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SUPPLEMENT TO
OBG MANAGEMENT
December 2020

Applying Guidelines in Practice: Noninvasive Prenatal Testing

This activity is supported by an independent educational grant from Roche Diagnostics.

OVERVIEW:

This supplement is designed to provide ObGyn clinicians with current information on the cell-free DNA screening test options available for fetal chromosomal abnormalities. These screening tests are commonly referred to as Noninvasive Prenatal Screening (NIPS). In August 2020, the American College of Obstetricians and Gynecologists (ACOG) issued a Practice Bulletin entitled "Screening for Fetal Chromosomal Abnormalities" (PB #226). This Practice Bulletin included expanded information regarding the use of NIPS in all patients regardless of maternal age or baseline risk. It also identified NIPS as the most sensitive and specific test for screening for the most common aneuploidies. The authors of this supplement provide additional information on the technology, performance, and clinical utilization of NIPS testing.

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LEARNING OBJECTIVES:

After participating in this educational activity, participants should be better able to:

- Overcome barriers and demonstrate competency in integrating ACOG/ Society for Maternal-Fetal Medicine Noninvasive Prenatal Testing Committee Opinions/Practice Bulletins recommendations into clinical decision-making surrounding prenatal visits for all pregnant patients.
- Explain the benefits and disadvantages of traditional fetal chromosomal aneuploidy screening tests compared with noninvasive screening tests.
- Define the technology that is the basis of the various noninvasive screening tests, including the role that fetal fraction plays in influencing results.
- Explain the expanding role of NIPS in the general obstetrical population.

ACCREDITATION AND CREDIT DESIGNATION STATEMENTS:

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TARGET AUDIENCE:

This activity is designed to meet the educational needs of the obstetrician and gynecologist, family physician, internal medicine physician, physician assistant, nurse practitioner, and certified nurse midwife.

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COMMERCIAL SUPPORT:

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Preamble

Approximately 6 million pregnancies occur each year in the United States. Of this number, approximately 4 million result in live births and 600,000 are lost due to miscarriage. Genetic abnormalities are a common cause of miscarriage and congenital malformations. Nearly half of all miscarriages are associated with abnormal karyotypes and occur during the first few weeks of pregnancy. Genetic abnormalities also cause many birth defects.

Amniocentesis and chorionic villus sampling are among the screening tests traditionally used to identify fetal chromosomal aneuploidies (see **TABLE** on pages S4-S5). While they can provide a definitive diagnosis, these procedures are invasive and carry a small risk of miscarriage. Evolving diagnostic tests use maternal blood samples and are based on DNA sequencing technologies. These tests are noninvasive and do not carry the risk of miscarriage. Noninvasive cell-free DNA prenatal testing, or NIPT, is associated with nearly 100% sensitivity and specificity in the detection of trisomies 21, 18, and 13. It can be used in pregnant patients who are at both a normal or increased risk of fetal aneuploidies.

In August 2020, the American College of Obstetricians and Gynecologists (ACOG), in collaboration with the Society for Maternal-Fetal Medicine, published ACOG Practice Bulletin #226 entitled "Screening for Fetal Chromosomal Abnormalities." The purpose of this Practice Bulletin is to provide ObGyn clinicians with the most current information regarding the benefits, performance characteristics, and limitations of the available screening test options for fetal chromosomal abnormalities. The introduction to the Practice Bulletin states: *"This Practice Bulletin has been revised to further clarify methods of screening for fetal chromosomal abnormalities, including expanded information regarding the use of cell-free DNA in all patients regardless of maternal age or baseline risk, and to add guidance related to patient counseling."*

The articles that comprise this journal supplement will often reflect and expand upon the information presented in the ACOG Practice Bulletin. After reading this supplement, it is hoped that learners will possess the information necessary to better counsel their patients regarding NIPT and be able to more efficiently and effectively implement NIPT into their clinical practices.

—Omnia Education

TABLE Common Screening Tests for Chromosomal Abnormalities[1]

Screening Tests	Methodology	GA Range for Screening (weeks)	Detection Rate (%)/ Trisomy 21	Screen Positive Rate (%)	Strengths	Weakness(es)
Cell-free DNA	MPSS SNP Microarray	9-10 to term	99	2-4	Highest DR Can be performed at any GA ≥9-10 weeks Lowest screen-positive rate	Results may reflect underlying maternal aneuploidy or maternal disease
First Trimester	NT+PAPP-A, Freeβ-hCG, +/- AFP	10-13$^{6/7}$	82-87	5	Early screening Single time-point test	↓ DR than tests with 1st & 2nd trimester component NT required
Quad Screen	hCG, AFP, uE3, DIA	15-22	81	5	Single time-point test No specialized US required	↓ DR than 1st trimester and 1st & 2nd trimester combined tests
Integrated	NT+ PAPP-A, then quad screen	10-13$^{6/7}$ then 15-22	96	5	↑ DR	Two samples needed; no 1st trimester results
Serum Integrated	PAPP-A, then quad screen	10-13$^{6/7}$ then 15-22	88	5	DR compares favorably with 1st trimester screening No specialized US required	Two samples needed; no 1st trimester results NT required
Sequential stepwise	NT+ freeβ-hCG+ PAPP-A, +/- AFP, then quad screen, NT+hCG+PAPP-A, +/- AFP, then quad screen	10-13$^{6/7}$ then 15-22	95	5	1st trimester results provided Comparable performance to integrated, but FTS results provided 1st trimester test result: **Positive**: diagnostic test or cell-free DNA offered **Negative**: no further testing **Intermediate**: 2nd trimester test offered **Final**: risk assessment incorporates 1st and 2nd trimester results	Two samples needed NT required

TABLE Common Screening Tests for Chromosomal Abnormalities[1] *continued*

Screening Tests	Methodology	GA Range for Screening (weeks)	Detection Rate (%)/ Trisomy 21	Screen Positive Rate (%)	Strengths	Weakness(es)
Contingent screening	NT+ freeβ-hCG+ PAPP-A, +/- AFP, then quad screen, NT+hCG+PAPP-A, +/- AFP, then quad screen	10-13$^{6/7}$ then 15-22	88-94	5	1st trimester results provided Comparable performance to integrated, but FTS results provided 1st trimester test result: **Positive**: diagnostic test or cell-free DNA offered **Negative**: no further testing **Intermediate**: 2nd trimester test offered **Final**: risk assessment incorporates 1st and 2nd trimester results	Possibly two samples needed NT required
Nuchal translucency alone	US only	10-13$^{6/7}$	70	5	Allows individual fetus assessment in multiple gestations Provides additional screening for fetal anomalies	Poor sensitivity and specificity in isolation NT required

[1]Adapted from the ACOG NIPT Practice Bulletin Number 226 *Screening for Fetal Chromosomal Abnormalities* Vol. 136, No. 4, October 2020.
Abbreviations: AFP, alpha-fetoprotein; DIA, dimeric inhibin-A; DR, detetcion rate; FTS, first-trimester screening; GA, gestational age; hCG, human chorionic gonadotropin; MPSS, massively parallel signature sequencing; NT, nuchal translucency; PAPP-A, pregancy-associated plasma protein A; SNP, single-nucleotide polymorphism; uE3, unconjugated estriol; US, ultrasonography.

NIPT: Overview of Technology

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Since its introduction and support by the American College of Obstetricians and Gynecologists in 2011, noninvasive prenatal testing (NIPT) has undergone rapid adoption and evolution.[1] NIPT rests on a history of prenatal diagnostics to detect chromosomal disorders that began in the 1960s.[2] Since that time, the addition of chorionic villus sampling (CVS) to the list of invasive procedure options fueled the desire to obtain prenatal assessments at earlier stages of pregnancy. This advance was followed by the development of noninvasive approaches to avoid the procedural risks and, for many women, the discomforts of both amniocentesis and CVS. The desire for improved sensitivities and specificities of screening converged with a nascent body of work regarding the presence and nature of circulating cell-free DNA (cfDNA),[3-5] the development of massively parallel sequencing (MPS),[6,7] and techniques to count DNA fragments. This convergence and continued advancement of technologies, coupled with a deepening understanding of cfDNA, has given women's health care providers a powerful and expanding screening tool to assess the genomic status of a developing fetus. With new technology advancements, NIPT using cfDNA and fetal cells will further evolve to replace amniocentesis and CVS in the future.

Biologic Basis of NIPT

The presence of cfDNA has been known for about 70 years.[8] Approximately 10% of the DNA in maternal circulation is of fetal origin, although that proportion ranges from <3% to >20% in any individual[9]; the remaining ~90% of circulating DNA is maternal. Most of this cfDNA is derived from the placenta,[5] with a considerably lesser contribution from the fetus itself. The DNA in circulation is typically found as small fragments of 150 to 200 base pairs,[10] which is thought to be derived mostly from DNA generated by apoptosis (programmed cell death) of placental cells, but may also be from live cells in a much smaller quantity.

In addition to the size of the DNA fragments in circulation, 2 features of cfDNA are of particular importance to the design and implementation of technologies for the noninvasive assessment of fetal status: the proportion of fetal DNA relative to the maternal contribution in circulation during pregnancy, known as the fetal fraction, and the timing of a detectable level of fetal DNA in circulation, which is usually by 7 weeks.[11,12] Additionally, fetal DNA is continually being refreshed with a half-life of <20 minutes and disappears from maternal circulation within a few hours postpartum, which eliminates concerns of a "carryover" effect from one pregnancy to the next.[12]

The presence of fetal cells in maternal circulation is much rarer compared to cfDNA. The number of fetal cells that can be successfully identified and isolated from 10 mL of maternal blood is often reported as well below 100. The presence of fetal cells in maternal circulation has been reported as nucleated red blood cells, trophoblasts, lymphocytes, and granulocytes.[13] The process of fetal cell isolation is generally much more tedious than cfDNA, and the efficiency of isolation can be inconsistent from sample to sample.

Despite these challenges, fetal cells, once isolated, likely contain the complete genetic information of the fetus without maternal background and limitations from the short DNA fragmentation in cfDNA. Fetal cells in maternal circulation provide a path for an accurate noninvasive analysis of all genetic diseases beyond aneuploidy.

Measuring DNA From Maternal Circulation: Technologies

The goals of any NIPT technology are to determine if the cfDNA from the fetus is in proportion to all of the anticipated 46 chromosomes and, consequently, to assess the likelihood that one or more of the chromosomes are in excess or absent. This capability would permit the inference that the fetus, from which the DNA is derived, has an aneuploid chromosome constitution. More specifically, the challenging question is: Can we assess the cfDNA molecules from each of the 46 fetal chromosomes by counting, or some other strategy, with sufficient accuracy and

reliability to make this determination? And can this assessment be done against a background of maternal DNA also representing a complete, and presumably normal, chromosome complement? While the answers to those questions have been affirmative, they are qualified ones; despite the impressive evolution of the technologies to perform NIPT, the assessment of cfDNA for aneuploidy is a screening strategy with embedded sources of error and not a diagnostic test.[1]

However, several different approaches have been successful in accomplishing a noninvasive screening test with a higher degree of accuracy than has been realized by other noninvasive prenatal strategies. To date, the approaches fall into 2 categories of techniques: MPS and targeted sequencing.

Massive Parallel Sequencing

Also referred to as next-generation sequencing (NGS), MPS is a high-throughput strategy that involves the concurrent sequencing of spatially separated single or highly amplified DNA templates.[14] The "shotgun" approach to NGS is that it sequences the entire genome; this strategy contrasts with individual sequencing reactions called Sanger sequencing. The parallel sequencing reactions of the total cfDNA by this type of NGS generates tens of millions of sequence reads that span the entire genome. These reads can then be aligned and "tagged" (or mapped) to locations on a reference human genome to identify their chromosome of origin. Once this mapping occurs, the tagged DNA fragments can be counted.[15,16]

Translating the counted, mapped fragments into a determination of the "ploidy" status is conceptually simple, although it requires a technically sensitive capability. If aneuploidy is present, then there is an increase (trisomy) or decrease (monosomy) in the number of mapped tags on an affected chromosome relative to the other euploid chromosomes. In principle, any segment of a chromosome can be assessed for the presence of microduplication or microdeletion; in fact, this has been accomplished with a current resolution of detection below that of a constitutional karyotype, but not yet at the level of chromosomal microarrays.

Interpretation of the mapping data has been aided by various algorithms that make comparisons of the tags on a particular chromosome (eg, 21 or 18) to multiple other chromosomes in the genome and that take into account variations in DNA arising from technical or sample-to-sample variations in sequencing. For example, the distribution of the 4 bases of DNA—guanine, cytosine, thymine, and adenine—are not consistent or uniform across fragments of genomic DNA nor from chromosome to chromosome, and this inconsistency can affect the efficiency of the sequencing itself.[17] There have been different approaches to the design and application of the data analysis algorithms, but they all have the same desired outcomes: to discriminate between true-positive and true-negative results. These algorithms should do so with the most clinically informative statistics, that is, high detection rates with low false-positive findings (sensitivity) and with low false-negative rates (specificity). They should also provide clinically meaningful positive and negative predictive values.

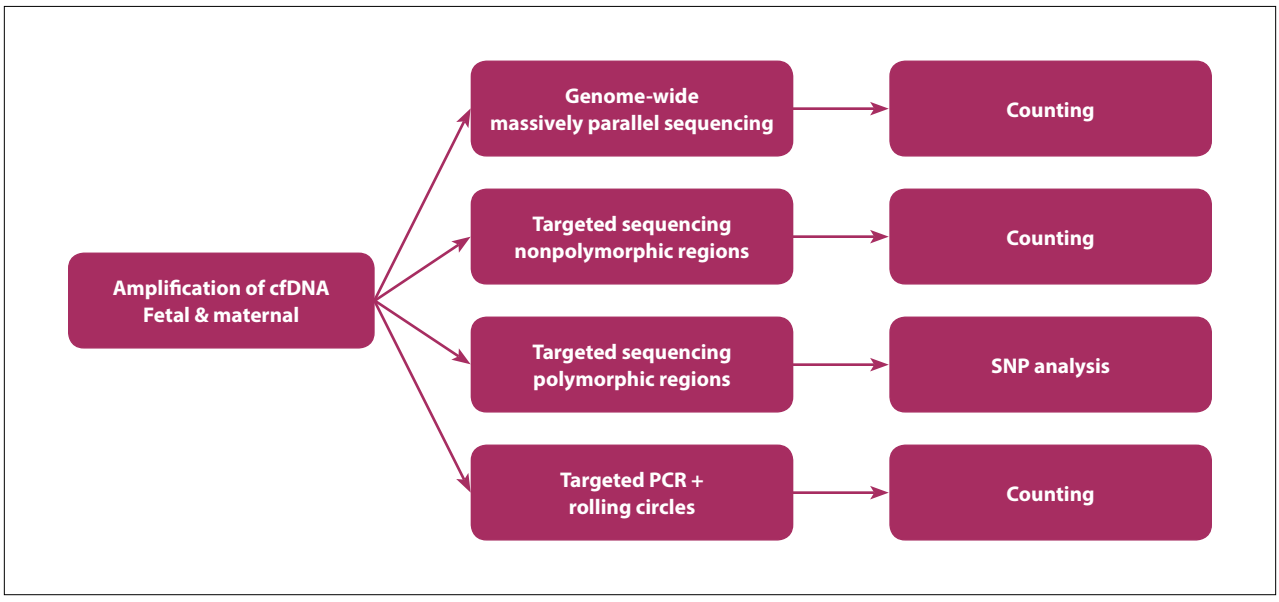
Targeted Sequencing

A second methodology for NIPT involves a targeted sequencing approach. This is an adaptation of NGS that specifically sequences only the chromosomes of interest for a focused and deep analysis.[14,18-20] The targeted sequencing technique has been applied to 2 different NIPT strategies: counting (in a manner similar to the MPS technology) and an elaborate analysis of single nucleotide polymorphism (SNP) data generated by the targeted sequencing that compares fetal and maternal DNA. SNPs are changes in a single base pair of DNA; such changes are normal and occur in every individual, providing markers of individual differences from one person to another.

The targeted adaptation of NGS has been focused on assessing the 3 most common sources of aneuploidy: chromosomes 13, 18, and 21, as well as the sex chromosomes.[21] In principle, as with the NGS approach, data from any portion of the genome can be evaluated; the targeted approach only requires that nonpolymorphic regions of interest be amplified with a high degree of accuracy in both the maternal and fetal components of the cfDNA before being analyzed by a "counting" algorithm.

The targeted approach to NIPT lends itself to a more streamlined workflow than the whole genome approach, and being amenable to the use of a microarray technology allows for additional accuracy and efficiencies in the processing of a blood specimen from receipt to report.[22] Several characteristics of microarray usage contrast with NGS: (1) a capacity to improve the accuracy of assessing aneuploidy by simultaneously reducing assay variability; (2) lowering the fetal fraction requirement and thus mollifying some of the known factors that affect the proportion of fetal DNA in a maternal plasma specimen (eg, maternal weight, gestational age); and (3) improving assay turnaround time, in part by reducing the need for normalization protocols that accompany the multiplexing of specimens when analyzed by sequencing tech-

FIGURE Schematic of NIPT Methodologies



Abbreviations: cfDNA, cell-free DNA; NIPT, noninvasive prenatal testing; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

niques. However, the targeted approach is likely to prove more difficult for surveying the entire fetal genome, which is often cited as a desire of tomorrow's routine NIPT capabilities.

The other important application of targeted sequencing uses a sophisticated SNP analysis of the entire maternal blood specimen rather than chromosomal fragment counting of the cfDNA found only in the plasma fraction.[19,23] In addition to sequencing the entirety of the plasma-bearing cfDNA, which represents both the fetal and maternal contributions, the DNA from white cells of the buffy coat, which essentially represents only the maternal genome, is also sequenced. The targeted sequencing of polymorphic regions from both of these DNA sources entails capturing up to 20,000 SNPs. These sequencing steps generate a profile of maternal plus fetal genotypes and a separate maternal genotype. The analysis that follows requires the subtraction of the maternal SNP alleles from the data set, leaving only the fetal alleles that are then subjected to a series of hypotheses regarding the likelihood that an allele set is representing a given chromosome and is doing so in excess of a euploid distribution. The analysis also takes into account possible crossover events and can be enhanced if the paternal SNP distribution is also available and included in the targeted sequencing reactions. In addition to being capable of assessing chromosomal aneuploidy, this application of targeted sequencing can detect a balanced triploid fetus.[23]

A recent entry into the targeted approach to NIPT combines the targeted polymerase chain reaction (PCR) amplification of specific sequences on each chromosome of interest (eg, 13, 18, 21, X, Y) with a counting analysis. This methodology, which functions well at levels of fetal fraction below the current norm, replaces sequencing and the related need to align and analyze DNA fragments with a "rolling circle" PCR strategy[24,25] that involves tagging each of the amplified segments with a distinct fluorescent dye and then counting each of the resultant "colors." The frequencies of each set of fluorophores, which represent the targeted chromosomes, are compared, and the presence of a relative excess or absence is equated to the presence of an aneuploidy. This technology, which was recently validated clinically for its capacity to assess trisomies 13, 18, and 21,[26] also appears to be considerably more cost effective than other strategies described here (**FIGURE**).

Factors Affecting the Analysis and Interpretation of Data

Regardless of the technology a laboratory uses to generate its screening results, a variety of factors enter into the calculation of the results, as well as technological limitations that may cause some patients to be identified as inappropriate for aneuploid screening by NIPT. As mentioned previously, the choice of technology leads to different statistical requirements for discriminating a likely euploid (normal) specimen from a finding of aneuploidy. While

trisomy 21 was initially the target for NIPT development, adjustments to counting algorithms, which used z-score statistics, allowed probability determinations to be made for additional chromosomes (13, 18, X, and Y) and eventually to findings across the genome.[26-28] Targeted sequencing strategies have also relied on alternative algorithms that draw on other factors in the patient profile.

The factor most common to the reliability of all NIPT technologies is the fetal fraction,[9,29] which is the proportion of fetal DNA in the total cfDNA extracted from a maternal specimen. Fetal fraction is a noncontrollable variable that can affect an individual NIPT outcome. It is a general rule that NIPT technologies using NGS perform best with increasing fetal fraction because it leads to increased reliability in the read depth of the sequencing reactions, which in turn allows for more precise counting. In a euploid fetal karyotype, chromosome 21 constitutes 1.5% of the total DNA. If the fetal fraction was 0%, a trisomy 21 would be missed because it would appear that chromosome 21 still constituted 1.5% of the total DNA. At 4% fetal fraction, however, that figure rises to 1.53%, and at 10% fetal fraction in a trisomy 21 pregnancy, the quantity of chromosome 21 DNA rises to 1.6%.[30] As moderate as that distinction between 1.53% and 1.6% over the euploid quantity appears to be, it is not only possible to make the discrimination, but it is now done routinely. Similar values and their changes hold true for trisomies 13 and 18 as well, given their size and DNA quantity in the context of the whole genome.

A number of studies have pointed to a fetal fraction value of 4% as a reliable standard for the generation of meaningful screening results from NIPT using NGS and a counting strategy.[31] However, it is important to recognize that all NIPT strategies may not have the same threshold and that, ultimately, it is a question of test validation by a laboratory that supports the minimal standards for fetal fraction as well as other analytic parameters. These data and their role in the test quality are typically described on a patient's results report along with the description of the test results as a positive or negative screen.

Various factors can—and do—affect the fetal fraction as well as the interpretation of cfDNA data for any given individual,[31-33] including maternal weight (increasing body mass index correlates with decreasing fetal fraction), placental mosaicism, an unrecognized or vanishing twin, gestational age, maternal medical conditions, maternal mosaicism, fetal aneuploidy itself, and in vitro fertilization with a donor egg. Each of these parameters can contribute to a "no-call" or difficult-to-interpret result and should be understood as the consequence of the specific technology that the NIPT rests on to provide its aneuploidy screening as well as the biology of the individual being screened.

Fetal fraction analysis is integrated into SNP-targeted NIPT, as many SNPs from each sample will be heterozygous for the fetus while homozygous for the mother. The ratio between the detected paternal and maternal alleles can accurately determine fetal fraction. A shotgun-based NIPT could estimate fetal fraction for male pregnancies by comparing the amount of DNA detected on Y chromosomes to the overall DNA amount, but an additional method to measure fetal fraction for female pregnancies will be needed.

Analyzing Fetal Cells From Maternal Circulation

A complete fetal cell assay consists of the following steps: debulking, fetal cell identification, and disease detection in isolated fetal cells.

Debulking is a process to remove maternal red blood cells (RBC) and the majority of nucleated maternal cells. It can be achieved by several strategies; some strategies are based on markers that are potentially unique to fetal cells, while others are based on removing maternal cells based on their unique markers and properties. Debulking is also the least controllable process given the fact that maternal blood has complex variabilities from one pregnancy to another and that the blood properties can further change after the blood draw.

Fetal cell identification and downstream disease detection require the ability to accurately analyze DNA at the single-cell level. Detecting the presence of paternal alleles is the most reliable approach to identify fetal cells compared with morphologic confirmation. The DNA from the confirmed fetal cells will typically be amplified by commonly available technologies (eg, sequencing, microarray, etc.) before disease detection. Similar to cfDNA analysis, the amplification on fetal cells can be arranged for the whole genome or for targeted regions relevant to diseases of interest. However, the amplification on single cells can generate artificial errors for final analysis if not properly designed.

Before commercialization, fetal cell assay development will also need to address its higher cost, lower throughput, and more complex clinical validations compared to cfDNA assays. It is broadly believed that as the next generation of NIPT, these challenges in fetal cell assay will be resolved in the not too distant future.

Conclusion

As NIPT continues to evolve, there are likely to be several modifications to the current technology. Among these predicted transitions are the simplification and scaling

of technologies through the application of engineering strategies; reductions in costs brought about by continued innovations; a crossing of the borders from screening to diagnostics; and analytic insights into the prediction of polygenic effects on prenatal and postnatal development. There will no doubt also be unpredictable insights into the biology of fetal growth and maternal-fetal interactions that will drive a need for techniques to capture and assess their significance so that these tests can better benefit pregnant women and physicians.

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Noninvasive Prenatal Screening: Development of Cell-Free DNA Technology and Its Move to Become a Standard of Care

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Introduction

Screening for fetal chromosomal abnormalities, a part of clinical obstetric practice since the late 1980s, provides families with information to assist them with reassurance, pregnancy and delivery planning, and decision-making.[1] Initially, these screens were used to assess risks for open neural tube defects and to measure placental and fetal markers during the second trimester to identify an age-related risk for Down syndrome and trisomy 18 (Edwards syndrome).[2-4] Advances in ultrasound technology with the nuchal translucency (NT) measurement have expanded such screening into the first trimester.[5,6] The laboratory-derived cell-free DNA (cfDNA) screening was made available in the United States in November 2011 with the aim of making these screens more sensitive and lowering their false-positive rate.[7] This new form of DNA sequencing technology has rapidly expanded into clinical practice as a reliable, accurate, and popular screen. In response, the American College of Obstetricians and Gynecologists (ACOG) and American College of Medical Genetics and Genomics (ACMG) have published practice bulletins and position statements giving guidance to their membership on how best to integrate this screening into their clinical practice for the benefit of their patients.[8,9]

Screens and Diagnostic Tests

The concept of screening for chromosomal abnormalities came from the observation that as a woman ages, her chance of conceiving a fetus with chromosomal aneuploidy increases. The initial screen was as simple as asking for the patient's date of birth. The prenatal diagnostic techniques of amniocentesis and chorionic villus sampling (CVS) were developed as ways to sample prenatally derived cells to obtain a chromosomal karyotype.[10,11] As ultrasound technology and its use became more widespread and as education on how to perform these procedures became a standard part of obstetrics training, it became the standard of care to offer such tests to this group of high-risk pregnant women.[12,13] However, not every woman was comfortable with the risk of a procedure-related loss. Therefore, screens were developed to assess and personalize risks for chromosomal abnormalities.

The purpose of screens and tests is to give a pregnant woman information, but the results of each are reported in different ways. The prenatal diagnostic tests CVS and amniocentesis are the gold standard and provide definitive results.[14] Screens, on the other hand, are not definitive and traditionally provide a numeral risk assessment representing a woman's chances of carrying a fetus with the chromosomal abnormality in question (**TABLE 1**, page S12). By stratifying risk, many women rely on screens to determine if they should undergo one of the diagnostic options.[8] They may choose to have one of the diagnostic tests initially, and this option is acceptable regardless of risk. In 2007, ACOG's recommendations through practice bulletins on screening and diagnostic testing explained that all screens and tests should be made available to all women regardless of age.[15,16]

As these screens developed, their accuracy improved. Traditionally, the serum screens in the first trimester, second trimester (triple, quad), and those integrating first- and second-trimester components (sequential, integrated) all used a screen (false) positive rate of 5%.[6] Over time, various technologies allowed for improvements in sensitivity (detection rate) from 69% to 96%. All of these screens utilized markers of placental- or fetal-derived proteins in the maternal serum with or without a specific ultrasound measurement (NT in the first trimester). There are a multitude of reasons why these values can be increased or decreased compared to a normal value, and these can be unrelated to the presence of chromosomal aneuploidy in the fetus.[17]

Cell-Free DNA Testing

The advent of cfDNA testing completely changed the clinicians' approach to screening. Instead of looking at proteins in maternal serum, this technology analyzes actual

TABLE 1 Differences Between Screens and Diagnostic Tests

Screens	Diagnostic Tests
Traditionally offered to low-risk population	Traditionally performed on at-risk population
Inexpensive	Expensive
Easy to perform	Have risks
Quick to perform	
Reliable	
Help to define at-risk population	
Do not provide a definitive answer	**Provide a definitive answer**

fragments of DNA not bound in cells. These fragments primarily come from the placenta through cellular apoptosis and circulate freely in maternal blood. DNA amplification techniques like massive parallel (next-generation) sequencing of these fragments or of single nucleotide polymorphisms have allowed testing to compare expected amounts of this DNA to what is present in the sample.[9] When originating from particular chromosomes (21, 18, 13, X, and Y), excess amounts of DNA are highly associated with the presence of fetal aneuploidy. Along with greatly increased detection rates, the false-positive rates are consistently much lower. These rates are not the same for each chromosome evaluated and vary based on DNA amplification and the frequency of these chromosomal abnormalities in the general population. False-positive rates are not zero but can be attributed to confined placental mosaicism, a vanishing twin pregnancy, maternal aneuploidy, or maternal malignancy.[8,18]

In addition to the statistical concepts of sensitivity and false-positive rates, we must also take into account the positive predictive value (PPV). PPV is defined as the chance that a positive screen occurs when there is a true positive. As a screen, cfDNA can have some false positives, and this rate is stable across chromosomes. Less frequent chromosomal abnormalities will result in a lower PPV due to their lower prevalence. Similarly, as the prevalence of chromosomal abnormalities is lower in women of younger ages, the PPV will be lower. However, cfDNA screening consistently outperforms the other aneuploidy screens. As a result, this technology has expanded beyond those women thought to be at a higher risk who were originally studied.[19] The lower PPV can also be attributed to other areas on chromosomes, such as the common yet rare microdeletion/duplication syndromes (eg, DiGeorge, Prader-Willi, and Angelman) or areas on other chromosomes.[20,21]

Another important concept which may affect the accuracy of the screen or the ability of the testing laboratory to give an accurate result is fetal fraction, which is the percentage of the total cfDNA that is placental or fetal in origin. The pregnant woman's serum contains cfDNA from her own cells, but the pregnancy-related content is 10% on average.[22] Depending on the testing laboratory, fetal fraction may be part of the calculation.[23] Low fetal fractions can affect the results, leading to a "no-call" or "nonreportable" result, and can be associated with the presence of aneuploidy, certain medications like low-molecular-weight heparin, and high body mass index.[22] These are not direct contraindications, but they must be considered when ordering these screens.

Society Recommendations

The ease of screening, the ability to give results as early as 10 weeks of gestation, the noninvasive nature, marketing by the laboratories, and the ability to predict gender as a side effect of identifying sex chromosome abnormalities made cfDNA screening a very popular choice.[24] The rapid incorporation of the screening by clinicians led to the need for societies such as ACOG, ACMG, the Society for Maternal-Fetal Medicine (SMFM), the International Society for Prenatal Diagnosis (ISPD), and the National Society of Genetic Counselors (NSGC) to publish documents in order to give recommendations on how to order these screens, who the most appropriate candidates are, how expansive these screens should be, and future outlooks for the technology.

The first society to publish a statement was NSGC in November 2012, which was followed by a white paper in January 2013.[25,26] This event was of particular note given that pretest and posttest genetic counseling is integral to ensuring patient understanding and acceptance of the test results. Genetic counselors within the prenatal health care team are in a prime position to explain these complex concepts in an understandable fashion. At the time of the recommendations by NSGC, the screening was available and validated only for Down syndrome, tri-

TABLE 2 Characteristics of Prenatal Screening Options[8,31]

Screen	Gestational Age Range (Weeks)	Detection Rate for Down Syndrome (%)	Screen Positive Rate (%)	Method
First trimester	10-13$^{6/7}$	82-87	5	NT + PAPP-A, free βhCG ± msAFP
Quad screen	15-22	81	5	msAFP, βhCG, uE3, DIA
Integrated screen	10-13$^{6/7}$ then 15-22	96	5	NT + PAPP-A, then quad screen
cfDNA	9-10 to term	99	2-4 (including no-call results)	Several molecular analyses of cfDNA

Abbreviations: βhCG, beta-human chorionic gonadotropin; cfDNA, cell-free DNA; DIA, dimeric inhibin A; msAFP, maternal serum alpha-fetoprotein; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein A; uE3, unconjugated estriol.

somy 18, and trisomy 13 (Patau syndrome) in a high-risk population.

Shortly thereafter in December 2012, ACOG and SMFM published a committee opinion as a quick response to the development of this technology.[19] Based on the studies available, high-risk criteria for who should be offered cfDNA screening were devised as follows: (1) advanced maternal age; (2) previous pregnancy with a trisomy; (3) either parent with a Robertsonian translocation involving chromosome 13 or 21; (4) an increased risk for aneuploidy based on a traditional screening method; or (5) an abnormality seen on ultrasound that indicated an increased risk for aneuploidy. At this point, the document by ACOG and SMFM was not recommending that cfDNA screening be a part of routine screening.

ACMG followed with their first policy statement on the screening in February 2013.[27] Since a portion of ACMG's membership works in clinical laboratories, much of the document focused on the bioinformatics and statistical accuracies of the technology as well as its growth. When ISPD published their first policy statement mentioning cfDNA screening, they were able to put it into context with the various screens already available.[28]

With 2 to 3 further years of experience, along with expansion of what the screening could offer, all of the aforementioned societies published new documents. ISPD came out with a new policy statement in April 2015.[29] This was the first major society document to advocate offering cfDNA screening to all pregnant women regardless of age or pregnancy or family history. They importantly reiterated that amniocentesis and CVS were the only definitive ways to diagnose chromosomal aneuploidy prenatally. The document also reviewed the importance of genetic counseling during the pretest, consenting, and posttest disclosure of results, especially when the risk for aneuploidy is higher than normal.

ACOG and SMFM updated their previous committee opinion in September 2015.[30] The update provided a critical analysis and explanation of the statistics, especially that of PPV. With limited prospective data on the low-risk population (those not mentioned in the previous committee opinion), the document stated that the traditional screening methods were the "most appropriate choice for first-line screening for most women in the general obstetric population." The next recommendation stated that "any patient" had the option to choose cfDNA screening given an explanation and understanding of the benefits and limitations. The committee opinion mentioned that cfDNA screening is most accurate for chromosome 21, 18, 13, X, and Y and that screening for microdeletions was not recommended. The document also did not recommend use of cfDNA screening for twin pregnancies due to limited data. Because many families choose this screen due to its high accuracy and to potentially avoid a diagnostic test, the document also stated that pregnancy management decisions including pregnancy termination should not be based solely on these results because cfDNA screening is not 100% accurate. The committee opinion reiterated the point of residual risk in which a low risk or negative result does not exclude a chromosomal abnormality. Because some women may enter into aneuploidy screening when receiving an anatomy scan, the recommendation was that diagnostic testing should be offered when an abnormality is visualized and should not be replaced by cfDNA screening. Finally, as all screens and tests are performed based on the decision of the pregnant woman, she has the option to decline these options.

ACOG and SMFM revised their practice bulletin on screening options in May 2016.[31] In addition to discussing first trimester, second trimester, and combined screens as well as ultrasound markers, the bulletin also

focused on cfDNA screening. Many of the points in this section of the article reviewed what was discussed by ACOG above. The bulletin also compared and contrasted cfDNA screening with the traditional screening methods (**TABLE 2**, page S13). Importantly, the bulletin still stated that every woman, regardless of age, has the option of screening or diagnostic testing. As cfDNA screening is not definitive, prenatal diagnostic testing should be offered when a result is high risk or positive for aneuploidy. In July 2018, the previous committee opinion was withdrawn by ACOG's Committee on Genetics.

In October 2016, ACMG published an update to their statement on noninvasive prenatal screening.[9] The statement focused on the multifaceted nature in which screening should be implemented into clinical practice, especially considering the importance of the genetic counseling process. As in any nondirective counseling process, the decision to proceed with any screen or test is the option of the pregnant woman. The only differentiating factor regarding maternal age is that the statement made is in terms of PPV. They did not recommend aneuploidy screening beyond chromosomes 21, 18, 13, X, and Y, and finding out the sex should not be the sole reason to screen for sex chromosome abnormalities. The document recommended that clinicians contact the testing laboratory for more information about the reliability of testing in multiple gestations prior to offering such tests in practice. The ACMG also noted that cfDNA screening does not assess the risk for open neural tube defects or adverse pregnancy outcomes, nor will it replace ultrasound.

Most recently in August 2020, ACOG and SMFM updated their practice bulletin on screening for fetal chromosomal abnormalities.[8] The new document is very similar to the previous one, but there are a few changes due to more years of experience with the technology. Most notably, further data showed that cfDNA screening could be offered in twin pregnancies. Also, the management and follow-up of no-call results is discussed in more detail. Screening for microdeletions is still not recommended, although a more detailed discussion of such screening by cfDNA is included.

Conclusions

Screening with cfDNA has revolutionized prenatal assessment of chromosomal abnormalities because it is available starting in the first trimester, uses only a maternal blood sample, and has high statistical accuracy. Like any new technology, cfDNA screening is not just a simple blood test and carries with it all of the ethical and psychosocial concerns that occur when a woman finds out that her pregnancy may be affected by aneuploidy. Genetic counseling is an invaluable part of the discussion, consenting, and disclosure process. cfDNA is now available to pregnant women of all ages, and while the number of women choosing this option is increasing, diagnostic testing through CVS and amniocentesis will always be the gold standard to determine the chromosomal makeup of the pregnancy.

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NIPT: A Clinical Update

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Introduction

Evaluating pregnancies for fetal abnormalities has been a mainstay of prenatal care since the 1960s. From the introduction of amniocentesis for the detection of fetal chromosomal abnormalities to the initial screenings for Tay-Sachs disease and sickle cell disease among individuals of Eastern European Jewish (Ashkenazi) and African ancestries, respectively, the advancement of prenatal screening modalities has sought to develop highly effective screening protocols to identify women and couples who are at an increased risk for detectable fetal abnormalities. With the growing selection of screening tests that evaluate pregnancies for Down syndrome (trisomy 21) and other fetal chromosomal and genomic abnormalities, the development of more effective screening protocols has been a long sought-after goal—one that was achieved with the development of noninvasive prenatal testing (NIPT).

Screening Versus Diagnosis

Despite the frequent interchange of the 2 words by patients and clinicians alike, understanding the difference between screening and diagnosis is critical to empowering women and couples to make truly informed prenatal care decisions that are right for them and for the prenatal information that they wish to acquire. Screening is a risk-adjustment process through which clinicians can determine whether to offer diagnostic testing to patients. Residual risk always exists regardless of the actual screening outcome; that is, in no instance is there a guarantee that no fetal chromosomal abnormality exists or is completely ruled out. Diagnosis, on the other hand, relates to a process that determines the presence or absence of a disease state. Screening results should be communicated as either a "positive" or "negative," whereas diagnostic results are communicated as "normal" or "abnormal." Examples of screening tests in use in reproductive medicine include second trimester quad-analyte screening and nuchal translucency measurements; examples of diagnostic tests include chorionic villus sampling (CVS) and amniocentesis.

The distinction between screening and diagnostic testing modalities is a critical aspect of the process by which patients decide what testing, if any, they wish to undergo to evaluate their pregnancies. The choice of a screening test provides an adjusted risk for the more common fetal chromosomal abnormalities. Negative results indicate a markedly reduced, but not eliminated, chance for a common fetal chromosomal abnormality, whereas a positive result is indicative of a considerably increased risk for that specific fetal chromosomal abnormality. However, that positive result is not a guarantee that the fetus is so affected, even if other signs are present (eg, an abnormal ultrasound examination) that are associated with fetal abnormality. Accordingly, positive results alone should never be used for pregnancy management decisions; diagnostic testing is strongly supported in all such situations.

In addition, screening algorithms do not provide a comprehensive assessment of fetal chromosomal abnormalities; therefore, a "negative" result could miss an aberration of a chromosome that is not evaluated in the screening test.[1,2] Conversely, diagnostic testing provides a more comprehensive assessment of the fetus. Screening tests invariably do not increase the risk for fetal loss, because they do not involve the acquisition of fetal tissue. However, CVS and amniocentesis are associated with a very small increased risk for fetal loss, a value that is less than the risk of detecting a fetal abnormality in essentially all cases.

NIPT

NIPT is the latest technology used to screen for fetal chromosomal abnormalities. Prior to its introduction, a variety of technologies were used, and continue to be used, for prenatal screening. These screening technologies include first- or second-trimester measurement of maternal serum biomarkers (eg, alpha-fetoprotein [AFP], human chorionic gonadotropin [hCG]) as either single-analyte or multivariate risk algorithms and ultrasonographic measurement of certain fetal anatomic features. This is most commonly the measurement of the fetal nuchal translucency in the late first trimester, as well as algorithmic combinations of biomarker and ultrasound measurements. All of these technologies pro-

vide an adjusted risk for fetal trisomy 21 alone, although a few also provide an adjusted risk for fetal trisomies 18 and 13. The ongoing development of these technologies resulted in an increasing detection rate for fetal trisomy 21, although all are associated with relatively low (3%-5%) positive predictive vales (PPVs).[3] As such, the development of more effective and expansive screening protocols for fetal chromosomal abnormalities is, and continues to be, highly desired.

Unlike the earlier prenatal screening modalities that used maternal serum biomarker and anatomic measurements, NIPT evaluates cell-free nucleic acid in maternal blood to assess the relative ratio of chromosome-specific sequences, comparing the patient sample to what is expected to be found in a euploid mother carrying a euploid fetus. This process provides a more accurate and specific risk assessment for common fetal chromosomal abnormalities as well as for some select genomic microdeletion syndromes and for larger deletions and duplications of the fetal genome. However, not all commercially available NIPT tests offer the same chromosomal and genomic screening targets. Clinicians are strongly advised to educate themselves about the specifics of the NIPT test that they use, including the appropriate gestational ages for evaluation, which fetal conditions are screened, the percentage of tests that result in an indeterminate result, and the typical turnaround time. Regardless of what screening targets are chosen, clinicians must also be able to counsel their patients about the clinical ramifications of a positive, negative, or indeterminate test. The latest Practice Bulletin from the American College of Obstetricians and Gynecologists (ACOG), published in August 2020, entitled "Screening for Fetal Chromosomal Abnormalities," puts particular emphasis on patient counseling, both pretest and posttest. In fact, ACOG defined such counseling as "essential."[4] Specific information about such counseling can be found in the Practice Bulletin.

In a comparison of NIPT with serum analyte testing, McLennan and colleagues showed that NIPT was superior to first-trimester maternal serum analyte-based screening for identifying women at risk for carrying fetuses with trisomies 21, 18, and 13.[5] The technologies used to accomplish NIPT show comparable capabilities for detecting fetal trisomies 21, 18, and 13, with approximate detection rates of 99%, 97%, and 90% and PPVs of 84%, 76%, and 45%, respectively.[6,7] Detection rates and PPVs for sex chromosome abnormalities are somewhat lower than those observed with aneuploidy screening.[7,8] To provide a comparison to conventional screening, the detection rate for fetal trisomy 21 by sequential screening is approximately 93% with a PPV of 3%.[9]

NIPT can also be used to screen for other fetal chromosomal and genomic abnormalities. For instance, Lefkowitz and colleagues showed the ability of NIPT to detect fetal subchromosomal abnormalities as well as chromosomal microdeletions.[10] In another study, Gross and colleagues showed that the PPV for NIPT when evaluating maternal blood for the most common microdeletion syndrome, 22q11.2 or DiGeorge syndrome, was 18%, a figure supported by a study from Petersen and colleagues.[7,11] In the study by Petersen et al, the PPVs for other well-characterized but less common microdeletion syndromes ranged from 0% to 14%.[7] Despite the increasingly expansive applications of NIPT for chromosomal and subchromosomal fetal abnormalities, it should be noted again that NIPT is a screening exam and that not all chromosomal, let alone genomic, abnormalities will be detected by NIPT. In fact, Chen and colleagues estimated that 12.4% of fetal chromosome abnormalities would be missed by NIPT but would be detected by diagnostic procedures.[2]

The use of NIPT was initially offered solely to women at increased risk for fetal chromosomal abnormalities, eg, women at advanced maternal age (≥35 years) and women found to be at an increased risk based on "positive" conventional screening outcomes. Over the past several years, NIPT has been directed to a low-risk obstetric population, although the screening characteristics of NIPT are different in the "general-risk" obstetric population.[4] In support of this idea, Norton and colleagues showed that NIPT was superior to conventional maternal analyte/nuchal translucency measurement screening with regard to fetal trisomy 21 in a low-risk cohort.[12] No other fetal trisomies were detected in the low-risk cohort, thus precluding an assessment of NIPT screening for fetal aneuploidies other than Down syndrome in a low-risk population.

In light of these findings, ACOG's August 2020 Practice Bulletin states:

"This Practice Bulletin has been revised to further clarify methods of screening for fetal chromosomal abnormalities, including expanded information regarding the use of cell-free DNA in all patients regardless of maternal age or baseline risk, and to add guidance related to patient counseling."[4]

With regard to the above, the Practice Bulletin specifically noted:

"Prenatal genetic screening (serum screening with or without nuchal translucency [NT] ultrasound or cell-free DNA screening) and diagnostic testing (CVS or amniocente-

sis) options should be discussed and offered to all pregnant patients regardless of age or risk for chromosomal abnormality. After review and discussion, every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing. Pretest and posttest counseling is essential."[4]

The Practice Bulletin goes on to make a number of clinical recommendations based on good and consistent scientific evidence (deemed Level A). Among these are[4]:

- Cell-free DNA (cfDNA) is the most sensitive and specific screening test for the common fetal aneuploidies.
- Patients whose cfDNA screening test results are not reported by the laboratory or are uninterpretable (a "no-call result") should be informed that the test failure is associated with an increased risk of aneuploidy, receive further genetic counseling, and be offered comprehensive ultrasound evaluation and diagnostic testing.
- If screening is accepted, patients should have one prenatal screening approach and should not have multiple screening tests performed simultaneously.

The Practice Bulletin also discussed the role of fetal fraction (FF) and cfDNA testing. The Bulletin noted that for cfDNA testing to be accurate, a minimum FF level is required, most commonly reported as 2% to 4%. At 10 to 14 weeks of pregnancy, the median FF level is approximately 10%. In light of the effect of FF on test accuracy, the Practice Bulletin commented that cfDNA testing should preferably be done in a laboratory that reports FF.

Finally, the role of cfDNA in evaluating fetal abnormality in twin gestations has often been raised in the scientific literature. The Practice Bulletin made a recommendation based on limited/inconsistent data (Level B). This recommendation noted that cfDNA can be performed in twin pregnancies, with screening for trisomy 21 being labeled "encouraging."[4]

Clinical Management

NIPT is performed on women during the late first or early second trimester of pregnancy. NIPT is performed by the evaluation of a peripheral blood sample obtained from the pregnant woman, usually 8 to 10 cc of blood. Results are usually available in 3 to 7 calendar days and are communicated as negative, positive, or indeterminate (a no-call result). Specific criteria for the blood sample, turnaround time, and categorization of screening outcomes are unique to each lab performing NIPT; clinicians are strongly encouraged to be well versed in the specific instructions for sample collection, transportation, and interpretation of the lab(s) that they use for NIPT.

FF, or the percent of fetal cell-free nucleic acid in a blood sample, is a critical aspect of the quality control used by laboratories to assure accuracy in their NIPT assays. FF increases with gestational age but decreases with increasing maternal weight. While there is no optimal FF, most consider an FF of 8% as providing a strong foundation for accurate screening. Each laboratory incorporates FF assessment in their proprietary screening algorithm, with some using the figure as an absolute determinant of screening success, while other laboratories use FF as one of several variables within their screening algorithms.

All NIPT results are risk-adjustment outcomes so that the clinical implication of each result is based on the specific clinical presentation of each patient. A negative result indicates a considerable reduction in the risk for the chromosomal abnormalities being screened for in the assay. A positive result will be positive for a specific chromosomal or subchromosomal abnormality and indicates a considerably increased risk for that specific chromosomal abnormality in the fetus. As NIPT typically screens for only a limited number of chromosomal abnormalities, a positive or negative result may or may not provide the requisite information to clarify the clinical presentation of the individual woman undergoing NIPT. For example, a woman presenting with a fetus with cri-du-chat syndrome (5p–syndrome) will likely have a negative NIPT test if that test screens only for chromosomes 13, 18, 21, X, and Y.

As opposed to serum- and ultrasound-based screening algorithms, NIPT testing can return an indeterminate (or no-call) outcome. The rates for these outcomes differ from one laboratory to another, but generally occur in <5% of samples.[13] In addition, the reasons for a laboratory to characterize a sample as "failed" or "indeterminate" are unique to each laboratory and can include low FF, sequencing failures, or sequencing outcomes that do not correlate with defined clinical outcomes. ACOG currently recommends that women who obtain such a result with NIPT screening be offered genetic counseling and diagnostic testing because of an increased risk for fetal aneuploidy.[4,12] Yaron affirms that such cases are characterized by a higher risk for fetal aneuploidy.[13] However, as there are no head-to-head trials of any of the available NIPT products, it is not possible to ascribe superiority of any one test over another. For now, it remains appropriate to offer genetic counseling and consideration of diagnostic testing to women with an indeterminate NIPT result, although consideration of a repeat test is warranted if the indeterminate result is due to a low FF.[4] In such cases, the test should always be

repeated using the same laboratory that evaluated the initial sample. Repeating the test will delay the performance of diagnostic testing, although most repeated samples are returned as negative. In addition, although a negative result on the second specimen can be managed as a negative screening outcome, because of the increased risk for fetal aneuploidy, a positive or indeterminate result on the second analyzed specimen should be managed by both the offering of genetic counseling and the consideration of diagnostic testing.

Several other clinical scenarios and NIPT tests warrant mention: While it is well accepted that prenatal aneuploidy screening modalities are less effective in multiple gestations than singleton pregnancies,[4] Yang and colleagues showed that NIPT worked well in twin pregnancies with no false-positive results for trisomies 21 and 18.[14] ACOG's 2020 Practice Bulletin recommendation noting the "encouraging" performance of NIPT in twin pregnancies for trisomy 21/18 appears consistent with these findings.[4]

In addition, Beulen and colleagues underscored the screening nature of NIPT and its limitations compared with prenatal diagnostic testing.[1] In pregnancies characterized by ultrasound-detected fetal abnormalities, NIPT should "not be recommended for the genetic evaluation of the etiology of ultrasound anomalies, as both resolution and sensitivity, or negative predictive value, are inferior to those of conventional karyotyping and microarray analysis."[1] Nonetheless, some pregnant women will still consider NIPT to be an acceptable alternative to diagnostic testing despite the clearly demonstrated inferiority of NIPT for the assessment of fetuses with ultrasound-detected abnormalities. That is why counseling is a foundational part of the process of offering prenatal screening and diagnosis to all women regardless of *a priori* risk.

Conclusions

Counseling has always been, and should remain, an essential component in the process by which prenatal screening and diagnostic testing is offered. However, owing to the complexities of new technologies like NIPT, as well as misperceptions as to the actual capabilities of these new algorithms and the safety of diagnostic testing, counseling has become an even more important part of the process by which women and couples choose what, if any, prenatal testing to undergo prior to and during their pregnancies.

The misperceptions that surround NIPT and other prenatal testing options have arisen from a variety of sources, including the relatively rapid introduction of these tests into clinical care, aggressive marketing practices, the internet, "word-of-mouth," and suboptimal professional educational programs. All of these sources have made pretest and posttest counseling even more vital, given the great potential for misinterpretation of screening results.[15]

Future applications of NIPT will likely involve the screening of a more expanded prenatal genome, although validating such a screening protocol will be challenging because of the relative rarity of an individual deletion/duplication of genomic aberrations.[16] In addition to fetal chromosomal and genomic abnormalities, several laboratories have launched NIPT screening assays for fetal single-gene disorders (eg, cystic fibrosis). Perhaps the most intriguing potential applications of NIPT involve screening, diagnosis, and management of malignancies. Regardless of the future clinical applications of circulating cell-free nucleic acid analysis, the integration of this technology into clinical care will continue to require the counseling of women before and after testing.

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How Community-Based ObGyns Implement Noninvasive Prenatal Testing into an Effective Process

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"Prenatal testing for chromosomal abnormalities is designed to provide an accurate assessment of a patient's risk of carrying a fetus with a chromosomal disorder."[1] So begins the latest American College of Obstetricians and Gynecologists (ACOG) Practice Bulletin on screening for fetal aneuploidy. Most women opt for prenatal aneuploidy screening, indicating just how valuable this information is to them.

Community ObGyns have the dual obligations of offering their patients a timely aneuploidy screening test that maximizes the chance of detecting an affected fetus and offering a test that minimizes false-positive results. False-positive results lead to emotionally exhausting and expensive medical odysseys that may conclude with a needless invasive test that interrupts a pregnancy.

In 2017, there were 3.86 million births in the United States, and 82.4% of these births were to women younger than 35.[2] Because of the higher birth rate in younger women, 80% of babies with trisomy 21 were born to women under age 35.[3] Standard screening modalities place this low-risk cohort at increased risk for unnecessary invasive diagnostic testing.

The challenge for practitioners is to determine how best to identify affected pregnancies while minimizing risk and emotional discomfort to most pregnant women. Understanding the benefits and limitations of the testing modalities and recognizing the importance of positive predictive value (PPV) allows providers to select the ideal screening test.

Prior to the advent of cell-free DNA (cfDNA), standard aneuploidy screening tests possessed an intrinsic 5% false-positive rate. The 1-in-20 false-positive rate meant that an average 26-year-old pregnant woman, with a risk of 1:1,290 for a trisomy 21-affected pregnancy,[1] would find that a positive test result was 65 times more likely to be a false positive than a true positive. Paradoxically, offering pregnant women under age 35 a standard screening test with a 5% intrinsic false-positive rate means that they are more likely to receive a positive screen than a woman older than 35 who is offered a cfDNA-based test.

In making the case for using cfDNA aneuploidy testing in the general risk population, both the benefits and limitations of the test should be explored. The efficacy of a test is reflected in the sensitivity. cfDNA screens detect true positives at a rate of >99%. The accuracy of a test is reflected statistically with specificity. The rise in specificity reflects the decrease in the false-positive rate. False-positive rates are additive, so it is critical for the general ObGyn to judiciously avoid testing for rare conditions that needlessly increase the false-positive rate without materially adding benefit to the low-risk patient.

Workflow in a community setting rests on several factors: testing parameters, test limitations, timeliness, accuracy, technical resources, and cost.

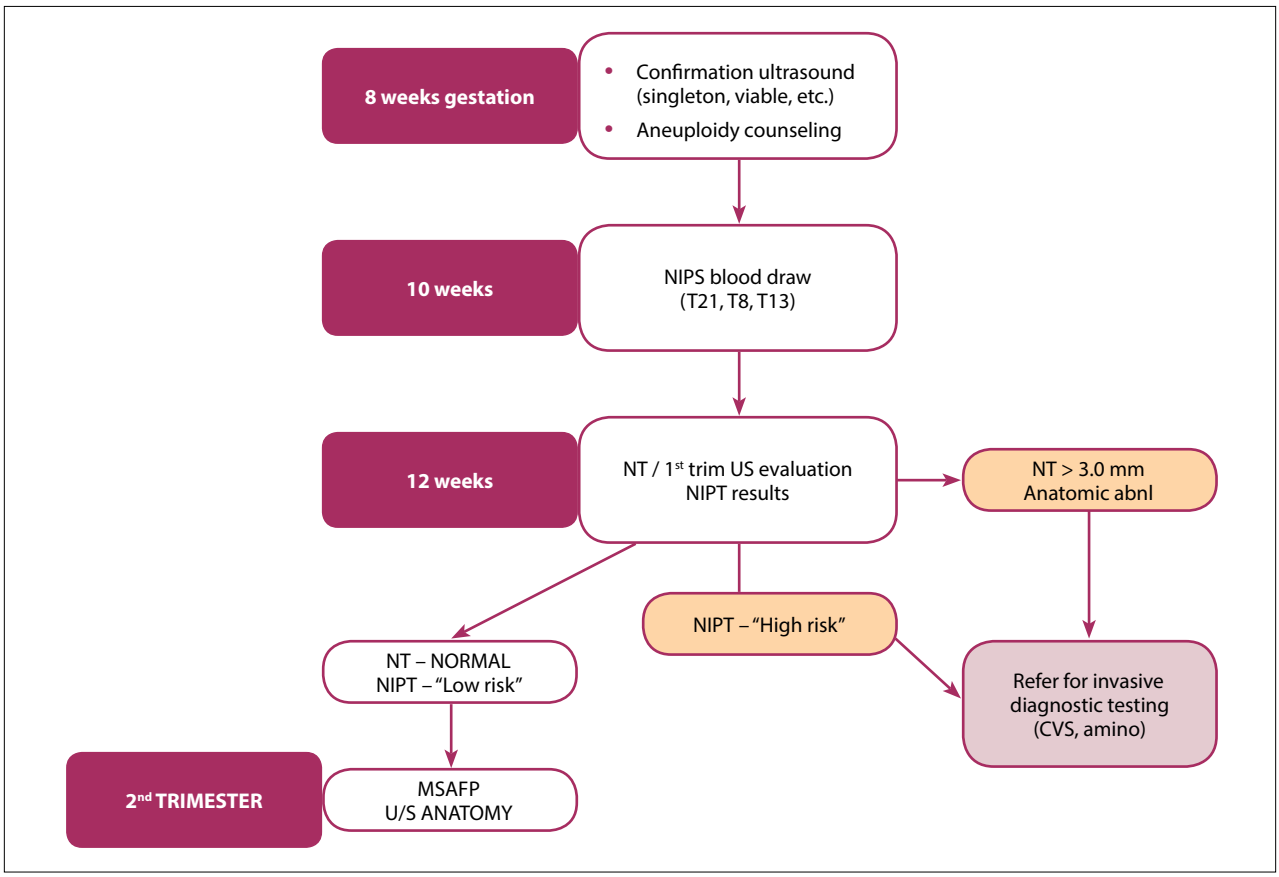
Testing Parameters

1. Screening is performed at ≥10 weeks. Results are available in 7 to 10 days.*
2. cfDNA aneuploidy screening can be performed on any singleton pregnancies, including donor egg and surrogate pregnancies.
3. Screening can be performed on all biologic twin pregnancies as well as donor egg and surrogate twin pregnancies.
4. Sex can be determined on singleton and twin gestations. Note that for twins, sex is identified as two female fetuses or "there is at least one male."

Test Limitations

1. Screening should NOT be done on a pregnancy where a demise has occurred in the pregnancy. During the process of reabsorption, a deceased twin sheds more DNA into the maternal system than the living twin. The test result is more likely to be aneuploid, thereby producing a false-positive result.
2. Other sources for false positives are rare. When they do occur, it is usually due to irreducible biologic factors such as aneuploidy or mosaicism in the placenta, silent maternal chromosomal abnormalities, or as a result of an organ transplant in the mother.
3. "No result" occurs 2%-3% of the time. The test requires a minimum of 4% fetal fraction of the cfDNA for

FIGURE General Risk: Aneuploidy Screening Protocol



Abbreviations: abnl, abnormal; amnio, amniocentesis; CVS, chorionic villus sampling; MSAFP, maternal serum alpha-fetoprotein; NIPS, noninvasive prenatal screening; NIPT, noninvasive prenatal testing; NT, nuchal translucency; US, ultrasound.

accurate analysis. In women weighing more than 250 lb, there is a reduced probability that there will be an adequate amount of cf-fetal DNA to analyze. It is appropriate to redraw at 12 weeks in the obese gravida. An inadequate sample in a woman with a normal body mass index may reflect aneuploidy, and invasive screening should be considered.

4. An 11- to 13-week ultrasound (US) for nuchal translucency (NT) is still important for detecting fetal abnormalities such as anencephaly, cystic hygromas, cardiac defects, abdominal wall defects, and aneuploidy syndromes not otherwise detected by cfDNA noninvasive prenatal testing (NIPT).

Timeliness

NIPT can be performed as early as 10 weeks.* It can also be performed at any time during pregnancy. Standard screening modalities that have been validated during discrete gestational time frames are NT between 11 and 13 weeks' gestation (fetal crown-rump length roughly equaling 45-84 mm)[4] and alpha-fetal protein (AFP)[1] between 15 and 21[5] weeks.

Accuracy

PPV is a population statistic that applies to specified populations. PPV is essentially a way of quantifying the chance a test is accurate when it indicates there is a problem. In a cohort of gravidas age 30.7 years, the PPV for cfDNA screening was 80.9% for trisomy 21, compared with 3.4% for standard screening.[5]

Technical Resources

Phlebotomy is all that is required to perform cfDNA NIPT.

Cost

The upfront test is more expensive than standard screening, but at current pricing, the total cost for a population is more economical than with older methods based on the earlier availability of test results and the lower false-positive rate. A false-positive result incurs costs related to genetic counseling and unnecessary referrals to high-risk specialists.[6]

The presence or absence of aneuploidy is binary—a fetus is either affected or unaffected. All screening tests are nonbinary and carry an error rate. The decision that needs to be made based on a screening result is whether to pursue a diagnostic test. For the patient who finds the uncertainty inherent in a screening test unacceptable, a diagnostic test should be offered along with an explanation of risk.

For most patients, the currently available cfDNA screening tests are "accurate enough," convenient, timely, and carry an acceptable error rate.

Here are some suggested workflows depending on gestational age at intake (**FIGURE**, page S22):

- If the patient presents at 8 weeks' gestation, provide a confirmation US to verify dating, exclude pregnancies with a twin demise, and allow for consultation regarding aneuploidy screening.
- Return visit at 10 weeks for a cfDNA NIPT blood draw. If there is no result and the patient weighs >250 lb or is obese, consider repeating the screen at 12 weeks. If the patient is not at risk for the 2% to 3% no-call result due to dilutional low fetal fraction, refer to a maternal-fetal medicine specialist (MFM) for counseling and chorionic villus sampling (CVS) testing.
- At 12 weeks, return for an NT US. If the NT is normal and the cfDNA NIPT is low risk for aneuploidy, continue with routine prenatal care with second trimester anatomy evaluation and maternal serum alpha-fetoprotein (MSAFP) testing.
- If the cfDNA NIPT is high risk for aneuploidy or the NT is ≥3 mm or there is an anatomic abnormality, refer to an MFM for diagnostic testing.

For later gestational age at intake, modify the above screening protocol as follows:

- Intake between 11 and 13 weeks: Provide an US for NT thickness, verify dating, and confirm that there is no evidence of an early twin demise. If NT measurement is ≥3 mm, refer directly to an MFM. If thickness is <3 mm, draw blood for NIPT. If there is evidence of a twin demise resulting in a singleton, obtain NT and standard screening labs.
- If the cfDNA NIPT is used and returns a no-call result, evaluate for dilutional etiologies. If the patient weighs >250 lb or is obese, consider repeating the screen in 2 weeks. If the patient is not at risk for the 2% to 3% no-call result due to dilutional low fetal fraction, refer to an MFM for counseling and CVS testing. If a patient qualifies for repeat screening due to obesity and the result of this screen is again a no-call result, the patient should be referred to an MFM.

If a patient presents in the second trimester after the window for NT evaluation has closed, obtain an US to verify dating, confirm singleton/multiple status, and ensure that there is no evidence of early twin demise. Offer a combination of cfDNA NIPT and MSAFP if <23 weeks' gestation[6] or cfDNA NIPT alone if gestation is ≥24 weeks.

Conclusion

Community ObGyns have 2 obligations to their pregnant patients when it comes to the use of NIPT in detecting fetal aneuploidy. The first obligation is to use a test with the greatest probability of detecting a fetal aneuploidy, and the second is to use a test that minimizes the risk of a false-positive result. Before the development of NIPT, blood analyte screening tests were associated with a false-positive rate of approximately 5%. NIPT is associated with false-positive rates of <1% and sensitivity rates of >99%.

The benefits of NIPT extend beyond the sensitivity and specificity of the tests. NIPT can be performed as early as 10 weeks* into the pregnancy, and results are usually available within 7 to 10 days of the lab receiving the sample. The accuracy of the test results, which are expressed in terms of PPV, is high. Research has demonstrated that the PPV for trisomy 21 in a cohort of 30-year-olds was ~80% for NIPT versus ~3% for standard blood analyte testing. A concern with NIPT is the issue of a no-call result. Management of this outcome will vary based on patient factors.

*Based on the ACOG NIPT Practice Bulletin Number 226 *Screening for Fetal Chromosomal Abnormalities* Vol. 136, No. 4, October 2020 that states that cf DNA can be performed as early as "9-10 weeks of gestation…"[1]

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