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# Noninvasive prenatal testing

## Background

Noninvasive prenatal testing (NIPT) has marked a revolution in aneuploidy screening because it allows a simple maternal blood test to detect Down syndrome in a fetus with a very high level of accuracy (at least 99.5% with a false-positive rate of 0.2%).

## Objective

To describe the new tests that have become available and their place in antenatal screening to help GPs and their patients make informed decisions about their use.

## Discussion

Results are available from 12 weeks gestation, giving a high level of reassurance for Down syndrome early in pregnancy. There are concerns, however, that the test is being offered without proper counselling and that women may not have a 12-week ultrasound to exclude significant structural abnormalities, therefore decreasing the early detection of severe abnormalities. In addition, the test is expensive and therefore prohibitive for many women and their families.

## Keywords

genetic testing; antenatal screening



Noninvasive prenatal testing (NIPT) is a new term used to describe a very exciting new set of technologies that enable a simple maternal blood test to identify whether a fetus has Down syndrome. This test has been validated in a high-risk population in more than 10 clinical trials, showing an accuracy of detection for Down syndrome (trisomy 21) of 99.5% (false positive rate 0.2%), Edward syndrome (trisomy 18) of 99% and Patau syndrome (trisomy 13) of 79–92%.<sup>1</sup> This level of accuracy has not been seen before in prenatal aneuploidy screening.

There have now been five clinical trials evaluating NIPT in a population of low- and high-risk women from China, the United Kingdom and the United States. These large trials confirm similar high detection rates and low false-positive rates for the test in a general obstetric population.<sup>2–6</sup>

## Logistics of the test

Pregnant women who elect to have NIPT can have a blood test from 10 weeks gestation. All samples are sent offshore to America or China at a cost to the patient of \$500–1400, depending on the provider. The result is available within 10–14 days and reports a risk assessment for trisomy 21, 18 and 13. In addition, if the patient requests analysis of the sex chromosomes, the test shows the sex of the fetus and some sex chromosome abnormalities.

## What if the test is positive?

The false-positive rate is low but significant and, therefore, invasive testing is always recommended to confirm a positive result.<sup>7</sup> A karyotype will provide diagnostic information, reveal an unbalanced translocation and provide more information for mosaic cases.

## What if no result is issued?

There is also a percentage of tests that fail to yield a result, usually because of low fetal DNA fraction (when <4% of the total maternal free DNA pool is of fetal origin) or from assay failure. Total test failure rates are usually less than 5%, but this depends on patient characteristics, particularly maternal weight.<sup>8</sup> The fetal fraction decreases with increased maternal weight; for a maternal weight of



160 kg, in 50% of samples the fetal fraction will be less than 4% and the assay will give a failed result.<sup>8</sup>

## What is cell-free DNA?

NIPT involves the testing of cell-free DNA in the maternal plasma. Cell-free DNA was first discovered in 1997 by Lo et al.<sup>9</sup> They found that small fragments of DNA are released from the placental cells through apoptosis and subsequently appear in the maternal circulation. These fragments are not contained within a cell so they are unstable and have a short half-life of the order of 4–30 minutes.<sup>10</sup> These fragments of DNA are extracted from maternal plasma and used to assess the fetal genetic material.

## Developments in sequencing and bioinformatics systems

The DNA fragments need to be multiplied millions of times per patient sample. This has been made possible, in the short time frame required, by recent developments in sequencing technologies that combine complex bioinformatics systems. Massively parallel sequencing or next generation sequencing was the first to become commercially available in 2005. The chromosomal origin of DNA fragments is identified and then the relative quantity of that chromosome is compared to a reference genome. In this way over-representation of one chromosome can be determined.

Targeted sequencing has now been developed. Fragments of DNA from only chromosomes 13, 18, 21, X and Y are typically multiplied and analysed. This is more cost-effective and has translated into a reduction in cost to the patient; however, information from the whole genome is not available using this technique. Typically, the maternal genome is used as the reference genome so this type of testing should not be used if the pregnancy has been conceived with a donor egg.

## Australia and NIPT

NIPT has become readily available in Australia with the development of the Streck tube, which is a blood collection tube containing a preservative that allows the DNA to remain stable in ambient temperatures for offshore testing. Although NIPT is available in all capital cities in Australia, the cost remains at \$500–1400 to the patient, which is financially prohibitive for many women and their families.

## What benefits does the current Down syndrome screening program offer?

The current aneuploidy screening program at 11–13 weeks gestation involves both an ultrasound and a blood test for placental protein A (PaPP-A) and free human chorionic gonadotrophin (hCG). This first trimester combined screening has a detection rate for Down syndrome of 90% and a false-positive rate of 3%.<sup>11</sup> However, it provides more information than only the risk of the three most common trisomies. An ultrasound alone in the first trimester ensures correct dating, diagnosis of multiple pregnancies, and chorionicity and anatomy assessment to detect major abnormalities. All cases of acrania,

alobar holoprosencephaly, exomphalos, gastrschisis, megacystis and body stalk anomalies were detected in a prospective series of 44,859 ultrasounds at 11–13 weeks gestation.<sup>12</sup> In this series, 44% of non-chromosomal fetal abnormalities detectable by prenatal ultrasound were picked up in the first-trimester ultrasound. An increased nuchal translucency measurement is a marker for many structural abnormalities, most notably cardiac abnormalities, as well as aneuploidies.<sup>13</sup> Low PaPP-A levels have been associated with adverse pregnancy outcomes, including fetal loss, preterm birth, intrauterine growth restriction and preeclampsia.<sup>14</sup> It is important to recognise and understand the value of the currently offered screening programs and be aware that NIPT is not a replacement for first-trimester ultrasound and blood testing at 11–13 weeks. Although the patient will often incur an out-of-pocket cost for first trimester combined screening, it is substantially less than that for NIPT at the present time.

## Incorporating NIPT into current clinical practice

Currently in Australia, the uptake of NIPT is small; however, awareness among consumers is rapidly increasing. Use of NIPT will continue to be determined by patient and doctor awareness of the availability of the test and cost. There are no current Australian guidelines as to who should be offered the test. The issue of affordability presents significant ethical concerns. *Table 1* lists important counselling points for women who are considering NIPT.

The American College of Obstetricians and Gynaecologists advises that NIPT should be considered only in pregnancies at high-risk of aneuploidy.<sup>15</sup> This is a logical first step for incorporation into our current screening program as an option instead of invasive testing after combined first trimester screening. If used in this manner NIPT reduces the number of invasive tests but does not improve detection rates and decreases rates of identifying other chromosomal abnormalities that would have been picked up on a formal karyotype.

**Table 1. Important counselling points**

- The test is very accurate for detection of Down syndrome (sensitivity 99.5%) and Edward syndrome (99%). It is less accurate for the detection of Patau syndrome (79–92%).
- It is unlikely to give a false-positive result (0.2%) but all positive results need to be confirmed by an invasive test (amniocentesis or chorionic villus sampling).
- The cost is \$500–1400.
- There is a test failure rate of up to 4% (this is higher as body mass index increases: the test failure rate is likely to be 50% at a maternal weight of 160 kg).
- Ultrasound to exclude structural fetal abnormalities is still very important; nuchal translucency and morphology ultrasounds are recommended.
- NIPT can also test for fetal sex and some sex chromosome abnormalities.



Nicolaides initially proposed an alternative approach of routine NIPT at 10 weeks followed by a 12-week ultrasound. This method increases the sensitivity of screening for trisomy 21 to 99% and reduces the invasive testing rate by > 90%, but is expensive.<sup>2</sup> NIPT has not been validated as yet for multiple pregnancies, although some companies still offer the test for twins and higher order multiples.

## Ethical issues

Many concerns are emerging already and must be addressed as this test is incorporated into clinical practice:

- Will women be adequately informed and counselled?
- Will providers undertake independent advertising?
- Will women not see the need for a first-trimester scan?
- Will women feel that combined first-trimester screening is an inferior test if they cannot afford NIPT?
- Will terminations of pregnancy occur because of early determination of fetal sex?

## Future developments

NIPT has appeared in Australia without any controls or guidelines for use. Women have taken it up in an ad hoc way according to whether they or their doctor have heard about the test and whether they can afford it. Although the cost has been decreasing over the past 12 months, it is not subject to a Medicare rebate so women must pay the full price in advance.

There is an urgent need to have a unified approach with consistency of information and control of the way NIPT is incorporated into our current first-trimester screening program.

## Summary

NIPT is available in all capital cities of Australia. It may be provided by maternal fetal medicine specialists in tertiary hospitals, some obstetricians, specialised obstetric ultrasound facilities or pathology companies.

NIPT has been validated for women at high risk for Down syndrome (due to high-risk first-trimester screening or maternal age); however, women not in a high-risk group can also have the test with appropriate counselling. Women with a history of infertility, recurrent miscarriages or a previous child with Down syndrome, for example, may choose to have the test before their nuchal translucency ultrasound.

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