, 18, and 21, and fetal sex. Many also include results for sex chromosome aneuploidy, but these tests are less reliable. In some rare cases, cfDNA may also return an unexpected result that shows multiple increases and decreases in cfDNA. Although such results were originally not understood, they may signal the presence of a maternal malignancy.[3] When maternal tumors are present, they overexpress DNA that is released into the circulation, and they can mimic the findings seen in a fetus with multiple aneuploidies.

Test failures occur when the laboratory determines that the fraction of fetal DNA is too low to reliably screen for aneuploidy. In most laboratories, a fetal fraction cutoff of 4% is used. If the fetal fraction of cfDNA is less than 4%, the laboratory will not proceed with

testing. The most common causes of low fetal fraction include aneuploidy (some forms of aneuploidy may cause a low fraction), triploidy, young gestational age (<10 weeks), and high maternal body mass index (>35).[4] A positive or failed cfDNA test result should be followed up with genetic counseling and confirmatory testing by chorionic villus sampling or amniocentesis.

Several professional society guidelines endorse genetic counseling prior to prenatal screening.[1,5,6] The discussion in genetic counseling should include the following: (1) the exact nature of the conditions being screened; (2) whether patients desire fetal sex information; (3) the possibility of false-positive or inconclusive results; (4) the need for follow-up diagnostic testing in the event of a positive result; (5) the possibility that the screen may detect maternal characteristics such as 47,XXX; (6) and how and when test results will be returned.·

Before recommending cfDNA screening, local variation in public and private insurance reimbursement policies for noninvasive genetic screening should be considered. Patients and clinicians should verify health insurance coverage prior to cfDNA screening. Lack of insurance authorization may result in high out-of-pocket expenses for the patient.

Value

Laboratory prices for cfDNA screening vary (range, $500-$2100).[7] This is considerably more expensive than traditional screening approaches, which yield similar information and many of which are subsidized or completely covered by public health screening programs. Currently, cfDNA testing is not cost-effective as a first-tier screen for all pregnancies.

When cfDNA is compared with traditional first- and second-trimester screening (eg, the ultrasound method of nuchal translucency measurement [subdermal fluid at the back of the fetus' neck] and serum analyte screening) for the detection of common aneuploidies (trisomies 13, 18, and 21), cfDNA screening has a higher detection rate, higher positive and negative predictive values, and lower false-positive rate. However, traditional screening may actually have a higher overall detection rate for chromosomal abnormalities because it detects many chromosomal problems other than just the common aneuploidies. One study found that 17% of chromosomal abnormalities detected by a traditional screening test were not detectable by cfDNA screening[5,8]; these abnormalities included rare trisomies, unbalanced rearrangements, and large deletions or duplications. Thus, fetal cfDNA is considered to be a screening modality and should not be used to make pregnancy-altering decisions. Chromosome microarray analysis of specimens obtained via chorionic villus sampling or amniocentesis is recommended as the definitive test to be used after an abnormal cfDNA result. Chromosome microarray analysis is also the preferred test for patients requesting complete aneuploidy information.

Evidence Base

Several large-scale clinical trials have validated cfDNA testing for the detection of trisomies 13, 18, and 21.[9] Additional trials validated the method for the presence or aneuploidy of sex chromosomes, which suggests fetal sex as well as the presence of 47,X and 47,XXY and other sex chromosome variations.[10]

Current guidelines from the American College of Medical Genetics, the American College of Obstetricians and Gynecologists, and the Society for Maternal Fetal Medicine advise that cfDNA may be offered to all women to screen for common aneuploidies and for fetal sex, but it is not considered the standard approach in pregnancies that are not considered high risk.[1]

Bottom Line

cfDNA screening can detect chromosome aneuploidy in pregnancy after 10 weeks' gestation but is less effective at screening for other genetic abnormalities. cfDNA tests are more expensive than other approaches. Positive results require secondary testing with more definitive techniques. Genetic counseling should be offered whenever genetic testing is conducted.

ARTICLE INFORMATION

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REFERENCES

1. Gregg AR, Skotko BG, Benkendorf JL, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update. *Genet Med*. 2016;18(10):1056-1065.

2. Mackie FL, Hemming K, Allen S, Morris RK, Kilby MD. The accuracy of cell-free fetal DNA-based non-invasive prenatal testing in singleton pregnancies. *BJOG*. 2017;124(1):32-46.

3. Bianchi DW, Chudova D, Sehnert AJ, et al. Noninvasive prenatal testing and incidental detection of occult maternal malignancies. *JAMA*. 2015;314(2):162-169.

4. Hudecova I, Sahota D, Heung MMS, et al. Maternal plasma fetal DNA fractions in pregnancies with low and high risks for fetal chromosomal aneuploidies. *PLoS One*. 2014;9(2):e88484.

5. Oepkes D, Tabor A, Yaron Y. Prenatal aneuploidy screening using cell free DNA. *Am J Obstet Gynecol*. 2015;213(4):596-597.

6. Committee on Practice Bulletins—Obstetrics, Committee on Genetics, Society for Maternal-Fetal Medicine. Practice bulletin No. 163: screening for fetal aneuploidy. *Obstet Gynecol*. 2016;127(5):e123-e137.

7. Walker BS, Jackson BR, LaGrave D, Ashwood ER, Schmidt RL. A cost-effectiveness analysis of cell free DNA as a replacement for serum screening for Down syndrome. *Prenat Diagn*. 2015;35(5):440-446.

8. Norton ME, Jelliffe-Pawlowski LL, Currier RJ. Chromosome abnormalities detected by current prenatal screening and noninvasive prenatal testing. *Obstet Gynecol*. 2014;124(5):979-986.

9. Norton ME, Brar H, Weiss J, et al. Non-Invasive Chromosomal Evaluation (NICE) study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. *Am J Obstet Gynecol*. 2012;207(2):137.e1-137.e8.

10. Bianchi DW, Parsa S, Bhatt S, et al. Fetal sex chromosome testing by maternal plasma DNA sequencing. *Obstet Gynecol*. 2015;125(2):375-382.