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Title: Rational Testing: Non-invasive Prenatal Testing (NIPT) for aneuploidy screening

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Contributors and sources: All authors contributed to the design of the article, to critically revising the manuscript and have approved the manuscript for publication. The manuscript is based on current published literature. Rebecca Spencer is a Clinical Lecturer in Obstetrics and Gynaecology and subspecialty trainee in Maternal and Fetal Medicine. She drafted the original manuscript, liaised with patient representatives, revised the manuscript in light of their feedback, and acts as guarantor for the paper. Hilary Hewitt is a screening co-ordinator and fetal medicine midwife. She made a particular contribution to the clinical scenario and discussion of pre- and post-test counselling. Laura

McCarthy is a GP and a previous trainee in Obstetrics and Gynaecology. Her contribution was especially important for ensuring the manuscript was relevant and accessible to a non-specialist audience. Ruwan Wimalasundera and Pranav Pandya are Consultant Obstetricians and Fetal Medicine specialists and Pranav Pandya is the Chair of the Fetal Anomaly Screening Programme Advisory Group. They have provided expert clinical input and oversight.

The case

A 36 year old woman attends her GP at 8 weeks in her second pregnancy. She has heard there is a new blood test she can have which will test for chromosomal anomalies and wants to find out more.

Aneuploidy screening

In many countries, screening for trisomies 21 (Down's syndrome), 18 (Edward's syndrome) and 13 (Patau's syndrome) is offered as part of routine antenatal care. This can be performed from 11+2-14+1 weeks gestation using combined screening (nuchal translucency [NT], pregnancy-associated plasma protein A [PAPP-A], human chorionic gonadotrophin beta subunit [βHCG] and maternal age) or from 14+2-20+0 weeks using quadruple screening (βHCG, unconjugated estriol, alpha-fetoprotein and inhibin A). About 3% of women will receive a high-chance result from one of these screening tests and are traditionally offered an invasive procedure to confirm the result - either chorionic villus sampling (from 11+2 weeks) or amniocentesis (from 15+0 weeks). The disadvantage of these invasive procedures is the procedure-related risk of miscarriage of about 0.3%. [1] Non-invasive prenatal testing (NIPT) is a newer screening test with high detection rates and low false-positive rates.

Of note, whether or not a pregnant woman chooses to have aneuploidy screening, the first trimester ultrasound remains an important test for dating the pregnancy and diagnosing multiple pregnancy, missed miscarriage and early structural anomalies. [2]

What is non-invasive prenatal testing (NIPT)?

NIPT is a maternal blood test that makes use of cell-free fetal DNA (cffDNA), fragments of DNA that are released from the placenta into the pregnant woman's circulation (Figure 1). [3] By 11-13 weeks of gestation, cffDNA makes up an average of 10% of cell-free DNA in the mother's blood. [4] This is called the fetal fraction.

There are several commercial providers and laboratories offering NIPT screening for trisomies 21, 18 and 13, many of which include the option of reporting fetal sex. Some also offer screening for sex chromosome aneuploidies, such as Turner (45 X) and Klinefelter (47 XXY) syndromes, or microdeletions and duplications, such as DiGeorge syndrome (22q11 deletion). NIPT screening for sex chromosome aneuploidies, microdeletions and duplications is based on limited published evidence, raises a number of concerns and is not currently supported by professional societies. [5-10] Cell-free fetal DNA can also be used to diagnose fetal rhesus D status and an increasing number of single gene conditions. [11] A full discussion cffDNA-based diagnostic testing is beyond the scope of this article.

Pre- and post-test counselling for NIPT

Women must be empowered to make free and informed choices based on objective information about all antenatal screening tests. Healthcare professionals may have a tendency to focus on medical complications of a condition, but the information given should also include the broader experiences of individuals and families living with the condition. [7] Where trisomy screening is offered as part of standard antenatal care, there is a risk that women accept because they feel it is expected of them. There is also a risk, especially with expanded screening for conditions other than trisomies 21, 18 and 13, that women accept screening without full consideration of the conditions screened for or next steps in the event of a positive result.

The aims of pre-test counselling are to explore a woman's expectations of NIPT as well as her understanding of and attitude towards the potential outcomes (Box 1). Of particular importance are pregnant patients' understanding of the benefits and limitations of NIPT screening, including the possibility of test failure.

It can be difficult for women accessing NIPT privately to find unbiased information, since most test information is produced by commercial companies and tends to emphasise perceived advantages over limitations. Box 2 lists some of the questions women might ask a potential NIPT provider.[12] Women receiving a high-chance NIPT result or positive diagnostic test need to receive up-to-date and balanced information about what this result means and the possible next steps, along with sign-posting to additional sources of information and support.

Who should be offered NIPT screening?

Ideally, all pregnant women who choose to have aneuploidy screening for trisomies 21, 18 and 13 would be given the option of NIPT as a primary screening test, which can be performed from 10 weeks of pregnancy. Compared with combined screening, NIPT has higher detection rates and much lower false positive rates (Table 1). This means that fewer pregnant women with euploid pregnancies would have to experience the discomfort and miscarriage risk of invasive testing in order to rule out an aneuploidy.

One of the most important things to discuss with women choosing between NIPT and combined or quadruple screening as their primary screening test is the possibility of a 'failed' test due to low fetal fraction or processing issues resulting in a 'no-call' NIPT result. About 1-8% of women will not get a result from their first NIPT test, and 15-50% of these women will not get a result after a second test. [13 14 15 16] A no-call result because of low fetal fraction is more likely to occur at earlier gestations, with a high maternal weight and with dichorionic twin pregnancies. [4 11 15] There is also evidence that a no-call NIPT result is associated with an increased chance of aneuploidy, so women with no-call NIPT results may want to consider invasive testing. [13] If invasive testing were offered to all women with a no-call result as well as women with a high-chance NIPT result, the screen positive rate of NIPT would be only 0.5% lower than traditional combined screening (2.4% v 2.9%). [14] Therefore, women who are more likely to receive a no-call result, e.g. those with a very high maternal weight, may want to consider alternatives such as combined screening.

NIPT is currently more expensive than combined or quadruple screening. As a result, it is more cost-effective to offer NIPT only to women with an increased chance of trisomy based on their combined or quadruple screening test results ("contingent testing") instead of using NIPT as a primary screening tool. [2 17] With contingent testing, women with a high-chance initial screening test result may choose no further testing, NIPT as a second line screening test or invasive testing. A woman's choice may depend on factors such as her wish to continue the pregnancy, the perceived risk of miscarriage and the importance she places on receiving a definitive result. In the context of any structural anomaly, including an increased nuchal translucency (>3.5mm), invasive testing would allow additional diagnostic testing beyond the three main trisomies, including chromosomal microarray. [9 10] The use of NIPT in a high-chance population reduces invasive procedure rates (and their associated costs) and increases aneuploidy detection rates because some women who would not want an invasive test will opt for NIPT. [18 19 20]

Who can access NIPT screening?

For many women, financial access to NIPT is a major limitation of this test. NIPT is available privately in most countries, with reimbursement rates varying between private medical insurers. Publicly

funded NIPT provision differs between healthcare systems, with some countries, such as Belgium, funding primary NIPT screening for all pregnant women, while others, such as Switzerland, offer contingent screening. [21 22] In 2016 the UK National Screening Committee recommended a national evaluation of contingent NIPT screening for women who receive a high-chance (1:2-1:150) trisomy result on combined or quadruple screening. [8] The three-year evaluation process is expected to start in April 2021.

What do the results of NIPT aneuploidy screening mean?

Unlike combined and quadruple screening, which give results as a chance of '1 out of X' on a continuous scale, NIPT results are often reported as a low-chance, high-chance or no-call. As with all screening tests, the positive predictive value (PPV) of a high-chance result is lower when the prevalence of a condition is lower. This is demonstrated in Table 1 with the lower PPVs for trisomies 18 and 13 compared with trisomy 21 and lower PPVs in a general obstetric population compared with a high-chance population. [23-25] This distinction should be clearly explained as part of the pre-test counselling process, particularly for women in the general obstetric population who choose NIPT for first line aneuploidy screening. If a woman receives a high-chance or no-call NIPT result and wants a definite diagnosis, she can be offered invasive testing, as discussed above.

What are the limitations of NIPT?

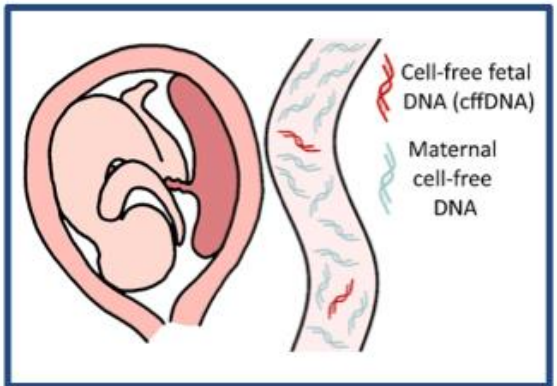
The major limitations of NIPT are cost and the chance of a no-call result. The proportion of women with a fetal fraction <4% is estimated to increase from 0.7% at a maternal weight of 60kg to 7.1% at 100kg and 41.2% at 150kg.[4] One study reported no-call results after initial testing in 11.3% of dichorionic twin pregnancies compared with 4.9% of monochorionic twin pregnancies and 3.4% of singleton pregnancies.[15] Dichorionic twins also raise the possibility of false negative results if one twin is affected by a trisomy but contributes a lower fetal fraction. Still, much of the evidence for NIPT in dichorionic pregnancies is encouraging, and they will be included in the NHS evaluation study.[26]

Because cffDNA is produced by the placenta rather than the fetus, false-positive results can also be caused by confined placental mosaicism or a 'vanishing twin' (a twin pregnancy where demise of one twin results in a singleton pregnancy). False-positive and no-call results can also occur in maternal cancer.[7]

Outcome

After further discussion with her midwife and accessing non-commercial online information, the woman decided to accept the aneuploidy screening offered as part of standard care. In her healthcare system this was combined screening as a first line and she received a low-chance result for trisomies 21, 18 and 13.

Figure 1: Cell-free fetal DNA for non-invasive prenatal testing (NIPT) (adapted from Spencer & Pandya[3])



Advantages

- Excellent screening test for T21, 18 and 13
- Low false positive rate gives potential to avoid an invasive test, which carries a risk of miscarriage
- Clarity of reported results - low chance or high chance

Limitations

- May be a false negative or no result with a low fetal fraction
- May be a false positive with 'vanishing' twin, maternal cancer, or placental mosaicism
- Cost of assay

Box 1: Recommendations for pre-test counselling (adapted from Bianchi & Chiu[27])

- State that screening is optional
- Clarify that it is a screening test not a diagnostic test
- Describe the limitations of the test (i.e. what it does not screen for)
- Review the clinical features and variability of the conditions being screened for
- Briefly review the test methods and reporting formats
- Define positive and negative predictive values and their clinical significance
- Explain that definitive diagnosis would require further testing (either invasive testing during pregnancy or neonatal testing)
- Mention the possibility of incidental findings related to maternal health

Box 2: Questions to ask private NIPT providers when considering testing (adapted from Antenatal Results and Choices[12])

- Which conditions will be screened for?
- How long will I wait for a result?
- How will I be given my result?
- What happens if I get a high-chance result?
- What is the chance of an inconclusive (no-call) result?
- How much will it cost?
    - Does this include the cost of an ultrasound scan?
    - Does this include a repeat test if the result is inconclusive?
- What are the alternatives to NIPT?

Table 1: Performance of antenatal screening for trisomies 21, 18 and 13: the observed performances of combined first trimester screening (NT, PAPP-A, freeβHCG, maternal age) compared with the modelled performances of non-invasive prenatal testing (NIPT), based on meta-analysis and modelled for general and high-chance populations.

	Screening test	Modelled prevalence	DR	FPR	PPV
Trisomy 21	Combined first trimester screening[23]		85.0%	2.0%	
	NIPT high-chance population[24]	3.33%	97%	0.31%	91%
	NIPT general obstetric population[24]	0.43%	95.9%	0.09%	82%
Trisomy 18	Combined first trimester screening[25]		91.9%	3.5%	
	NIPT high-chance population[24]	1.50%	93%	0.26%	84%
	NIPT general obstetric population[24]	0.10%	86.5%	0.15%	37%
Trisomy 13	Combined first trimester screening[25]		83.1%	4.4%	
	NIPT high-chance population[24]	0.50%	95.0%	0.07%	87%
	NIPT general obstetric population[24]	0.05%	77.5%	0.42%	49%

NT=nuchal translucency, PAPP-A=pregnancy-associated plasma protein A, HCG=human chorionic gonadotrophin, DR=detection rate=sensitivity, FPR=false positive rate=(1-specificity), PPV=positive predictive value.

What you need to know

- NIPT relies on fragments of DNA from the placenta, called cell-free fetal DNA
- NIPT is a screening test for fetal aneuploidy with high negative predictive rates, making it a valuable alternative to combined or quadruple screening
- NIPT can either be offered to all pregnant women as a primary screening test or contingent upon initial combined or quadruple screening
- Unbiased information on the conditions being screened for as well as the advantages and limitations of different screening approaches is essential for women to make an informed choice

How patients were involved in the creation of this article

Representatives from the Down's Syndrome Association and Antenatal Results and Choices (ARC) were consulted on the design of this article and provided feedback on a draft version. Their input included:

- Emphasising that women must understand the nature of the conditions being screened for to make truly informed choices
- Recommending the use of the phrase 'high-chance' instead of the more pejorative 'high-risk'
- Making sure women are aware of the limitations of NIPT for chromosomal anomalies and are aware what questions they should ask of any private provider
- Including what should happen in the event of a high-chance result

Rational testing into practice

If aneuploidy screening is available to your pregnant patients, consider how this is offered. What sources of information do they have access to? Do they receive individual counselling? Do you think they receive enough information to make a truly informed choice? If not, how could this be improved?

Additional resources

More information about NIPD can be found on the NHS RAPID website http://www.rapid.nhs.uk/guides-to-nipd-nipt/ and the Antenatal Results and Choices charity website http://www.arc-uk.org/tests-explained/non-invasive-prenatal-testing-nipt. Information about Down's Syndrome, including antenatal screening tests, can be found on the Down's Syndrome Association website https://www.downs-syndrome.org.uk/about/pre-natal-faqs/.

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