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Non-invasive prenatal testing - a new method in improving first trimester screening for chromosome-related abnormalities

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Abstract

In the last 15 years, major advances have been made in prenatal screening. Cohort studies have led to an understanding of the causes of many common diseases that are determined by the combined effects of genetic and phenotypic factors. Non-invasive prenatal testing (NIPT) is a technology used to isolate deoxyribonucleic acid (DNA) placental fragments from the mother's blood at as early as 10 weeks of gestation, using cell free fetal DNA technology. NIPT screens for trisomy 13,18 and 21 for sex chromosome abnormalities with a high detection rate. Cohort studies have shown a high detection rate and a low false positive rate for NIPT, but it is still considered a screening test and not diagnostic. It is recommended confirmation in case of a positive test, with a diagnostic procedure as chorionic villus sampling or amniocentesis. It is essential that NIPT be used ethically and effectively. Because of its high sensitivity (true positive rate) and its specificity (true negative rate) many recommend that NIPT should be used as a diagnostic method. However, today NIPT is used as a screening method, an attractive alternative to the serum screens and invasive test currently in use. There is a continuing decline in sequencing costs and hopefully, soon, the cost will be reduced.
Keywords: *NIPT, Down syndrome, chromosome abnormalities*

Introduction

In the last 15 years, major advances have been made in prenatal screening. Cohort studies have led to an understanding of the causes of many common diseases that are determined by the combined effects of genetic and phenotypic factors[1].

Prenatal screening for genetic conditions has become in nowadays an overwhelming process with many tests to choose from. Because of the advancing technology the process is complex, however both physician and the patient would opt for a low-cost test with increased effectiveness[2,3].

Prenatal screening for chromosome abnormalities should be available to all women during pregnancies[4,5]. An accurate diagnosis aids women in taking one of the following decisions: some may want to have this information to prepare for when the baby is born, other women may use it to guide decisions such as termination and adoption[6,7].

A trisomy can occur in any chromosome pair, but most of the fetuses are not compatible with life resulting in a miscarriage. The ones compatible with life are trisomy 18 and 13. These two are more severe than Down syndrome and they will result in most cases with a miscarriage, but some may make it to term. These babies usually won't live past 1 year of life[8,9].

As women get older, the chances for chromosome abnormalities increase[10].

An essential test in screening for chromosome-related abnormalities in the first trimester is the ultrasound examination between 11 weeks and 13 weeks and 6 days and particularly analyzing nuchal translucency. This represents an accumulation of fluid at the base of the fetus's neck. If a measurement above 3.5 mm is found, it is considered to be abnormal, and it is usually associated with a higher risk of chromosome abnormalities and structural defects. Detecting the serum levels of pregnancy-associated plasma protein A and beta-human chorionic gonadotrophin (B-HCG) is also important. With these information and other factors such as maternal age, diabetes and ethnicity, a risk assessment for the pregnancy will be calculated. The test is performed for Down syndrome and trisomy 18. The detection rate for Down syndrome is 83% with a false positive of 5%, and for trisomy 18 is 80% with a false positivetly below 1%. The test will be marked as positive for Down syndrome if the risk is greater than 1 in 270. A positive result does not mean the fetus has a chromosome abnormality, it means that the risk is increased[11,12].

In the second trimester besides trisomy 18 and 21 screening for neural tube defects is also recommended.

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During 14 and 22 weeks of gestations by determining the serum levels of 4 proteins (alfa-fetal protein (AFP), B-HCG, unconjugated estriol and dimeric inhibin A) a risk for chromosome related-abnormalities and neural tube defects can be established. The detection rates for trisomy 18 and 21 are the same as in the first trimester. The detection rate for open neural tube defects is calculated by measurement of the AFP, and consists in a rate of 80%[13].

Screening Methods

Multiple screening methods that combine the tests briefly described above have been proposed:

- Full integrated screening: it combines both 1st and 2nd trimester screening tests to increase the overall detection rate. The downside is that the diagnosis is established late in the second trimester.
- Serum integrated screening: it involves just the blood samples analysis from the 1st and 2nd trimester without the ultrasonography.
- Sequential screening: this is basically the same with the full integrated screening but the patient will receive preliminary results after the first trimester screening.
- Triple screening: analyzing only 3 instead of 4 proteins from the 2nd trimester test. The detection rate is slightly decreased.
- AFP only: calculated in the 2nd trimester, it will screen only for neural tube defects.
- 2nd trimester targeted ultrasound: ultrasound performed between 18 and 29 weeks of gestation. Usualy a normal second trimester ultrasound certainly decreases the risk that the fetus has a chromosome abnormality[14,15].

Non-invasive prenatal testing (NIPT) is a technology used to isolate deoxyribonucleic acid (DNA) placental fragments from the mother's blood at as early as 10 weeks of gestation, using cell free fetal DNA technology. The first NIPT was introduced to clinical practice in Honk Kong in 2011. NIPT screens for trisomy 13,18 and 21, for sex chromosome abnormalities with a high detection rate.

Cohort studies have shown a high detection rate and a low false positive rate for NIPT, but it is still considered a screening test and not diagnostic.

It is still recommended confirmation in case of a positive test, with a diagnostic procedure as chorionic villus sampling (CVS) or amniocentesis. Even a negative test cannot rule out chromosome abnormality. Placental mosaicism can explain a false positive test, because NIPT tests placental DNA and not fetal DNA. In a low percentage of cases, below 3%, the genetics of the placenta can be different from the ones of the fetus wich can lead to false positive or false negative results[16,17].

Non-invasive prenatal diagnosis of aneuploidy has been a challenging problem because fetal DNA consitutes a small percatage of total DNA in maternal blood, and intact fetal cells are even rarer[18,19]. Successful development of a truly universal, polymorhism-independent noninvasive test for fetal aneuploidy is near. By direct sequency of maternal plasma DNA, as one study shows, fetal trisomy 21 as early as 14th weeksof gestation can be detected[20,21]. The same study atests that fetal cell-free DNA clears from the blood to undetectable levels within a few hours after delivery and therefore is not carried forward from one pregnancy to the next[22,23,24].

Sex chromosomal aneuploidies (SCA) include full-blown and mosaic numerical abnormalities leading to syndromes interfering with normal sexual development. These include Turner syndrome (45,X) and sex-chromosomal trisomies, such as Klinefelter (XXY) and triple X-syndrome (XXX)[25]. There are many individuals with sex chromosomal aneuploidies that remain undiagnosed. There is a small amount of data regarding NIPT and SCA. However actual data indicates that NIPT has a lower accuracy in detecting SCA than for trisomies 21 and 18[26,27,28].

The possible sources of error of NIPT test (HarmonyTM, PanoramaTM) are represented by: early gestational age, maternal obesity, multiple pregnancies, placental mosaicism and maternal conditions such as chromosomes abnormalities or malignant disease.

The amount of cffDNA in maternal blood increases with gestational age and if samples are taken too early in pregnancy, false-negative results are likely. Maternal obesity is associated with lower fetal DNA percentage, the reason is unclear but it is believed that the high adipose turnover increasing maternal DNA to be responsible by a dilution effect[29].

If a twin pregnancy is monochorionic both fetuses may have chromosomal abnormalities. The amount of cffDNA is almost double that of a singleton pregnancy, so the cffDNA aneuploidy testing will not only be possible but probably more effective than in singletons. If the twins are dichorionic the maternal plasma DNA testing would not be as straightforward[29,30].

Porreco et al. published a prospective study that demonstrates that noninvasive prenatal analysis of cell-free deoxyribonucleic acid from maternal plasma is an accurate advanced screening test with extremely high sensitivity and specificity for trisomy 21 (>99%) but with less sensitivity for trisomies 18 and 13. Despite high sensitivity, they observed that the test has modest positive predictive values for the small number of common sex chromosome aneuploidies because of their very low prevalence rate.

This study provides the largest prospectively number of collected samples concurrently processed and analyzed by this sequencing technology to date (Table 1). It demonstrates that noninvasive prenatal analysis of cfDNA from maternal plasma is an accurate advanced screening test with extremely high sensitivity and specificity for trisomy 21 (>99%) but with somewhat less sensitivity (although high specificity) for trisomies 18 and 13[31].

Other authors describes an ethical issue related with NIPT regarding sex selection for non-medical reasons.

Table 1 Harmony™ and Panorama™ - NIPT comparison[33]

Harmony and Panorama test comparison		Harmony test	Panorama test
Down Syndrome (trisomy 21)	Sensitivity	>99%	>99%
	False positive rate	0.1%	0%
Edward syndrome (trisomy 18)	Sensitivity	98%	>99%
	False positive rate	0.1%	<0.1%
Patau's syndrome (trisomy 13)	Sensitivity	80%	>99%
	False positive rate	0.05%	0%
Turner's syndrome (monosomy X) (optional)	Sensitivity	96.7%	91.7%
	False positive rate	Unreported	<0.1%
Triploidy (optional)	Sensitivity	Uneable to detect	>99%
Gender (optional)	Sensitivity	>99%	>99%
	False positive rate	Unreported	0%
Redraw Rate	Sensitivity	3-5%	6%
Results available		10-14 working days	10-14 working days
Fetal fraction reported		Yes	Yes
Available for twins		Yes	No
Available for donor eggs		Yes	No
Available from		10 weeks gestation	9 weekds gestation

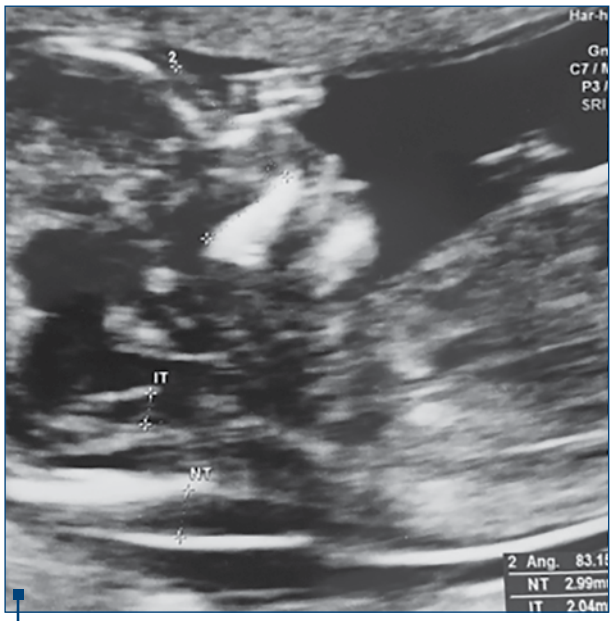


Figure 1. Ultrasound 12W- Nuchal translucence 2.99mm

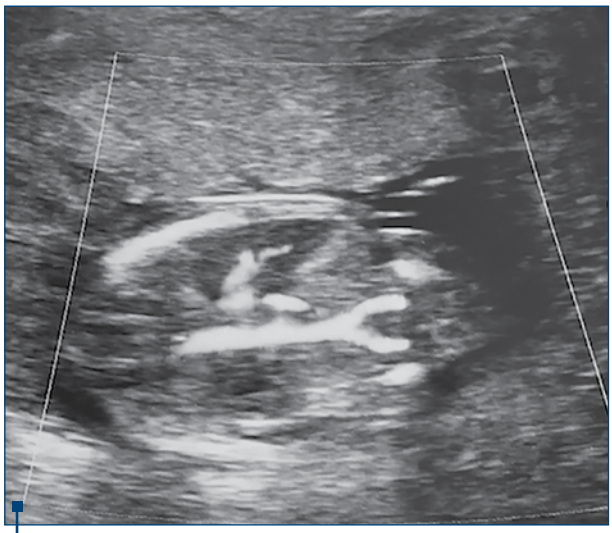
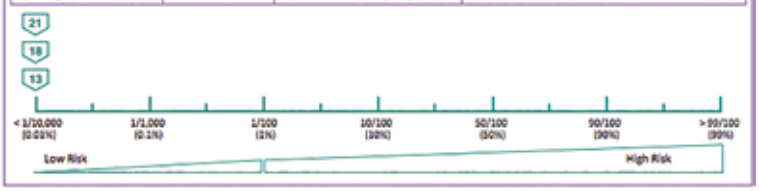


Figure 2. Ultrasound-Bilateral renal agenesis

Figure 3. NIPT - Harmony™ test result of the patient diagnosed with bilateral renal agenesis

Test Results — **Fetal cfDNA Percentage: 10%**

CHROMOSOME	RESULT	PROBABILITY	RECOMMENDATION
Trisomy 21 (T21)	Low Risk	Less than 1/10,000 (0.01%)	Review results with patient
Trisomy 18 (T18)	Low Risk	Less than 1/10,000 (0.01%)	Review results with patient
Trisomy 13 (T13)	Low Risk	Less than 1/10,000 (0.01%)	Review results with patient
Fetal Sex	Female Fetus	Greater than 99/100 (99%)	Review results with patient
X,Y Analysis	XX	Greater than 99/100 (99%)	Review results with patient



NIPT lead to information about fetal sex and there is a concern that some pregnant women and their partners may use this to have an abortion if the sex of the fetus does not match their preference.

That is also a cultural and social factor involved; in some Asian countries selection for males has led to marked disturbance of the sex ration with serious social effects[31,32,33].

At 12 weeks of pregnancy ultrasound, of a 38 years old primigravida, we detected a nuchal translucence of 2.99 mm and bilateral renal agenesis (Figures 1 and 2). A NIPT was performed (HARMONY™) in order to detect chromosomal abnormalities. NIPT showed a female fetus with low risk for chromosomal abnormalities. We decided to terminate the pregnancy because of renal agenesis (Figure 3).

Conclusions

It is essential that NIPT is used ethically and effectively. Because of its high sensitivity (true positive rate) and its specificity (true negative rate) many recommend that NIPT should be used as a diagnostic method.

However, today NIPT is used as a screening method, an attractive alternative to the serum screens and invasive test currently in use.

Because of areas where sex-based abortions are prevalent, particular attention to returning fetal sex information should be given.

Currently, NIPT is too expensive for the majority of people, and health care systems are not offering NIPT as free screening. There is a continuing decline in sequencing costs and hopefully, soon, the cost will be reduced. ■

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