Editorials

Non-invasive prenatal testing:

use of cell-free fetal DNA in Down syndrome screening

BACKGROUND

Non-invasive prenatal testing (NIPT) is based on analysis of cell-free DNA (cfDNA) in maternal blood. The majority of cfDNA in maternal blood originates from the mother herself, with the fetal component (cffDNA) contributing approximately 10–20% of the total. cffDNA is present in maternal blood from early pregnancy.[1] It emanates from the placenta, but represents the entire fetal genotype and is rapidly cleared from the maternal circulation with hours of delivery, making it pregnancy specific. If the fetus has Down syndrome (DS), there will be slightly more chromosome 21-specific DNA in the maternal circulation. With technological advances it has become possible to deliver highly accurate single-molecule counting and thereby detect small changes in the number of sequences on the chromosome of interest in blood.[2] This approach forms the basis of NIPT for aneuploidy, a maternal blood test that can be performed in early pregnancy to significantly refine the DS risk, and reduce the need for invasive testing such as chorionic villus sampling (CVS) or amniocentesis.

NIPT became available in Asia and the US in 2011 and, subsequent to significant commercial drive, is now available, largely in the private sector, throughout the world.[3] NIPT has been widely validated, including comparison with standard prenatal aneuploidy screening,[4] and has been shown to be a highly accurate screening test with high sensitivity (99%) and specificity (99.5%),[5] which can be used from 10 weeks in pregnancy to determine risk of DS. NIPT can be used to screen for the other common chromosomal aneuploidies, trisomy 18 (Edwards syndrome) and trisomy 13 (Patau syndrome), albeit with lesser degrees of accuracy.[5]

As NIPT tests all cfDNA in maternal blood (fetal and maternal) and the cffDNA comes from the placenta, results that are discordant with the fetal karyotype can arise from detection of maternal chromosomal rearrangements or mosaicism, maternal malignancy, confined placental mosaicism, or vanishing twin pregnancies.[6] False negatives can also occur through low levels of cffDNA or laboratory technical issues. As such, NIPT is not diagnostic and confirmation of a positive result by invasive testing (CVS or amniocentesis) is required.

NIPT has a much greater sensitivity than traditional screening methods and significantly reduces the need for invasive testing.[7] NIPT as a screening test has been endorsed by professional bodies from several countries, including the UK.[3] In 2016 following a systematic review[8] and a study of NIPT in routine NHS maternity care,[9] the UK National Screening Committee (UKNSC) recommended NHS implementation as a contingent test to refine the aneuploidy screening risk for women who have a high-risk screening result for Down, Edward, or Patau syndromes, following the current screening test. A ministerial decision later in 2016 approved an evaluative roll-out in England from 2018.

There are other uses for analysis of cffDNA already in NHS clinical care, including determining fetal RhD status in RhD negative mothers, fetal sex determination for sex-linked single-gene disorders, and diagnosis of single-gene disorders such as cystic fibrosis. These applications are diagnostic as they target specific genes in high-risk pregnancies, but the focus of this article is the place of NIPT in DS screening.

SCREENING FOR DOWN SYNDROME

Current methods used and recommended by the NSC. The first trimester combined test, which uses ultrasound to date the pregnancy and measure nuchal thickness with maternal serum hormone levels, is the screening test currently recommended by the NSC for women booking in the first trimester, with the quadruple test in the second trimester. All pregnant women having antenatal care in the NHS are offered DS screening. The first trimester combined test, which uses ultrasound to date the pregnancy, confirm the number of fetuses and measure nuchal thickness.

Box 1 highlights the components of the tests included in these risk assessments. In the UK if the threshold of 1 in 150 risk at term is reached women are offered an invasive test, either CVS or amniocentesis, both of which carry around a 0.5% miscarriage risk. The detection rate or screening performance for DS depends on the test used, but rates of around 85–90% with false positive rates between 2–5% have been cited.[10]

Box 1. Components of antenatal screening for Down syndrome

- Maternal age (all tests take account of this)

Screening in first trimester

- Nuchal translucency measurement based on ultrasound
- Maternal serum concentrations of free beta-subunit HCG
- Serum concentrations pregnancy — associated plasma protein A (PAPP-A)

Screening in second trimester

- Quadruple test
  Includes triple test hormones and inhibin A

VALIDATION OF NON-INVASIVE PRENATAL TESTING

There has been a worldwide effort to validate NIPT as a screening tool in pregnancy for DS and the other common aneuploidies, and NIPT has indeed been shown to be highly accurate.[5] Initial validation studies were performed in high-risk populations, but more recently NIPT has been shown to be highly accurate in the general population as well.[4] For example, a study of nearly 16 000 unselected pregnancies confirmed the higher detection rates using NIPT for DS compared with first trimester combined screening with a false positive rate of 0.06% compared with 5.4% and positive predictive value of 80.9% compared with 3.4% respectively.[4]

Data from a large prospective cohort study, involving eight maternity units in the UK,[10] helped inform the NSC decision to implement NIPT in England. The aim of the study was to evaluate how NIPT could be incorporated into the NHS DS screening pathway, elucidate test performance and

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acceptability, and provide data on the economic costs of implementation. NIPT was offered to all women with a DS risk from the combined or quadruple test greater than 1 in 1000; those with a risk greater than 1 in 150 had the option of NIPT or invasive testing. Using the real-life data to create a national model, offering NIPT to women with a DS screening risk of at least 1 in 150 was predicted to increase detection by 195 and result in 3368 fewer invasive tests and 17 fewer procedure-related miscarriages. It was also calculated that implementation of NIPT as a contingent test would be cost neutral compared with current screening at a screening threshold of 1 in 150, if the cost of NIPT was less than £256.

ETHICAL AND SOCIAL IMPLICATIONS

There is a growing body of research looking at the social implications of the use of NIPT.[11,12] Stakeholders are positive about the clinical benefits NIPT brings in terms of ease of access and increased accuracy resulting in decreased need for invasive compared with traditional screening tests. Uptake of NIPT has generally been high and this includes parents who would not have previously considered prenatal testing due to the risk of miscarriage, and who use the information, not for decisions about termination, but to continue the pregnancy prepared for the birth of a child with DS. The most common concerns are that NIPT may be seen as a routine blood test for which the full ramifications of the test are not discussed to enable informed choice, or that parents may feel societal pressure to have NIPT simply because it is safe and easy to perform. For the most part, stakeholders are confident that these concerns can be addressed through training of health professionals to deliver expert pre- and post-test counselling and adherence to guidance from professional and regulatory bodies. Another common concern is that ease of access to safer screening may increase the number of terminations for DS, but some studies suggest that a significant number of women using NIPT will continue the pregnancy after a diagnosis of DS. For example, Chitty *et al*[9] found that 13 out of 42 (31%) women who had NIPT that predicted their baby had DS continued the pregnancy, in comparison with the national figure of 8% reported for diagnoses following traditional DSS and invasive tests.[13]

NHS implementation will ensure equity of access to NIPT and prevent the inequalities that currently exist while it is mainly available in the private sector. However, careful training of health professionals offering screening will be required to ensure women are given balanced information upon which to make informed choices regarding screening.

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