South African Society for Ultrasound in Obstetrics and Gynaecology

BEST PRACTICE GUIDELINE – NON-INVASIVE PRENATAL TESTING (NIPT) by means of cell-free DNA (cfDNA)

Endorsed by the Medical Geneticist Group of the SA Society of Human Genetics

INTRODUCTION

NIPT is now widely available in South Africa in the private funded sector but different tests are being offered, using different molecular technologies and with different advantages and disadvantages, different indications and contraindications.

- It is therefore essential that a **proper assessment of each case precedes the decision to request a NIPT and the decision of which specific NIPT test to select**, to ensure that NIPT is appropriate and that the chosen test is appropriate for the specifics of the case.

As far as trisomy 21 (T21) is concerned, clinical studies have shown several NIPT methods to be excellent screening tests.

- **Not all cfDNA test methods can be used in all clinical situations** e.g. with donor eggs, surrogacy, or twin pregnancies (including vanishing twins, which need to be ruled out by scan). In tests that can be used for twins, cfDNA is also less accurate than in singletons. However, as other screening tests are also inferior in twins, cfDNA may add clinical value.
- **Test success** depends in part on the characteristics of the specific test method, but is also strongly influenced by fetal fraction (the fraction of cfDNA that originates from the fetus – or actually from the cytotrophoblast cells of the placenta). Fetal fraction depends on gestational age and maternal body habitus. For this reason it is **recommended that NIPT is not performed before 10 weeks gestation (confirmed by US) and that women over 120 kg are warned about high failure rates** (i.e. no NIPT result).

Parents should receive pre-test counselling by a knowledgeable professional regarding the alternative approaches (including the option of no screening) and the risks and benefits of each. A guideline for this counselling process is provided in APPENDIX.

Although excellent, NIPT remains a screening test as results are NOT conclusive. NIPT results for T21 are very reliable and *in general* the detection rate is > 99%, the false positive rate approx. 0.1% and test failure rate below 5%. The positive predictive value however varies with the a priori risk and can be quite low in low risk pregnancies.

- **Post-test genetic counselling is strongly recommended for all positive ("high risk") NIPT results.**
- **ALL positive NIPT results should be followed up with confirmatory testing** (karyotype, FISH or qfPCR) on a fetal sample, as it would be ill advised to terminate a pregnancy without ruling out the possibility that the NIPT result is false positive. Confirmation of the fetal karyotype is also recommended in order to identify the underlying mechanism for the chromosomal imbalance as this influences counselling and recurrence risk.
- **Post-test genetic counselling is strongly recommended for all cfDNA results which are not reported, are indeterminate or uninterpretable** (possibly after a repeat sample is examined or an alternative NIPT test has been performed) and comprehensive ultrasound/invasive testing for karyotype need to be considered (increased failure rate for repeat NIPT, and higher risk of underlying aneuploidy).

NIPT has primarily been developed as a very accurate screening test for T21 but **screening for other conditions has become possible with the same technologies**. Most tests include screening for T13 and T18 but they differ considerably in the **additional conditions** that can be tested for.

- Most of these conditions are unknown to the general public and **extensive genetic counselling is advised** to inform the parents of the scope of the test and the test performance for each condition, which is invariably poorer than for T21, and sometimes very significantly so.
- Also the implications of such "other conditions" differ greatly from that of T21: The clinical significance varies from more disabling than Down syndrome for some of the rare microdeletions, to significantly less severe than Down syndrome for the more common conditions e.g. most cases of sex chromosome abnormality (SCA) or 22q11.2 deletion syndrome.
- It is also important to note that NIPT for microdeletion syndromes or "all chromosome testing" has not yet been validated in large cohorts of patients and has not been assessed for cost-benefit.
- As such the direct-to-consumer marketing of this kind of NIPT is strongly opposed as it would lack truly informed consent.

- It is important that it is communicated to the parents that such an extended panel would significantly increase the need for confirmatory invasive testing.
- As this type of NIPT is more expensive (except for gender determination) and less well proven than the "standard" NIPT (for T21, 13, 18), and as it increases the need for invasive testing, **cfDNA for microdeletion syndromes, all chromosome testing and SCA is currently not recommended for routine practice**.
- It is strongly recommended that a request for NIPT to detect microdeletions or SCA or for all chromosome testing should be from a knoweldgeable professional based on clinical directives.

The current guideline is therefore primarily focused on the major autosomal trisomies (13, 18, 21).

NIPT PRIMARILY TO RULE OUT T21 (and T13 and T18)

As all NIPT tests currently are still quite expensive in spite of a significant reduction in cost in recent years, the question arises for which patients NIPT for T21 is a "good investment". Several implementation strategies can be considered, depending on which alternative screening modalities are available.

1. <u>OPTION 1: "Blanket" cfDNA for T13, 18, 21 for everyone</u>

This would obviously have the highest detection rate (>99%) for DS but the highest total cost and cost per case detected.

As it would not identify all genetic abnormalities that are detectable on full karyotyping or all structural anomalies or genetic conditions that could be diagnosed with expert ultrasound, it is **currently not recommended for low risk women who have other screening options**.

This recommendation may change in the future should costs reduce sufficiently to make it cost-effective to offer cfDNA in addition to expert ultrasound.

It should always be preceded by counselling, a 'viability scan' for dating and exclusion of twins/vanishing twin and parents should be made aware that the NIPT test may fail and that the pregnancy may be too advanced by then for a "first trimester combined T21 screen".

2. cfDNA for T13, 18, 21 if alternative screening tests indicate a risk above a pre-defined cut-off value

2.1. OPTION 2: Risk based on comprehensive screening by Fetal Medicine Foundation accredited operators including ultrasound and serum biochemistry (i.e. the 'combined test').

2.1.1. This would be **the ideal primary screening method as the detection rate without NIPT is already in excess of 90%** and most parents would be sufficiently reassured by a low risk result.

2.1.2. The benefits of this approach would be a lower total cost and a lower cost per detected case of DS, but a somewhat higher number of missed cases of DS (approx. 1 in every 10.000 women screened) while having the **advantage of a high early detection of many structural anomalies (far more common than T21) and a significant number of genetic conditions not diagnosable with cfDNA** (Norton 2014). For these reasons, ACOG (2015) still recommends this approach above "blanket cfDNA for all".

2.1.3. There is however the **question of accessibility** as typically no more than 30 fully accredited practitioners with an up-to-date FMF license provide NT screening in private practice in SA and the service is generally limited to the major centres.

2.1.4. After disclosure of the risk estimate resulting from such "ideal" screening parents who are paying out of pocket can certainly decide for themselves whether they are sufficiently reassured or not but if external funding is requested, it would be reasonable to **decide (on a cost-benefit analysis) what level of risk is deemed reasonable to proceed with further cfDNA testing**.

2.1.5. **Recommended guideline for cfDNA after such ideal screening**:

2.1.5.1. **VERY HIGH RISK: Risk higher than 1:10** (above approx. 99th centile):

Counselling should primarily be towards invasive testing as the parents may not want to wait for a NIPT result and may not be fully reassured by a negative cfDNA while karyotyping for confirmation of a positive cfDNA results is likely to be needed anyway, given the high prior risk. cfDNA testing in all these cases may therefore not be cost-effective (although it may still be useful in specific individual clinical scenarios).

2.1.5.2. **HIGH RISK: Risk between 1:10 and 1:100** (approx. P95th to 99th):

Offer either invasive testing OR cfDNA (with subsequent karyotyping only if cfDNA abnormal)

2.1.5.3. **INTERMEDIATE RISK: Risk lower than 1:100 but higher than 1:1000-2500:**

[the cut-off depends strongly on available resources as the group between 1:1000 and 1:2500 probably amounts to approx. 15% of pregnancies]: **Offer cfDNA** (with subsequent karyotyping if cfDNA abnormal).

2.1.5.4. **LOW RISK: Risk lower than 1:1000-2500: cfDNA is unlikely to be cost-effective** as the chance for a positive result is very low and the chance that a positive result would be a false positive would be significant.

2.2. OPTION 3: When/where FMF accredited combined screening is not available for all patients, it is recommended that biochemical screening be used as first line for triage purposes (first trimester PAPP-A, free b-HCG and/or second trimester AFP, E3 and HCG).

2.2.1. NT scanning by non-FMF-accredited operators has a low detection rate and generally inferior performance compared to serum screening hence biochemical screening is preferred as first line.

2.2.2. Compared to FMF-accredited combined screening, serum screening is far **less efficient** as the detection rate is only in the order of 60-65% with current cut-off values (slightly higher if serum screening is done in both first and second trimester) and many more patients will receive a result that is not very low (higher than 1:1000 or 2500). Far **more parents (numbers depending on the exact cut-off used) would therefore require NIPT while many fetal anomalies and other genetic disorders would remain undetected** by this approach. As such it is not the preferred strategy if option 1 is available.

2.2.3. NIPT together with serum screening will have a lower detection rate and result in a higher number of missed diagnoses of T21, at a higher total cost than FMF accredited screening for all, but would be **more feasible in the local setting**.

2.2.4. Recommended guideline for cfDNA after serum screening only:

2.2.4.1. **VERY HIGH RISK: Serum risk higher than 1:10:**

If possible refer to a fetal medicine unit for full assessment and recalculation of the risk estimate based on ultrasound findings - if the risk remains high, **counselling should primarily be towards invasive testing** as cfDNA is unlikely to be cost-effective (although it may still be useful in specific individual clinical scenarios).

2.2.4.2. **HIGH RISK: Serum risk between 1:10 and 1:100:**

Parents can choose between referral to a fetal medicine unit for full assessment (esp. useful < 13 weeks) OR cfDNA (with subsequent karyotyping only if cfDNA abnormal)

2.2.4.3. **INTERMEDIATE RISK: Serum risk lower than 1:100 but higher than 1:1000–1:2500** [the cut-off depends strongly on available resources as the group between 1:1000 and 1:2500 probably amounts to approx. 15% of pregnancies]:

Offer cfDNA (with subsequent karyotyping only if cfDNA abnormal)

2.2.4.4. **LOW RISK: Risk lower than 1:1000-1:2500:**

cfDNA is unlikely to be cost-effective as the chance for a positive result is very low and the chance that a positive result would be a false positive would be significant.

Note: If biochemical screening is done at 10 weeks, referral to a FMF accredited centre should ideally be attempted for all patients with a serum risk above 1:100 (depending on resources & availability) as this would significantly reduce the total cost of NIPT. If the serum screening is done in the second trimester (50% of cases) cfDNA (rather than referral) may be preferred for risks higher than 1:100 as the potential for significant risk reduction by an expert would be limited.

Recommended indications for Karyotyping

1. **All cases of positive ("high risk") cfDNA screening require FULL karyotyping:** not only to confirm the diagnosis but also to determine the mechanism (non-dysjunction or translocation) as this affects recurrence risk and counselling.
    1.1. If time is an issue qfPCR may be requested to allow for early TOP if abnormal
2. **High risk for non-trisomic chromosomal anomaly** (full karyotyping and/or microarray testing is preferred as neither qfPCR nor cfDNA would provide sufficient information), e.g:
    2.1. Recurrent miscarriages
    2.2. Family history suggesting a familial chromosome abnormality or known translocation
    2.3. Structural fetal anomaly (including cystic hygroma)
    Note: *These cases are usually more genetically complex, and should be referred for full genetic counselling by a medical geneticist or genetic counsellor.*

After wide consultation, this guideline was compiled by the following professionals who acknowledge that this is a rapidly evolving field and that the guideline may need to be reviewed whenever significant progress is made in this area.

- Prof Lut Geerts, MRCOG, on behalf of SASUOG
- Dr Mike Urban, FCPaed, Cert (Med Genetics), PhD, on behalf of SASHG-MGG
- Mrs Mardelle Schoeman, MSc (Med) Genetic Counselling and Ms Noelene Kinsley, Msc (Med) Genetic Counselling on behalf of SASHG - GC-SA

 March 2019

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APPENDIX: Pre-test counselling

In order to ensure informed and autonomous parental consent, international societies recommend accurate and individualized non-directive pre-test counselling for NIPT by **appropriately skilled professionals** (ACOG, 2015; Gregg et al, 2013; Devers et al, 2013) such as genetic counsellors, medical geneticists, certain obstetricians, fetal medicine specialist or midwives. Patients should receive **current, balanced and accurate information on the nature of the test, the possible outcomes and the options available** to them. Pre-test genetic counselling for NIPT should therefore include the following:

1.**Review of the background information, to assess the risk for birth defects and genetic conditions in this particular pregnancy**:

- Family history, previous and current pregnancy history, ultrasound findings and maternal serum screen results (if available)

2.**Review whether an invasive test may be more appropriate than NIPT**

- NIPT is less informative than a full karyotype on fetal cells, and may miss about 30-50% of karyotypically abnormal fetuses. This figure is even higher (up to 75%) in women with a lower prior risk for T21 (e.g. younger than 35 years) (Gregg et al 2013)Karyotyping is preferred over NIPT in the presence of factors suggesting a genetic condition other than T21, such as a structural abnormality on ultrasound, a family history of a chromosome abnormality or recurrent miscarriages

3. **Discuss the prior risk of T21, T18, T13 and SCA in this particular pregnancy**
4. **Discuss where NIPT fits into the screening process**
   - NIPT is a screening test, **not diagnostic**. A negative test result therefore does not completely rule out T21.
   - NIPT does not replace the utility of ultrasound as this provides information not detectable by NIPT for conditions that are far more common and sometimes more serious than T21 (e.g. 2-3% of all fetuses have major structural anomalies detectable on scan).
   - Comparison with existing screening methods – brief discussion of alternatives
5. **Discuss the advantages of NIPT**
   - It is the most accurate screening test for T21
   - High sensitivity (>99%) means that fewer cases of T21 will be missed. Although not a 100% guarantee, the risk of a missed diagnosis is very low (high negative predictive value)
   - High specificity (>99.9%) means that fewer false positive results occur, leading to less anxiety and unnecessary invasive tests, with their associated cost and risk of miscarriage.
   - NIPT can be performed from 10 weeks gestational age throughout pregnancy
6. **Provide information on the conditions NIPT can and can't test for**
   - This should include a brief discussion on the natural history and expected outcome for <u>each of the conditions tested for</u>. It should be noted that the nature and severity of the different conditions vary widely.
   - Detection rates and false positive rates vary for the different conditions tested for and accuracy data must be presented per condition as test performance varies widely.
   - Parents have a choice to test for SCA or not. They need to understand that the implications of Turner or Klinefelter syndrome are very different from the significant intellectual disability associated with T21. In addition, sex chromosome analysis may detect conditions with very few clinical implications, e.g. 47,XYY or 47,XXX. A particular ethical concern is that NIPT should not be used for sex selection with a view to termination on the basis of gender alone (except in the situation of an X-linked condition).

- Parents have a choice to test for the extended panel of "other conditions" or not. As most of these conditions are not known to the general public, the counselling can be tricky and extensive, in particular because test performance for this panel is significantly poorer than for T21.
- Parents should understand that the NIPT does not test for conditions other than those included in the panel. In particular NIPT does not test for single gene disorders.

7. Discuss the positive and negative predictive value of NIPT

- The negative predictive value (NPV; the chance that a normal result indeed means that the fetus does not have the abnormality tested for) of NIPT is very high for T21 (close to 100%) but is lower for other conditions.
- The positive predictive value (PPV; the chance that an abnormal result indicates that the fetus indeed has the abnormality tested for) is also very high for T21 but depends on how common T21 is in the group of women tested. This varies according to the mother's age and the prior risk for DS – the younger the patient or the lower the prior risk, the higher is the chance that an abnormal NIPT result is a false positive result.
- False positive results (i.e. the NIPT indicates T21 but the fetus is actually normal) may be due to confined placental mosaicism, a vanishing twin or other maternal factors and lead to unnecessary anxiety and invasive testing. The false positive rate of NIPT is higher for conditions other than T21.
- Since false positive results for the different conditions are cumulative, the more conditions one tests for, the more likely it becomes that NIPT shows an abnormal result that is not due to an abnormality in the fetus.

8. Provide test-specific information

- NIPT platforms vary in the methodology they use and therefore also differ in the conditions that can be tested for, detection rates and contraindications for testing. This is pertinent for example to testing of twin pregnancies or pregnancies with donor eggs.

9. Discuss reporting and the need for confirmation of screen positive results with a diagnostic test

- Reporting does not confirm a diagnosis but reports a screen positive result, such as 'high risk' or 'aneuploidy detected' or screen negative result such as 'low risk' or 'no aneuploidy detected'.
- Delivery of a negative result should be accompanied by a comment on the residual risk of T21 or other genetic conditions and the value of continued prenatal management.
- A positive result necessitates detailed individualised post-test counselling by an appropriately skilled professional, ideally a genetic counsellor or clinical geneticist (ACOG, NSGC).
- Recommendation that a screen positive result be confirmed with a diagnostic test, not only to rule out a false positive result but also to identify the underlying chromosomal mechanism, e.g. DS due to trisomy 21 versus Robertsonian translocation involving chromosome 21, which have different implications for families and recurrence risk.

10. Explore reasons for wanting NIPT

- The decision to proceed with NIPT remains the choice of the patient.

11. Explore the parents' experiences with/views on T21 or disability in general

12. **Explore how parents would act on a positive result** - attitudes about termination of pregnancy

13. Discuss practical issues

- Turn-around time, failure rate, cost

14. Informed consent:

- Verbal, laboratory request form.