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Noninvasive prenatal testing for aneuploidy using cell-free DNA – New implications for maternal health

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Abstract

The rapid global uptake of noninvasive prenatal testing for Down syndrome based on maternal plasma cell-free DNA has provided new data on the interrelationship between cell-free DNA and maternal health. Specific maternal conditions that can affect the performance of noninvasive prenatal testing include obesity, active autoimmune disease and low molecular weight heparin treatment. There is also a growing appreciation of the implications of discordant noninvasive prenatal testing results for maternal health, including unexpected diagnoses of maternal chromosomal conditions, or rarely, occult cancer. The interrelatedness of noninvasive prenatal testing and maternal health mean that the longstanding principles underpinning prenatal screening – voluntary testing, informed decision making, availability of specialist genetic counselling and well-defined clinical pathways – are more important than ever before.

Keywords

Noninvasive prenatal testing, cell-free DNA, maternal health, Down syndrome screening

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Introduction

Cell-free (cf) nucleic acids circulating in peripheral blood are the basis of a relatively new and flourishing field in human health and disease.[1] cfDNA and RNA fragments are released into extracellular spaces and body fluids as a result of cellular turnover (apoptosis and necrosis), and actively regulated cellular processes (exosome secretion). While cfDNA is present in a wide variety of biofluids, including saliva, amniotic fluid and urine, the vast majority of research has been performed on nucleic acids in plasma and serum.

The majority of circulating cfDNA derives from hematopoietic cells,[2] but it also derives from solid tissues, including neoplastic cells. The so-called liquid tumour biopsy, utilizing circulating tumour (ct)DNA, has been widely studied for applications in oncology.[3]

The most rapid translation of cfDNA into clinical practice, however, has been in the field of obstetrics where cfDNA of placental origin has revolutionized prenatal screening for Down syndrome (trisomy 21). The technical fundamentals of cfDNA-based screening for aneuploidy, also called noninvasive prenatal testing (NIPT), have been recently summarized elsewhere.[4] Instead, this review focuses on the novel data emerging on the interrelationship between maternal health and NIPT, including the impact of maternal health on the performance of NIPT, and the secondary findings obtained about maternal health as a consequence of cfDNA screening.

Plasma cfDNA during pregnancy – The basics

The presence of fetal DNA in maternal plasma was first reported in 1997 using conventional polymerase chain reaction techniques to identify Y chromosome-specific DNA sequences.[5] The primary source of circulating fetal DNA is the placenta, specifically the outer cytotrophoblastic layer, rather than the mesenchymal core.[6] Although the term 'cell-free fetal DNA' is commonly used, it is important to remember that the pregnancy-derived DNA in maternal plasma reflects only the placental genome, as there can be situations of discordance between the fetal and placental karyotypes due to confined placental mosaicism.

cfDNA of placental origin is detectable in maternal blood from early pregnancy and its quantity rises with gestation. The average proportion of total cfDNA in maternal blood that derives from the placenta (the so called fetal fraction) is approximately 10% at 11–13 weeks gestation.[7] Placental cfDNA is cleared rapidly after birth, so the cfDNA detectable in a pregnant woman represents that of the current conceptus only.[8,9]

cfDNA from the placenta differs in its size distribution from the cfDNA derived from maternal tissues. Placenta-derived DNA fragments are shorter in length than maternally derived DNA fragments (size peak 143 base pairs (bp) vs. 166 bp). This difference in the fragment length profile is now being employed to improve the accuracy of prenatal aneuploidy screening.[10]

Prenatal aneuploidy screening and NIPT

Screening for fetal chromosome abnormalities is a well-established part of antenatal care in most developed countries. Trisomy 21 is the most common chromosome condition seen in newborns, with an overall birth prevalence of 1 in 434 (23/10,000).[11] The two next most common chromosome conditions with serious perinatal morbidity and mortality are trisomy 18 (Edwards syndrome) and trisomy 13 (Patau syndrome).

Until recently, the most accurate screening test for these aneuploidies was combined first trimester screening (CFTS), which can only be performed at 11+0 to 13+6 weeks gestational age. CFTS involves an algorithm that incorporates the results of first trimester ultrasound measurement of the fetal nuchal translucency, levels of maternal

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serum biochemical markers, and maternal age to produce an individualized risk figure for each trisomy. Detection rates of 85%–90% for trisomy 21 can be achieved for a fixed false positive rate of ~5%.[12,13] Women found to be at increased risk according to local thresholds are typically offered confirmatory invasive diagnostic testing with amniocentesis or chorionic villus sampling.

In pregnancies affected by Down syndrome, the presence of three copies of chromosome 21 in the cytotrophoblast results in a higher amount of chromosome 21 DNA fragments being released into maternal blood. Massively parallel sequencing of plasma cfDNA allows the chromosome of origin to be determined for each DNA fragment by mapping its base sequence to a reference genome. Fetal trisomy is presumed if a higher than expected number of cfDNA fragments originating from a particular chromosome are counted. The detection of fetal monosomy, conversely, involves the detection of less than expected DNA fragments from a particular chromosome.

Multiple clinical validity studies have demonstrated the superior accuracy of NIPT over CFTS for trisomy 21, 18 and 13 screening. A meta analysis calculated pooled sensitivities and specificities for trisomy 21 detection of 99.2% and 99.91%, respectively.[14] NIPT also has the added benefit of a wider gestational age window for screening (any time from 10 postmenstrual weeks) compared with CFTS.

The very low false positive rates (<1%) associated with the high specificity of NIPT has been a major benefit of NIPT, reducing unnecessary invasive testing in many women that would have been categorized as high risk by other forms of screening. Professional societies now support the use of cfDNA for aneuploidy screening, but emphasize that confirmation with prenatal or postnatal karyotyping is still required as false positive results still occur (Table 1).[15] The probability of an affected fetus after a high-risk NIPT result for trisomy 21 (positive predictive value, PPV) varies according to the background risk of the population, ranging from 45.5% in low-risk populations[16] to over 90% in high-risk women. These figures are a marked improvement on the PPVs for CFTS and other forms of screening, which are typically less than 10%–20% depending on background prevalence.

The scope of NIPT continues to expand with advances in sequencing and bioinformatics. Women are now routinely offered information on fetal sex and sex chromosome aneuploidy (SCA) on most commercial NIPT assays, in addition to screening for trisomies 21, 18 and 13. NIPT can also detect genome-wide subchromosomal copy number variations (CNVs), including the 22q11.2 deletion syndrome.[17] The main challenges for this technology now lie in its responsible and equitable clinical implementation.[18,19]

The accumulated global clinical experience of NIPT has lead to a growing awareness of its interrelationship with maternal biology. Two main issues have emerged with respect to NIPT and maternal health: (i) maternal factors that impede the technical performance of NIPT and (ii) the detection of undiagnosed maternal medical conditions as a direct result of sequencing maternal plasma DNA.

Table 1. Biological causes of false positive noninvasive prenatal test (NIPT) results (high-risk NIPT result, but normal fetal karyotype).

Placental/fetal causes
Confined placental mosaicism
In uterine demise of an aneuploid twin fetus ('vanishing twin')
Maternal causes
Maternal aneuploidy (e.g., 47,XXX)
Maternal mosaicism (e.g., 45X/46XX)
Maternal copy number variations (e.g., subchromosomal duplications or deletions, including 22q11.2 microdeletion carriers)
Maternal neoplasms (benign or malignant)
Prior organ transplant[a]

[a]May cause false positive result for male fetal sex.

Effect of maternal biology on the performance of NIPT

A small proportion of samples submitted for NIPT will not return an interpretable result. The most common reason for these 'no call' results is a relatively low amount of placental cfDNA in maternal blood, or low fetal fraction [fetal fraction = placental DNA/(placental DNA + maternal DNA)]. Most NIPT assays require a minimum fetal fraction of 2%–4% for a reportable result. Any condition which increases maternal cell turnover without increasing placental cell turnover could theoretically reduce the fetal fraction and increase NIPT failure rates. While approximately half of women with a 'no call' result will obtain a successful NIPT result on redraw, those that do not obtain a result on repeat testing may lose the opportunity to access CFTS if their gestation has advanced past 13+6 weeks. This has important implications for pre-test counselling and choice of screening test for women at increased risk of failed NIPT.

Maternal obesity

Obesity is known to be associated with a low-grade systemic inflammatory state and increased cell turnover in white adipose tissue. A case–control study comparing adipose tissue and cfDNA levels in obese and lean pregnant women found that the obese women had more apoptosis and necrosis in their adipose cells.[20] This was associated with a twofold increase in plasma levels of cfDNA of maternal origin, but no significance difference in the cfDNA of fetal origin. These findings suggested that obese women may have lower fetal fractions due to the increased apoptosis in adipose tissue.

In a clinical study of 1949 women undergoing NIPT, increasing maternal weight was confirmed to be significantly associated a lower fetal fraction.[7] The proportion of women with a fetal fraction <4% (and thus more likely to have a 'no call' result) increased from <1% at maternal weight of 60 kg to >50% at 160 kg. Maternal weight, body mass index and blood volume have been confirmed in other studies as the most important maternal factors negatively associated with fetal fraction.[21] Clinicians should consider this information in their pre-test counselling and have contingency plans for alternative forms of screening if referring obese women for NIPT.

Autoimmune disease

Autoimmune disease is also a known cause of increased cell turnover and non-pregnant patients with systemic lupus erythematosus (SLE) have elevated levels of circulating cfDNA. A recent study utilizing massively parallel sequencing has characterized in detail the abnormalities in plasma cfDNA in SLE patients.[22] The abnormalities observed compared to healthy controls included DNA fragment size shortening, over- and under-representation of genomic regions, and hypomethylation. Furthermore, these aberrations were associated with clinical disease activity and binding of anti-double stranded DNA antibody to plasma DNA. The authors concluded that caution should be exercised when interpreting results of NIPT in pregnant women with SLE. A case report of a pregnant woman with severe autoimmune disease (including severe thrombocytopenia and neutropenia) provides a clinical example of the adverse effect of active maternal disease on the performance of NIPT. In this patient, two attempts at NIPT returned 'no call' results due to low fetal fraction. However, when maternal disease activity was suppressed with oral steroids to improve the woman's platelet count in preparation for amniocentesis, the fetal fraction rose sufficiently to facilitate a third successful NIPT attempt.[23]

Table 2. Documented maternal and fetal influences on fetal fraction (FF).[7,21,41–43]

Positive correlation (associated with increased FF)
Gestational age
Crown-rump length
Maternal serum pregnancy-associated plasma protein A (PaPP-A)
Maternal serum free beta-human chorionic gonadotropin (beta-hCG)
Fetal trisomy 21
Negative correlation (associated with reduced FF)
Maternal weight
Maternal body mass index
Maternal blood volume
Multiple pregnancy (reduced 'per fetus' FF)
Fetal mosaicism
Fetal trisomy 18, trisomy 13
Digynic triploid pregnancy
Pre-existing hypertension
No correlation (no impact on FF)
Maternal age
Fetal sex
Nuchal translucency measurement
Pre-existing diabetes mellitus
Hyperthyroidism

Other maternal influences on fetal fraction

Other statistically significant associations of maternal factors with fetal fraction have been variously reported, including maternal race, smoking and pre-existing hypertension (Table 2). However, the literature contains conflicting findings on some of these maternal factors, and their clinical relevance is less significant compared with the effect of maternal weight or fetal factors.

Maternal medications

It is now apparent that some maternal medications can also interfere with NIPT performance. Low molecular weight heparin (LMWH) is the first drug reported to have an adverse effect on NIPT performance.[24] In the experience of one commercial laboratory, 9 of 12 women with 'no call' NIPT results were retrospectively determined to be on LMWH treatment at the time of testing. Their plasma samples were subsequently found to have an unusually high proportion of small DNA fragments. Repeat blood sampling timed during the LMWH 'trough' was associated with successful results in all five women who underwent repeat NIPT. While the exact mechanism of LMWH interference is unknown, this experience suggests that blood collection for NIPT should be timed as far as possible from the most recent dose.

Very little information about other therapies and their effect on cfDNA and NIPT currently exist. While intravenous immunoglobulin (IVIG) appeared to cause increased variance in cfDNA in the autoimmune case report above,[23] IVIG was not confirmed to be associated with any clinically significant change in fetal fraction or NIPT assay performance in an otherwise healthy woman requiring IVIG for fetal indications.[25]

Organ transplant recipients

Women who have had an organ transplant also require special consideration prior to NIPT. Transplanted organs from male donors can cause a false diagnosis of a male fetus due to the release of Y-chromosome-specific DNA sequences.[26] Women with a prior stem cell transplant or pregnancies conceived with donor eggs are unsuitable for single nucleotide polymorphism (SNP)-based methods of NIPT and should be referred for random massively parallel sequencing based assays.

Unexpected secondary maternal findings from NIPT

DNA sequencing for NIPT analyses both maternal and fetal DNA fragments, without necessarily determining their origin. NIPT is generally contraindicated in women with known chromosome abnormalities because their genomic variation will obscure assessment of the fetal cfDNA contribution. However, unsuspected maternal genetic conditions are now a well-recognized biological cause of false positive NIPT results and are therefore an important issue to discuss with women during pre-test counselling.

Maternal sex chromosome conditions

The accuracy of NIPT for abnormalities of the X chromosome is intrinsically inferior to that for the autosomes due to greater biological variation in the X chromosome. There is a higher rate of fetal and placental mosaicism for X chromosome abnormalities compared with other aneuploidies. There is also a normal phenomenon of age-related maternal loss of X chromosome, converting the maternal blood karyotype from normal XX to low-level XO/XX mosaic in some women.[27] Furthermore, as many SCAs have a mild phenotype (including normal fertility), there are now many instances of unsuspected maternal SCAs underlying discordant NIPT results.

One study that examined 181 cases of suspected SCA detected by NIPT found that 16 (8.5%) were due to abnormal maternal karyotypes, including mosaic Turner syndrome and 47 XXX.[28] Another large study reanalyzed stored NIPT samples to estimate the population-based prevalence of SCA.[29] These investigators, who also collected paternal samples in a proportion of cases, found 119 X and Y chromosomal abnormalities in their study population of 141,918 fertile women and 29,581 fertile men (overall prevalence of SCA: 1 in 1441). These results showed that fertility in women with mosaic Turner syndrome (45,X/46,XX) was higher than previously assumed.

Maternal autosomal abnormalities

Unsuspected abnormalities in the maternal autosomes (non-sex chromosomes) have also been reported as biological causes of false positive NIPT results. These abnormalities include low-level maternal mosaicism for trisomy 18,[30] and maternal partial duplications on chromosomes 18 and 13.[31,32]

With the recent expansion of some NIPT assays to include selected microdeletion syndromes, unexpected detection of maternal carriers of the 22q11.2 deletion syndrome (diGeorge syndrome) is also becoming a clinically significant issue. In one study of 175,393 women screened for fetal 22q11.2 deletion syndrome, over 60% (20/32) of women with high-risk results for the microdeletion had a suspected maternal component.[33] In contrast, another NIPT provider detected maternal deletions in only 2% of their high-risk calls.[17] These large differences in maternal carrier rates are likely due to different NIPT methods, referral bias and population characteristics, making meaningful comparisons between commercial assays difficult.

Epidemiological studies using NIPT samples

Large datasets accumulated by clinical NIPT providers are now being employed to provide information on genomic CNVs on a population level. In one study of over 51,000 women, more than 40% had at least

one putative maternal CNV detected.[34] Some of these CNVs were considered pathogenic (i.e., recognized deletion syndromes) or potentially pathogenic (e.g., CNVs in regions of known cancer genes). In another study, reanalysis of sequencing data from a Chinese biobank of maternal samples was utilized to create a genetic map of SNPs and genetic variants in a Han population.[35] This dataset of 150,000 women was able to provide new estimates of the molecular prevalence of single gene disorders such as thalassemia, Duchenne muscular dystrophy and spinal muscular atrophy. The rapid uptake of NIPT around the world has thus created new opportunities for large-scale epidemiological studies, but also highlights the challenges of ensuring the ethical conduct of genetic research using samples retained from clinical testing.

Maternal malignancy

Tumour cells with whole or subchromosomal abnormalities may release ctDNA that is detectable in plasma using routine NIPT workflows. A 2012 case report first described maternal malignancy as a cause of discordant NIPT results.[36] Subsequently, a case series obtained from a total cohort of 125,426 women provided details on 10 women that had discordant NIPT results due to an undiagnosed maternal cancer (prevalence 1 in 12,500).[37] The cancer types included lymphoma, leukaemia, colorectal and anal cancers. Detailed sequencing on 8 of these 10 cases showed that they were most commonly associated with an NIPT result indicating multiple aneuploidies, a highly unusual situation generally incompatible with a live pregnancy. Of the 39 cases in the total cohort that had an NIPT result indicating multiple aneuploidies, seven were due to asymptomatic maternal malignancies. These women also showed non-specific CNVs across multiple chromosomes on further bioinformatics analysis. In one woman with serial blood samples, the plasma DNA aberrations seen on NIPT cleared after cancer treatment, confirming the relationship between ctDNA, tumour load and NIPT result.

However, not all neoplastic causes of abnormal NIPT results are malignant; some may be due to benign tumours such as uterine leiomyomata. One woman with a large fibroid had an unusual NIPT result of 'monosomy 13', and partial/complete losses on chromosomes 1p, 14 and Xq on two separate analyses.[38] The maternal origin of these plasma DNA abnormalities was confirmed on molecular testing of the fibroid. The laboratory involved subsequently identified 15 other cases of discordant NIPT results attributable to leiomyomata among approximately 400,000 of their tests, giving an observed prevalence of 3.75/100,000.

In light of this accumulating data on maternal cancers, counselling women with unexplained false positive NIPT results can be difficult. A balance must be struck between timely cancer detection, and minimizing unnecessary maternal anxiety and costs. In the series by Bianchi et al, the 10 reported cases of maternal cancer represented only 0.008% of the total laboratory case volume, highlighting the rarity of maternal cancer as a cause of false positive NIPT.

As the unusual finding of multiple aneuploidies on NIPT is the abnormality most closely associated with maternal cancers, these cases should be targeted for careful maternal assessment and pregnancy follow up. In the presence of a euploid, structurally normal fetus and a normal maternal karyotype, maternal malignancy should be among the differential diagnoses for these women. If the same suspicious genomic representation is reproduced on repeat maternal plasma testing, then referral for oncological assessment may be warranted.[39]

Summary

Our growing experience of NIPT in clinical practice has provided us with intriguing evidence of its close interrelationship with maternal health. Clinicians should be aware of specific maternal conditions that may affect the performance of NIPT, such as obesity, active autoimmune disease and LMWH treatment. It is also an important ethical consideration to inform women about the risk of secondary maternal findings as a result of plasma DNA sequencing.[40] These implications for maternal health mean that the longstanding principles underpinning prenatal screening – voluntary testing, informed decision making, availability of specialist genetic counselling and well-defined clinical pathways – are more important in the genomic era than ever before.

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