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Newsletter

NON-INVASIVE PRENATAL SCREENING(NIPS)

Compiled by: Dr HE van Deventer, Dr A Glass and Dr V Sahibdeen

2nd Quarter 2023

Sample requirements:
- Maternal blood sample
- MUST be collected in a Streck tube.

Required documents:
- Genetic Test Request form (G1012).
- Informed Consent and Clinical Information for Genetic Testing form (EP01145 G1012).

Turnaround time (TAT):
7-10 days

Contact details:
- nips@lancet.co.za
- +2711 358 0800

Introduction

Non-invasive prenatal screening (NIPS) offers high sensitivity and specificity as a screening test for the common foetal aneuploidies. Due to advances in genomic technologies, we can detect the presence of foetal cell-free DNA (cfDNA), derived from the placenta, circulating in the maternal blood. The preferred nomenclature for what is often referred to as NIPT is NIPS ('S' for screening) to emphasise that utilising a placenta-derived cell-free DNA source, makes this a screening rather than a diagnostic test. The purpose of prenatal aneuploidy screening is to provide an assessment of a woman's risk that the foetus she is carrying has one of the more common foetal aneuploidies. This is in contrast to invasive prenatal diagnostic testing for genetic disorders, in which the foetal chromosomes are evaluated for the presence or absence of abnormalities in chromosome number, deletions and duplications, or the foetal DNA is evaluated for specific genetic disorders.

Recent international guidelines advocate the following:
- NIPS should be offered to all pregnant women regardless of risk.
- Patients should have the opportunity to make an informed choice to decline or accept testing.
- Pre- and post-test counselling regarding the benefits, risks and limitations is essential.

Current Screening options available

Lancet Laboratories offers First Trimester Down Syndrome Screen, Second Trimester Down Syndrome Screen and NIPS. Each screening option has relative advantages and disadvantages. It is important that obstetric care providers be prepared to discuss not only the risk of aneuploidy, but also the benefits, risks, and limitations of available screening tests. Screening for aneuploidy should be an informed patient choice, with an underlying foundation of shared decision making that fits the patient's clinical circumstances, values, interests, and goals. All patients should still be offered a second trimester ultrasound for foetal structural defects since these may occur with or without foetal aneuploidy.

Introduction to NIPS methodology

NIPS is a blood test that assesses cfDNA from a maternal blood sample (mixture of foetal and maternal DNA) together with next-generation sequencing (NGS) technologies to predict the risk for foetal genetic disorders during the pregnancy. This includes screening for common chromosomal conditions, including Trisomy 21 (Down Syndrome), Trisomy 18 (Edwards Syndrome), and Trisomy 13 (Patau Syndrome).

What is cell-free foetal DNA?

Segmented cfDNA molecules are continuously shed into human body fluids such as blood through various pathways of cell death, degradation, and regulated extrusion, containing partial or complete genomes of various origins (e.g. host cells, foetal cells, and infiltrating viruses and microbes). The foetal component of cfDNA is derived from placental trophoblasts that are released into the maternal circulation. Maternal cfDNA is approximately 166 bp in length, and foetal cell-free DNA (cffDNA) is approximately 143 bp in length. The foetal component of the total cfDNA is known as the foetal fraction, and comprises approximately 3 – 13% of the total cfDNA. The quantity of cffDNA increases throughout gestation. Accurate cfDNA screening requires a minimum foetal fraction of about 2 – 4%.

NIPS Technology

Generally, NIPS utilises NGS and bioinformatics algorithms to interrogate cfDNA fragments that have been extracted from maternal samples. **There are 3 main approaches for NIPS commercially available:**

- Genome-wide sequencing may or may not use PCR to amplify starting material, however both methods capture genetic information spanning the **entire genome.**
- Targeted-sequencing uses PCR to capture and amplify genetic information in specific regions of the genome.
- Non-sequencing methods use PCR of rolling circle amplification (RCA) to make copies of genetic information from targeted regions of the genome and fluorescently label the copies.

There has been a rapid progression in the number of NIPS tests available on the market. One important consideration is reducing cost to make NIPS testing more readily available to more patients. Different technologies offer some subtle differences in the information reported. Laboratory reporting information, such as positive predictive value (PPV) is not standardised, however important quality indicators such as foetal fraction should be reported. Screening performance of each approach also depends on the criteria being utilised and how no-call results are categorised. These are important considerations when evaluating available NIPS options.

Lancet Laboratories offers the Illumina VeriSeq™ NIPS Solution. VeriSeq™ uses whole-genome sequencing (WGS) with NGS technology to analyse cfDNA fragments across the whole genome. Test failure rates (no-call results) are substantially lower with WGS versus other methodologies. This is important as high test failure rates may lead to more invasive procedures, increased parental anxiety and aneuploidies being missed. The reported VeriSeq™ failure rate is as low as 0.7%. Testing can be done from 10 weeks' gestation.

Lancet Laboratories offers two NIPS test options:

- **Basic NIPS**
  Screens for trisomies 21, 18, 13 and sex chromosomal aneuploidies (SCAs). Sensitivities are >98.89% and specificities ≥99.73% with an overall positive predictive value of 84.8%.
- **Genome-wide NIPS**
  Screens all chromosomes for atypical chromosomal anomalies, SCAs, rare autosomal aneuploidies and partial duplications/deletions >7Mb with an overall sensitivity of 88.1% and specificity of 99.3%.

Measuring foetal fraction is critical for high-confidence NIPS results

The American College of Obstetricians and Gynecologists (ACOG) emphasises the importance of foetal fraction as an essential quality indicator for accurate test results. Failure to measure foetal fraction can correlate with false-negative results.

Counselling Considerations

Who should be offered testing for chromosomal abnormalities?

Screening (serum screening with or without nuchal translucency (NT) ultrasound or cfDNA screening) and diagnostic testing (karyotyping following CVS or amniocentesis) for chromosomal abnormalities are all available at Lancet Laboratories, and should be discussed and offered to all patients early in pregnancy regardless of maternal age or baseline risk. NIPS is a sensitive and specific screening test for the common foetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, cfDNA testing is not equivalent to diagnostic testing. Prior to testing, counselling should include the possibility of incidental findings affecting the patient, including medical conditions such as her own chromosomal aneuploidy, mosaicism, or malignancy. If foetal sex determination is elected, the risk of maternal and foetal sex chromosome aneuploidy should be discussed as a potential finding.

Follow-up for a 'No Call Result'

A potential limitation of NIPS cfDNA testing is the failure to provide a result. There are essentially three reasons for such a failure:

- Problems with blood collection and transportation of the samples to the laboratory, including inadequate blood volume, haemolysis and delay in arrival at the laboratory.
- Assay failure for a variety of reasons, including failed DNA extraction, amplification and sequencing.
- Low foetal fraction.

Reasons for a low foetal fraction include:

- Small placental mass.
- Impaired placentation.
- Maternal therapy with low molecular weight heparin.
- Compared to natural conception, pregnancies conceived with in-vitro fertilisation have a 3.8 times higher risk for low foetal fraction.
- Genetic conditions, particularly Trisomy 13 or 18, are linked to a low foetal fraction.
- An increased BMI.
- Advanced maternal age.
- Other contributors to test failure are Black and South Asian ethnic origins, which by comparison with White ethinicities, increase the risk by 2.0 and 1.7 times, respectively.

Considerations for discussion with the patient in case of a low foetal fraction:

- Inform the patient that there is an increased risk of aneuploidy.
- Offer genetic counselling and detailed ultrasound evaluation.
- Offer diagnostic testing for a no-call NIPS result.
- Repeat screening may be considered and, if appropriate, will be done at no-cost to the patient. Success rate of repeat screening is 75 to 80% (less with high BMI).

Repeat screening is not advised if:

- Ultrasound anomalies are present.
- If late gestational age where further delay may complicate access to reproductive options.

Considerations for discussion with the patient in case of a high risk result

All women with a high risk NIPS test result should be offered a diagnostic procedure before any irreversible action is taken.

Positive predictive value (PPV) is the probability that patients with a positive screening test truly have the condition for which testing was done. With NIPS, it is the likelihood that the foetus has a particular condition if the screening test returns a high risk result. For NIPS testing the PPV for Trisomy 21 is about 90% depending on maternal age. Age-specific PPV values can be calculated using the following calculator: http://secure.itswebs.com/nsgc/niptcalculator/index.html. The false-positive rate is calculated as the ratio between the number of negative events wrongly categorised as positive (false-positives) and the total number of actual negative events (regardless of classification). In a series of 15 841 patients for whom cfDNA results could be obtained, when cfDNA screening for Trisomy 21 was compared with first-trimester screening (NT and serum analytes) in a general population (mean maternal age 30.7 years), cfDNA screening had a lower false-positive rate (0.06% cfDNA versus 5.4% for serum screening) and a higher PPV (80.9% versus 3.4%).

False-positive cfDNA test results can occur because of confined placental mosaicism. When a screen positive cfDNA result differs from the foetal karyotype or QF-PCR result, the aetiology may also include maternal mosaicism, or in rare instances, it can occur secondary to a maternal malignancy. Of the reported cases, most malignancies have been haematological, but other types of cancer, such as anal and colorectal malignancies, were also identified. If unusual or multiple aneuploidies are noted, a family history should be obtained for familial cancer syndromes, and a physical examination for lymphadenopathy, breast and thyroid masses should be performed. A review of the patient's full blood count, complete metabolic profile, Pap smear, and faecal occult blood testing, followed by oncology consultation and imaging studies, should be considered.

Consideration for discussion with the patient in case of a low risk result

In the setting of a low risk NIPS test result, discussion should include the concept of residual risk, which is defined as the chance that an abnormality may still be present even if the screening test result indicates a low risk. Patients with a low risk screening test result should be made aware that this substantially decreases their risk for the targeted aneuploidy, but does not ensure that the foetus is unaffected. The potential for a foetus to be affected by genetic disorders that are not evaluated by the screening or diagnostic test should also be reviewed. Even if patients have a low risk screening test result they may choose diagnostic testing later in pregnancy, particularly if additional findings become evident such as foetal anomalies identified on ultrasound examination.

Twin Pregnancies

No method of aneuploidy screening is as accurate in twin pregnancies as it is in singleton pregnancies. cfDNA screening can be performed in twin gestations. Twin foetuses in a single pregnancy each contribute different amounts of cfDNA into the maternal circulation. It is possible that an aneuploid foetus would contribute less foetal DNA, therefore masking the aneuploid test result. The biological sex of individual foetuses in a twin pregnancy cannot be determined with Lancet Laboratories NIPS test and can only be determined by SNP-based NIPS testing.

Vanishing Twin

In multi-foetal gestations, if foetal demise or an anomaly is identified in one foetus, NIPS aneuploidy screening should be discouraged and cannot be performed. There is a significant risk of an inaccurate test result in these circumstances.

Cautions

- NIPS does not screen for neural tube defects; therefore, maternal serum α-fetoprotein testing to screen for neural tube defects should still be performed at 15 – 20 weeks of gestation.
- NIPS does not replace routine foetal anatomical screening using ultrasound.

Key Points

- All patients should be counselled as to the risks, benefits, and alternatives of various methods of prenatal screening and diagnostic testing, including the option of no testing. The decision as to whether to undergo prenatal genetic testing should be an informed choice.
- Management decisions, including termination of the pregnancy, should NOT be based on the results of the cfDNA screening alone.
- Diagnostic testing should be performed to confirm high risk cfDNA screening results.
- "No Call" results may indicate a higher chance of a chromosomal disorder, and patients who receive these results should be offered further assessment.

References available on request.

JOHANNESBURG	**0027 (0) 11 358 0800**	KWA-ZULU NATAL	0027 (0) 31 308 6500
BLOEMFONTEIN	0027 (0) 51 410 1700	NELSPRUIT	0027 (0) 13 745 9000
CAPE TOWN	0027 (0) 21 673 1700	POLOKWANE	0027 (0) 15 294 0400
EAST LONDON	0027 (0) 43 708 1570	PRETORIA	0027 (0) 12 483 0100
GEORGE	0027 (0) 44 805 0630	RUSTENBURG	0027 (0) 14 597 8500
KIMBERLEY	0027 (0) 53 836 4460	WELKOM	0027 (0) 57 355 9003

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