

# Noninvasive prenatal testing of aneuploidies: where are we now?

*Testes não invasivos para aneuploidias no pré-natal: onde estamos agora?*

## Editorial

### Introduction

Prenatal diagnosis of chromosomal aneuploidies is the most frequent prenatal test offered to pregnant women. In most cases, they are recommended in the following circumstances: maternal age of 35 years or above; positive first- or second-trimester screening test results, and increased risk of fetal aneuploidies due to family history. During the first trimester, screening tests include: nuchal translucency (NT) combined with maternal age; levels of maternal serum pregnancy associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotropin ( $\beta$ -hCG) combined with maternal age; combination of NT measurement, the first trimester maternal serum analytes (PAPP-A, and free  $\beta$ -hCG or total hCG) and maternal age, referred to as combined first trimester screening. The NT measurement is valid when crown-rump length (CRL) is 45–84 mm, corresponding to 11–13+6 week of gestation, while PAPP-A and free  $\beta$ -hCG may be measured between 9–13+6 week of gestation<sup>1</sup>.

More recently, another option, which is the detection of an increased amount of chromosomal material in maternal blood, became available to screen for chromosome aneuploidy. This is called Non-invasive Prenatal Testing (NIPT). Recently, different tests are available, depending on the employed methodologies and algorithms for data analysis. These may involve massively parallel sequencing (MPS), targeted sequencing of specific chromosomal segments, or directed sequence analysis of single nucleotide polymorphisms (SNPs)<sup>2</sup>.

While all these testing methods have limitations, healthcare providers need to be aware of them in order to give their patients reliable information and genetic counseling. In this paper, we focused on NIPT because it is the most promising screening option.

Among the above-mentioned tests, combined first trimester screening has been demonstrated to have higher detection rates for Down Syndrome (78–91%) and trisomy 18 (91–96%) compared to NT only or serum analytes methods<sup>3–5</sup>. Since pregnancies affected with trisomy 13 have PAPP-A, free  $\beta$ -hCG, and NT patterns similar to trisomy 18, this screening is also used to screen for trisomy 13<sup>6</sup>.

#### Correspondence

Aleksandra Jezela-Stanek  
Department of Medical Genetics,  
The Children's Memorial Health Institute  
Aleja Dzieci Polskich 20, 04-736  
Warsaw, Poland

#### Received

07/27/2014

#### Accepted with modifications

08/22/2014

DOI: 10.1590/S0100-720320140005099

<sup>1</sup>Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland.  
Conflict of interests: none.

Obviously, the great advantage of screening options for chromosome aneuploidy is that they are non-invasive. Hence, they are recommended for all pregnancies and usually precede a decision about whether or not to undergo invasive diagnostic testing. On the other hand, screenings have some limitations. The main one is that they do not provide a definitive diagnosis. Furthermore, they have lower detection rates in multiple pregnancies; variability in the detections rates of trisomy 21, 18, 13, while no information on fetal monosomy X, as well as false-positive results that, in most laboratories, are higher than 5%<sup>7</sup>.

## Results

The newest and recently introduced prenatal screening method is NIPT, which uses circulating cell-free fetal DNA (cffDNA) in maternal plasma to estimate risk for Down (trisomy 21), Edwards (trisomy 18), and Patau Syndrome (trisomy 13). cffDNA in the plasma of pregnant women was discovered by Lo et al.<sup>8</sup>. Later, in 2008, MPS of the maternal plasma was used to detect material from fetus with trisomy 21<sup>9,10</sup>. During the following years, the same technique also detected fetal trisomy 18 and 13<sup>11-13</sup>, as well as monosomy X<sup>14</sup> in high-risk pregnancies. The results obtained in the mentioned studies can be seen in Table 1.

In 2012, preliminary results were presented and concluded that cffDNA-based tests may have similar sensitivity and specificity in an average risk population<sup>15</sup>. The study of Nicolaides et al.<sup>1</sup> was conducted in 2,049 pregnant women undergoing routine screening for aneuploidies at 11w0d – 13w6d weeks' gestation. Trisomy risk scores were given for 95.1% (1,949 of 2,049) of the cases,

including all eight with trisomy 21 and two among the three with trisomy 18. The trisomy risk score was >99% in the eight cases of trisomy 21 and two of trisomy 18 and <1% in 99.9% (1,937 of 1,939) of the euploid cases. Results of the study presented by Norton et al.<sup>15</sup> showed: for trisomy 21, a sensitivity of 100% (95.5–100%) and a false-positive rate of 0.03% (95%CI 0.002–0.20); for trisomy 18, a sensitivity of 97.4% (86.5–99.9%) and a false-positive rate of 0.07% (95%CI 0.02–0.25).

Later, Sparks et al.<sup>16</sup> evaluated a novel biochemical assay and algorithm for the prenatal evaluation of risk for fetal trisomy 21 and 18 in a blinded analysis with 167 pregnant women. They performed a digital analysis of the selected regions (DANSR), in combination with a novel algorithm, fetal-fraction optimized risk of trisomy evaluation (FORTE). It allows correctly identifying all aneuploid cases (36 trisomies 21 and 8 trisomies 18)<sup>16</sup>.

Moreover, the investigators assayed cell-free DNA from a training set and a blinded validation set of pregnant women: 250 euploidies, 72 trisomies 21, and 16 trisomies 18. All 167 cases in the blinded validation and 163/171 in the training set passed through the quality control criteria. FORTE produced an individualized trisomy risk score for each subject, which correctly discriminated all T21 and T18 cases from the disomic ones. The authors concluded that DANSR and FORTE enable accurate non-invasive fetal aneuploidy detection in a high-risk population, and stated that larger studies including low- and average-risk pregnancies are needed.

Recently, in 2013, studies evaluating the performance of the Harmony Prenatal Test and Panorama Prenatal Test were reported<sup>17</sup>. Ashoor et al.<sup>17</sup> assessed the performance of the Harmony Prenatal Test for the detection of trisomy

**Table 1.** Results from the last published clinical trials that measured the sensitivity and specificity of Noninvasive Prenatal Testing in the diagnostics of common aneuploidies

	Palomaki et al. <sup>11</sup>	Palomaki et al. <sup>12</sup>	Bianchi et al. <sup>14</sup>	Ashoor et al. <sup>17</sup>
<b>Trisomy 21</b>				
Sensitivity	98.6% (95.9–99.7)		100% (95.9–100)	
Specificity	99.8% (99.4–99.9)	na	100% (99.1–100)	na
<b>Trisomy 18</b>				
Sensitivity	na	100% (93.9–100)	97.2% (85.5–99.9)	na
Specificity		99.7% (99.3–99.9)	100% (99.2–100)	
<b>Trisomy 13</b>				
Sensitivity	na	91.7% (61.0–99.0)	78.6% (49.2–99.9)	80% (49.0–94.3)
Specificity		99.1% (98.5–99.5)	100% (99.2–100)	99.9% (99.7–99.9)
<b>Monosomy X</b>				
Sensitivity	na	na	93.8% (69.8–99.8)	na
Specificity			99.8% (98.7–99.9)	

na: not analyzed.

13 in a two-phase, blinded, case-control study. In the second phase, after modification of trisomy 13 algorithm based on data from the first phase, the test was used to detect trisomy 13 risk scores for 10 cases of trisomy 13 and 1,939 euploid cases. The trisomy 13 risk scores were >99% in eight (80.0%) cases of trisomy 13. In the 1,939 euploid cases, the risk score for trisomy 13 was <0.01% in 1,937 (99.9%), 0.79% in one and >99.0% in one.

The Panorama Prenatal Test was validated by Nicolaides et al.<sup>18</sup> in a population of 242 women with singleton pregnancies, who had been submitted to chorionic villus sampling (CVS) from 11 to 13 weeks. They were referred because first-trimester screening indicated an increased risk for trisomy 21, 18 or 13<sup>18</sup>. Results were provided for 94.6% (229 cases): 32 cases were correctly identified as aneuploid, including trisomy 21 (n=25; sensitivity=100%, specificity=100%), trisomy 18 (n=3), trisomy 13 (n=1), Turner Syndrome (n=2) and triploidy (n=1), with no false-positive or -negative results. In all these studies (apart of Fairbrother et al.<sup>19</sup>), NIPT was integrated as a primary screening test for pregnant women at high-risk of aneuploidy.

## Discussion and conclusions

Testing can be done after the tenth week and typically it is performed between 10 to 22 weeks. Interestingly, as concluded by Norton et al.<sup>20</sup> from a population perspective, a better option for NIPT may be a second-tier test for those patients who screen positive by conventional aneuploidy screening<sup>20</sup>. However, before routine MPS-based population screening for fetal trisomy 21 are widely introduced, additional trials are needed. According to the International Society for Prenatal Diagnosis (ISPD), they should include i. a.: efficacy in low-risk populations or suitable for the diverse sub-populations, such as twins and IVF donor pregnancies<sup>21,22</sup>.

Unfortunately, data regarding the clinical validity of NIPT are still limited. These are available only for studies examining the early clinical experience of the Harmony and Verifi Prenatal Tests. The limitations are associated with the lack of follow-up information for the majority of studies on pregnancies. For example, in the investigation by Fairbrother et al.<sup>19</sup> with 284 obstetrical patients who were evaluated by both the Harmony Prenatal Test and traditional first-trimester screening, only one woman, who had a first-trimester screening result of one in five for trisomy 21, elected to have invasive prenatal diagnosis, which revealed a normal fetal karyotype<sup>19</sup>. Another study, presented by Futch et al.<sup>23</sup>, involved 6,123 patients tested with the Verifi Prenatal Test. Of 280 fetuses with aneuploidy detected by the NIPT, 94 (33.6%) were confirmed or the pregnancies resulted in miscarriage, and 14

(0.2%) yielded discordant (likely false-positive) results. Unclassifiable results were obtained in up to 1% of cases for each of the analyzed chromosomes. As a result, it was assumed that the pregnancies that had not yet delivered did not have an undiagnosed aneuploidy (missed by both NIPT and first-trimester screening).

At this time, NIPT is only recommended for patients from high-risk populations, including advanced maternal age, positive screening test, abnormal ultrasound suggestive of aneuploidy, or prior pregnancy with chromosome aneuploidy<sup>21,22,24</sup>. It is also recommended that a positive NIPT conclusion, due to occasional false-positive results, be followed by confirmatory diagnostic testing (chorionic villus sampling, CVS or amniocentesis) prior to making pregnancy decisions<sup>21,22,24</sup>. The invasive tests, apart from giving an accurate diagnosis, also provide important information about the cause and type of trisomy. When Down Syndrome is due to a 21 chromosome translocation, this has important recurrence risk implications for the parents and other family members.

Other risks concerning the NIPT are “unreportable”. From 0.5 to 7% of women who undergo NIPT will not get a result<sup>25</sup> (fact sheet published in 2012 by the National Coalition for Health Professional Education in Genetics and NSGC). This often happens due to the low amount of fetal DNA in the sample (high maternal weight or early gestational age). Moreover, some laboratories may decline to report results that are near the cutoff.

As one can see, there are still some questions regarding the introduction of NIPT into routine practice. In 2014, in the United Kingdom, this resulted in initiating a national project called “Evaluation of NIPT for aneuploidy in an NHS setting: a reliable accurate prenatal non-invasive diagnosis (RAPID) protocol”<sup>26</sup>. The collaborators expect that this study may be a significant contribution for policing decisions around the implementation of NIPT for aneuploidies and allow developing the laboratory standards for testing and reporting, education materials, and counselling strategies.

Non-invasive tests based on the presence of cfDNA in maternal plasma carry a promise for the future and will probably replace other screening methods as the standard of care. However, there is still much to learn about such technology and its clinical utility. Pretest genetic counseling should be given and must clearly state that cfDNA test, despite having the highest sensitivity and specificity among screening methods, is not a diagnostic test and does not detect all cases of fetal Down Syndrome. It only screens for the most common fetal trisomies (21, 18 and 13) and may give information on the sex chromosomes (X, Y – i.a. monosomy X). As for today, NIPT should not be proposed to low-risk pregnancies or multiple gestations because there is not enough evaluation in these groups.

## References

- Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. *Prenat Diagn.* 2011;31(1):7-15.
- Hayes, Inc. GTE report. Noninvasive prenatal testing (NIPT) for fetal aneuploidy. Lansdale: Hayes, Inc; 2013.
- Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, et al. First-trimester or second-trimester screening, or both, for Down's syndrome. *N Engl J Med.* 2005;353(19):2001-11.
- Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *J Med Screen.* 2003;10(2):56-104.
- Wapner R, Thom E, Simpson JL, Pergament E, Silver R, Filkins K, et al. First-trimester screening for trisomies 21 and 18. *N Engl J Med.* 2003;349(15):1405-13.
- Spencer K, Ong C, Skentou H, Liao AW, Nicolaides KH. Screening for trisomy 13 by fetal nuchal translucency and maternal serum free beta-hCG and PAPP-A at 10-14 weeks of gestation. *Prenat Diagn.* 2000;20(5):411-6.
- Wilson KL, Czerwinski JL, Hoskovec JM, Noblin SJ, Sullivan CM, Harbison A, et al. NSGC practice guideline: prenatal screening and diagnostic testing options for chromosome aneuploidy. *J Genet Couns.* 2013;22(1):4-15.
- Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. *Lancet.* 1997;350(9076):485-7.
- Chiu RW, Chan KC, Gao Y, Lau VY, Zheng W, Leung TY, et al. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. *Proc Natl Acad Sci USA.* 2008;105(51):20458-63.
- Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. *Proc Natl Acad Sci USA.* 2008;105(42):16266-71.
- Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med.* 2011;13(11):913-20.
- Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, et al. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genet Med.* 2012;14(3):296-305.
- Ashoor G, Syngelaki A, Wagner M, Birdir C, Nicolaides KH. Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. *Am J Obstet Gynecol.* 2012;206(4):322.e1-5.
- Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol.* 2012;119(5):890-901.
- Norton ME, Brar H, Weiss J, Karimi A, Laurent LC, Caughey AB, et al. Non-Invasive Chromosomal Evaluation (NICE) study: results of a multicenter, prospective, Cohort study for detection of fetal trisomy 21 and trisomy 18. *Am J Obstet Gynecol.* 2012;207(2):137.e1-8.
- Sparks AB, Struble CA, Wang ET, Song K, Oliphant A. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. *Am J Obstet Gynecol.* 2012;206(4):319.e1-9.
- Ashoor G, Syngelaki A, Wang E, Struble C, Oliphant A, Song K, et al. Trisomy 13 detection in the first trimester of pregnancy using a chromosome-selective cell-free DNA analysis method. *Ultrasound Obstet Gynecol.* 2013;41(1):21-5.
- Nicolaides KH, Syngelaki A, Gil M, Atanasova V, Markova D. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. *Prenat Diagn.* 2013;33(6):575-9.
- Fairbrother G, Johnson S, Musci TJ, Song K. Clinical experience of noninvasive prenatal testing with cell-free DNA for fetal trisomies 21, 18, and 13, in a general screening population. *Prenat Diagn.* 2013;33(6):580-3.
- Norton ME, Rose NC, Benn P. Noninvasive prenatal testing for fetal aneuploidy: clinical assessment and a plea for restraint. *Obstet Gynecol.* 2013;121(4):847-50.
- Benn P, Borrell A, Crossley J, Cuckle H, Dugoff L, Gross S, et al. Aneuploidy screening: a position statement from a committee on behalf of the Board of the International Society for Prenatal Diagnosis, January 2011. *Prenat Diagn.* 2011;31(6):519-22.
- Benn P, Borrell A, Cuckle H, Dugoff L, Gross S, Johnson JA, et al. Prenatal detection of Down syndrome using Massively Parallel Sequencing (MPS): a rapid response statement from a committee on behalf of the Board of the International Society for Prenatal Diagnosis, 24 October 2011. *Prenat Diagn.* 2012;32(1):1-2.
- Futch T, Spinosa J, Bhatt S, de Feo E, Rava RP, Sehnert AJ. Initial clinical laboratory experience in noninvasive prenatal testing for fetal aneuploidy from maternal plasma DNA samples. *Prenat Diagn.* 2013;33(6):569-74.
- Devers PL, Cronister A, Ormond KE, Facio F, Brasington CK, Flodman P. Noninvasive prenatal testing/noninvasive prenatal diagnosis: the position of the National Society of Genetic Counselors. *J Genet Couns.* 2013;22(3):291-5.
- National Coalition for Health Professional Education in Genetics [Internet]. Non-Invasive Prenatal Testing (NIPT) FactSheet. 2012 [cited 2014 Apr 10]. Available from: <[http://www.nchpeg.org/index.php?option=com\\_content&view=article&id=384&Itemid=255](http://www.nchpeg.org/index.php?option=com_content&view=article&id=384&Itemid=255)>
- Hill M, Wright D, Daley R, Lewis C, McKay F, Mason S, et al. Evaluation of non-invasive prenatal testing (NIPT) for aneuploidy in an NHS setting: a reliable accurate prenatal non-invasive diagnosis (RAPID) protocol. *BMC Pregnancy Childbirth.* 2014;14:229.