Positive or negative

ANTENATAL CARE

What will be the place for new methods of non-invasive testing in prenatal screening for chromosomal abnormalities?

DR GREG KESBY AND DR ROB ROBERTSON

TESTING of cell-free fetal DNA in maternal plasma is a screening test for Down syndrome and other trisomies which is now available in Australia. It has garnered high levels of public interest and expectation. However, although this form of testing appears to show great promise, there are currently no Australian guidelines defining its place in prenatal assessment. In this article, we explore the methodology of non-invasive prenatal testing (NIPT), its uses and limitations, and give recommendations as to when, and in whom, it is best used.

How testing works

Non-invasive prenatal testing for Down syndrome (and other aneuploidies) involves the molecular analysis of fragments of fetal DNA released into the maternal circulation as a result of apoptotic cell destruction occurring within the placenta. Generating a result from NIPT relies on there being enough high-quality fetal DNA to be differentiated and extracted from maternal plasma. The proportion of free fetal DNA circulating in maternal blood is known to increase with gestation (ie, with placental mass). This means the test can usually be performed from approximately 10 weeks' gestation.

About 7-10mL of maternal venous blood is collected into Streck tubes, which contain cellular-stabilising agents to prevent release of maternal genomic material into the plasma. The sample is transported at room temperature and should preferably be analysed no more than 72 hours later.

Currently available tests — VerifiTM (Verinata), MaterniT21TM (Sequenom), HarmonyTM (Ariosa), iGeneScreenTM (BGI/INEX) and PanoramaTM (Natera) — vary in their methodology but essentially analyse random fragments of cell-free fetal DNA isolated from maternal plasma. The generated sequences are then subjected to data analysis algorithms that enable identification of relative variations in counts, facilitating the detection of imbalances and, consequently, the diagnosis of trisomy (if internal statistical confidence benchmarks are satisfied).

The methodology can be applied across the whole genome, but is currently commercially available for the detection of trisomies 21 (Down syndrome), 18 (Edward syndrome) and 13 (Patau syndrome), as well as sex aneuploidies. All tests are being offered to women regardless of risk. However, while all providers have validated their tests for detection of the most common trisomies in high-risk women, the only test validated in a low-risk population is HarmonyTM. MaterniT21TM is available for use in twins on the basis of a small validation study, although caution should generally be exercised when using NIPT in the setting of a multiple pregnancy.

Although NIPT is a welcome addition to the clinical armamentarium ... it must still be considered a screening test, not a diagnostic test.

Screening options

Although NIPT is a welcome addition to the clinical armamentarium available for prenatal assessment, it must still be considered a screening test, *not a diagnostic* test. NIPT performs best in screening for Down syndrome, where the sensitivity is reported at about 99.5% and specificity 99.8%. The positive likelihood ratio generated from a positive test result is about 497.5. The negative likelihood ratio for a negative result is 0.005. The detection rate for trisomy 18 has also been shown to be about 99%. However, detection rates for trisomy 13 are currently lower (79-92%), though are expected to rapidly improve in line with development of the technology.

Consequently, the information provided by NIPT will significantly affect decision-making for a woman considering pursuing invasive diagnostic testing. However, it is crucial to understand that negative results — while strongly reassuring — do not exclude the presence of aneuploidy. Equally, it is essential that decisions regarding interruption of a pregnancy should not be made in women with a positive NIPT result without confirming the presence of aneuploidy by invasive diagnostic testing (usually by amniocentesis).

Cost and the absence of any Medicare rebate will limit the utility of NIPT in clinical practice. With the exception of HarmonyTM, the NIPTs cost about $1200-$1400, although intense competition is likely to decrease this to the more affordable $500-$700 range. Indeed, HarmonyTM has just entered the Australian market at about $600.

Where NIPT fits in

Regardless of cost, the swelling enthusiasm for the use of NIPT in antenatal care needs to be tempered with an understanding by both

cont'd next page

from previous page

patients and doctors of the test's limitations, and the need to integrate it with other screening modalities.

This 'non-invasive test for Down syndrome' should not be perceived as a valid replacement for combined nuchal translucency and first-trimester serum screening. It should also not be seen as being equivalent to amniocentesis in addressing concerns regarding normality of the karyotype.

Unlike NIPT, amniocentesis with either traditional cytogenetic analysis (G-banded karyotype; capable of detecting aneuploidy, rearrangements, insertions/deletions of greater than 5000 kilobase pairs) or the newer, higher resolution molecular karyotype analysis (arrayCGH; capable of detecting aneuploidy, microinsertions/microdeletions of less than 400 kilobase pairs) is not limited to an assessment of Trisomies 13, 18 and 21 and the sex aneuploidies (see table 1). Indeed, women assessed as being at high risk of carrying a fetus with Down syndrome on the basis of combined nuchal translucency first-trimester serum screening, and who elect for NIPT in preference to amniocentesis, need to be aware of the possibility of missing non-T13/18/21 chromosome abnormalities that may be present in this group. In other words, by avoiding the less than 1 in 200 risk of miscarriage associated with amniocentesis, they instead assume a 1.5-2.5% risk of failing to identify a significant atypical chromosome abnormality known to be identifiable by combined qfPCR and array CGH in this population (see box next page).

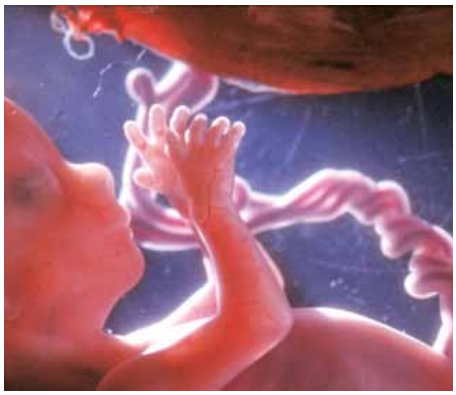

TABLE 1

		Molecular tests		
Detection	**Cytogenetic karyotype**	**qfPCR**	**ArrayCGH**	**Maternal blood NIPT**
All chromosomes examined	√	X	√	X
Trisomies 13/18/21	√	√	√	√
45X, 47XXX, 47XYY	√	√	√	√
Polyploidy (eg, triploidy)	√	√	X	X
Deletions/duplications	√	X	√	X
Microdeletions Microduplications	X	X	√	X
Balanced translocations	√	X	X	X
Unbalanced translocations	√	X	√	X
Risk of miscarriage	Yes	Yes	Yes	No

Furthermore, in addition to combined first-trimester screening's role in assessing risk for chromosome abnormality, the ultrasound assessment permits other important diagnoses. These include multiple pregnancy, accurate assignment of chorionicity, early detection of about 50% of the structural abnormalities expected to be evident at the 19-week morphology assessment, and the identification of fetuses at high risk for structural cardiac abnormality.

In addition, first-trimester serum screening is also now being used to determine the risk of early-onset pre-eclampsia and intrauterine growth restriction.

However, in practice we would advise it be considered as an adjunct to the traditional approach of combined first-trimester ultrasound and serum screening at 12 weeks' gestation.

NIPT use in practice

While NIPT is now available in Australia, clinical guidelines have not yet been formulated to define the test's place in prenatal screening and diagnosis. Until this void is filled, NIPT will simply be available to all who can afford it. However, in practice we would advise it be considered as an adjunct to the traditional approach of combined first-trimester ultrasound and serum screening at 12 weeks' gestation. The model we have adopted is based on counselling women on the basis of

nuchal translucency and first-trimester serum screening risk estimates (see figure 1).

Although NIPT is available to all women regardless of risk stratification, our working guidance favours the use of diagnostic testing (CVS, amniocentesis) rather than NIPT in those women at very high risk of chromosome abnormality (greater than 1 in 50). This is because the majority of Down syndrome and other chromosome abnormalities will be found in this group when tissue samples are analysed using molecular methods such as arrayCGH (whole genome analysis with results in less than two weeks). The remainder of women in the traditional high-risk group (ie, 1 in 50 to 1 in 300) may still opt for CVS/amniocentesis, but due to the much lower incidence of aneuploidy in this cohort, may prefer to seek direction from NIPT before committing to the risk of invasive testing. NIPT is also offered as a reasonable investigation in women whose risks extend down to 1 in 1000, but at risks lower than this, the possibility of a common trisomy is so low that it would be difficult to justify advising NIPT on cost–benefit grounds.

Conclusion

Although testing of cell-free fetal DNA in maternal plasma appears to show great promise as a screening test for Down syndrome and other trisomies, it has been most extensively studied in a high-risk setting. Confidence in its efficacy in average-risk pregnancies, as well as a significant reduction in the cost of the technology, are needed before this approach can replace current maternal screening using nuchal translucency and first-trimester serum screening.

Our suggested approach is to consider NIPT for secondary risk stratification following combined first-trimester nuchal translucency and first-trimester serum screening. This will allow the benefits of first-trimester ultrasound and serum screening to be preserved, while potentially reducing invasive testing by about 40%, and increasing detection of Down syndrome from around 94%, based on combined first-trimester ultrasound and serum screening alone, to about 98% with the addition of NIPT for secondary risk stratification.

Dr Kesby is a specialist in maternal–fetal medicine at Royal Prince Alfred Hospital. Dr Robertson is a clinical geneticist and specialist in obstetric & gynaecologic ultrasound.

FIGURE 1

Current/traditional risk groups based on nuchal translucency and first trimester serum stratification

HIGH-RISK GROUP		LOW-RISK GROUP	
> 1 in 50 2.3% population 90.5% T21 cases	1 in 50 to 1 in 300 1.7% population 3.8% T21 cases	1 in 300 to 1 in 1000 6.3% population 3.8% T21 cases	< 1 in 1000 89.7% population 1.9% T21 cases

New risk groups based on combined nuchal translucency and first trimester serum screening with the inclusion of NIPT

HIGH-RISK GROUP	INTERMEDIATE-RISK GROUP	LOW-RISK GROUP
> 1 in 50 High risk – advised to have invasive test in preference to NIPT	1 in 50 to 1 in 1000: total of 8% of patients NIPT offered with invasive tests reserved for NIPT positive women	< 1 in 1000 Reassured but NIPT not recommended

New stratification Approximately 40% reduction in CVS/amniocentesis Increase in detection of T21 from 94% to 98%

QFPCR

QfPCR analysis involves molecular amplification and analysis of chromosome-specific DNA sequences known as small tandem repeats to assess the number and nature of chromosomes. In practice, it is used to provide rapid information about trisomies 13, 18 and 21, polyploidy, gender and sex aneuploidies within 48 hours, while waiting for results of the more extensive cytogenetic or molecular karyotyping. Turnaround time is about two weeks.