Prenatal Cell-Free DNA Screening

Q&A for Healthcare Providers

What is cell-free DNA screening (cfDNA)?

cfDNA screening (also referred to as non-invasive prenatal testing, NIPT, or non-invasive prenatal screening, NIPS) is a screening test that utilizes bioinformatic algorithms and next generation sequencing of fragments of DNA in maternal serum to determine the probability of certain chromosome conditions in a pregnancy. All individuals have their own cell-free DNA in their blood stream. During pregnancy, cell-free DNA from the placenta (predominantly trophoblast cells) also enters the maternal blood stream and mixes with maternal cell-free DNA. The DNA of the trophoblast cells usually reflects the chromosomal make-up of the fetus.

What does cfDNA screen for?

cfDNA routinely screens for trisomy 21, trisomy 18 and trisomy 13. Screening for fetal sex, sex chromosome aneuploidy, other aneuploidies, triploidy, and specific microdeletion conditions is also available. Conditions included on the cfDNA panel vary based upon the performing laboratory. cfDNA cannot screen for all chromosome or genetic conditions.

How are results reported?

cfDNA results are reported differently, depending upon the performing laboratory. Some laboratories describe an increased risk as "Aneuploidy Detected" or "Positive for Trisomy". Others describe an increased risk as a risk score, which is often >99%. Low risk results may be described as "Negative", "No Aneuploidy Detected", or a risk score such as "<1 in 10,000". These results represent findings in the cfDNA and may not represent the chromosomal make-up of the fetus. A >99% risk score does <u>not</u> mean there is a greater than 99% chance that the pregnancy is affected with a condition. A "Positive" or "Aneuploidy Detected" does not mean the fetus definitively has a chromosome condition. A "Negative", "No Aneuploidy Detected", or "<1 in 10,000" result does not definitively rule out a chromosome condition.

How accurate are the results?

These tests are often advertised to patients and healthcare providers as >99% accurate. It is important to recognize this is a population-level statistic and only applies to the entire population of women screened. It does not apply to an individual's result. Since most pregnancies are unaffected and most results are "low risk" this test is correct 99% of the time for all women. However, the probability a high risk result indicates an affected fetus is not 99% in the majority of cases. In order to determine how likely a high risk result indicates an affected fetus, you need to know the positive predictive value (PPV). The PPV reflects the chance that a positive test result is a true positive. This statistic depends on the sensitivity and specificity of the screen as well as the incidence of a condition in a population. Data from five different studies evaluating PPV of cfDNA screening are summarized below.

Positive Predictive Value	Wang et al.	Bianchi et al.	Choy et al.	Meck et al.	Norton et al.
Trisomy 21	38/41 (93%)	5/11 (45.5%)	52/55 (95%)	29/30 (97%)	9/47 (80.1%)
Trisomy 18	16/25 (64%)	2/5 (40%)	6/12 (50%)	3/5 (60%)	9/10 (90%)
Trisomy 13	7/16 (44%)		4/7 (57%)	1/4 (25%)	2/4 (50%)
Sex Chromosome Aneuploidy	6/16 (38%)		4/6 (67%)	1/7 (14%)	

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As demonstrated by these studies, PPV varies by condition, the study population and incidence of a condition in that population (the a priori risk), as well as the sensitivity and specificity of the cfDNA screen. While studies support that cfDNA screening has a higher PPV than traditional screening tests it is important to note that these PPVs cannot be universally applied to patients. The PPV will be higher for patients who have a higher a priori probability based on age or other screening results; the PPV will be lower in women with a lower a priori risk. For instance, with all else equal, the PPV is higher for women at age 40 than it is at age 20 because the a prior risk of aneuploidy increases with maternal age.

How does cfDNA compare to other screening tests?

cfDNA has both advantages and disadvantages compared to traditional screening. cfDNA has higher sensitivity and specificity for trisomy 21 than traditional biochemical screening (i.e. quad, integrated, sequential, first trimester screen, etc.), leading to fewer false positives and false negatives. More data is needed to evaluate the ability of cfDNA to identify an increased risk for additional conditions, including other autosomal aneuploidies, sex chromosome aneuploidy, triploidy and microdeletion conditions. cfDNA screening should not be conducted in parallel with other biochemical Down syndrome screening tests.

Conversely, cfDNA does not screen for open neural tube defects and abdominal wall defects. MSAFP screening and/or a second trimester anatomy scan is recommended for all women undergoing cfDNA screening.

How does cfDNA compare to diagnostic tests, such as amniocentesis?

It is essential providers recognize cfDNA is a *screening* test and does not replace diagnostic testing. Unlike diagnostic testing, cfDNA results can be confounded by low fetal fraction, vanishing twins, unrecognized maternal conditions, and other unknown biological confounders. The accuracy of cfDNA and CVS may also be affected by placental mosaicism. Amniocentesis remains the most accurate diagnostic option with an accuracy of 99.9%. Furthermore, diagnostic testing will detect additional conditions, undetectable by cfDNA.

What should I tell my patients when offering cfDNA screening?

Pre-test counseling for cfDNA screening should include:

- cfDNA appears to be the most accurate *screening* test for trisomy 21.
- False positive and false negative results do occur with cfDNA.
- Patients who desire definitive information about chromosome conditions in their pregnancy should be offered the option of amniocentesis or CVS.
- Confirmatory diagnostic testing is recommended for abnormal cfDNA results.
- A negative cfDNA result indicates a decreased risk and does not definitively rule out chromosome conditions.
- cfDNA screening may not yield a result, which may indicate an increased risk for a chromosome condition.
- cfDNA does not evaluate for all chromosome conditions.
- All genetic screening is optional and may be declined.

How can I find a genetic counselor?

Genetic counselors are health care professionals with specialized training in cfDNA screening and the psychosocial complexities surrounding genetic testing and screening. They can help your patient understand their options and facilitate a decision about testing. A genetic counselor can be located using the “Find a Genetic Counselor” link on the nsgc.org website. You can also find more information about individual genetic conditions and national advocacy organizations for these conditions at www.lettercase.org/prenataltesting/.

References:

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