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NON-INVASIVE PRENATAL TESTING FOR COMMON CHROMOSOMAL ABNORMALITIES: FREQUENTLY ASKED QUESTIONS

Introduction

Non-invasive prenatal testing (NIPT) is rapidly being adopted as a screening tool for Down syndrome and other common chromosomal abnormalities. This is a simple guide to address frequently asked questions about NIPT and to address issues that may encountered by those offering more traditional forms of Down syndrome screening.

What is NIPT?

NIPT, which identifies cell free fetal DNA in a maternal blood sample, is a highly effective screening tool for common chromosomal aneuploidies including trisomies 21, 18 and 13. NIPT involves collection of a maternal blood sample and can be performed from 10 weeks' gestation (though some providers offer it from 9 weeks). The test can be extended to determine fetal gender and to identify sex chromosome anomalies (SCAs) and other chromosomal abnormalities, such as the relatively common microdeletion del22q11.

The cell free fetal DNA assessed in this test originates from the outer cytotrophoblast of the placenta. Whilst this is a true representation of the fetal karyotype in 98% of pregnancies there is a small risk that the result could be impacted by placental anomalies such as confined placental mosaicism (CPM).

Test performance:

NIPT has been shown to be highly effective in screening for Down syndrome with very high sensitivity and specificity in reportable cases (Table 1).¹

| | Detection rate | FPR | Positive LHR | Negative LHR |
|------------|----------------|-------|--------------|--------------|
| Trisomy 21 | 99.7% | 0.04% | 2506 | 0.003 |
| Trisomy 18 | 97.9% | 0.04% | 2330 | 0.022 |
| Trisomy 13 | 99.0% | 0.04% | 2819 | 0.01 |
| 45X | 95.8% | 0.14% | 694 | 0.04 |

FPR; false positive rate. LHR; likelihood ratio

Most test providers report a 1-3% failure rate; where risk assessment cannot be completed. Approximately 50% of these cases will obtain a result from repeat testing. Test failure may be associated with increased rates of aneuploidy – so the option of proceeding with an invasive test (like amniocentesis) should be considered.²

Although there are fewer reported data, NIPT also appears to be very effective in assessing the risk of Down syndrome in twin pregnancies and identifies at least 90% of pregnancies where one fetus is affected by Down Syndrome.^{1, 3}

Who should be offered NIPT?

NIPT has been validated in both high-risk and unselected populations and performs well in both groups. NIPT can be offered to any pregnant woman who wishes to have Down Syndrome screening. Women should be advised that:

This is a screening, not a diagnostic, test

That high-risk results require further validation before action

That the test may be inconclusive

That the test examines all fragments of DNA (maternal and placental) and can, in rare circumstances, identify maternal chromosomal abnormalities.

NIPT is best performed after an ultrasound scan has been used to define viability, gestational age and plurality. If the scan identifies a major structural abnormality then invasive testing would be preferred.

NIPT tests are available from 10 weeks gestation, but there may be a higher failure rate at early gestations and testing at >10 weeks gestation may be preferred.

Are there any contraindications to NIPT testing?

Absolute contraindications:

- a. Women who do not wish to have Down Syndrome screening.
- b. Women who wish to have a diagnostic, rather than a screening test.
- c. Women carrying triplets or higher order multiples.
- d. Women that have a high-risk of uncommon forms of aneuploidy (not tested by NIPT) e.g.: known parental translocations / fetal structural anomalies.

- e. Women with history of ovum donation or of organ, stem cell or marrow transplant (contraindicated for some forms of NIPT).

The chance of test failure is also increased:

- a. In women of high BMI.
- b. In women that have a low PaPP-A result through combined first trimester screening.
- c. In women with active autoimmune disorders.
- d. In women on therapeutic heparin/LMWH.
- e. In women with a recent cancer diagnosis.

Interpreting results

High risk results: NIPT tests have very high positive predictive values.⁴ A positive test result requires further action. Women should be informed of the results and the potential consequences of this condition as soon as possible. The findings may correlate with other ultrasound / biochemical results. As the positive predictive value is not 100% women should be offered confirmatory invasive testing before deciding to interrupt the pregnancy. False positive tests can occur and may be related to confined placental mosaicism or a vanishing twin. Amniocentesis may therefore be the preferred testing option to guard against false positive diagnosis, particularly in relation to high-risk results for sex chromosome aneuploidy. Positive predictive values are typically higher for trisomy 21 than for trisomies 18 and 13, but all require further discussion / action. Invasive testing can be delayed for women who would not contemplate interrupting the pregnancy, but a detailed anomaly scan may help identify any underlying structural anomalies that will require acute neonatal intervention.

Low risk results: The negative predictive value of NIPT is extremely high and should be regarded as reassuring requiring no further testing. Further (invasive testing) may be considered if:

- There are obvious structural abnormalities seen on subsequent ultrasound.
- The fetal fraction is low (<4%) in a high-risk setting (see below).

Can I use NIPT to screen for sex chromosome aneuploidy and microdeletions?

Various NIPT providers will report fetal sex, common forms of sex chromosome aneuploidy (monosomy X, 47XXX, 47 XXY and 47XYY) if requested. Similarly various providers will report a number of microdeletions, such as del22q11, if requested.

These forms of aneuploidy were not previously screened for and clinicians need to adapt pretest counseling to ensure patients are appropriately informed about the basis for screening and consent to extended screening before testing.

There are fewer data validating extended forms of NIPT but clinicians should be aware that performance is not uniform across all providers and that screening accuracy is poorer (sensitivity and positive predictive values are lower) compared to screening for common trisomies. Confirmatory invasive testing by amniocentesis is always recommended before any irreversible pregnancy decisions are made.

If fetal sex is determined through NIPT this should be correlated with the phenotypic appearance of the fetus during the morphology scan.

What should I do if NIPT provides no result?

No result (test failure) is typically reported if either the quality of DNA in the blood sample is poor or the relative proportion of cell free DNA fragments from the placenta is low (a low fetal fraction).

One large series reported in the literature had a test failure rate of 3% and 1 in 30 of these cases had a chromosomal abnormality (either common trisomies or other atypical forms of aneuploidy).⁵ This was supported by a recent Australian series reporting 6% prevalence of chromosomal abnormality in this group.²

If NIPT is repeated (a redraw), 50-70% of women will receive a result through a second round of testing. These patients should be aware that NIPT will not detect any atypical chromosomal abnormality that was the basis of previous test failure.

Alternative strategies include reverting to the use of combined first trimester screening (using ultrasound based NT and biochemical markers free BhCG, PaPP-A and PIGF) or offering an invasive test (on the basis that 1 in 15 to 1 in 30 may have aneuploidy – which would be considered to be within the threshold of a high risk group).

Some NIPT providers report inconclusive as well as failed results. In this circumstance it is best to have a direct conversation with the provider to establish why the test was inconclusive and form a plan about further assessment. Further advice can also be sought from clinical geneticists, genetic pathologists or MFM / COGU sub-specialists.

Is a first trimester ultrasound still of value?

NIPT is best performed after an ultrasound has confirmed viability, gestational age and plurality. The placenta should also be assessed to check there is no obvious evidence of triploidy – which is not detected by most NIPT tests. Evidence for a ‘vanishing’ twin – which may affect NIPT results, should also be assessed.

First trimester (11-13+6 week) ultrasound has many values that are not represented through NIPT screening and this scan should still be offered to all women. These values include:

- a. Accurate pregnancy dating (impacting future obstetric management).
- b. Sequential structural evaluation of the fetus (which will detect >50% of major structural anomalies). These may be associated with atypical chromosomal abnormalities not detected by NIPT.
- c. Assessment of nuchal translucency (measures >3.5mm may be associated with structural anomalies or atypical chromosomal abnormalities not detected by NIPT).
- d. Defining chorionicity, and ongoing risks, for twin pregnancies.
- e. Identification of concurrent maternal pathologies (e.g. uterine fibroids, ovarian pathologies).
- f. Defining risks of later adverse pregnancy outcome (e.g. uterine artery Doppler assessment for pre-eclampsia and IUGR screening).

Failure to provide appropriate first trimester ultrasound assessment for a patient choosing NIPT as a primary form of screening for Down syndrome risks failing to identify this range of pregnancy complications.

Can combined first trimester screening and NIPT be combined?

Whilst NIPT is clearly a more effective means of screening for Down syndrome, it is not of significant value in screening for a wider range of adverse pregnancy outcomes and combined first trimester screening (cFTS) still has a role to play in early assessment and management of pregnancy.

One method of combining both tests is to use a 'contingent' model. In this model, all women are first offered cFTS. Rather than using the traditional 1 in 300 risk cut-off to define a high-risk group, women are split in to three cohorts. Those with a risk >1 in 50 are considered to be high risk and should be offered invasive testing. Those with a risk of 1 in 50 to 1 in 1000 are considered intermediate risk and are offered NIPT. Those with a risk >1 in 1000 are considered low risk and are reassured that no further testing is needed. This model has the advantage that it improves overall detection rates for trisomy 21 (to 97-98%), reduces invasive testing rates and limits NIPT to 15-20% of the population (cost saving). The cut-offs (1 in 50, 1 in 1000) can be adjusted according to local providers requirements for screening performance.

Is first trimester biochemistry still of value?

If NIPT is being used as the primary form of screening for Down syndrome then traditional biochemistry is not needed. If NIPT is being offered in a contingent model, then traditional first trimester biochemical markers should still be included as part of the primary cFTS test.

The first trimester serum screening markers free BhCG, PaPP-A and PIGF do have some other values in the screening process:

- Polarized free BhCG ($>3.0\text{MoM}$ or $<0.2\text{MoM}$) and PaPP-A ($<0.2\text{MoM}$) measures are recognized as being associated with atypical chromosomal abnormality and may lead to invasive testing rather than NIPT in a contingent screening model

- A low PaPP-A result is associated with low fetal fraction and poorer performance of NIPT tests; if NIPT is offered after cFDS in circumstances where the PaPP-A was low then reviewing fetal fraction is worthwhile.
- PaPP-A and PIGF both add significant value within a validated multi-marker algorithm for pre-eclampsia.

References

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