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Non-invasive prenatal screening for trisomy in maternal blood (NIPT): is it fulfilling its promise?

Conception is imperfect. Many human embryos have an abnormal chromosomal constitution (aneuploidy) and are lost to early abortion. Embryos with trisomy 21 (T21) and also T18, T13 and sex chromosome aneuploidy have a high chance of ongoing pregnancy and live birth. T21 causes Down syndrome, T18 and T13 the lethal Edwards and Patau syndromes; sex chromosome aneuploidies (SCA) are associated with conditions that are generally much less severe and that may even go undetected throughout life. The risk of having an aneuploid foetus is affected by maternal age, increasingly rapidly after 32 years.

Elective prenatal testing for aneuploidy has been a feature of healthcare for decades. Invasive testing and karyotyping permits "gold-standard" diagnosis, accurate to 99% or higher. However, the risk of procedure-related pregnancy loss, generally cited as 0,5%, discourages its use in all but high-risk pregnancies. Prenatal screening is therefore used to identify high-risk pregnancies for invasive diagnosis. The first-trimester combined test (1TT) determines the risks of T21, T18 and T13 via a combination of maternal serum testing (β-HCG and PAPP-A), nuchal transparency and maternal age. 1TT is performed in the 11th–12th week of pregnancy; with a threshold of 1/380 the detection rate for T21 is 85% but the false-positive rate (FPR) is high at about 5% (specificity of 95%). Consequences of the low specificity are increased anxiety and many unnecessary invasive tests (in theory up to 4000 per year in Switzerland) with the associated risk of pregnancy loss.

What is NIPT?

The presence in the maternal circulation of trophoblast cells was first described in 1893, but attempts to use circulating cells for non-invasive prenatal diagnosis were abandoned because cells persist from one pregnancy to the next and because cell recovery is unreliable. The current revolution in NIPT – non-invasive prenatal testing by analysis of cell-free DNA (cfDNA) from maternal blood – was made possible by two advances: 1) the discovery by Dennis Lo in 1997 of cell-free "fetal" DNA (actually of placental origin) in maternal plasma during pregnancy and 2) the rapid evolution of massively multiplex technologies for characterizing and quantifying DNA. Many tests are available but all share a basic work-flow:

1. Maternal blood is sampled and cfDNA is purified from plasma.
2. cfDNA fragments (mostly maternal, plus the fetal fraction) are characterized by next-generation sequencing or by microarray, with other techniques on the horizon. Some tests target specific chromosomes whereas others analyse all chromosomes. The fetal fraction should be accurately quantified in all cases.
3. Bioinformatic analysis classifies each cfDNA molecule according to chromosome. Statistical analysis looks for significant over- or under-representation of target regions, ideally taking into account the fetal fraction.

Accuracy

The aims of NIPT are to improve the **sensitivity** to detect a higher proportion of affected foetuses, and to increase the **specificity** to reduce false-positive results, lowering anxiety and the number of invasive tests.

NIPT is an *excellent* screening test for T21 (Table). The sensitivity is close to that of karyotyping and false-negative results should be extremely rare, while the very high specificity also reduces the FPR to less than 1 per 1000 tests. To illustrate the value of such accuracy, if all 85 000 annual Swiss pregnancies were tested for T21, only 1 or 2 T21 pregnancies would be missed (15–20 with 1TT) and just 75 normal pregnancies would receive false-positive results (3400 for 1TT). NIPT is also a *good* screening test for T18 and T13 but as the Table shows, 1TT actually has a better detection rate and FPR.

The performance for **SCA** is good but there are important reserves: firstly, it is debatable whether it is appropriate to offer screening for SCA given their low clinical impact; secondly, NIPT can incidentally reveal SCA in mothers (often in mosaic); thirdly, testing for SCA reveals fetal sex, which should not be communicated before the 12th week of pregnancy (except when required diagnostically). Some tests also offer screening for **microdeletions** but there is little published data on performance. Wapner et al. reported detection of 5/6 affected pregnancies for a FPR of 1,2% with a PPV of only about 4%.

Limitations

For the gynaecologist, **positive and negative predictive values** (PPV, NPV) are more important than sensitivity and specificity. PPV represents the proportion of "positive" results that are true and is a function of the specificity of the test and the prevalence of the condition; the rarer the condition or the lower the specificity, the lower the PPV. The Table shows that even in the best case only 9/10 "positive" NIPT results are correct (in all six scenarios the negative predictive value (NPV) was ≥99,9%). These PPVs highlight two key limitations of NIPT:

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		Analytical accuracy		Diagnostic accuracy	
		DR	FPR	PPV-HR*	PPV-LR*
1TT	T21	~ 90%	4,0%	20%	2%
	T13, T18	~ 95%	+0,1%	5%	1%
NIPT	T21	≥ 99,2%	0,09%	92%	53%
	T18	≥ 96.3%	0,12%	63%	22%
	T13	≥ 91,0%	0,13%	43%	13%
	SCA	≥ 90–94%	0,23%	68%	47%

Table: **Performance of first-trimester combined testing (1TT) and NIPT, for multiple providers between 2011 and 2015** (after Gil et al. 2015, Morris & Meyer-Kleine 2014). DR = detection rate (sensitivity); FPR = false-positive rate (1-specificity); SCA = sex-chromosome aneuploidy. ***PPV** = positive predictive value, calculated according to the following prevalences: HR (high risk): T21 1/100, T18 1/500, T13 1/1000, SCA 1/200. LR (low risk): T21 1/1000, T18 1/3000, T13 1/5000, SCA 1/2000. The figures concern only successful NIPT tests; the failure rate has not been included.

1. NIPT is not diagnostic but a screening test with a PPV less than 100%; **all "positive" results must be confirmed** before considering interruption of pregnancy.
2. The prior risk of the pregnancy affects the clinical accuracy: **NIPT is significantly less accurate in low-risk situations** than in high-risk ones.

Many NIPT tests have been clinically validated only in high-risk groups and in such cases performance figures should be treated with caution.

NIPT is also susceptible to **test failure**. The rate varies from 1 to 10% (up to 17% for SCA) and depends on the test used, the fetal fraction and pre-analytical variables. Test failures are more common in aneuploid pregnancies and reduce the real-world detection rate of aneuploidy. There is a debate about whether to consider failures as "screen-positive" and this will need to be resolved before relying on NIPT as a primary screening method (Norton et al. 2016).

Some tests have been clinically validated for **twin pregnancies** but published data is limited. The test failure rate is higher and it is prudent to assume that accuracy will be lower than for singleton pregnancies. There is very little published data for triplet pregnancies or vanishing twins, and the use of NIPT is not recommended.

Implementation, present and future

NIPT is not diagnostic but has an important role to play in screening, at least for T21. In the future its value will certainly extend to other aneuploidies including microdeletions, but this is not justifiable scientifically until specificity is improved and clinical data is available.

Switzerland is the first European country to reimburse NIPT via basic health insurance, provisional to restrictions including: singleton pregnancy; from 12th week; after 1TT; risk for T21, 18 or 13 ≥1/1000 (see OPAS/KLV/OPRe Art. 13, let. b^{ter}). Conditions for reimbursement will be reevaluated in 2017. Experience tells us that many pregnant women opt for NIPT at their own expense.

There are essentially three future scenarios for clinical implementation:

1. NIPT for all pregnancies would offer early reassurance or diagnosis, but stopping 1TT would reduce the overall detection rate of fetal anomalies.
2. NIPT following 1TT (the current Swiss option) has high accuracy and reasonable cost, with the detriment of slight delay.
3. Simultaneous NIPT and 1TT would provide the most accurate and earliest testing, at a higher cost.

It will also be necessary to define the screening targets (only T21?, T18, T13, SCA, microdeletions?) on objective medical, scientific and ethical grounds and also to include "safety net" features for exceptional situations which are currently unfairly disadvantaged, for example twin pregnancies, women who did not undergo first-trimester screening, or women with particularly high-risk pregnancies who do not want to wait for their screening test.

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Le dépistage prénatal non invasif de la trisomie dans le sang maternel (NIPT): a-t-il rempli sa promesse?

Le test combiné du premier trimestre (1TT) pour le dépistage de trisomie 21, 18 et 13 est un élément clé du suivi des grossesses mais reste limité par sa sensibilité faible (85–90% pour la T21) et le taux élevé de faux positifs (4–5%). Les fournisseurs NIPT promettent une meilleure performance; est-ce confirmé dans la réalité? Pour la T21, le NIPT s'est montré extrêmement exact, avec une sensibilité >99% et moins de 1/1000 résultats faux-positifs. La performance pour T18 et T13 est bonne mais pas au même niveau que le 1TT. La VPP est un élément clé des tests de dépistage et nous montre que 1) l'exactitude du NIPT est moins bonne dans les grossesses à bas risque et 2) chaque résultat «positif» de NIPT doit être confirmé par un test diagnostique. Ces éléments essentiels doivent être clairement communiqués aux médecins et aux patientes. Enfin les échecs de NIPT restent toujours un problème, ce qui augmente le stress des patientes et diminue le taux effectif de détection d'aneuploïdie.

References

Allyse, Chandrasekharan. "Too much, too soon? Commercial provision of noninvasive prenatal screening for subchromosomal abnormalities and beyond". *Genet Med* 2015 17:958–961.

Gil et al. "Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis". *Ultrasound Obstet Gynecol* 2015;45:249–266.

Morris, Meyer-Kleine. «Wie verlässlich ist der Nachweis fetaler Trisomien aus mütterlichem Blut?» *gynäkologie + geburtshilfe* 2014; 19:22–27.

Norton et al. "Cell-free DNA vs sequential screening for the detection of fetal chromosomal abnormalities". *Am J Obstet Gynecol* 2016.

Quezada et al. "Screening for trisomies 21, 18 and 13 by cell-free DNA analysis of maternal blood at 10–11 weeks' gestation and the combined test at 11–13 weeks". *Ultrasound Obstet Gynecol* 2015;45:36–41.

Wapner et al. "Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes". *Am J Obstet Gynecol*. 2015 Mar;212(3):332.e1–9.