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Non-invasive prenatal testing for Down syndrome

SUMMARY

Fetal DNA can be detected in maternal plasma. This can be used to identify chromosomal and genetic abnormalities.

The concentration of free fetal DNA increases with advancing gestation. Non-invasive prenatal testing should not be performed before 10 weeks.

Non-invasive prenatal testing has more than 99% sensitivity and specificity for trisomy 21. It can also be used to identify trisomy 18, trisomy 13 and 45X.

Non-invasive prenatal testing will not detect all chromosomal abnormalities found by amniocentesis.

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Introduction

The identification of cell-free fetal DNA in maternal plasma[1] has enabled identification of genetic differences between mother and fetus. This allows fetal sex or rhesus D blood group to be determined without recourse to invasive prenatal diagnosis.[2,3] A highly sensitive and specific screening test for Down syndrome, called non-invasive prenatal testing, has been developed.[4-14] The test is likely to improve prenatal care.

Fetal DNA

Fetal DNA is thought to be derived from the placenta, which undergoes continual remodelling throughout pregnancy.[15] Once a mother delivers, fetal DNA is rapidly cleared. This means that any fetal DNA present originates from the current rather than previous pregnancies.[16]

Most cell-free DNA in plasma (85–90%) is maternal. Tests designed to identify fetal fragments have to focus on parts of the genome that are unique to the fetus. An example would be to look for the male sex determining region Y gene, which, if present, must be fetal rather than maternal.[2] This is the basis of testing designed to identify genetic differences or disorders in the fetus, but it is not readily applied to identification of chromosomal abnormalities.

Non-invasive prenatal testing

'Next generation' sequencing generates masses of DNA sequence data at relatively low cost. It is the most common method used to identify numeric chromosomal abnormalities. This relatively new technology is used to define the relative proportion of DNA fragments originating from different chromosomes. If a fetus is trisomic, then the proportion of DNA fragments related to that specific chromosome will be increased relative to other chromosomes.

A positive result is reported when the number of fragments of an individual chromosome is more than three standard deviations from the mean of reference chromosomes. The absolute difference in the proportion of fragments is very small as the abnormal fetal genome is diluted by normal maternal genome. However, the advantage of sequencing technology is that millions of fragments are counted, allowing these small differences to be resolved. After sequencing, bioinformatic analysis determines whether there is evidence of a numeric chromosomal abnormality.

Non-invasive prenatal testing does not necessarily differentiate between fragments of maternal and fetal DNA, although at least 4% of cell-free DNA needs to be fetal in origin to be able to resolve differences between euploid and trisomic samples.[17] Approximately 2–5% of samples will have lower levels of fetal DNA and in these circumstances it is not possible to report a result.

What does the test screen for?

As well as trisomy 21, non-invasive prenatal testing can report trisomies 13 and 18 and 45X (Turner syndrome). This means that the test covers about 80–90% of anomalies that would be detected using traditional cytogenetic karyotyping. The test does not pick up all chromosomal abnormalities that would be reported by amniocentesis.

It is possible to sequence the fetal genome in more detail (described as deep sequencing). In the future non-invasive prenatal testing may be used to screen the whole genome in higher resolution.[18]

Currently, non-invasive prenatal testing cannot detect single gene disorders such as cystic fibrosis, beta-thalassaemia and sickle cell anaemia, which can only be identified by invasive testing. The test will not detect triploidy or molar placenta.

How accurate is the test?

It is important to recognise that non-invasive prenatal testing is not a diagnostic test, but a very effective screening test. A number of studies have shown that it is highly sensitive (99.6%) and specific (99.9%) as a screening tool for trisomy 21 (Table).[4-14] Most studies were performed in 'high-risk' populations (advanced maternal age, previous history, abnormal ultrasound, increased risk after routine screening) but there are also data that support testing in an unselected population.[10,11] Based on sensitivity and specificity results, likelihood ratios can be calculated – a positive result effectively increases a patient's a priori risk of having an affected pregnancy almost 1000-fold and a negative test reduces a patient's risk 250-fold.

The sensitivity and specificity for trisomies 18 and 13 appears to be lower as sequencing is less accurate for fragments of these chromosomes. There are, however, recent datasets that report 98.4% sensitivity for trisomy 18 and 85% sensitivity for trisomy 13 (Table).[6-9,12-14] An alternative approach, based on single nucleotide polymorphism analysis rather than just counting DNA fragments, may improve the efficacy of non-invasive prenatal testing for trisomies 18 and 13 and for sex chromosome aneuploidy.[9]

Although most commercial laboratories are able to report fetal sex with this technology and offer non-invasive prenatal testing for sex chromosome aneuploidy (often 45X), there is little published data describing its effectiveness.[9,13] Sensitivity for 45X currently appears to be 90.5% (Table).

Current screening for Down syndrome

The current 'gold standard' for Down syndrome screening is combined first trimester screening. This is performed between 11 weeks and 13 weeks 6 days of pregnancy and involves risk assessment based on:

- maternal age (Fig. 1)[19]
- ultrasound measurement of nuchal translucency thickness
- maternal serum analytes – free beta human chorionic gonadotrophin and pregnancy-associated plasma protein A.

This assessment has 90% sensitivity and 95% specificity for Down syndrome.

How will the test fit into practice?

When the new test is compared to combined first trimester screening purely on sensitivity and specificity results, non-invasive prenatal testing appears to be better.[20] Combined first trimester

Table **Studies reporting the effectiveness of non-invasive prenatal testing for trisomy 21 in high-risk populations‡**

Study	Trisomy 21		Trisomy 18	Trisomy 13	45X
	sensitivity	specificity	sensitivity	sensitivity	sensitivity
Chiu et al. 2011[4]	100% (86/86)	97.9% (143/146)	–	–	–
Palomaki et al. 2011[5]	98.6% (209/212)	99.8% (1468/1471)	–	–	–
Ashoor et al. 2012[6]	100% (50/50)	100% (297/297)	98% (49/50)	–	–
Bianchi et al. 2012[7]	100% (89/89)	100% (404/404)	97.2% (35/36)	78.6% (11/14)	93.8% (15/16)
Norton et al. 2012[8]	100% (81/81)	99.9% (2887/2888)	97.4% (37/38)	–	–
Zimmermann et al. 2012[9]	100% (11/11)	100% (126/126)	100% (3/3)	100% (2/2)	100% (1/1)
Dan et al. 2012[10]	100% (143/143)	99.9% (10914/10915)	100% (47/47)	–	–
Nicolaides et al. 2012[11]	100% (8/8)	99.9% (1937/1939)	66.7% (2/3)	–	–
Palomaki et al. 2012[12]	–	–	100% (59/59)	91.7% (11/12)	–
Ashoor et al. 2013[13]	–	–	–	80% (8/10)	–
Jiang et al. 2012[14]	100% (16/16)	–	100% (12/12)	100% (2/2)	75% (3/4)
Overall	**99.6% (693/696)**	**99.9% (18176/18186)**	**98.4% (244/248)**	**85% (34/40)**	**90.5% (19/21)**

‡ high-risk populations are variously described in these studies on the basis of maternal age (>35 years), findings of first and/or second trimester screening and a previous or family history of a chromosomal abnormality

screening does, however, provide other information. Ultrasound screening allows accurate dating of the pregnancy, recognition of structural (rather than chromosomal) anomalies and identification of multiple pregnancies. It may also identify pregnancies at risk of other adverse obstetric outcomes such as pre-eclampsia and fetal growth restriction.

At present, most national and international guidelines suggest that non-invasive prenatal testing should be restricted to women with a high risk of an affected pregnancy.[21-23] Although it is highly specific, the prevalence of a Down syndrome pregnancy is low in women who have not had previous screening or who are considered to have a low risk after prenatal screening. The positive predictive value (proportion of positive results that are true positives) in an unselected population is at best 50%. In other words, one in two positive test results in low-risk women are likely to be false positives – and test results need to be confirmed by amniocentesis before any intervention.

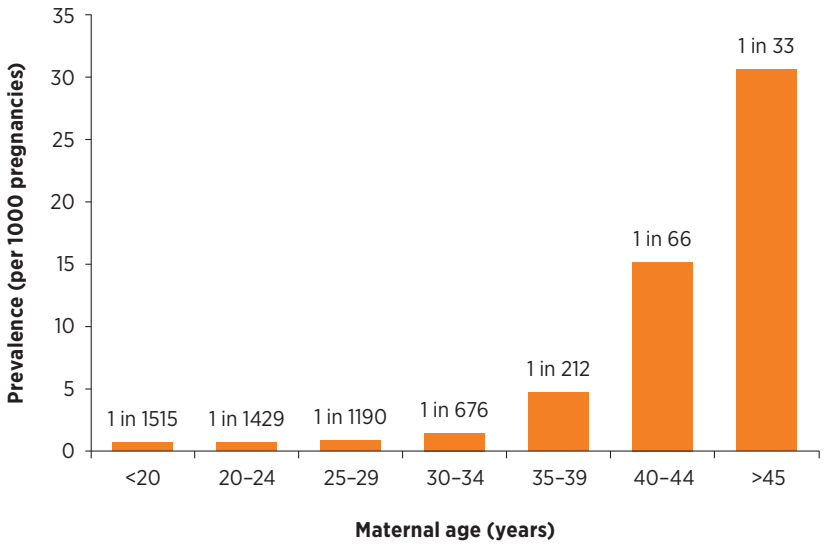
If non-invasive prenatal testing is restricted to patients who have previously been screened for Down syndrome and found to have a high risk, then a positive result will imply that the fetus is indeed affected, and a negative result will imply the fetus is unlikely to be affected. False positive results have been reported and all positive results should be confirmed by amniocentesis. Using quantitative fluorescent polymerase chain reaction, the result can be confirmed within 24 hours. If women have not had any previous screening or are considered to be low risk after prenatal screening, confirmation of a positive result will be more important.

One attraction of this test is that the sample is very stable so it can be transported long distances to a centralised facility. Combined first trimester screening relies on the ability to provide high quality obstetric ultrasound facilities locally. Non-invasive prenatal screening may help to reduce the inequality of access in rural areas.[24] However, at present the test is not reimbursed on the Medicare Benefits Schedule and may cost a patient over $500.

Options for screening strategies

As non-invasive prenatal testing is so sensitive, one option is to offer this test to women who have had a high-risk result from combined first trimester screening. It has been suggested that this may lead to 80% reduction in the current invasive testing rate. While this will improve the overall specificity of the screening strategy, it does not take advantage of the high sensitivity of non-invasive prenatal testing for the population as a whole.

Fig. 1 **Maternal age-related risk for Down syndrome** [19]



An alternative strategy is to offer all women non-invasive prenatal testing and an ultrasound scan. However, this will increase the cost of the screening program quite significantly.

A third strategy would be to change the reporting strategy of combined first trimester screening to identify three groups:

- a high-risk group (>1 in 50) offered invasive testing
- a low-risk group (<1 in 1000) reassured and advised no further screening is necessary
- an intermediate-risk group (1 in 50 to 1 in 1000) who would be advised about the availability of, and offered, non-invasive prenatal testing.

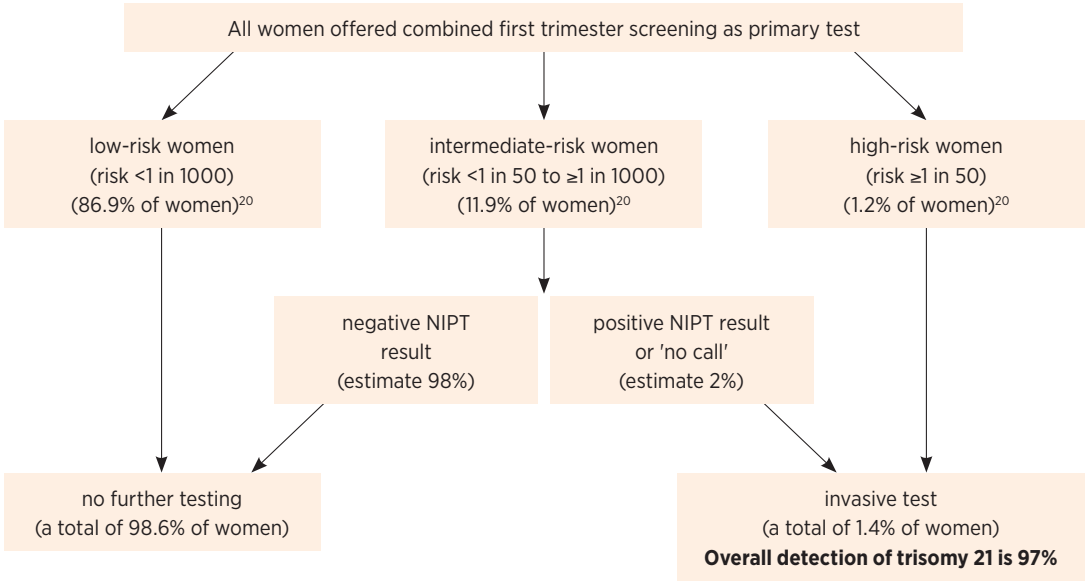
false positive results have been reported and all positive results should be confirmed by amniocentesis

This is described as a contingent screening model with the use of the test being contingent on the results of combined first trimester screening. The advantage of this strategy would be an overall increase in detection of trisomy 21 (97% sensitivity) with a reduction in the false positive rate (<1.5%). This model is outlined in more detail in Fig. 2.

When combined first trimester screening is not possible

Sometimes combined first trimester screening is not available, for example for those living in remote areas or presenting at more than 14 weeks gestation. In these circumstances non-invasive prenatal testing could be used, but only after an ultrasound scan to check that the pregnancy is viable and that the placenta has a normal appearance.

Fig. 2 **Contingent screening model for Down syndrome**



NIPT non-invasive prenatal test
'no call' means the test could not be reported

Non-invasive prenatal testing may be best used in a contingent approach. Here, combined first trimester screening (maternal age, ultrasound, serum analytes) is offered to all women as an initial screening tool. From this, women are stratified by risk to determine further management. Women with a high risk are offered an invasive test (chorionic villus sampling or amniocentesis). Women with a low risk are reassured and advised that no further testing is needed. Women with an intermediate level of risk are offered a non-invasive prenatal test. Contingent screening allows highest detection (an estimated sensitivity of 97%) while reducing the false positive rate to 1.4%.

Limitations of the test – informing the patient

Women who choose to have non-invasive prenatal testing rather than amniocentesis need to appreciate that some chromosomal abnormalities that would have been an incidental finding of a cytogenetic test will not be detected.

If there is a low fraction of fetal DNA in the sample (<4%), the non-invasive prenatal test cannot be reported (described as a 'no call'). Test failure (due to low fetal fraction – occurring in 2–5% of cases) is more likely at early gestations (for example at 10 weeks) and in obese patients.[25] However, it is not indicative of an abnormal result. As the test examines free DNA from both the mother and the fetus, there is a small risk that a maternal chromosomal abnormality could be identified and reported.

Women need to be aware that non-invasive prenatal testing is not a diagnostic test. While a positive result should be confirmed by amniocentesis, a negative result should be interpreted as meaning that it is very unlikely that the fetus is affected.

Conclusion

Non-invasive prenatal testing has the potential to change the established paradigm of prenatal screening. This test performs an order of magnitude better in terms of sensitivity and specificity for common forms of aneuploidy. At current prices, it is difficult to see how this will be a cost-effective tool for population screening. However, it is a viable alternative to amniocentesis for detecting Down syndrome in high-risk pregnancies (identified from combined first trimester screening).

However, some 'atypical' chromosomal abnormalities that are identified through amniocentesis will be missed using this new technique. Parents need to be counselled as to the relative advantages and disadvantages of non-invasive prenatal testing when deciding which prenatal tests are of value. ◁

Conflict of interest: none declared

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