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Non-invasive prenatal screening of trisomy 13, 18 and 21

NIPT

The discovery of foetal DNA in maternal blood combined with a new and very-high output sequencing method (NGS - Next Generation Sequencing) has opened numerous perspectives in the field of non-invasive prenatal testing (NIPT). One of the main applications of this testing method is in the screening of the main foetal aneuploidies i.e trisomy 13, 18 and 21.

NIPT: a revolution for prenatal diagnostic testing

In France, the decree of 23 June 2009 defines the precise screening procedures for trisomy 21, including the quantification assay of serum markers during the first trimester (or second trimester) combined with a nuchal translucency measurement during the ultra sound scan. According to an article released by the Collège National des Gynécologues et Obstétriciens Français (French National College of Gynaecologists and Obstetricians) on 29 January 2013, 'the successful implementation of this screening method has led to a considerable reduction in the number of invasive samples collected (amniocentesis and trophoblast biopsies) on a national scale leading to a similar decrease in the numbers of foetal loss caused by sampling (risk estimated at 1%)'. NIPT has been implemented for trisomy 21, using a method that is based on the analysis of circulating foetal DNA in maternal blood and which thus enables the number of invasive samples needed in women at high-risk (› 1/250) to be decreased even further. We estimate that some 90 to 95% of invasive samples collected could be avoided. As such, NIPT is a reassuring tool. Yet, should the NIPT highlight an abnormality, this result should be confirmed on an invasive collected sample in order to analyse the foetal karyotype.

The creation of very high output sequencing platforms in combination with a high-performance bioinformatics tool, means that the main foetal aneuploidies can be detected with excellent performance results. (See Table 1 and Table 2). Thanks to this innovating new technology, a simple blood sample is now enough to perform genetic testing for trisomy 21, ensuing no risk for the foetus and also being highly reliable.

NIPT: screening of circulating foetal DNA in maternal blood

We now know that maternal blood contains both maternal DNA and foetal DNA, which comes from the trophoblast cells in the placenta. This DNA is released into the maternal blood as small DNA fragments (150-200 base pairs). Foetal DNA can be detected from the 7th week of pregnancy. The quantity of foetal DNA varies according to the gestational age: 2-6% at 5 weeks, then the percentage increases throughout the pregnancy. The life expectancy of the foetal DNA in the mother's blood is only a few minutes. It disappears approximately 30 minutes after labour, which eliminates any contamination by a prior pregnancy.

In practical terms, the prenatal screening for trisomy 21 can be performed from the 12th WA.

The foetal DNA fraction correlates with the development of the pregnancy, but it is independent of maternal age, ethnicity or even any medical treatments. It decreases slightly with an increase in BMI.

The foetal DNA under investigation is, in reality, a 'placental DNA'. Indeed, it is essential that a true foetal disease is distinguishable from a mosaic confined to the placenta. This therefore requires that every positive result be confirmed through a karyotype investigation performed on an invasive sample.

NIPT: Technological aspects

Currently, this screening test is performed using two approaches: a targeted approach (on chromosomes 13, 18 and 21) and a more general genome-wide approach, which is based on massive sequencing of DNA (Massively-Parallel Sequencing or MPS).

Biomnis has made the decision to collaborate with the company Illumina and use their technological know-how via the test Verifi® (Verinata). This massive DNA sequencing test is performed on a sequencer - HiSeqTM 2500 from Illumina. The principle used relies on reading a large number of target sequences for chromosomes 13, 18 and 21. These target sequences are read without any prior differentiation of the maternal fractions from the foetal fractions. Bioinformatic

capture on the chromosomes of interest and biostatistical exploitation provide aneuploidy results in the form of a standardised result relative to a genomic reference from the studied foetus.

Contrary to the targeted approach, the massive parallel DNA sequencing covers all of the genome and releases a result more quickly, with a failure rate seen at Biomnis (i.e. impossible to provide a result) of 0.4% - usually on women with a very high BMI score, which restricts the detection of the foetal fraction.

Table 1 below summarises the elements of the method in comparison to other NIPT methods; Table 2 indicates the performance scores obtained with the Verifi® used at Biomnis on a HiSeqTM 2500 Illumina sequencer.

Table 1: elements compared to other NIPT methods

	Verifi® Illumina	MaterniT21 Sequenom	Harmony Ariosa	Panorama Natera
Method	MPS*	MPS	Targeted	Targeted
Number of reads	› 19.0M	16.3M	1.1M	6.5M
Failure rate	0.07%	1.4%	4.6–4.9%	5.9–12.6%
Turn-around-time	3–6 days	7–10 days	7–10 days	7–10 days
Sample	2 tubes of the mother's blood	2 tubes of the mother's blood	2 tubes of the mother's blood	2 tubes of the mother's blood + 2 tubes of the father's blood

** MPS: Massively-Parallel Sequencing*

- *Rabinowitz, et al. ASHG Abstract 2012.; Presented data at NSGC AEC 2012*
- *Norton ME, et al. Am J Obstet Gynecol. 2012 doi:10.1016/j.ajog.2012.05.021*
- *Palomaki GE, et al. Genet Med. 2012 Mar;14(3):296-305*
- *Futch et al., Prenat Diagn 2013 Apr [Epub ahead of print]*

Table 2: data obtained from using the Verifi® test during the MELISSA trial (Maternal Blood Is Source to Accurately Diagnose Fetal Aneuploidy). Bianchi DW, Platt LD, Goldberg JD, et al. Genome-Wide Fetal Aneuploidy detection by maternal plasma DNA sequencing, Obstet Gynecol. 2012 May; 119(5): 890-901. *DNA sequencing, Obstet Gynecol.* 2012 May; 119(5): 890-901.

Performance	Sensitivity	95% CI	Specificity	95% CI
T21 (n=493)	100% (89/89)	95.9–100.0	100% (404/404)	91.1–100.0
T18 (n=496)	97.2% (35/36)	85.5–99.9	100% (460/460)	99.2–100.0
T13 (n=499)	78.6% (11/14)	49.2–99.9	100% (485/485)	99.2–100.0

In terms of result interpretation, the limits are currently well known; false positives (estimated at 0.2%) could be the result of a mosaic confined to the placenta, an anomaly in the number of maternal copies (maternal mosaic) or maternal neoplasia. False negatives (0.08%) are caused by either a very weak mosaic aneuploidies for 13, 18 and 21, structural anomalies involving small chromosomal segments (13, 18 and 21) or chromosomes other than 13,18 and 21 (0.2%) or even a triploidy (69XXX).

Conclusion

NIPT for trisomy 13, 18 and 21 is available for routine screening. A consistent high-performance has been seen in numerous studies.

This non-invasive test is now available at Biomnis. It is not reimbursed by the French National Health Service and is awaiting recommendations from the French Health Authorities.

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