REVIEW | Genetics inMedicine

Noninvasive prenatal testing: limitations and unanswered questions

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The clinical use of noninvasive prenatal testing to screen high-risk patients for fetal aneuploidy is becoming increasingly common. Initial studies have demonstrated high sensitivity and specificity, and there is hope that these tests will result in a reduction of invasive diagnostic procedures as well as their associated risks. Guidelines on the use of this testing in clinical practice have been published; however, data on actual test performance in a clinical setting are lacking, and there are no guidelines on quality control and assurance. The different noninvasive prenatal tests employ complex methodologies, which may be challenging for health-care providers to understand and utilize in counseling patients, particularly as the field continues to evolve. How these new tests should be integrated into current screening programs and their effect on health-care costs remain uncertain.

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SCREENING FOR ANEUPLOIDY IN PREGNANCY

The past 2 decades have seen major advances in prenatal screening for chromosomal conditions, most recently in the realm of noninvasive prenatal testing (NIPT). These tests evaluate circulating cell-free DNA (cfDNA) to determine the risk of fetal aneuploidy. In pregnancy, cfDNA in maternal blood is a mixture of maternal DNA and placental DNA from apoptosis of placental cytotrophoblasts. The circulating cfDNA can be analyzed to identify qualitative and quantitative differences between the maternal and the placental DNA.

Qualitative differences in the DNA allow for the detection of paternally transmitted mutations such as RhD typing (for an RhD-negative mother and an RhD-positive father).[1] Quantitative differences allow for the detection of trisomies or monosomies by detecting a quantitative difference in the total number of expected chromosomes (e.g., an increased total number of chromosome 21 in the case of Down syndrome). Detection of quantitative differences in DNA can be accomplished by one of two methods: massively parallel sequencing, in which all free DNA in the maternal blood is sequenced and then the number of each chromosome of interest is quantitatively compared to the number expected,[2,3] or by a directed approach, in which specific sequences from the chromosomes of interest are recovered, and selected sequences are then analyzed.[4–6] Once the genetic data are obtained, the results are analyzed by either quantitative read counting or by analyzing single-nucleotide polymorphisms.[5,6] Quantitative read counting compares the number of chromosome fragments of interest to a euploid reference sample to calculate the expected proportion from each chromosome. Single-nucleotide polymorphisms can be detected and analyzed using bioinformatic algorithms that account for the fetal cfDNA and maternal and paternal DNA, and then calculate the statistical chances of an outcome.[5,6]

Prior screening algorithms for aneuploidy involve ultrasound and/or serum screening in the first and/or second trimesters, with trisomy 21 detection rates of 81–96% with false-positive rates set at 5%.[7] Initial reports of NIPT describe detection rates of 98–99% or higher for trisomy 21, 44–91% for trisomy 13, and 83–95% for trisomy 18, with false-positive rates of 1–2%.[8–11] Sensitivity and specificity are high; however, the positive predictive value (reliability of a positive test) and the negative predictive value (reliability of a negative test) are affected by the prevalence of the disease in the population tested. The lower the disease prevalence, the higher the negative predictive value (true negatives) and the lower the positive predictive value (true positives). For example, with sensitivity of 100% and specificity of 99% (1 false-positive in 100), if the disease prevalence is high (risk of Down syndrome of 10 per 100, 10%), screening 1,000 patients would result in a positive predictive value of 91%. With a lower disease prevalence (risk of Down syndrome of 1 per 1,000), screening 1,000 patients would result in a positive predictive value of 9%. Therefore, the prevalence of the condition being screened for must be taken into account when interpreting test results, particularly the positive predictive value, as with a lower disease prevalence, a positive result is less reliable (more likely to be a false-positive result).

Many professional organizations have released position statements regarding NIPT. The Committee Opinion on Noninvasive

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Prenatal Testing for Fetal Aneuploidy published by the American College of Obstetricians and Gynecologists and the Society of Maternal Fetal Medicine states that patients with increased risk for fetal aneuploidy can be offered testing with cfDNA. High-risk women were defined as those of maternal age 35 years or older at delivery, with fetal ultrasonographic findings indicating an increased risk of aneuploidy, with a history of prior pregnancy with trisomy, with a positive maternal serum screen for aneuploidy, or with parental balanced robertsonian translocation with increased risk for fetal trisomy 13 or trisomy 21.[12] The American College of Obstetricians and Gynecologists and the Society of Maternal Fetal Medicine recommend pretest counseling, which should include information regarding the nondiagnostic nature of the test, as well as a review of the family history to assess for other chromosome or single-gene disorders. They also recommend referral for genetic counseling in the event of a positive result. The National Society of Genetic Counselors Position Statement on NIPT echoes these sentiments, with additional emphasis on providing pretest counseling in a nondirective way.[13]

The International Society for Prenatal Diagnosis recommends more limited use of NIPT, with first-tier prenatal screening recommended using serum analyte and ultrasound screening for all women, including those above the age of 35.[14] The International Society for Prenatal Diagnosis recommends consideration of NIPT only as a second-tier test for women who have increased risk for aneuploidy determined through serum analyte and ultrasound markers, or for women who present to care too late to undergo serum or ultrasound screening that depends on gestational age.

In its published policy statement, the American College of Medical Genetics and Genomics (ACMG) recently advocated changing the terminology from NIPT to noninvasive prenatal screening (NIPS) to emphasize the limitations of cfDNA testing.[15] This policy statement also provides an overview of the limitations of this new technology and guidance on important points for pretest and post-test counseling. Of note, the ACMG guideline does not define a target "high risk" population for which NIPT is indicated.

IMPLICATIONS OF TESTING FOR CLINICIANS

Benefits of testing

Based on initial studies, NIPT has the potential to offer improved sensitivity and specificity for prenatal screening, and lower false-positive rates as compared with maternal serum analyte screening. In addition, NIPT has the potential for earlier results in the first trimester without multiple blood draws, and may result in a decrease in invasive diagnostic procedures, which are associated with a risk of pregnancy loss.

Current limitations of research

Despite the above potential benefits associated with NIPT, notable limitations exist. False-positive and false-negative test results do occur and in clinical practice may occur at a higher rate than reported in carefully controlled, small clinical trials. Current studies have been completed in predominantly high-risk women already scheduled to undergo invasive diagnostic testing. Studies examining the use of NIPT in screening low-risk populations have included between 289[16] and 2,049 patients.[17] In the largest trial, by Nicolaides et al.[17], the overall trisomy detection rate was 100% with a combined false-positive rate of 0.1% and 4.8% no test rate. However, this study did not confirm findings with karyotyping, and euploidy was assumed based on lack of phenotypic features of aneuploidy. As discussed above, a lower prevalence of trisomies in the population being screened will lead to a lower positive predictive value (fewer true positives).

There have been no large-scale clinical validation studies of NIPT to date. In most of the studies, the confirmatory test was chorionic villus sampling (CVS) or amniocentesis; however, no studies correlated test results with actual maternal chromosomes and fetal chromosomes at delivery. This is important because cfDNA is maternal and placental in origin, thus maternal somatic mosaicism and confined placental mosaicism (CPM) can affect results, and there have been reports of false-positive NIPT results due to CPM including trisomy 21.[15,18]

In many studies, the confirmatory diagnostic test most often used was CVS, which involves karyotype analysis of placental tissue.[19] The possibility of ploidy discordance between fetal and placental tissues has been well documented in the medical literature, with CPM typically thought to occur in 1–2% of CVS cases.[20] However, CVS analyzes a small sample of placental cells, and it is possible that the incidence of placental mosaicism (PM) may be higher, with one study reporting that 4.8% of term placentas contained PM.[21] It is unclear how the type of mosaicism and/or mosaic cell distribution in the placenta may affect cfDNA testing. Cases have been reported in the literature in which CPM was identified with NIPT, and it has been suggested that NIPT may be useful in screening for placental dysfunction.[22] Although PM can lead to adverse pregnancy outcomes, including intrauterine growth restriction and intrauterine fetal demise, it can be associated with normal pregnancy outcome. The overall effect of CPM on a pregnancy is dependent on the timing, type, and amount of the chromosome involved.[23] Artan et al.[24] reported site-specific variation of chromosomal mosaicism in term placentas, with mosaicism scattered throughout the placenta in some, and limited to some areas in others. The cfDNA studies that have assessed products of conception following pregnancy termination have not separated placental and fetal tissue, thus abnormal karyotype following pregnancy termination does not verify whether the source was fetal or placental. In addition, normal NIPT results were not verified with confirmatory amniocentesis, CVS, or post-delivery data in some studies,[4,25,26] thus determination of euploidy was solely based on NIPT results—essentially using a screening as a diagnostic test.

The studies on NIPT also assumed a normal maternal karyotype. Low-level maternal mosaicism will result in variations in the maternal contribution to circulating cfDNA, which may also impact NIPT results.[9] The potential for discordance among maternal, fetal, and placental chromosomes requires additional research and large studies assessing the correlation between

NIPT and actual maternal and fetal karyotype. As NIPT is employed in clinical practice in large numbers of patients, some of these more rare situations are likely to be encountered. This may affect the test's clinical performance and simultaneously present a clinical conundrum to providers and patients alike.

In the majority of studies, the tests were run on archived or accumulated samples, and it is unclear how these tests will perform (or are performing) in real-life clinical situations. Noninformative results reportedly occur in 3–7% of tests.[9–11,26–28] In the studies reported to date, noninformative results include samples that do not meet quality control, have low fetal fraction (typically <4%), or are due to collection/sampling errors. Some noninformative results or failures are known to include samples with aneuploidy,[11,27,28] and these failures were excluded from determination of the sensitivity and specificity for each disorder in some studies.[11,28] In some reports, aneuploidy was present in a high proportion of the excluded cases,[10,28] but the true association between aneuploidy and test failure is unknown. Conflicting data exist as to whether repeating the test overcomes initial test failure, and one study demonstrated that fetal fraction does not increase appreciably until the third trimester.[28] There is also a reported association between increased body mass index and decreased fetal fraction;[29] however, the overall impact of body mass index is unclear. None of the previously published studies have defined the potential impact of obesity on the ability of NIPT to provide results.

Of note, the majority of the published literature on the performance of NIPT includes authors affiliated with or studies funded by commercial laboratories currently offering NIPT on a clinical basis or companies that are financially invested in the technology used to perform NIPT. Although some of the studies were blinded and used independent labs and research companies for interpretation of results, this represents a shift from public to private funding of such research. The competitive commercial environment surrounding NIPT, including direct marketing to patients and health-care providers, may have contributed to the early introduction of NIPT into clinical practice despite limited evidence to support broad use of this technology. There is increasing concern that the limitations and shortcomings of NIPT may be underappreciated by clinicians and the public.[30,31]

ADEQUATE INFORMED CONSENT

The ACMG statement on NIP screening states that "aneuploidy screening is not routine; it is acceptable for patients to decline screening."[15] As with prior screening modalities, the decision about whether or not to undergo aneuploidy screening should be made in the context of the patient's needs and values. Pretest counseling should include discussion about the potential benefits and limitations of screening options as well as diagnostic testing.

The possibility that a NIPT result may not correlate with the fetal chromosomes should be clearly discussed with patients, and they should be counseled about the limitations of follow-up diagnostic testing with CVS, which may not reflect the actual fetal karyotype in cases of CPM. In addition to a discussion of PM, pretest counseling should include information about the possibility of discovering maternal chromosomal variations not previously detected. Although it is state dependent whether informed consent is required before genetic testing, appropriate counseling and documentation of informed consent are recommended, as with any medical test or procedure.

Another important consideration that must be adequately addressed in pretest counseling includes the patient's desires related to diagnosis of aneuploidy versus screening for aneuploidy. If patients ultimately desire diagnostic testing, NIPT cannot supplant amniocentesis. Because NIPT is a screening test, using it as a "secondary screen" following abnormal serum screening or abnormal ultrasound may actually delay definitive diagnostic testing.

Patients with "screen-negative" results need to be counseled about the possibility of a false-negative result, and ideally a residual risk should be provided. Genetic counseling and consideration of diagnostic testing is recommended to follow up a screen-positive result, and accurate and up-to-date information about the conditions being tested should be made available to patients with "screen-positive" results. The ACMG policy statement on NIP screening provides a list of specific resource recommendations.[15]

REGULATORY OVERSIGHT

The US Food and Drug Administration (FDA) is the federal agency responsible for oversight and regulation of in vitro diagnostic (IVD) products, which are reagents, instruments, and systems intended for use in diagnosis of disease, in order to cure, mitigate, treat, or prevent disease or its sequelae. IVDs are medical devices, subject to pre- and postmarket controls, as well as the Clinical Laboratory Improvement Amendments (CLIA) of 1988. Lab-developed tests (LDTs) are local, noncommercial tests used in a hospital setting for the diagnosis and monitoring of rare diseases, with small numbers of tests and expert oversight/interpretation. LDTs are laboratory tests that are developed for use in a particular laboratory. The lab then offers the testing service, which is performed in house, and is thus not typically subject to FDA approval. LDTs may be used for research only, for guiding patient treatment, or as "companion diagnostics"—tests that are used to determine the safe and effective use of corresponding therapeutic products.

Currently, LDTs fall under FDA regulation; however, due to enforcement discretion the FDA has the option not to actively regulate LDTs. Federal oversight occurs under the CLIA legislation, which is managed by the Centers for Medicare and Medicaid Services. The companies that perform NIPT claim that their tests are laboratory-derived tests and that they are functioning as a CLIA lab. New LDTs are often a site of entry for novel tests developed by companies, then licensed to a lab. LDTs are dependent on components (e.g., machines, reagents) that are assembled and marketed by other companies. There are now more of these LDTs in commercial labs and biotech companies, and these tests are developed for broad commercial use with aggressive direct marketing to health-care practitioners

and patients. Companies are factoring in the FDA's enforcement discretion policy and using this loophole to gain rapid market access for their test(s) without FDA oversight. There is no well-defined clinician–pathologist–patient relationship, and expert oversight of these individual tests cannot be assured.

Other differences exist between regulation of IVDs by the FDA versus the CLIA. The FDA requires a research phase in which the investigational use of the IVD is defined, research subjects must be protected by an institutional review board, and adequate informed consent must be obtained. Following this, the FDA requires analytical and clinical validation and reporting of adverse events to the FDA. Final results are published and available to the public. By contrast, CLIA regulation of IVDs does not require a research phase. Analytical validation can be attained through post hoc sampling, and clinical validation is not required. No requirement for reporting of adverse events exists, and, moreover, no system is in place to track, record, or report adverse events. In the event of a problem, no formal mechanism for recall of devices exists. Quality control and quality assurance testing is of critical importance for NIPT, particularly with the varied and complex nature of these tests. Currently, oversight of quality assurance is lacking. In essence, NIPT is now commercially available and is aggressively marketed to health-care providers and patients. The lack of clinical validation is not widely publicized. Patients and providers may be unwittingly participating in a large phase IV clinical trial without formal, centralized tracking of adverse events (including false-positive and false-negative test results). Although the testing is not diagnostic, some patients may choose to act on results without confirmatory invasive diagnostic testing.

In the face of new developments and rapidly changing genetic tests for prenatal screening, as well as other diagnostic and screening modalities, the current regulatory landscape may evolve and change with time. There are likely many factors influencing the regulation of genetic tests, including financial pressures, rapid developments and advancements in technologies, and political pressures.

FUTURE DIRECTIONS

More research is needed to address questions of clinical performance/accuracy and how best to implement the testing in the clinical setting. Additional considerations also include adequate regulatory oversight and quality control to ensure patients and clinicians can rely on NIPT results.

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DISCLOSURE

The authors declare no conflict of interest.

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