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Targeted approach versus genome-wide non-invasive prenatal testing

By Associate Professor Mirette Saad

Since the discovery of the fetal cell-free DNA (cfDNA) in maternal plasma, large progress has been made in the development of non-invasive prenatal screening tests.

Non-Invasive Prenatal Testing (NIPT), based on circulating free DNA, has been available in Australia since 2012. It has been broadly adopted by clinicians and patients due to its high analytical sensitivity and specificity in screening for the most common fetal autosomal aneuploidies, including Trisomy 21, 18 and 13. It can be offered to all pregnant women from 10 weeks of pregnancy onwards, in naturally conceived or in vitro fertilisation (IVF) singleton or twin pregnancies (including those with egg donors). NIPT can also be used to screen for fetal gender and, in singleton pregnancy, for sex chromosomal aneuploidies (SCAs), and, if selected, micro-deletions.

NIPT methods have capabilities and limitations along with associated challenges for diagnostic services and healthcare providers. There are currently two major cfDNA NIPT technologies: “Genome Wide (GW)” and “Targeted” detection methods.

Targeted cfDNA prenatal screening approach for the common trisomies provides the highest accuracy and sensitivity of this non-invasive screening test with a high detection rate and a very low false-positive rate (<0.1%). The test offers high analysis depth across the clinically relevant chromosome alleles within the targeted region. Focused NIPT screening approach, therefore, reduces unnecessary invasive follow-up diagnostic techniques which is the main advantage of cfDNA non-invasive screening compared to the conventional combined first trimester screening (cFTS).

On the other hand, Genome-Wide (GW) cfDNA analysis represents an enhanced screening tool for prenatal detection of chromosomal abnormalities, allowing identification of clinically relevant imbalances, that are not detectable by conventional cfDNA testing, rather than being confined to screening for the three major trisomies. The rationale for such a policy is that GW testing has the potential to identify rare autosomal trisomies (RATs) (other than 13, 18, 21 and the sex chromosomes) and rare additional fetal segmental imbalances (SIs), but, with shallow analysis depth across all chromosomes.

This article highlights some points of concern in using cfDNA GW NIPT approach for fetal aneuploidy screening.

Benefits and Limitations The basic principle of prenatal screening is to offer a safe, accessible and accurate test to all pregnant women in order to identify those women with an increased likelihood of having a baby with a chromosomal aneuploidy that can cause birth defects.

This principle seems to be applicable through targeted NIPT screening. However, so far, the benefits of GW screening for all genetic chromosomal abnormalities and imbalances do not seem to outweigh the potential harms. Therefore, clinical implementation, even in a research setting, may be questionable ethically.

Complex Counselling There are ethical and legal issues (including costs and availability) around the complexity of counselling procedures required before and after GW cfDNA NIPT regarding those rare conditions and patients’ consent for the future plan of management. For GW NIPT patients, the potential for other unanticipated findings of relevance to maternal health (including maternal genomic imbalances) should be included in pre-test counselling.

Patients undergoing a GW antenatal screening should be clearly informed of the capabilities and limitations of this test, including the possible difficult clinical decisions if positive findings of unknown significant chromosomal abnormalities were obtained.

Higher False-Positive Rates Literature shows that using GW-cfDNA analysis may fail the main goal of targeted screening method of antenatal screening. Wider, less targeted, screening results in increased false-positive findings of rare chromosomal abnormalities, resulting in an increased rate of unnecessary invasive follow-up diagnostic procedures for conditions of unknown significance.

Guidelines The HGSA/RANZCOG, along with international guidelines, recommend Down Syndrome screening in the first trimester to all pregnant women by either cFTS or cfDNA NIPT depending on local resources, patient demographics, and individual patient characteristics.

Currently, a broader GW-cfDNA NIPT approach is *not recommended by clinical guidelines* and may violate World Health Organisation (WHO) screening principles. Updated guidelines by HGSA/RANZCOG 2018, state that *“routine population-based screening for genome-wide chromosome abnormalities are not recommended due to the absence of well-performed clinical validation studies”* (HGSA/RANZCOG 2018).

This is clearly due to the uncertainty as to the clinical significance of a heterogeneous set of chromosomal abnormalities and how best to manage a positive result. Therefore, follow-up care for positive cases has not been adopted by clinical guidelines.

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Higher Failure Rates and TAT While both targeted and GW-cfDNA NIPT methods have, overall, similar sensitivity, the targeted NIPT test demonstrates a significantly lower failure (no call) rate and a shorter Turn Around Time (TAT) compared to GW testing.

Targeted NIPT is the Preferred Patients' Choice
Large cohort surveys of pregnant women showed they would prefer the use of targeted over GW NIPT methods. False-positive results are always associated with inevitable anxiety that, in some cases, leads to pregnancy termination even after a normal diagnostic result is received.

In summary, it is clear that GW testing can potentially detect some additional clinically significant unbalanced chromosome abnormalities which would otherwise be undetectable except through an invasive test or, perhaps, ultrasound abnormalities. However, the implementation of genome-wide NIPT is under debate because the benefits of detecting other fetal chromosomal aberrations must be balanced against the risks of discordant positives, parental anxiety, and a potential increase in invasive diagnostic procedures. More follow-up studies on the use of genome-wide screening using cfDNA from maternal plasma is required.

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