Expanding the indications for cell-free DNA in the maternal circulation: clinical considerations and implications

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Noninvasive prenatal testing for fetal aneuploidy using cell-free DNA has been widely integrated into routine obstetrical care. The scope of cell-free DNA testing has expanded from trisomies 21, 18, and 13 to include sex chromosome conditions, panels of specific microdeletions, and more recently genome-wide copy number variants and rare autosomal trisomies. Because the technical ability to test for a condition does not necessarily correspond with a clinical benefit to a population or to individual pregnant women, the benefits and harms of screening programs must be carefully weighed before implementation. Application of the World Health Organization criteria to cell-free DNA screening is informative when considering implementation of expanded cell-free DNA test menus. Most microdeletions and duplications are rare to the point that the prevalence has not even been defined and their natural history cannot be reliably predicted in the prenatal period. At the current time, scientific evidence regarding clinical performance of expanded cell-free DNA panels is lacking. Expanded cell-free DNA menus therefore create a dilemma for diagnosis, treatment, and counseling of patients. The clinical utility of expanding cell-free DNA testing to include panels of microdeletions and genome-wide assessment of large chromosomal imbalances has yet to be demonstrated; as such, the clinical implementation of this testing is premature.

Key words: cell-free DNA, copy number variants, duplication, genome-wide copy number variant, microdeletion, noninvasive prenatal testing, prenatal screening, rare autosomal trisomies

Noninvasive prenatal testing (NIPT) for fetal aneuploidy using cell-free DNA (cfDNA) has been widely integrated into routine obstetrical care.[1] Initially, cfDNA tests focused on chromosomal aberrations addressed by conventional prenatal screening methods, namely trisomy 21, trisomy 18, and trisomy 13. The scope soon expanded to include sex chromosome aneuploidy and microdeletion panels. Recently genome-wide analysis has become available, expanding the scope of NIPT to address rare autosomal trisomies and large copy number variants (CNVs), although detection of smaller CNVs remains technically challenging.

NIPT using cfDNA may have the potential to address a wide variety of conditions during pregnancy. However, the technical ability to test for a condition does not necessarily correspond with a clinical benefit to a population or to individual pregnant women.

The benefits and harms of screening should be considered

Biological limitations exist that preclude cfDNA testing from being a diagnostic test. The cfDNA in maternal circulation that originates from the pregnancy is derived primarily from placental tissue and may not necessarily represent fetal genetic status.[2–4] cfDNA testing is therefore able to identify only pregnancies at high risk for certain conditions and confirmatory testing such as chorionic villus sampling (CVS) or amniocentesis is required for definitive diagnosis.[1,5]

The benefits and harms of screening programs must be carefully weighed before implementation. The World Health Organization has developed criteria to guide the selection of conditions that are suitable for screening programs (Table 1).[6] These criteria have recently been updated to include emerging considerations in the era of genetic and genomic testing[7] and are useful to consider when implementing expanded cfDNA test menus.

Most microdeletions and duplications are rare to the point that the prevalence has not even been defined

Clinically significant CNVs, including microdeletions and microduplications, occur in close to 1.7% of pregnancies.[8] If all were detectable, the combined incidence is significant; however, the incidence of CNVs that are included in currently available microdeletion panels is quite low.

The most common microdeletion is 22q11.2 deletion, which has been estimated to occur in at least 1 in 4000 to 1 in 6000 live births[9,10] and has been documented in as many as 1 in 1000 pregnancies undergoing prenatal diagnosis.[8,11] The 22q11.2 deletion is the second most common genetic cause of developmental delay and congenital heart defects after trisomy 21.[12]

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TABLE 1
Criteria for the development of screening programs

Excerpted from Wilson and Jungner, WHO commission[6]

- The condition should be an important health problem.
- The natural history of the condition should be adequately understood.
- Facilities for diagnosis and treatment should be available.

Emerging screening criteria over the past 40 years[7]

- The screening program should respond to a recognized need.
- The objectives of screening should be defined at the outset.
- There should be scientific evidence regarding screening program effectiveness.
- The overall benefits of screening should outweigh the harm.

WHO, World Health Organization.

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Although screening for 22q11.2 deletion may be desirable because of its prevalence, the rationale for including other specific microdeletions is less apparent. For example, Jacobsen syndrome has a frequency of 1 in 100,000 (Table 2).

So-called whole genome or genome-wide cfDNA analyses claim to look beyond specific microdeletions to CNVs throughout the genome, including deletions and duplications as well as rare autosomal trisomies (RATs) involving chromosomes other than 21, 18, or 13. The majority of these conditions are exceedingly rare to the point at which the prevalence has not even been defined. It is extremely difficult to make the argument that these conditions are a significant enough health problem to warrant screening.

The natural history of CNVs and RATs cannot be reliably predicted

In the field of prenatal screening, prospective parents must be counseled regarding what the condition may mean for their unborn child. For many CNVs and RATs, the natural history is not well understood or cannot be reliably predicted in any individual case because of the variable clinical presentation. They may present clinically with anatomic abnormalities or adverse pregnancy outcome or be completely inconsequential.[20]

Some CNVs, such as 22q11.2 deletions, recur because of mutation hot spots and have been well described, but others have not been previously described and phenotype is unknown. RATs are often present in mosaic form, and it is not possible to predict the clinical phenotype prenatally because of the unknown percentage and distribution of abnormal cell lines throughout the body tissues. A lack of information about natural history and highly variable presentation makes it impossible to counsel patients regarding predicted pregnancy outcome.

TABLE 2
Frequency of CNVs included on commercial cfDNA screening tests

CNV	Frequency	Associated condition
22q11.2 deletion	1 in 1,000[8,11]	22q11.2 deletion syndrome
1p36 deletion	1 in 10,000[13]	1p36 deletion syndrome
15q11.2	1 in 10,000[14,15]	Prader-Willi syndrome or Angelman syndrome
5p15.3 (5p minus)	1 in 50,000[16]	Cri du chat syndrome
4p16	1 in 50,000[17]	Wolf Hirschorn syndrome
11q23	1 in 100,000[18]	Jacobsen syndrome
8q23–24	Unknown (rare)[19]	Langer Giedion syndrome

cfDNA, cell-free DNA; *CNV*, copy number variant.

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Whole-genome cfDNA analysis is driven by feasibility rather than clinical need

Massively parallel shotgun sequencing sequences cfDNA fragments from the whole genome. When reporting results only for the common trisomies, large quantities of unused sequencing data are generated. Expanding massively parallel shotgun sequencing—based cfDNA test options to whole-genome analysis utilizes these data without any additional benchwork rather than responding to a specific clinical need.[21]

Collectively, CNVs are relevant to pregnancy care, with clinically significant microdeletions and microduplications occurring in 1.65% of pregnancies in the general population.[8] However, studies of whole-genome NIPT suggest low test sensitivity for small chromosomal imbalances such as microdeletions.[22] Sensitivity depends on the size of the region as well as the depth of sequencing and the fetal fraction.[23,24] Thus, detection of small imbalances such as microdeletions would require greater depth of sequencing than that required for aneuploidy detection and would be particularly challenging at lower fetal fractions.

The depth of sequencing that can be provided is limited by cost considerations. Commercial cfDNA tests offer insufficient depth of sequencing to consistently identify CNVs of 5 Mb or smaller.[24] In one validation study of genome-wide cfDNA analysis,[25] the researchers limited their scope to imbalances of 7 Mb or greater as well as select microdeletions of <7 Mb and acknowledge that imbalances of 7 Mb or greater represent only 30% of subchromosomal CNVs.[26]

In another study of whole-genome cfDNA analysis,[27] only 3 CNVs less than 7 Mb, including only 1 case of 22q11.2 deletion, were identified in

more than 12,000 subjects, despite a relatively high depth of sequencing. The low numbers of CNVs detected in this population suggest that only a small percentage of affected pregnancies were identified.[22]

If the objective of testing is the minimization of any genetic risk to the pregnancy, expanded cfDNA menus are not the most effective method of accomplishing this goal. To maximize the detection of fetal genetic diagnoses, fetal diagnostic testing (CVS or amniocentesis) with chromosomal microarray should be offered.[28]

Scientific evidence regarding clinical performance of expanded cfDNA panels is lacking

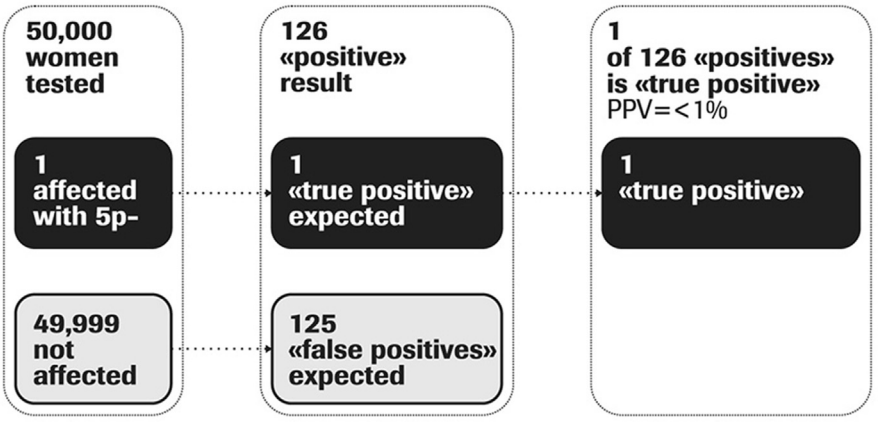
Despite the publication of proof-of-concept and analytical validation studies regarding cfDNA testing for panels of microdeletions[29,30] and genome-wide cfDNA analysis,[25,27,31–40] the clinical performance of these tests has not been described. Because of the rarity of individual CNVs and RATs, the determination of sensitivity and specificity in a prospective, blinded validation study would require enrollment of a prohibitively large number of patients.

There have been retrospective reports of laboratory experience,[30,41] but these do not report outcome data for all subjects, precluding determination of the detection rate (sensitivity) of the test and providing specificity that may be biased by ascertainment. In one report,[27] the authors claim 100% sensitivity for genome-wide cfDNA analysis, despite the lack of genetic test results or long-term follow-up for all subjects. Because some CNVs and mosaic aneuploidies may not be apparent from observing neonatal phenotype, long-term follow-up or genetic testing would be necessary to assess clinical performance.

The clinical utility of expanded cfDNA panels is limited in both low-risk and high-risk populations

The positive predictive value (PPV) for rare conditions will be low in routine clinical practice.[42–44] The PPV of a test refers to the proportion of positive test results that represent true positive results.

FIGURE 1
Positive predictive value is low for rare conditions



The PPV is low for rare conditions because true-positive results are expected to be less frequent than false-positive results. For example, the 5p15.3 deletion, which is included in cfDNA microdeletion panels, has a population frequency of close to 1 in 50,000. In a population of 50,000 pregnant women in the general screening population, it is expected that 1 fetus will be affected with 5p15.3 deletion. Based on a false-positive rate of 0.24%,[29] 125 false-positive results are expected in this same population of 50,000 women. If the affected pregnancy is detected by the cfDNA test and receives a positive result, 1 of 126 positive results will be true positives and 125 of 126 will be false positives. Thus, the likelihood of being truly affected with 5p15.3 deletion in the event of a positive cfDNA result is less than 1%.

cfDNA, cell-free DNA; *PPV*, positive predictive value.

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Thus, the PPV is dependent on the false-positive rate of the test (how common are false positives?) as well as the frequency of the condition in the population (how common are true positives?). For rare conditions in a low-risk population, false-positive results will be more common than true-positive results. For example, for 5p15.3 deletion, we would expect 125 false-positive results for every 1 true -positive result (Figure 1).

Expanded cfDNA test menus compound this problem. Each condition added to a screening panel adds to the overall false-positive rate of the test and decreases positive predictive value. This negates one of the most compelling benefits of NIPT, which is the significant reduction of false-positive results for the common trisomies as compared with conventional screening methods.

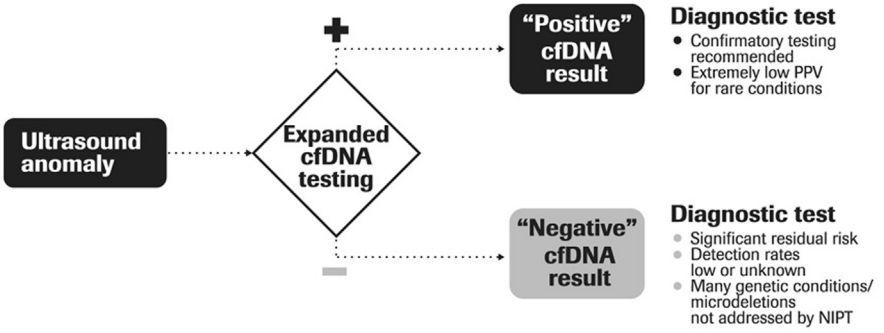
In high-risk pregnancies, such as cases of fetal anomalies detected by ultrasound, cfDNA testing may not alter medical management.[45–48] If the cfDNA result is positive, diagnostic testing is required to confirm a diagnosis because of low positive predictive value of cfDNA testing for rare conditions. If the cfDNA result is negative, a diagnostic test should still be offered (Figure 2).

CfDNA expanded panels do not address all conditions potentially detectable by diagnostic testing with chromosomal microarray, such as small CNVs and mosaicism, and detection rates may be relatively low for some conditions included on the cfDNA test panel. Thus, regardless of the cfDNA test result, a diagnostic test is indicated in high-risk pregnancies. The cfDNA testing in such cases increases cost and delays diagnosis without having an impact on the clinical outcome.

Expanded cfDNA menus create a dilemma for diagnosis and treatment

Because they have been associated with adverse pregnancy outcome, the identification of RATs has been presented as

FIGURE 2
CfDNA testing in high-risk pregnancies does not alter medical management



In the case of high-risk pregnancy, cfDNA testing is not expected to alter medical management. For example, in the case of an ultrasound anomaly identified by ultrasound, a diagnostic test is indicated, regardless of cfDNA result. If the cfDNA result is positive, diagnostic testing is required to confirm a diagnosis. If the cfDNA result is negative, a diagnostic test should still be offered because of significant residual risk.

cfDNA, cell-free DNA,

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potentially beneficial;[27,40] however, follow-up testing in such cases may be complex and of unclear benefit. RATs are most often mosaic and confined to the placenta. In one large study, only 5 of 60 of cases of cfDNA results indicating presence of a RAT (8%) were associated with true fetal mosaicism for a trisomy.[49]

The clinical significance of confined placental mosaicism (CPM) for RATs has not been well described. While there is compelling evidence that CPM for trisomy 16 detected by CVS can be associated with fetal abnormalities and pregnancy complications,[50] the risk of adverse pregnancy outcome associated with CPM for other specific chromosomes is not well established.

To complicate matters further, CVS and amniocentesis cannot definitively rule out mosaicism in the fetus or placenta, leaving patients and providers with uncertainty as to whether there may be increased cause for concern following a positive cfDNA test result with a normal diagnostic test result. In addition, CPM for specific chromosomes may prompt further genetic workup for uniparental disomy and possible imprinting disorders. Patients who are screen positive by cfDNA for a RAT face considerable uncertainty as to the clinical significance, and the optimal management of these pregnancies is currently unclear.[51–53]

TABLE 3
Pretest counseling guidelines

Pretest counseling should include:[1]

- Scope and nature of conditions being tested
- Performance of the test for each condition, including detection rate, false-positive rate, and no-call rate (no-call rate is dependent on gestational age and maternal weight[61])
- An explanation that false-positive results may be more common than true positive results for rare conditions
- The need to confirm results through additional testing
- The potential to detect maternal chromosome abnormalities and constitutional changes associated with malignancy
- Uncertainties associated with mosaicism and unexpected findings

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Counseling patients about the risks and benefits becomes increasingly challenging

Time and resources to adequately counsel patients about their options for prenatal screening are limited.[54,55] Counseling becomes more challenging with an increase in the complexity of testing options[56] and the potential of identifying conditions with an uncertain prognosis (Table 3). There is also the potential to identify previously undiagnosed genetic changes in the pregnant woman.[30]

Broadening the scope of cfDNA testing may undermine productive decision making.[57] Concerns regarding the routinization of complex genetic testing and equal access to testing options have also been raised.[58] Another aspect that deserves attention is the psychological burden that unexpected findings pose to women that chose cfDNA to avoid an invasive procedure but may end up having to undergo it for conditions of uncertain significance that may cast a shadow on their emotional experience around the pregnancy.

Current status

Professional societies do not currently recommend expanded cfDNA testing:

- The International Society for Prenatal Diagnosis recommends that "testing should be limited to clinically significant disorders with a well-defined severe phenotype".[1]
- The Society for Maternal-Fetal Medicine states that "routine screening for microdeletions with cfDNA is not recommended."[59,60]
- The European and American Societies for Human Genetics "argue for a cautious expansion of the scope of prenatal screening to serious congenital and childhood disorders, only following sound validation studies and a comprehensive evaluation of all relevant aspects."[58]
- The American College of Medical Genetics and Genomics "does not recommend [cfDNA testing] to

screen for autosomal aneuploidies other than those involving chromosomes 13, 18, and 21" and "does not recommend [cfDNA testing] to screen for genome-wide CNVs."[28] The authors go on to state that "if this level of information is desired, then diagnostic testing … is recommended."

Summary

Because cfDNA menus have expanded from the common trisomies (trisomy 21, trisomy 18, and trisomy 13) to include sex chromosome aneuploidy, panels of microdeletions and, more recently, genome-wide assessment, the clinical relevance of assessed conditions has decreased, validation data have become more scarce, and detection rates have decreased. Because false-positive rates are cumulative, the overall false-positive rate of cfDNA tests increases as more conditions are added to testing panels. In addition, patient counseling has become increasingly complex because of the variety of conditions tested as well as the potential for identifying conditions with uncertain prognosis. Another critical factor that has not been addressed in this analysis but that warrants further consideration is the cost-effectiveness of expanded screening panels.

The clinical utility of a test refers to its ability to improve health outcomes for patients. Specifically, the test results must change clinical decision making, and these changes should be beneficial to patients. The clinical utility of expanding cfDNA testing to include panels of microdeletions and genome-wide assessment of large chromosomal imbalances has yet to be demonstrated; as such, the clinical implementation of this testing is premature. ■

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