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Cell-Free DNA Screening: Complexities and Challenges of Clinical Implementation

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Abstract

Screening for fetal aneuploidy in pregnant women using cell-free DNA has increased dramatically since the technology became commercially available in 2011. Since that time, numerous trials have demonstrated high sensitivity and specificity to screen for common aneuploidies in high-risk populations. Studies assessing the performance of these tests in low-risk populations have also demonstrated improved detection rates compared with traditional, serum-based screening strategies. Concurrent with the increased use of this technology has been a decrease in invasive procedures (amniocentesis and chorionic villus sampling). As the technology becomes more widely understood, available, and utilized, challenges regarding its clinical implementation have become apparent. Some of these challenges include test failures, false-positive and false-negative results, limitations in positive predictive value in low-prevalence populations, and potential maternal health implications of abnormal results. In addition, commercial laboratories are expanding screening beyond common aneuploidies to include microdeletion screening and whole genome screening. This review article is intended to provide the practicing obstetrician with a summary of the complexities of cell-free DNA screening and the challenges of implementing it in the clinical setting.

Target Audience—Obstetricians and gynecologists, family physicians.

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Learning Objectives—After completing this activity, the learner should be better able to understand the complexities of cell-free DNA screening and describe considerations involved in its clinical implementation.

The American Congress of Obstetricians and Gynecologists (ACOG) recommends that all pregnant women, regardless of age, be offered aneuploidy screening before 20 weeks' gestation.[1] Before the development of first and second trimester screening tests, a woman's age and pregnancy history were the only means by which a provider could stratify a patient's risk for Down syndrome. Serum screening for Down syndrome became available in the 1980s, and since that time numerous screening options have evolved using a combination of serum analytes including alpha-fetoprotein, human chorionic gonadotropin, unconjugated estriol, inhibin A, and pregnancy-associated plasma protein A (PAPP-A), as well as ultrasound markers (such as first trimester nuchal translucency and presence or absence of nasal bone and second trimester markers such as an increased nuchal fold). In addition, screening has now expanded beyond Down syndrome to include trisomies 13 and 18.

Combinations of serum screening approaches and ultrasound have yielded the triple, quad, and penta screens; the first trimester combined test; the integrated screening test; and the sequential screening test (both stepwise and contingent). Invasive testing for aneuploidy, in the form of chorionic villus sampling or amniocentesis, has traditionally been offered to women who will be at least 35 years old at delivery or who have other risk factors for aneuploidy. However, practice has changed to make invasive diagnostic testing available to all pregnant women, regardless of age or other risk factors for aneuploidy.[2] This change is the result of an increased appreciation for the importance of patient preferences in decision making surrounding prenatal screening and diagnostic testing coupled with low loss rates from invasive diagnostic procedures and the ability to comprehensively screen for chromosome abnormalities and other genetic syndromes with microarray. As screening has evolved, the goal has been to maximize detection rate while minimizing the false-positive rate.

Discovery of Cell-Free DNA in Pregnancy

In 1997, researchers identified what they described as cell-free “fetal DNA” in maternal circulation by identifying Y-specific DNA fragments in serum and plasma samples collected from pregnant patients.[3] The following year, efforts to quantify the amount of cell-free DNA (cfDNA) from the pregnancy present in maternal serum using real-time polymerase chain reaction showed that it represented 3.4% to 6.2% of the total cfDNA in maternal plasma.[4] Subsequent studies have shown that the percentage may be as high as 10% to 20%.[5] Once the presence of cfDNA from a pregnancy was identified and quantified, it was immediately identified as a potential source of material for prenatal aneuploidy screening. In 1999, the first report that pregnancies with trisomy 21 had higher concentrations of circulating cell-free “fetal DNA” compared with those with euploid fetuses was published.[6] Thus began the race for laboratories to develop the technology to identify, quantify, and sequence cfDNA in pregnancies to screen for aneuploidy and other genetic conditions.

Technology

Cell-free DNA from a pregnancy is thought to come from apoptotic trophoblastic cells and is thus placental in origin. Some researchers have dropped the term “fetal” from current descriptions of the technology to reflect this understanding. Cell-free DNA circulates as very small fragments (less than 200 base pairs), is detectable in maternal serum as early as 7 weeks' gestation, and is rapidly cleared after delivery.[7,8] Numerous terms have been used to describe these tests, including noninvasive prenatal testing, noninvasive prenatal screening, noninvasive prenatal diagnosis, and cfDNA screening.

The first commercial screening test for aneuploidy using cfDNA became available to patients and providers in 2011. Since then, other platforms have become available, and now patients and providers may choose from several different commercial tests sold by various laboratories. Each of the platforms that provide these tests uses different applications of next-generation sequencing technologies. The first described technique was massively parallel shotgun sequencing, which refers to technology that counts and sequences millions of fragments of DNA in plasma, thereby yielding tens of millions of short sequence reads. The laboratory then maps these short sequences to the chromosome of origin.[9] If there is excess DNA present from the chromosome of interest (ie, chromosome 21), this result is consistent with aneuploidy for that chromosome. The approach is described as “shotgun” because all chromosomes are sequenced and mapped. A similar but alternative platform amplifies, sequences, and maps fragments from targeted chromosomes (eg, 13, 18, 21, and sex chromosomes).[10] This approach is called targeted massively parallel sequencing. Because massively parallel shotgun sequencing and targeted massively parallel sequencing compare actual to expected amounts of DNA, these techniques are not able to make a distinction between maternal and placental cfDNA. Alternatively, a third approach uses differences in single-nucleotide polymorphisms (SNPs) between maternal and placental DNA to determine risk for aneuploidy by performing targeted amplification and analysis of thousands of SNPs on chromosomes 21, 18, 13, X, and Y.[11]

Uptake and Impact on Invasive Procedures

Since its introduction to clinical practice, cfDNA screening for aneuploidy has seen extraordinary uptake among high-risk patients in the United States. [12–14] This has been observed among obstetricians and maternal fetal medicine specialists alike.[15] Regional differences in uptake in the United States have been documented with 1 study showing less frequent cfDNA screening in the Midwest compared with the East and West coasts.[16] The rapid application of cfDNA screening for aneuploidy has also been seen globally.[17–19]

Concurrent with the introduction of cfDNA screening in clinical practice has been a documented decrease in invasive procedures. Even before cfDNA screening was widely available, decision-analytic models predicted a decrease in both invasive procedures and pregnancy loss related to this testing. One model predicted that cfDNA screening would decrease invasive procedures in high-risk pregnancies by more than 95% and reduce euploid pregnancy loss rates by more than 99%.[20] Subsequent studies investigating these predictions confirmed these estimates. One large, retrospective study of more than 15,000 procedures

performed over 9 years at 1 hospital in the United States demonstrated a decrease in amniocentesis (76%) and chorionic villus sampling (54%) after the introduction of cfDNA screening in 2012.[21] Numerous other studies have confirmed a decrease in the number of invasive procedures that prenatal diagnosis centers are now performing.[22]

Professional Society Guidelines

Since 2011, numerous professional societies have published guidelines for the use of cfDNA screening in pregnancy, including ACOG, the Society for Maternal Fetal Medicine (SMFM), the National Society for Genetic Counselors, the International Society for Prenatal Diagnosis (ISPD), and the American College of Medical Genetics and Genomics. Initial guidelines from all major societies recommended limiting the use of cfDNA screening to only those pregnancies at increased risk of aneuploidy. Increased risk was typically defined as age 35 years or older at the time of delivery, ultrasound findings that suggest an increased risk of aneuploidy, positive first or second trimester screening tests for aneuploidy, a history of a previous pregnancy with trisomy, or a parental balanced Robertsonian translocation that increases the risk of trisomy 21 or 13.[23] As more research became available validating the performance of cfDNA screening in general obstetric populations, some societies softened previous recommendations to limit cfDNA screening to high-risk patient populations. In a 2012 Committee Opinion, ACOG recommended that cfDNA not be offered to low-risk women or women with multiple gestations.[23] In September 2015, a joint ACOG/SMFM Committee Opinion was published that stated “given the performance of traditional screening methods and the limitations of cfDNA, conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population.” ACOG and SMFM, however, acknowledged that patients may choose cfDNA screening regardless of their risk status, and in those cases, the limitations and benefits of this screening strategy should be discussed in the context of alternative screening and diagnostic options. ACOG and SMFM continue to recommend that patients should receive pretest counseling to discuss the risks and benefits of screening, that diagnostic testing be recommended to patients who receive a positive cfDNA screening result, and that pregnancy management decisions, including termination, should not be made based upon a cfDNA screen result alone.[24]

Earlier in 2015, ISPD updated its position statement on chromosome abnormality screening. Previously, the society recommended that cfDNA screening be limited to women at increased risk for aneuploidy only. In their updated guidelines, however, ISPD listed many different screening protocols that they consider appropriate including the use of cfDNA screening as a primary test offered to all pregnant women, or the use of cfDNA screening in combination with numerous other screening strategies.[25]

While some laboratories will report results on cfDNA screening tests performed on twin pregnancies, ACOG and SMFM do not recommend the use of cfDNA screening in multiple gestations. Instead, these organizations acknowledge that preliminary studies suggest that this form of screening is accurate but await the results of larger prospective studies before changing this recommendation.[24] ISPD states that if a cfDNA test is interpretable,

performance in a twin pregnancy is expected to be equivalent to that for singletons, but does not provide specific guidelines for or against the use of cfDNA in twin pregnancies.[25]

Performance of cfDNA Screening Tests

Validation studies published soon after cfDNA became widely available demonstrated high sensitivity and specificity to screen for aneuploidies in high-risk populations regardless of the technology or the platform studied.[10,11,26,27] This was especially true for trisomies 21 and 18. When comparing cfDNA screening tests in high-risk populations to other prenatal screening options, cfDNA screening has been shown to have higher detection rates and lower false-positive rates and can be performed at an earlier gestational age (Table 1). In subsequent studies investigating clinical performance in low-risk or general obstetrical populations, cfDNA screening continued to show higher sensitivity and specificity when compared with other screening tests.[12,32–34]

A recent meta-analysis of 37 studies in both high-risk and general-risk populations investigating cfDNA screening for aneuploidy found a pooled weighted detection rate (DR) for trisomy 21 of 99.2%, for trisomy 18 of 96.3%, and for trisomy 13 of 91.0%.[28] It is worth noting that for most validation and clinical studies, cases with mosaicism (potentially leading to a false-positive result), complex karyotypes, and samples with a low fetal fraction were excluded. This has the potential to overstate the detection rates and minimize the false-positive rates. The concept and implications of low fetal fraction will be described later in this review.

Overall, despite differences in the technology that is used, performance among the available platforms in clinical trials is similar, and as such, 1 platform is not recommended over another based upon peer-reviewed and published performance data.

Complexities and Challenges Of Clinical Implementation

Cost

One of the central tenets of medical screening is cost-effectiveness.[35] The cost of cfDNA screening can be one of the largest challenges of widespread clinical use. In the United States, the cost of the various cfDNA screening tests remains high, ranging from approximately $700 to over $2700.[36,37] Actual cost to the patient is a function of the laboratory used, the conditions screened for, whether or not the patient has insurance, and if she has insurance, the extent to which her insurance company reimburses the cost of the test.

While the actual costs to patients may not be high, widespread use at current prices have significant implications on the population level. Several researchers have performed decision-analytic and cost-effective modeling studies to investigate the economic impact of using cfDNA screening alongside or instead of traditional screening methods. These studies are challenging because the value of cfDNA screening must be considered from different standpoints (ie, patient, payer, governmental, or societal). In addition, the cost of cfDNA screening must be compared with the cost of several different serum screening strategies.[38–43] A recent economic decision analysis compared conventional screening to

cfDNA screening in a theoretical cohort of pregnant women in the United States and found that replacing conventional screening with cfDNA screening would reduce health care costs if the test could be provided for $744 or less in the general pregnancy population. Further, in this model, cfDNA had a higher detection rate compared with conventional screening (96.52% vs 85.9%), reduced the theoretical number of invasive procedures by 60%, and reduced the number of theoretical procedure-related euploid pregnancy losses by 73.5%.[38]

Notably, most studies investigating the cost-effectiveness of cfDNA screening have been performed by, or in association with, commercial laboratories. These studies are often limited in that they do not consider all potential outcomes of a prenatal screening or diagnostic testing strategy (eg, a failed test, an anomaly that would have been detected by another method of screening, or a chromosomal finding for which there is no current cfDNA-based screening). In 1 study, when a comprehensive set of outcomes was considered using 6 different testing strategies (diagnostic testing with chromosomal microarray, multiple marker screening, cfDNA screening, and nuchal translucency screening alone, in combination, or in sequence), the authors found that multiple marker screening with the option of diagnostic testing for screen-positive results was the optimal strategy for most women. In addition, they concluded that it is not until age 40 years that cfDNA screening became optimal and cost-effective as a primary screening test.[44] While the cost associated with cfDNA screening may not be prohibitive to an individual patient, the aggregate costs of using this technology will continue to influence public health, governmental agencies, and private payers.

Interpreting Results

One challenge in interpreting results of cfDNA screening test is the amount of variability in how results are reported to patients and providers. First, laboratories are not consistent with the language used to communicate results. Examples of screen-positive results include “positive,” “high risk,” “aneuploidy detected,” and “aneuploidy suspected.” Similar language is often used for negative results. Often, this language is paired with a numeric probability (often greater than 99/100 [99%]), which is reflective of the laboratory's confidence that the result is abnormal rather than the odds that the pregnancy is affected given the abnormal result. If a laboratory reports a “positive” result for trisomy 21 paired with a sensitivity and specificity of 99%, and a “probability” of 99/100, it is plausible that a patient or provider may interpret this result as nearly diagnostic. To appropriately interpret the results of a screening test, however, it is important to understand the relationship between a test's sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) (Table 2). In this setting, a test's sensitivity (or detection rate) is the number of affected pregnancies identified by the test divided by the total amount of affected pregnancies. The specificity of a test is the number of unaffected pregnancies identified by the test divided by the actual number of unaffected pregnancies. Neither the sensitivity nor the specificity of a test is affected by disease prevalence. Sensitivity and specificity are population measures that look backwards at results gathered over time and are of less use to clinicians and patients than the indices that assess the test's ability to accurately predict an actual result. The PPV and NPV of a test are of more use in the clinical setting as they are individual measures that are forward-looking.[35] The PPV, or the likelihood that a pregnancy

is affected if the test is positive, is a function of disease prevalence. Laboratories who perform cfDNA screening tests often tout their tests' sensitivity and specificity in direct-to-patient and direct-to-provider marketing as a measure of accuracy. False-negative and false-positive cfDNA screening results have been reported despite very high sensitivity and specificity.[45,46] ACOG and SMFM have encouraged laboratories to report both PPV and residual risk (the risk that a patient may have an affected pregnancy despite a negative test) on their reports but most laboratories do not currently report this information.[24] A positive result with a low, but undisclosed, PPV may be misinterpreted as diagnostic by some patients or providers. There is evidence that up to 6% of women who received positive cfDNA screening results proceeded with pregnancy termination without confirming results.[32]

A woman's a priori risk for aneuploidy, as determined by her age or other risk factors, is an important consideration when interpreting screening tests for aneuploidy. Screening performed in low-risk women will have a lower PPV than the same screening test performed in a high-risk woman. Because a priori risk affects the PPV of cfDNA and laboratories are inconsistent with reporting the PPV on reports, 2 Web-based calculators have been made available to estimate the PPV of cfDNA screening to assist providers in interpreting results and to enhance patient counseling.[47,48] The calculator developed by the Perinatal Quality Foundation can be found at https://www.perinatalquality.org/Vendors/NSGC/NIPT/. The calculator developed by the University of North Carolina Department of Obstetrics and Gynecology can be found at: http://www.mombaby.org/NIPS. Until laboratories consistently report PPV and residual risk, tools such as these will continue to be important assets to providers and genetic counselors who interpret and communicate results to patients.

Fetal Fraction

Fetal fraction, or the proportion of circulating cfDNA that is from the fetoplacental unit, is the single most important factor that determines the reliability of a cfDNA screening result.[49] Some laboratories measure and report fetal fraction and require at least 4% to report a result. A low fetal fraction is the most common reason for a screen failure and occurs from 1% to 8% of the time, depending on the platform and laboratory used.[24] Fetal fraction is known to be affected by gestational age, maternal weight, and the presence of aneuploidy.[50,51] Obesity is associated with increasing total cfDNA, which is thought to be from increased apoptosis of adipocytes. Because fetal fraction is the percent of cell-free fetal DNA divided by maternal cfDNA, the overabundance of total cfDNA in obese pregnant women results in lower relative amounts of cfDNA from the fetus and the placenta and may increase the risk of a test failure.[52] In a nested case-control study of almost 400 pregnancies that had cfDNA screening between 11 and 13 weeks gestation, increasing maternal weight from 60 to 120 kg decreased the mean fetal fraction from 12% to 6%. Among women who weigh more than 250 lb, 10% may have a fetal fraction of less than 4%.[53]

Another common cause for a screen failure due to low fetal fraction is the presence of a pregnancy affected by aneuploidy. Studies have reported an increased fetal fraction in a pregnancy affected by trisomy 21.[54,55] However, studies have reported a significantly decreased fetal fraction in pregnancy affected by trisomy 18, trisomy 13, monosomy X, and

triploidy [50] Some laboratories recommend repeating the cfDNA screen at a later gestational age after an initial failure. However, the rate of repeat test failure may be as high as 40% to 50%, and the rate of aneuploidy has been reported to be significantly higher (odds ratio, 9.2; confidence interval, 4.4–19.0) among women who receive an uninterpretable result from cfDNA screening.[24,33] For this reason, ACOG and SMFM suggest that women who receive an inconclusive or uninterpretable result should receive additional genetic counseling and be offered ultrasound screening and diagnostic testing. If the patient elects for repeat cfDNA screening, the increased risk for aneuploidy and the risk of a repeat test failure should be disclosed and discussed.

False-Positive Results

Discordancy (false-positive or false-negative) between cfDNA screening tests and true fetal karyotypes have been reported and remain an important consideration when utilizing cfDNA screening for aneuploidy. The actual frequency of discordant results in clinical practice is unknown. Laboratories do not routinely request that discordant results be reported to them and there is no central registry for reports of false-positive or false-negative results.[45] Despite this, numerous causes of discordant results have been reported. The following are some of the more commonly reported reasons for a discordant or false-positive result:

Placental Mosaicism—As previously discussed, the primary source of cfDNA is trophoblastic tissue. The chromosome complement of the placenta may not be the same as that of the fetus. This phenomenon is known as confined placental mosaicism and is a common reason for a cfDNA screening tests to yield a false-positive result.

Demise of Co-Twin—Another cause for an abnormal cfDNA screen in an unaffected singleton pregnancy is the presence of a demised co-twin, often described as a “vanishing twin.”[56] When products of conception are successfully karyotyped, approximately half have a chromosomal abnormality[57]. In 1 study, 15% of false-positive screen results were the result of a demised co-twin.[58] There are no formal guidelines to indicate how long after the demise of a co-twin cfDNA remains present from the co-twin. Therefore, cfDNA screening is contraindicated in this setting. If fact, some providers who routinely use cfDNA screening recommend or require that an ultrasound be performed at the time of cfDNA screening to rule out a demised co-twin or spontaneous abortion to minimize the risk of a discordant result or reporting a result on a nonviable pregnancy.

Maternal Karyotype Abnormality—Another important cause of discordant results of cfDNA screening is abnormal maternal chromosome complement. Previously unknown maternal mosaicism (low-level maternal Turner syndrome, for example) may yield a positive screen for sex aneuploidy in an unaffected pregnancy.[59,60] Maternal copy-number variations are other potential sources of discordant results.[61] Copy-number variants are structural alterations in a chromosome as a result of a duplication or deletion. Because most cfDNA is maternally derived, if a woman carries a previously unknown deletion or duplication on a targeted chromosome, it is plausible that some cfDNA platforms may interpret this underrepresentation or overrepresentation as fetal aneuploidy or a fetal microdeletion or duplication.

Maternal Malignancy—Another source of a positive cfDNA screen in an unaffected pregnancy is the presence of a maternal malignancy. The first report of a discordant cfDNA screening test and a subsequently diagnosed maternal malignancy was published in 2013. In this case, the patient's cfDNA screen returned positive for fetal trisomy 13 and monosomy 18. The patient elected for an amniocentesis, and karyotype and microarray were consistent with a normal male fetus (46,XY). The patient was subsequently diagnosed with a metastatic neuroendocrine carcinoma in the postpartum period.[62] After this case report, occult maternal malignancy became part of the differential diagnosis for discordant cfDNA screening results. Since then, a case series of 10 cases of patients with cancers diagnosed after discordant cfDNA screening has been published.[63] Seven of the 10 cases reported in this series had cfDNA results indicative of multiple aneuploidies detected, and 1 of the 10 had results consistent with a nonviable monosomy. It is presumed that the source of the abnormal cfDNA is the cytogenetic abnormalities in the tumor cells. In addition to the previously described neuroendocrine carcinoma (that was included in this case series), other occult malignancies included 3 cases of non-Hodgkin lymphoma, an acute T-cell lymphoblastic leukemia, anal cancer, and colorectal cancer. Using their data, the authors of this series estimated that there is between a 20% and 44% risk of maternal cancer if multiple aneuploidies are detected using cfDNA screening. These data need to be confirmed by additional studies before recommendations can be made about how to evaluate a cfDNA test that is positive for multiple aneuploidies.

Maternal Health Implications

As discussed previously, it is clear that cfDNA screening has the potential to discover previously unknown conditions in a pregnant woman. Some of these conditions may have no clinical significance or may have uncertain clinical significance. Other conditions, as in the case of an occult malignancy, have significant medical implications for a woman. The potential maternal health implications of cfDNA screening for aneuploidy continue to be researched and debated. However, there are as yet no guidelines for laboratories or providers regarding how incidental maternal health implications of cfDNA should be reported and what evaluation and follow-up is appropriate. In the interim, providers must be aware of the maternal health implications of cfDNA screening and must decide to what extent these possibilities should be addressed during pretest counseling.

Comparison to First Trimester Screening

It is important to remember that cfDNA screening does not screen for every possible chromosomal abnormality. Cell-free DNA screening is highly sensitive and specific in screening for trisomies 21 and 18, and to a lesser extent trisomy 13. However, a large retrospective cohort study of over 450,000 women in California participating in a state-wide prenatal screening program who received first trimester screening, second trimester screening, or both in an integrated risk assessment demonstrated that the program achieved high detection rates for trisomy 21 of 92%, for trisomy 18 of 93.2%, and for trisomy 13 of 80.4% while also detecting 80% of all chromosome abnormalities (including other trisomies, monosomy, translocations, additions, duplications, inversions, rings, polyploidy, triploidy, and other chromosome abnormalities).[64] Another study evaluated how many chromosome abnormalities were detected by diagnostic testing as compared with cfDNA screening

among women who were identified as screen-positive by traditional screening. Over 1.3 million patients were screened in a state-wide screening program, and 68,990 (5.2%) were screen-positive. Of the chromosome abnormalities detected among these screen-positive women, 83.1% had a chromosome abnormality that was predicted to be detectable with current cfDNA screening methods, whereas 16.9% had a chromosome abnormality considered not currently detectable.[65] Both of these studies underscore the fact that current cfDNA screening tests do not detect all chromosome abnormalities, some of which may be detected by conventional screening followed by invasive testing.

Screening Beyond Common Aneuploidies

Sex Chromosome Aneuploidy—Soon after the rapid adoption of cfDNA screening in high-risk populations, laboratories began investigating and reporting the expansion of the technology to screening for conditions beyond common aneuploidies. Cell-free DNA screening has remarkably high detection rates for fetal sex with 1 meta-analysis reporting a sensitivity of 94.4% and a specificity of 98.6% for detecting Y chromosome sequences among women pregnant with a male fetus.[66] Laboratories who report fetal sex chromosome complement now often report fetal sex chromosome aneuploidy (SCA), when suspected. Consequently, patients who may be motivated to pursue cfDNA screening for autosomal aneuploidies or for the purposes of determining fetal sex may also receive simultaneous SCA screening. The most common SCAs are 47,XXX, 47,XXY, 47,XYY, and 45,X and have a combined incidence of 1/350 to 1/400 live births.[67] Ultimately, clinical performance of cfDNA screening tests to detect SCAs in large populations is unknown. Most data regarding clinical performance centers on the sensitivity to detect nonmosaic monosomy X (45,X). Four published studies report detection rates for monosomy X ranging from 75% to 92%.[26,68–70] Robust performance data for the less common SCAs are not yet available.

The phenotypes of people with SCA are highly variable. Most people with SCAs other than 45,X have few serious physical abnormalities, especially when compared with autosomal trisomies. This makes prenatal counseling and decision making complex when a cfDNA screening test returns positive for an SCA. Further complicating this counseling is the fact that maternal mosaicism for an SCA may result in a false-positive cfDNA pregnancy screen.

Microdeletion Syndromes—In addition to autosomal and SCA, laboratories have expanded screening to include various microdeletion syndromes. Microdeletions are small deletions (<5 Mb) that often span several genes and are typically too small to be detected by traditional or high-resolution karyo-types. They differ from autosomal aneuploidies in 2 key ways: they are not related to maternal age, and individually, they are far less common than autosomal or sex aneuploidies. Expanded screening panels offered by some laboratories include screens for 22q11 deletion syndrome, cri-du-chat (5p-), Wolf-Hirschhorn syndrome (4p-), Prader Willi or Angelman syndrome, 11q deletion (Jacobsen syndrome), 8q deletion (Langer-Giedion syndrome), 1p36 deletion syndrome, and some rare trisomies often associated with nonviable pregnancies (trisomies 16 and 22). 22q11 deletion syndrome is the most common of the microdeletion syndromes screened by current expanded panels, with a prevalence of 1:4000 to 1:6000.[71] Other microdeletion syndromes have even lower prevalence. Cru-di-chat syndrome, for example, is estimated to be present in 1 in 50,000 live

births.[72] Screening for conditions with such low prevalence will inevitably result in false-positive results as the PPV will be low. One recent study investigating SNP-based methods to detect microdeletion syndromes found PPVs that ranged from 3.8% to 17%, depending on the syndrome.[73] When some laboratories began offering expanding microdeletion panels, they did so in an “opt-out” manner. Similar to SCAs, data on the clinical performance of cfDNA screening tests for microdeletion syndromes in large populations are not available. Due to lack of clinical data, ACOG and SMFM recommend against routine use of cfDNA screening for microdeletion syndromes.[24]

Whole Genome Screening—Thus far, expansion of cfDNA screening has been largely driven by clinical laboratories and the scope of this screening continues to expand. One of the cfDNA platforms is now offering clinical testing with whole genome screening and reporting any aneuploidy, select microdeletions, and any chromosomal gains or losses greater than 7 Mb. This is roughly on par with the resolution of a karyotype and does not provide the same resolution as a chromosomal microarray analysis.[74] Data demonstrating actual clinical performance of this test have not been published, and guidelines regarding its use in clinical practice have not been issued.

Ethical, Legal, and Social Considerations

Beyond the challenges of clinical implementation, the rapid update of prenatal cfDNA screening has raised numerous ethical and legal considerations. An early concern of noninvasive prenatal screening was that of burdening women with decision-making regarding results of cfDNA screening tests performed on early pregnancies that are destined to miscarry.[75] The fact that cfDNA screening can be carried out at a gestational age earlier than conventional screening may be a benefit to some but a liability to others. In addition, as the cost of cfDNA screening goes down, some have questioned whether the safety and accuracy of cfDNA screening may shift it from an option involving a patient's preferences and informed consent to a routine procedure included in initial prenatal laboratories.[76] This would represent a dramatic shift in the prenatal screening paradigm from one that was traditionally performed in targeted populations. If it is to remain a procedure that a well-informed woman can accept or reject, consideration must also be given to how the process of obtaining informed consent should evolve alongside the ever-increasing number of conditions that laboratories screen for using cfDNA; each with their own prevalence, detection rate, and PPV. Ethical and legal concerns increase substantially when consideration is given to the potential that cfDNA offers in the near future—from testing for disease predisposition (ie, breast cancer), to paternity, to late-onset conditions (ie, Huntington disease), to sequencing of the whole fetal genome. Some have questioned whether or not prenatal screening or testing for more minor conditions may trigger or exacerbate belief in “genetic determinism”: the belief that a person's genes determine their capacity and characteristics beyond that of environmental influences (social environment and upbringing).[77] Potential expansion of the technology to broad-scope screening (phenotypically mild conditions, variants of unknown significance, genetic predisposition for certain diseases, or susceptibilities for behavioral characteristics) has caused others to question the benefit-to-harm ratio for the child.[78] On one hand, there may be benefit to diagnosing a condition that may have otherwise gone unnoticed over the course of a child's

lifetime because the child may receive improved care. On the other hand, a child's future autonomy may be undermined by no longer making it possible for them to “not know.”[79] In addition, the child may be subjected to harm in the form of diminished self-esteem, parental overprotectiveness, and stigmatization.[78]

Another ethical concern of wide-spread use of cfDNA screening is that of utilizing the technology for the purposes of sex selection. Globally, sex selection and sex-selection abortion almost always results in the abortion of female fetuses.[80] Given the accuracy that cfDNA screening offers for detecting fetal sex at an early gestational age, the concern that this technology may be used solely for the purposes of sex selection seems well-founded.

Summary

The use of cfDNA in maternal blood for prenatal screening is rapidly evolving. Soon after this technology became available for clinical use, it expanded beyond screening for trisomy 21 to include screening for trisomy 18, trisomy 13, and fetal sex. Recently, commercial laboratories have begun to offer expanded cfDNA screening options that include sex aneuploidies, microdeletion syndromes, and even whole genome sequencing. While detection rates for common aneuploidies are high in clinical trials and the use of invasive testing had decreased since these screening tests became available, there continue to be clinical challenges and complexities that make the use of these tests in all women, for all conditions offered, anything but routine. Clinicians who elect to offer prenatal screening using cfDNA should be familiar with the performance of the test used, the role that a patient's age (or other risk factors for aneuploidy) may play in the test's performance, the conditions being screened for, the manner in which results are reported, and how to interpret results to patients. Thorough pretest and posttest counseling to discuss the risks and benefits to screening should be provided to patients who elect for cfDNA screening. Clinicians and counselors should have an understanding of the common reasons for false-positive and false-negative results and should offer patients confirmatory testing in the event of a positive screen. Providers should be familiar with society guidelines that provide guidance regarding the use of cfDNA screening in women at low-risk for aneuploidy and women with multiple gestations. Finally, physicians and genetic counselors should be familiar with the ethical and social considerations that are being raised as the use of this technology quickly moves beyond screening for common aneuploidies within the confines of clinical trials to wide-spread commercial use driven by industry.

Practical Pearls

- Cell-free DNA screening has a much higher detection rate for trisomy 21 than traditional, serum-based first or second trimester screening. Performance for trisomies 13 and 18 is somewhat less robust.
- Patients should receive pretest counseling detailing the risks, benefits, and alternatives to cfDNA screening. In addition, they should receive post-test counseling regarding the meaning of their results and the limitations of the test.

- Laboratories present positive and negative results of cfDNA screening in multiple different ways, and providers should be familiar with the meaning and implications of their laboratory's report.
- PPV is a function of disease prevalence. The probability that a positive result is a true positive is influenced by a patient's prior risk. A patient's age and other risk factors for aneuploidy should be taken into consideration when interpreting a positive result.
- An uninterpretable or inconclusive result increases a patient's risk for aneuploidy, and these patients should be offered comprehensive ultrasound and diagnostic testing.
- Positive screening results should be confirmed with diagnostic testing.
- cfDNA screening does not test for all genetic conditions, and a negative result does not ensure a pregnancy is unaffected.
- There is limited evidence for the clinical performance of cfDNA screening for microdeletion syndromes, and the routine use of cfDNA to screen for these conditions is not currently recommended.
- There are no peer-reviewed data available on whole genome screening using cfDNA.

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Table 1

Comparison of Prenatal Screening Options[1,28–31]

	cfDNA Screening	First Trimester Screening	Second Trimester Screening (Quad Screen)	Integrated Screen (NT, PAPP-A, Quad Screen)
Gestational age	>10 wk	11–14 wk	15–24 wk	PAPP-A: 9–13 wk NT: 10–13 wk Quad screen: 15–24 wk
Detection rate				
Trisomy 21	99.2	82–87	81	94–96 *
Trisomy 18	96.3	81	60	90 †
Trisomy 13	91.0	Limited data	N/A	Limited data

* Detection rate for trisomy 21 of a fully integrated screen that includes a nuchal translucency measurement.

† Detection rate of trisomy 18 of a serum integrated screen.

NT indicates nuchal translucency; N/A, not applicable.

Table 2

Important Screening Tests Indices

Sensitivity	Probability that a screening test will be positive if pregnancy is affected
Specificity	Probability that the screening test will be negative if pregnancy is unaffected
PPV *	The probability that a pregnancy is affected if the test is positive
NPV *	The probability that a pregnancy is unaffected if the test is negative

* Both PPV and NPV are dependent upon the disease prevalence or a priori risk.