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EDITORIAL

Has Noninvasive Prenatal Testing (NIPT) Come of Age?

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Introduction

Recent advances in genomic sequencing and bioinformatics have led to the development of noninvasive detection methods with detection rates approaching those obtained with amniocentesis and chorionic villus sampling (CVS) [1, 2]. Recently, a novel prenatal testing method has become available. This method, known as noninvasive prenatal testing (NIPT), is a molecular approach for assessing fetal aneuploidy using cell-free fetal deoxyribonucleic acid (cffDNA) from the plasma of pregnant women. NIPT has a false positive rate of about 0.2 % and detection rate of about 98 % for Down syndrome [1, 2]. NIPT has been used for assessing abnormalities such as trisomy 21, trisomy 18, and trisomy 13. Approximately 10–15 % of the cell-free deoxyribonucleic acid (cfDNA) in maternal blood comprises cffDNA [3, 4]. The half-life of cffDNA is short, and it clears from maternal circulation soon after delivery [3]. Hence, there is no risk of fetal DNA persisting from one pregnancy to the next and confounding test results. For women infected with hepatitis B, hepatitis C, and/or human immunodeficiency virus (HIV), the use of noninvasive methods of prenatal risk assessment is recommended, using tests with high sensitivity and low false-positive rates, such as serum screening combined (or not) with nuchal translucency, anatomic ultrasound, and noninvasive molecular prenatal testing [5]. Among other factors, cost implications for introducing this new technology in clinical practice will need to be considered.

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Discussion

Prenatal screening pathways, as nowadays offered in most Western countries, consist of similar tests. First, a risk-assessment test for major aneuploidies is offered to pregnant women. In case of an increased risk, invasive diagnostic tests, entailing a miscarriage risk, are offered. For decades, only conventional karyotyping was used for final diagnosis. Moreover, several fetal ultrasound scans are offered to detect major congenital anomalies, but the same scans also provide relevant information for optimal support of the pregnancy and the delivery. Noninvasive prenatal testing (NIPT) is an emerging form of prenatal genetic testing that provides information about the genetic constitution of a fetus without the risk of pregnancy loss as a direct result of the test procedure. As with other prenatal tests, information from NIPT can help one make a decision about termination of pregnancy, plan contingencies for birth or prepare parents to raise a child with a genetic condition. NIPT can also be used by women and couples to test purely 'for information'.

As the first laboratory to offer massively parallel sequencing (MPS)-based noninvasive prenatal testing (NIPT) for fetal aneuploidies, Sequenom Laboratories has been able to collect the largest clinical population experience data to date, including >100,000 clinical samples from all 50 U.S. states and 13 other countries. The objective of a recent study was to give a robust clinical picture of the current laboratory performance of the MaterniT21 PLUS LDT [6]. Samples were assessed for trisomies 13, 18, 21, and for the presence of chromosome Y-specific DNA. Sample data and ad hoc outcome information provided by the clinician were compiled and reviewed to determine the characteristics of this patient population, as well as estimate the assay performance in a clinical setting. NIPT patients most commonly undergo testing at an average gestation period of 15 weeks, 3 days and average age of 35.1 years. The average turnaround time is 4.54 business days and an overall not reportable rate 1.3 %. The positivity rate for Trisomy 21 was 1.51 %, followed by 0.45 and 0.21 % rates for Trisomies 18 and 13, respectively. NIPT positivity rates are similar to previous large clinical studies of aneuploidy in women of maternal age ≥35 undergoing amniocentesis. In this population, 3519 patients had multifetal gestations (3.5 %) with 2.61 % yielding a positive NIPT result [6]. NIPT has been commercially offered for just over 3 years, and the clinical use by patients and clinicians has increased significantly. The risks associated with invasive testing have been substantially reduced by providing another assessment of aneuploidy status in high-risk patients [6].

As the classical first trimester Down syndrome screening (FTS, combination test) has a false-negative rate of 20–25 % and >95 % of the abnormal FTS results are false-positive, Willems et al. [7] evaluated the NIPT in Belgium and The Netherlands. The study population consisted of 3000 consecutive pregnancies in Belgium and the Netherlands in which NIPT was performed using the Harmony test. In 57 (1.9 %) of the 3000 pregnancies, an abnormal NIPT result was found. This included 51 fetuses with trisomy 21, four fetuses with trisomy 18, and two fetuses with trisomy 13. In 47 of the 57, the NIPT result was confirmed by genetic testing of material obtained by amniocentesis or chorionic biopsy, and no false-positive results were recorded. The false-negative rate as determined on more than 2000 women who had delivered at the time of reporting was low, and so far only two false-negative results were reported (one trisomy 18 and one trisomy21). The failure rate where no NIPT result could be obtained after repeated sampling was 0.90 %. In this large clinical series, NIPT using the Harmony test proves to be a very reliable prenatal test to detect fetal trisomies 21, 18, and 13 in maternal blood in Belgium and The Netherlands [7].

Fairbrother et al. [8] set up a study to estimate the cost effectiveness of fetal aneuploidy screening in the general pregnancy population using NIPT compared with first trimester combined screening (FTS) with serum markers and NT ultrasound. Using a decision-analytic model, they estimated the numbers of fetal T21, T18, and T13 cases identified prenatally; the number of invasive procedures performed; corresponding normal fetal losses; and costs of screening using FTS or NIPT with cfDNA. Modeling was based on a 4-million pregnant women cohort, which represents annual births in the U.S. For the general pregnancy population, NIPT identified 15 % more trisomy cases, reduced invasive procedures by 88 %, and reduced iatrogenic fetal loss by 94 % compared with FTS [8]. The study concluded that NIPT in the general pregnancy population leads to more prenatal identification of fetal trisomy cases compared with FTS and is more economic at a NIPT unit cost of $453 [8].

A recent study by Li et al. [9] from Taiwan assessed the performance of noninvasive prenatal testing (NIPT) for fetal aneuploidies in a pregnancy population with mixed risk factors . Data review of 169 pregnant women undergoing prenatal aneuploidy screening in a single tertiary medical center was conducted. Indications included maternal anxiety, advanced maternal age, abnormal nuchal translucency, and high/moderate risk of first trimester Down syndrome screening. Multifetal pregnancies and patients receiving in vitro fertilization were also enrolled for analysis. A total of 169 patients were enrolled in this study over a time period from July 2012 to June 2014. For patients ≥34 years, anxiety about amniocentesis was the most common reason for patients selecting NIPT for fetal

aneuploidy screening, with 107 (88.4 %) patients choosing NIPT for this reason. Among the total patient population, two patients showed a positive result from NIPT. One patient displayed 47, XXY, which was confirmed to be a false-positive result. The other patient displayed trisomy 18, which was confirmed by an amniotic cell culture. The sensitivity for NIPT is 100 % with the specificity 99.4 %. NIPT for fetal aneuploidy in a pregnancy population with mixed risk factors showed high accuracy [9]. Li et al. also stated that NIPT applied to the low-risk population might reassure the anxious family [9].

The aim of a recent British study was to investigate aneuploidy detection using an approach based on nuchal translucency (NT) and noninvasive prenatal testing (NIPT) [10]. This was a cohort study including 5306 high-risk pregnancies with NT measurements and chorionic villus samples (CVS) tested for full karyotype. A policy of NIPT for increased-risk cases and CVS with full karyotype if the NT was ≥3.0 mm reduced the risk of miscarriage, yet still identified 95 % of clinically significant aneuploidy [10].

The objective of Lichtenbelt's study was to determine what percentage of fetal chromosomal anomalies remains undetected when first trimester combined testing is replaced by noninvasive prenatal testing for trisomies 13, 18, and 21 [11]. They focused on the added clinical value of nuchal translucency (NT) measurement. Data on fetal karyotype, ultrasound findings, and pregnancy outcomes of all pregnancies with an NT measurement ≥3.5 mm were retrospectively collected from a cohort of 25,057 singleton pregnancies in which first trimester combined testing was performed. Two hundred twenty-five fetuses (0.9 %) had an NT ≥ 3.5 mm. In 24 of these pregnancies, a chromosomal anomaly other than trisomy 13, 18, or 21 was detected. Eleven resulted in fetal demise, and ten showed fetal ultrasound anomalies. In three fetuses with normal ultrasound findings, a chromosomal anomaly was detected, of which one was a triple X. In three out of 25,057 pregnancies (0.01 %), noninvasive prenatal testing and fetal ultrasound would have missed a chromosomal anomaly that would have been identified by NT measurement [11].

Song et al. [12] evaluated the feasibility of noninvasive prenatal testing (NIPT) of maternal plasma samples collected from pregnant Chinese women in early pregnancy. In this pilot study, 212 women with high-risk pregnancies were recruited at a single Chinese Hospital. Fetal aneuploidies associated with chromosomes 21, 18, 13, X, and Y were detected by conducting MPS of maternal plasma DNA samples. Invasive prenatal diagnosis by either chorionic villus sampling or amniocentesis and then karyotyping were offered to all women to confirm both positive and negative NIPT results. All confirmed NIPT-negative pregnancies were followed up to birth, and neonates were clinically evaluated for any symptoms of chromosomal disease. Autosomal aneuploidies—trisomy 21 ($n = 2$), 18 ($n = 1$), and 13 ($n = 1$)—were detected by NIPT and confirmed by amniocentesis and karyotyping. There were one false-positive 45, X sample; and two false-negative samples associated with fetal karyotypes 47, XXY, and 45, X[16]/47, XXX[14]. The majority (95 %) of pregnancies had a fetal DNA fraction > 4 %, which is generally the limit for accurate aneuploidy detection by NIPT. Across this early gestational time period, there was a weak inverse relationship ($R = 0.186$) between fetal DNA fraction and maternal weight [2]. They concluded that NIPT is highly reliable and accurate when applied to maternal DNA samples collected from pregnant women in the first trimester between 8 and 12 weeks [12].

Zhang et al. [13] reported the clinical performance of MPS-based noninvasive prenatal testing (NIPT) in detecting trisomies 21, 18, and 13 in over 140,000 clinical samples and comparing its performance in low-risk and high-risk pregnancies. Between January 1, 2012 and August 31, 2013, 147 314 NIPT requests to screen for fetal trisomies 21, 18, and 13 using low-coverage whole-genome sequencing of plasma cfDNA were received. The results were validated by karyotyping or follow-up of clinical outcomes. NIPT was performed, and the results were obtained in 146,958 samples, out of which outcome data were available in 112,669 (76.7 %). Repeat blood sampling was required in 3213 cases, and 145 had test failure. Aneuploidy was confirmed in 720/781 cases positive for trisomy 21, 167/218 cases positive for trisomy 18, and 22/67 cases positive for trisomy 13 on NIPT. Nine false negatives were identified, including six cases of trisomy 21 and three of trisomy 18. The overall sensitivity scores of NIPT were 99.17, 98.24, and 100 % for trisomies 21, 18, and 13, respectively, and specificity scores were 99.95, 99.95, and 99.96 % for trisomies 21,18, and 13, respectively. There was no significant difference in test performance between the 72382 high-risk and 40287 low-risk subjects (sensitivity, 99.21 vs. 98.97 % ($P = 0.82$); specificity, 99.95 vs. 99.95 % ($P = 0.98$)). The major factors contributing to false-positive and false-negative NIPT results were maternal copy number variant and fetal/placental mosaicism, but fetal fraction had no effect. This landmark study concluded that using a stringent protocol, the good performance of NIPT shown by early validation studies can be maintained even in large clinical samples [13]. This technique can provide equally high sensitivity and specificity in screening for trisomy 21 in a low-risk compared with high-risk population.

Liu et al. from China developed a new method for noninvasive prenatal testing (NIPT) of paternally inherited fetal mutants for β-thalassemia (β-thal) [14]. For plasma DNA testing, the results detected by PIRA-PCR assay achieved 100.0 % consistency compared to those obtained

from the amniocentesis analysis. This new method could potentially be used for NIPT of paternally inherited fetal mutants for β-thalassemia [14].

Xiong et al. demonstrated that detection of paternal mutations using next-generation sequencing (NGS) can be readily achieved with high sensitivity and specificity, obviating the need for an invasive test in 50 % of pregnancies at risk of a thalassemia in cases where the father and mother carry different mutations [15].

Noninvasive fetal Rhesus (Rh) D genotyping, using cell-free fetal DNA (cffDNA) in the maternal blood, allows targeted antenatal anti-RhD prophylaxis in unsensitized RhD-negative pregnant women [16, 17]. The purpose of a recent Canadian study was to determine the cost and benefit of this approach compared with routine antenatal anti-RhD prophylaxis for all the unsensitized RhD-negative pregnant women [16]. Their data support the feasibility of a targeted antenatal anti-RhD prophylaxis program, at a lower cost than that of the existing routine prophylaxis program, with no increased risk of sensitization [16].

A prospective, interventional, cross-sectional observational study, to determine whether a policy of offering cffDNA testing to all RhD-negative women at about 16 weeks' gestation to avoid anti-D administration when the fetus is RhD-negative could be implemented successfully in the NHS without additional funding, was set-up by Soothill et al. [17].The total use of anti-D doses fell by about 29 % which equaled to about 35 % of RhD-negative women not receiving anti-D doses in their pregnancy unnecessarily [17]. The authors strongly recommended that this service be extended to all the UK NHS services.

Gil et al. recently reviewed the clinical validation and implementation studies of maternal blood cfDNA analysis to define the performance of screening for fetal trisomies 21, 18, and 13 and sex chromosome aneuploidies [18]. Searches of PubMed, EMBASE, and The Cochrane Library were performed to identify all peer-reviewed articles on cffDNA testing in screening for aneuploidies between January 2011, when the first such study was published, and January 4, 2015. In total, 37 relevant studies were identified, and these were used for the meta-analysis on the performance of cfDNA testing in screening for aneuploidies. These studies reported cfDNA results in relation to fetal karyotype from invasive testing or clinical outcome. Screening for trisomy 21 by analysis of cffDNA in maternal blood is superior to all other traditional methods of screening, with higher DR and lower FPR [18].

A 37-year-old primigravida, with a pregnancy conceived by intracytoplasmic sperm injection (ICSI), was offered NIPT) due to advanced maternal age. NIPT performed at 23 weeks' gestation reported a diagnosis of monosomy X. She was offered an amniocentesis, which revealed a euploid fetus with no sex-chromosome abnormalities. Even with single nucleotide polymorphism (SNP)-based NIPT, positive predictive value for detection of sex-chromosome abnormalities is around 50 % [19]. Positive results of NIPT should be heeded with caution, and an invasive diagnostic procedure should be performed, especially for rare chromosomal abnormalities and sex-chromosome abnormalities where NIPT performs sub-par compared with its performance for detection of trisomy 21 [19].

At 17(+4) week, noninvasive prenatal testing (NIPT) results of a 24-year-old mother showed high risk of monosomy X (45, X). Abnormally shaped head and cardiac defects were observed in prenatal ultrasound scan at 19(+3) week. Amniocentesis conducted at 19(+3) week identified karyotype 47, XX, +18, which suggested that the NIPT failed to detect trisomy 18 (T18) in this case [20]. With a further MPS of maternal blood, fetal, and placental tissues, Pan et al. [20] found a confined placental mosaicism (CPM) with non-mosaic T18 fetus and multiclonal placenta with high prevalence of 45, X and low level of T18 cells. FISH and SNP-array evidences from the placental tissue confirmed the genetic discrepancy between the fetus and placenta. Because the primary source of the fetal cfDNA that NIPT assesses does mostly originate from trophoblast cells, the level of T18 placental mosaicism may cause a false-negative NIPT result as in this rare case of double aneuploidy [20].

Although NIPT marks a notable development in the field of prenatal genetic testing, there are some physician liability considerations raised by this technology. If NIPT is discussed with patients, it is important to disclose the limitations of this technology with respect to its accuracy and the number of disorders that it can detect compared with invasive diagnostic options. A failure to sufficiently disclose these limitations could leave patients with false assurances about the health of their fetuses and could raise informed consent and liability issues, particularly if a child is born with a disability as a result [21].

A recent publication contains a joint ESHG/ASHG position document with recommendations regarding responsible innovation in prenatal screening with NIPT [22]. By virtue of its greater accuracy and safety with respect to prenatal screening for common autosomal aneuploidies, NIPT has the potential of helping the practice better achieve its aim of facilitating autonomous reproductive choices, provided that balanced pretest information and non-directive counseling are available as part of the screening offer. With improving screening technologies and decreasing costs of sequencing and analysis, it will become possible in the near future to significantly expand the scope of prenatal screening beyond common autosomal aneuploidies. Commercial providers have already begun expanding their tests to include sex-chromosomal abnormalities and microdeletions. However, multiple false

positives may undermine the main achievement of NIPT in the context of prenatal screening: the significant reduction of the invasive testing rate. This article argues for a cautious expansion of the scope of prenatal screening to serious congenital and childhood disorders, only following sound validation studies and a comprehensive evaluation of all relevant aspects [22].

Conclusion

Since its introduction to clinical practice in Hong Kong in 2011, NIPT has quickly spread its wings across the globe. While many professional societies currently recommend that NIPT be used as a screening method, and not as a diagnostic test, its high sensitivity (true positive rate) and specificity (true negative rate) make it an attractive alternative to the serum screenings and invasive tests currently in use. Professional societies also recommend that NIPT be accompanied by genetic counseling so that families can make informed reproductive choices [22]. Although there are additional challenges for NIPT uptake in the developing world, including the lack of healthcare professionals and infrastructure, the use of NIPT in low-resource settings could potentially reduce the need for skilled clinicians who perform invasive testing. Future advances in NIPT technology promise to expand the range of conditions that can be detected, including single-gene disorders. With these advances questions of how to handle incidental findings and variants of unknown significance do arise. Moving ahead, it is mandatory that all stakeholders have their voices heard in formulating policies to ensure the ethical and equitable use of NIPT across the world.

Conflict of interest None.

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