

Society for Maternal-Fetal Medicine (SMFM) Consult Series

#36: Prenatal aneuploidy screening using cell-free DNA

Society for Maternal-Fetal Medicine (SMFM) Publications Committee

Obstetric providers are challenged continuously with the evaluation of the potential benefits and harms of new diagnostic and therapeutic procedures or technologies for patients (mother and fetus), often in the setting of limited high-quality data (eg, randomized clinical trials). Although innovations in technology have created opportunities to expand and improve the genetic testing options that are available to women during pregnancy, the complexity of these technologies, combined with the commercial interest to implement these tests rapidly into clinical care, have created potential for misunderstanding, misuse, and unintended consequences of these tests.

The purpose of this document is to aid clinicians in counseling their patients regarding prenatal aneuploidy testing options with cell-free DNA (cfDNA) screening that includes the potential benefits and harms, a comparison to current screening tests, as well as the limitations and caveats. It is expected that guidelines of care and paradigms for application of cfDNA will evolve because of updated data regarding test performance, clinical experience, and cost-effectiveness.

What are cfDNA and cfDNA aneuploidy screenings?

CfDNA consists of small (<200 base pairs) fragments of DNA that are free floating in the plasma. During pregnancy, after 10 weeks of gestation, approximately 10-15% of the total cfDNA in the maternal plasma is of placental origin (ie, derived from trophoblast) and can be used therefore to test for fetal disorders.¹ Cell-

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Recent advances in technology have created exciting opportunities to expand and improve genetic testing options that are available to women during pregnancy. However, the novelty and complexity of these technologies, combined with the commercial interest to implement these tests rapidly into routine clinical care, have created challenges for physicians and patients and potentially will lead to misunderstanding, misuse, and unintended consequences. The purpose of this document was to aid clinicians in their day-to-day practice of counseling patients regarding prenatal aneuploidy testing options with cell-free DNA screening, which includes how it compares to current testing methods, potential benefits and harms, and its limitations and caveats.



EDITORS' ★ CHOICE

free DNA screening (also referred to as cfDNA testing, noninvasive prenatal testing, and noninvasive prenatal screening) is a test that uses next-generation sequencing of cfDNA in maternal plasma combined with bioinformatic algorithms to determine the probability of certain fetal chromosomal conditions in pregnancy.

Although such testing has been demonstrated to be possible for a variety of genetic conditions (which include blood type, autosomal single gene disorders, and determination of fetal sex to assess risk for X-linked diseases), the large majority of clinical tests are done to test for fetal chromosomal disorders. Initially focused only on Down syndrome, the laboratories that perform the testing all subsequently have added assessment for trisomies 13 and 18 and the sex chromosomes. In addition, some laboratories provide testing for additional trisomies and some micro-deletion syndromes. It is likely that the range of available conditions that can be tested for with the use of cfDNA screening will continue to increase over time.

How is cfDNA analysis performed?

CfDNA screening was made possible by 2 developments: advances in next-generation sequencing after

completion of the Human Genome Project in 2001 and the discovery that cfDNA of placental origin is present in the maternal circulation and can be analyzed from a plasma sample. Different laboratories use somewhat different platforms, but the common theme of next-generation sequencing is increased automation, which allows faster and cheaper sequencing than earlier methods.

Some cfDNA tests use an approach known as *massively parallel shotgun sequencing*, in which all of the cfDNA that is extracted from a maternal sample is sequenced.^{2,3} Each DNA fragment is localized to its chromosome of origin, and the number of fragments that arises from each chromosome is counted. Additional material that arises from a given chromosome increases the chance that the fetus carries an extra copy of that chromosome. Another method also counts chromosome fragments but includes those from a more limited subset of the total genome; this is more efficient than massively parallel shotgun sequencing and thereby allows for somewhat lower costs.⁴ A third approach involves the analysis of many thousands of single nucleotide polymorphisms to determine the genotype and relative copy number of each chromosome of interest.⁵ Each platform has limitations and

specific maternal or fetal criteria that must be met for optimal test performance.

In addition to differences in how the sequencing and laboratory analyses are performed by the different platforms, there are also differences in the bioinformatics analysis and interpretation. This interpretation and the presentation of results are important and complex parts of the comparison of the overall test characteristics.

How accurate is cfDNA aneuploidy screening?

CfDNA screens for trisomies 13, 18, and 21 and sex chromosomal abnormalities; the accuracy of screening for each of these conditions varies somewhat by condition and platform used.^{1-3,5-7} The ability of cfDNA to identify the presence (or absence) of a chromosomal aneuploidy depends on a number of factors, including, the amount of fetal DNA that is present, the a priori chance that a chromosome abnormality is present (that is, the woman's risk based on maternal age or results of other screening), and other factors such as the presence of a multifetal gestation or a nonviable second embryo/fetus or the presence of placental mosaicism.

Some laboratories report the probability of aneuploidy; most commonly, this is stated to be >99% in patients who are at increased risk and <1/10,000 in patients who are at low risk. Such results suggest a degree of certainty that is near diagnostic; however, this is a population statistic and applies only to the entire population of women who were screened and not to an individual's result. It is important for providers to recognize that a positive result for any of these aneuploidies confers a chance that the fetus is affected, which is usually far <99%, particularly in lower risk patients.⁸

To determine how likely it is that a positive result indicates an affected fetus, the positive predictive value (PPV) should be assessed. PPV is the proportion of positive results that are true positives and is dependent not only on the sensitivity and specificity of the test but also is highly dependent on the prevalence of the condition. When testing for rare conditions (such

as aneuploidy in younger women), the PPV is much lower than when testing for more common conditions (such as trisomy 21 in older women).⁹ Therefore, more false-positive results are expected in women who are at low risk or when screening is done for very rare conditions. The PPV for trisomy 21 has been reported as varying from 45% in low-risk patients to ≥96% in the highest risk patients. In one study of diagnostic testing after abnormal cfDNA screens, aneuploidy was confirmed in 93% of trisomy 21 cases, in 64% of trisomy 18 cases, in 44% of trisomy 13 cases, and in 38% of sex chromosomal abnormalities.¹⁰

How should a cfDNA "positive test" be interpreted?

Laboratory reports vary in how they report findings that are "positive" based on cfDNA analysis. Aneuploidy risk is generally reported as "positive" or "detected" or as a probability of >99% in patients who are at increased risk. After a positive test, patients should be referred for posttest counseling to a maternal-fetal medicine subspecialist, geneticist, and/or genetic counselor. Such counseling should include a discussion of the predictive value of cfDNA as a screening test (including the possibility that the result is a false positive) and the offer of diagnostic testing (chorionic villous sampling or amniocentesis) for confirmation, particularly in women who are considering pregnancy termination.

In part because of the manner in which cfDNA results are presented and how the tests are being marketed, there is some confusion by both providers and patients regarding the possibility of false-positive results.¹¹ Often, it is assumed mistakenly that this testing is diagnostic.¹² In a study that described outcomes of cfDNA testing from commercial testing of >30,000 women, approximately 6% of women with a positive cfDNA test result proceeded to pregnancy termination without confirmatory diagnostic testing.¹³ It is important that obstetric providers understand and appropriately interpret the results of cfDNA screening and accurately convey this information to their patients as part of pretest counseling (Table 1).¹⁴

What are the implications of a failed cfDNA test result and how should such cases be treated?

Because of the fact that screening requires a minimum amount of cfDNA, often referred to as the "fetal fraction (FF)," there is a risk for test failure because of low FF. In addition, some tests do not provide a result because of difficulties in interpretation of sequencing data. Although FF is relatively constant from 10-22 weeks of gestation, it is lower at <10 weeks of gestation and less likely to provide a result. Overall, the chance of test failure is reported at 0.9-8.1% and varies in part by whether the laboratory measures FF and requires a minimum concentration.^{1-3,5,6,10,15}

Low FF and failed results have been associated with fetal aneuploidy. In one recent study, 8% of the patients overall had failed testing; this increased to 16% in cases with fetal aneuploidy. In this study, the odds ratio for aneuploidy was 9.2 in cases with failed tests.¹⁵

Given this association, women with failed cfDNA screens should be counseled that they are at increased risk for trisomy, particularly trisomies 13 and 18, and triploidy. It is therefore appropriate to offer the option of diagnostic testing in these cases, given the increased risk. A repeat cfDNA screen will be successful in 50-80% of cases.^{16,17} Whether the patient chooses to attempt cfDNA screening again may depend in part on gestational age; a patient at a more advanced gestation may not wish to delay obtaining definitive information, given the increased risk.

What are other limitations of cfDNA aneuploidy screening?

Low FF has been associated with maternal obesity; in 1 study, cfDNA aneuploidy screening failed to provide a result in 20% of women >250 lb and 50% of women >350 lb.¹⁸ Therefore, in obese or morbidly obese women, cfDNA aneuploidy screening may not be the best screening option.

At present, there are limited data on the use of cfDNA aneuploidy screening in multifetal gestations; most published studies have included a small number of aneuploid fetuses.¹⁹⁻²¹ In these series, it has been noted that the failure rate is higher and the detection rate may be lower, although the number of cases is

very small. With regard to a nonviable cotwin, it is recognized that a high percentage of fetal losses are aneuploid, which is also true with a dead twin. The presence of a second gestational sac has been associated with false-positive cfDNA results; therefore, this test is not a good option for women with a “vanishing twin” or empty second sac. At this time, the data are too limited to recommend routine cfDNA aneuploidy screening in women with multifetal gestations.

What evaluation is appropriate for women with a false-positive cfDNA aneuploidy screening result?

Given that the cfDNA present in maternal plasma is a mixture of maternal and placental DNA, a number of biologic phenomena can cause a false-positive, or discordant, cfDNA result. Many cases are thought to result from confined placental mosaicism or a cotwin death or a vanishing twin. Cases of false-positive results for sex chromosomal aneuploidy have been reported in which pregnant women were found to have a sex chromosomal abnormality themselves, often in mosaic form.²² This has led to the discussion of the possible benefit of karyotyping of women who have a false-positive cfDNA screening result to rule out a mosaic chromosomal abnormality in the mother.^{23,24}

A few cases of maternal malignancies with chromosomal abnormalities within the tumor have been reported in patients with false-positive cfDNA screening results.²⁵ These reports have raised the question about the benefit of further evaluation for maternal malignancy in women with false-positive results. Although such cases of malignancy are of interest in considering the underlying biologic evidence of cfDNA, at this time the clinical utility and yield of an extensive work up (eg, multiple diagnostic imaging studies to search for undiagnosed maternal malignancy) are unknown.

Who are candidates for cfDNA aneuploidy screening, based on current Society of Maternal-Fetal Medicine/American College of Obstetrics and Gynecology recommendations?

In 2012, Society of Maternal-Fetal Medicine (SMFM) and American College of

TABLE 1

Checklist for pretest counseling for cell-free DNA aneuploidy screening

Cell-free DNA screening appears to be the most accurate screening test for trisomy 21.

Cell-free DNA does not screen for all chromosomal conditions.

Women who desire definitive information about chromosome conditions in their pregnancy should be offered the option of amniocentesis or chorionic villous sampling.

False-positive and false-negative results do occur with cell-free DNA.

Diagnostic confirmation with chorionic villous sampling or amniocentesis is recommended for women with abnormal cell-free DNA results.

A negative cell-free DNA result indicates a decreased risk and does not definitively rule out trisomy 21 or other chromosome conditions.

All genetic screening is elective; whether a woman chooses to have aneuploidy screening, prenatal diagnostic testing, or no testing is a personal decision, and any of these is a reasonable option.

SMFM. Prenatal aneuploidy screening with cfDNA. *Am J Obstet Gynecol* 2015.

Obstetrics and Gynecology (ACOG) published a joint Committee Opinion (no. 545) entitled “Noninvasive prenatal testing for fetal aneuploidy.”²⁶ Although likely to be updated as the evidence evolves, this guideline recommended that routine screening be limited to women at increased risk for fetal aneuploidy, such as those with (1) maternal age ≥ 35 years old at delivery, (2) fetal ultrasound finding that indicates an increased risk of aneuploidy, specifically for trisomies 13, 18, and 21, (3) a history of previous pregnancy with a trisomy detectable by cfDNA screening (trisomies 13, 18, or 21), (4) positive test results for aneuploidy that include a first-trimester, sequential, integrated, or quadruple screen, and (5) parental balanced Robertsonian translocation with increased risk of fetal trisomy 13 or 21.

Because cfDNA screening does not assess risk for open neural tube defects or other structural abnormalities that are associated with increased maternal serum alpha-fetoprotein, routine prenatal screening should also include second-trimester anatomy ultrasound scanning and/or maternal serum alpha-fetoprotein to assess for these conditions. It is also important to note that, although some sonographic findings (such as a thickened nuchal fold in the second trimester) are associated highly specifically with Down syndrome, most structural anomalies can be associated with a number of different aneuploidies.

Therefore, comprehensive diagnostic testing with karyotype or chromosomal microarray should be offered when a fetal malformation is identified.²⁷

Current guidelines from SMFM/ACOG, American College of Medical Genetics and National Society of Genetic Counselors all recommend against the use of cfDNA aneuploidy screening as part of routine prenatal care for all women without informed consent. SMFM/ACOG and National Society of Genetic Counselors guidelines suggest that this test not be used for “low risk” women. Although cfDNA aneuploidy screening appears to have similar sensitivity and specificity for identifying trisomy 21 and the common aneuploidies in both high risk and lower risk populations,^{2,28-30} several of these studies have been done by laboratories with a commercial interest and have had limited transparency of many details. In addition the costs and cost-effectiveness for society and the overall health care system of widespread implementation are unknown.

Given that the joint SMFM/ACOG committee opinion was published in 2012 and recognizing that the literature regarding test performance, expansion of testing for other conditions, and cost-effectiveness is rapidly evolving, it is expected that updates of the existing guideline will be forthcoming that will evaluate these new data and how they should be translated into clinical care.

TABLE 2

Comparison of prenatal screening and diagnostic testing options for fetal aneuploidy

| Test | DR T21, % | DR all aneuploidies, % | SPR, % ^a |
|------------------------------|-----------|------------------------|---------------------|
| First-trimester screen | 80 | 69 | 5 |
| Sequential/integrated screen | 93 | 82 | 5 |
| Cell-free DNA screen | 99 | 72 | 1-9 |
| Chorionic villus sampling | >99 | >99 | 1 ^b |
| Amniocentesis | >99 | >99 | 0.2 ^b |

DR, detection rate; SPR, screen positive rate.

^a Includes all results that required further follow up (ie, failed cell-free DNA tests and false-positive results); ^b Mosaicism, which included confined placental mosaicism.

SMFM. Prenatal aneuploidy screening with cfDNA. *Am J Obstet Gynecol* 2015.

How does cfDNA aneuploidy screening compare with traditional serum and ultrasound-based aneuploidy screening methods?

In considering the options for prenatal aneuploidy screening, the primary consideration is that patients be counseled adequately regarding the benefits and limitations of the various options. Although the risk of aneuploidy varies by maternal age, a woman of any age can have a fetus with trisomy or another chromosomal abnormality. In addition, some women may prefer diagnostic testing or no testing, regardless of age.³¹ It is appropriate therefore to offer diagnostic testing, screening, or the option of no testing to women of all ages.^{32,33} The offer of prenatal screening requires discussion of the pros and cons of all test options, including the detection rates, screen positive rates, and recommended follow up if an abnormal result is obtained (Table 2).

Compared with current aneuploidy screening tests, cfDNA screening has a higher sensitivity and specificity for trisomy 21 and therefore results in fewer false-positive and false-negative test results than traditional serum-based biochemical screening for trisomy 21. Although many women undergo aneuploidy screening with tests other than integrated (or sequential) screening (eg, first-trimester only or second-trimester screening), integrated screening with first- and second-trimester serum and nuchal translucency ultrasound scanning has the best performance of the traditional screening

options. Because cfDNA has a lower screen positive rate, its implementation has led to lower use of invasive testing.³⁴ However, approximately 0.8-8% cases of cfDNA tests will report “no results,” and thus the number of women who require follow-up or additional testing may be comparable depending on the platform used. Diagnostic testing should be considered on all cases where a non-reportable result is obtained when cfDNA is used as a screening test.

In prenatal series, which include primarily women who are at high risk, trisomies 13, 18, and 21 make up approximately 70% of all aneuploidies; sex chromosomal abnormalities comprise another 10-13%.³⁵⁻³⁷ Because cfDNA has a more narrow focus of screening (trisomy 21, 18, 13, and sex chromosomes), the detection rate for other chromosomal abnormalities is lower than with current screening methods, in which many “screen positive” cases ultimately are found to have other, less common chromosomal abnormalities. In a recent study of 450,000 women who underwent sequential screening in California, the detection rate for all chromosomal abnormalities was 81.6%, with a false-positive rate of 4.5%.²⁸ It was estimated that cfDNA potentially would have detected 70-75% of these abnormalities. In a separate analysis of data from the California Prenatal Screening program that included >1.3 million women, of all chromosomal abnormalities that were identified by traditional aneuploidy screening followed by diagnostic testing, 16.9% would not have been

detected had cfDNA analysis been performed instead of chorionic villus sampling or amniocentesis. In women who had positive serum screening, it was estimated that, after a normal cfDNA screen, the residual risk of a chromosomal abnormality was 1 in 50 (2%).³⁸ The detection rate in this study was associated inversely with maternal age; the lowest detection rate for cfDNA occurred in women who were <25 years old, because the relative percentage of chromosomal abnormalities other than trisomy 21, 18, or 13 is greater in younger women.

Is it appropriate to perform both maternal serum screening and cfDNA aneuploidy screening at the same time?

Traditional aneuploidy screening and cfDNA analysis are both primarily intended to be screening tests for the same chromosomal aneuploidies. Although the tests have differing performance characteristics, there are no data to support an approach in which both tests are done concurrently (eg, first-trimester screening and cfDNA screening). An appropriate approach is to offer patients the option of either traditional aneuploidy screening or cfDNA screening as a first-line test. In patients who are screen positive by traditional screening, diagnostic testing or cfDNA screening may be offered. If cfDNA screening is selected, recognition that its detection is limited generally to trisomy 21, 18, 13 and the sex chromosomes chromosomal abnormalities is important. Although 1 or 2 laboratories also assess risk for conditions such as trisomy 9, 16, or 22, these are quite rare and the detection rates are unknown; in most cases, these abnormalities result in miscarriage very early in gestation.

The option of the performance of cfDNA screening and first-trimester nuchal translucency measurement concurrently has also been suggested. At 11-13 weeks of gestation, some structural abnormalities can be identified by ultrasound scanning, which includes cystic hygroma, anencephaly, and other fetal abnormalities.³⁹⁻⁴¹ Nuchal translucency measurement screens mainly for the common trisomies (which would be screened by cfDNA screening as well),

and the additional clinical utility of nuchal translucency to detect other chromosomal or structural abnormalities is unknown but appears to be limited.³⁸

What is the benefit of cfDNA screening for microdeletions?

Some laboratories have added testing for chromosomal microdeletions to their cfDNA screening panels. In some laboratories, such expanded panels are a standard component of the test and require the ordering provider to opt out if they do not desire the information; although for other laboratories, they constitute an additional test with added cost.⁴² Pathogenic chromosomal microdeletions or microduplications (copy number variants) overall occur in approximately 1.2% of fetuses and newborn infants, and many of these are associated with significant neurodevelopmental and other disabilities.⁴³ However, this number refers to all possible copy number variants in a population, although cfDNA currently screens for only 4 or 5 such disorders (although this number is rapidly expanding). Therefore, women who desire testing for copy number variants or who prefer comprehensive testing for as many disorders as possible should be offered diagnostic testing with chromosomal microarray. Although the well-recognized 22q microdeletion that is included on cfDNA expanded screening panels is relatively common and affects about 1 in 4000-5000 individuals, most others are far less common with incidences closer to 1 in 30,000-50,000.⁴⁴ Such testing has not been validated in clinical trials; rather, proof-of-principle studies have included the mixing of normal and abnormal DNA in laboratory samples at ratios thought to represent typical FE.⁴⁵ Even with very high sensitivity and specificity, at such low prevalence, the PPV of such testing is likely very low, and the clinical utility is unclear. At this time, routine screening for microdeletions with cfDNA is not recommended.²⁷ ■

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Summary recommendations

| No. | Recommendations | GRADE |
|-----|--|--|
| 1 | Optimal candidates for routine cfDNA aneuploidy screening are women with: Maternal age ≥ 35 years at delivery. Fetal ultrasound finding that indicates an increased risk of aneuploidy, specifically for trisomies 13, 18, or 21. History of previous pregnancy with a trisomy detectable by cfDNA screening (trisomies 13, 18, or 21). Positive screening results for aneuploidy that include a first-trimester, sequential, integrated, or quadruple screen. Parental balanced Robertsonian translocation with increased risk of fetal trisomy 13 or 21. | 1B: Strong recommendation, moderate quality evidence |
| 2 | Routine screening for microdeletions with cfDNA is not recommended. | 1B: Strong recommendation, moderate quality evidence |
| 3 | For women who desire comprehensive testing for chromosomal disorders, diagnostic testing should be offered. | 1B: Strong recommendation, moderate quality evidence |
| 4 | For women who undergo cfDNA aneuploidy screening, maternal serum alpha-fetoprotein, and/or second-trimester anatomy ultrasound scan should also be performed. | Best practice |
| 5 | Formal genetic counseling by maternal-fetal medicine subspecialist, geneticist, or genetic counselor after a positive cfDNA test is recommended | Best practice |
| 6 | Chorionic villous sampling or amniocentesis should be offered after a positive cfDNA screen to confirm the diagnosis. | Best practice |
| 7 | Traditional aneuploidy screening and cfDNA aneuploidy screening should not be performed at the same time. | Best practice |
| 8 | After a failed cfDNA test, genetic counseling should be performed that includes offering diagnostic testing (chorionic villous sampling or amniocentesis) and repeat cfDNA screening. | Best practice |

cfDNA, cell-free DNA.

SMFM. Prenatal aneuploidy screening with cfDNA. *Am J Obstet Gynecol* 2015.

2015. All Committee members (Sean Blackwell, MD, Vincenzo Berghella, MD, Joseph Biggio, MD, Aaron Caughey, MD, PhD, Sabrina Craigo, MD, Jodi Dashe, MD, Cynthia Gyamfi-Bannerman, MD, Judith Hibbard, MD, Jamie Lo, MD, Tracy Manuck, MD, Mary Norton, MD, Luis Pacheco, MD, Lauren Plante, MD, Eva Pressman, MD, Laura Riley, MD, Anthony Sciscione, MD, Neil Silverman, MD, Methodius Tuuli, MD, Christopher Robinson, MD, George Saade, MD, Priya Rajan, MD, George Wendel, MD, Jeffrey Ecker, MD) have filed conflict of interest disclosures delineating personal, professional, and/or business interests that might be perceived as a

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