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Newsletter

LANCET LABORATORIES Ghana

Non-invasive prenatal screening (NIPS)

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Background

Non-invasive prenatal screening (NIPS) is the most sensitive and specific screening test for the common foetal aneuploidies. Due to advances in genomic technologies, we can detect the presence of foetal cell-free DNA, derived from the placenta, circulating in the maternal blood to screen for common foetal aneuploidies. The preferred nomenclature changed from NIPT to NIPS (**'S'** for screening) to emphasise that utilising a cell-free DNA approach is a screening test and not diagnostic. The purpose of prenatal screening for aneuploidy is to provide an assessment of the woman's risk of carrying a foetus with one of the more common foetal aneuploidies. This is in contrast to 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 (ref.1-4) advocate the following:

- NIPS 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 offer First Trimester Down Syndrome Screen, Second Trimester Down Syndrome Screen and NIPS. Each screening option has relative advantages and disadvantages. It is important that obstetrician-gynaecologists and other 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 (ref.2).

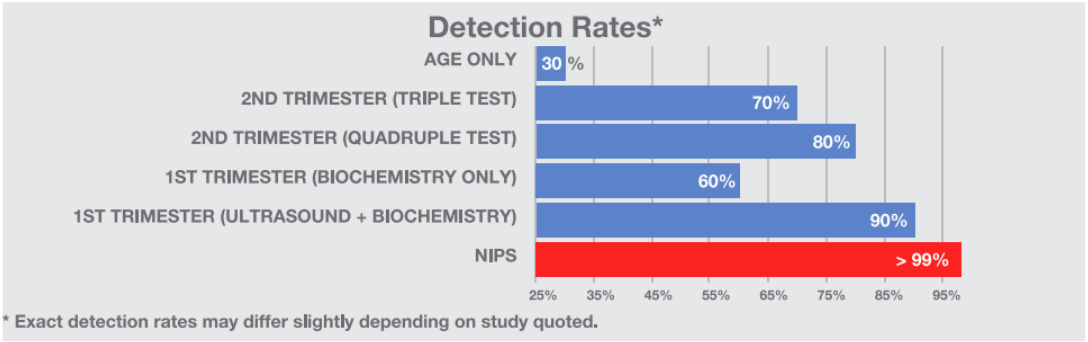

Figure 1. Detection rates for various risks and screening options available

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Table 1. Available screening test options

Screening Approach	Gestational Age Detection for Screening	Detection for Trisomy 21	Screen positive Rate	Advantages	Disadvantages
First Trimester (Biochemistry)	8 weeks to 13 weeks 6 days	60%	5%	May detect placental disorders	Lower detection rate than with ultrasonography. Higher false-positive rate than NIPS.
First Trimester (Biochemistry + Ultrasound)	8 weeks to 13 weeks 6 days	90%	5%	Early screening	Accurate ultrasonography required. Higher false-positive rate than NIPS.
Second Trimester (Triple Test)	15 weeks to 20 weeks 6 days	70%	5%	Does not require specialised ultrasonography for nuchal translucency (NT) measurement	Lower detection rate than First Trimester. Screen with ultrasonography. Higher false-positive rate than NIPS.
Second Trimester (Quadruple Test)	15 weeks to 20 weeks 6 days	80%	5%		
NIPS	10 weeks	> 99.9%	< 1%	Highest detection rate. Lowest false-positive rate.	More expensive than First Trimester and Second Trimester screen. Results may be affected by placental mosaicism, maternal aneuploidy or maternal disease.

Introduction to NIPS methodology

NIPS is a blood test that utilises cell-free DNA technology (cfDNA) to predict the risk for foetal genetic disorders during pregnancy. Cell-free DNA is the most sensitive and specific screening test for the common foetal aneuploidies. NIPS analyses cell-free DNA from a maternal blood sample (mixture of foetal and maternal DNA) to screen for common chromosomal conditions including Trisomy 21 (Down Syndrome), Trisomy 18 (Edwards Syndrome), and Trisomy 13 (Patau Syndrome).

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What is Cell-free DNA?

The foetal component of cfDNA is derived from placental trophoblasts that are released into the maternal circulation. Maternal cell-free DNA is approximately 166 bp in length, and foetal cell-free DNA (cffDNA) is approximately 143 bp in length. The foetal component of the total cell-free DNA is known as the foetal fraction and comprises approximately 3 — 13% of the total cell-free DNA. The quantity of cff DNA increases throughout gestation. Accurate cell-free DNA screening requires a minimum foetal fraction, most commonly estimated at about 2 — 4%. (ref.2)

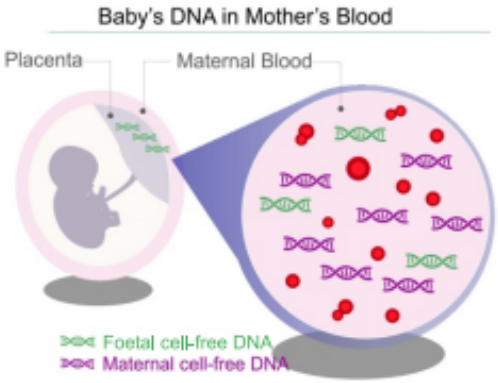

Figure 2. Cell-free DNA comprising both maternal cell-free DNA and a small fraction of foetal cell-free DNA

NIPS Technology

Generally, NIPS utilises next-generation sequencing and bio-informatics algorithms to interrogate cell-free DNA fragments that have been extracted from maternal samples. Broadly it is divided into counting methods (CM) or SNP-based methods (SBM) using either:

- Whole-genome massively parallel shotgun sequencing (CM)
- Targeted sequencing — also known as chromosome-specific sequencing (CM)
- Single nucleotide polymorphism (SNP) sequencing (SBM)

There has been a rapid progression in the number of NIPS tests being available to the market. One important consideration is reducing costs in order 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) and foetal fraction, is not standardised. 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.

CentoNIPT uses underlying technology from the Illumina VeriSeg(TM) NIPT Solution. The Illumina VeriSeq'(TM) NIPS solution uses whole-genome sequencing (WGS) with next-generation sequencing (NGS) technology to analyse cfDNA fragments across the whole genome. Test failure rates are substantially lower with WGS versus other methodologies. High test failure rate may lead to more invasive procedures, increased parental anxiety and aneuploidies being missed. With CentoNIPT reported assay failure rate is as low as 0.7%. Testing can be done from **10** weeks' gestation.

Measuring Foetal Fraction is critical for high-confidence NIPS results

- ACOG emphasises the importance of foetal fraction as essential for accurate test results.
- Failure to measure foetal fraction can correlate to false-negative results.

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Counselling Considerations

Who should be offered testing for chromosomal abnormalities?

Screening (serum screening with or without NT ultrasound or cell-free DNA screening) and diagnostic testing (CVS or amniocentesis) for chromosomal abnormalities should be discussed and offered to all patients early in pregnancy regardless of maternal age or baseline risk. (ref.1)

Cell-free DNA is the most sensitive and specific screening test for the common foetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, cell-free DNA 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'

One issue with cfDNA testing as a method of screening for aneuploidy is 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 to 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 increases the risk of a low foetal fraction (ref.5)
- Compared to natural conception, pregnancies conceived with in-vitro fertilisation has a 3.8 times higher risk of having a low foetal fraction (ref.6)
- Genetic conditions, particularly Trisomy 13 or 18 are linked to having a low foetal fraction (ref.2)

- The risk of low foetal fraction increases with increased BM (ref.6)
- The risk of low foetal fraction increases with advanced maternal ages (ref.6)
- Other contributors to test failure are Black and South Asian racial origin, which by comparison with White origin, increase the risk by 2.0 and 1.7 times, respectively. (ref.6)

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 will be done at no-cost to the patient.
  - Success rate is 75 to 80% (less with high BMI).
  - Repeat screening is not advised for the following:
- Ultrasound anomalies present
- Later gestational age where further delay may complicate access to reproductive options

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

All women with a high risk / positive cell-free DNA test result should be offered a diagnostic procedure before any irreversible action is taken. (ref.7)

Positive predictive value is the probability that subjects with a positive screening test truly have the condition tested for. With NIPS testing it is the likelihood that the foetus has a particular condition if the screen returned a high-risk / positive result. For NIPS testing the positive predictive value 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*

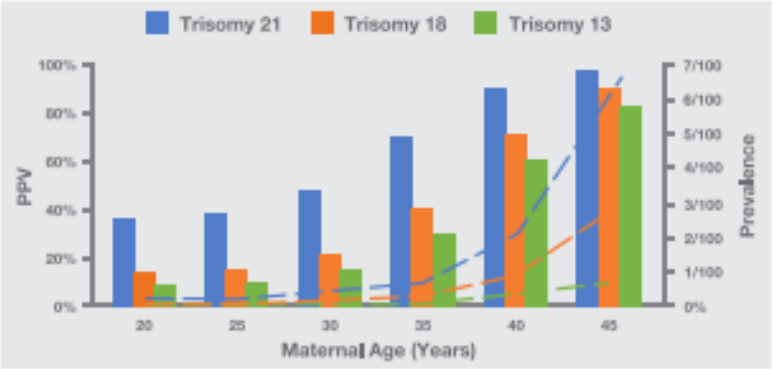

Figure 3. PPV of NIPS testing dependent on underlying prevalence of condition. (Adapted from Reference 8)

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 which cell-free DNA results could be obtained, when cell-free DNA screening for Trisomy 21 was compared with first-trimester screening (NT and serum analytes) in a general population (mean maternal age 30.7 years), cell-free DNA screening had a lower false-positive rate (0.06% cell-free 9 DNA versus 5.4% for serum screening) and a higher PPV (80.9% versus 3.4%).(ref.9)

False-positive cell-free DNA test results can occur because of confined placental mosaicism (ref.2). When a screen positive cell-free DNA result differs from the foetal karyotype, the aetiology may also include maternal (ref.2) mosaicism, or in rare instances, it can occur secondary to a maternal malignancy (ref.2) Of the reported cases, the majority of 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 test, and faecal occult blood testing followed by oncology consultation and imaging studies should be considered. (ref10)

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

In the setting of a low-risk 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 is negative. Patients with a low-risk / negative screening test result should be made aware that this substantially decreases their risk of 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 negative 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. (Ref.2)

Twin Pregnancies

No method of aneuploidy screening is as accurate in twin pregnancies as it is in singleton pregnancies. Cell-free DNA screening can be performed in twin gestations. Twin foetuses in a single pregnancy each contribute different amounts of cell-free DNA into the maternal circulation. It is possible that an aneuploid foetus would contribute less foetal DNA, therefore masking the aneuploid test result.

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. (Ref.7)

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 anatomic 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 about whether or not 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 is recommended to confirm high risk / positive cfDNA screening results.
- "No Call" results indicate a higher chance of a chromosomal disorder, and patients who receive these results should be offered further assessment

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