Aktuální gynekologie a porodnictví

AGP

www.actualgyn.com

LETTER TO THE EDITOR

Non invasive prenatal testing of the most frequent chromosomal aneuploidies – from the theory to the practice

Neinvazivní prenatální diagnostika nejčastějších chromozomálních aneuploidií – od teorie k praxi

Jaroslav Loucký, Michal Zemánek
IMALAB s.r.o., Zlín

Correspondence: RNDr. Jaroslav Loucký, IMALAB s.r.o., U Lomu 638, 760 01 Zlín, tel.: +420 602 303 098, e-mail: loucky@imalab.cz

Published: 5. 11. 2012 Received: 4. 10. 2012 Accepted: 30. 10. 2012
Actual Gyn 2012, 4, 99-100 ISSN 1803-9588

Free fulltext article at www.actualgyn.com

Cite as: Loucky J, Zemanek M. Non invasive prenatal testing of the most frequent chromosomal aneuploidies – from the theory to the practice. Actual Gyn. 2012; 4:99-100

Dear Editors,
over the course of the years 2008 to 2011, we participated in the international clinical validation study InFANet, whose task was to verify the possibility of implementing new, non-invasive prenatal procedures, based on the detection of free fetal DNA in the maternal plasma.

Thanks to this participation and studies of the available literature, we discovered that the progressively-developing field of Molecular Biology has significant potential for the future. Contemporary prenatal screening possibilities, thanks to the combination of biochemical testing of the mother with the ultrasound examination of the fetus, enables a greater than 90% chance of capturing of the most frequent forms of chromosomal aberrations. Unfortunately, for current screening methods, a relatively large number of pregnant women are classified as positive after undergoing such the screening, and – as a result, they undergo one of the invasive procedures with the aim of acquiring fetal cells and the subsequent performance of their genetic analysis. These invasive methods carry with themselves a slight, but objective risk of loss of the fetus, and otherwise, these are often a very unpleasant subjective experience for many pregnant women.

In 1997, an article by Dennis Lo (1) was published, which described the occurrence of free fetal DNA in the mother's blood circulation (cffDNA) for the first time. Thanks to this discovery, great efforts were made over the ensuing years by many scientific teams in order to exploit this fact for the direct detection of the genetic information of the fetus by means of a simple-to-do phlebotomy of the maternal blood. Free fetal DNA fragments can be detected in the maternal blood, roughly from the fourth week of pregnancy. Their representation in ratio to the free maternal DNA is, on average, about 10% (2). An important characteristic, from the practical usage point-of-view, is the fact that the period of their persistence in the maternal circulation is about 24 hours. Fetal cells also occur in the mother's circulation, but – unlike the free DNA, they are represented in a much smaller measure relative to the maternal cells and, in addition, it is possible to identify them in the mother's blood up to 27 years after giving birth to the fetus (3). This fact however limits to use fetal cells in non-invasive prenatal diagnostics, since we are unable to differentiate from which pregnancy they came from.

Since 2008, our medical genetics laboratory – Imalab s.r.o., together with the Prediko s.r.o. Centre for Prenatal Testing have engaged in research in the field of exploiting free fetal nucleic acids in prenatal diagnostics. This study was originally designated as an RNA Study. Later, after changes of the evaluation methodology, it was renamed as the InFANet Study. The research was led by Doctors Jacob Canick and Glenn Palomaki of the Laboratories of Medical Screening and Specialised Testing, of the Department of Patology and Laboratory Medicine in the Women & Infants Hospital and The Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA and 27 centres from all around the world participated in it. In the context of the research, pregnant women under-

going on the basis of a high risk of aneuploidies in the fetus procedures (CVS or AMC), were offered the chance to participate in this study.

On the basis of the informed consent of the patients, 3x10 ml of peripheral blood was taken into a tube containing EDTA, then a two-stage separation of plasma was carried out in the laboratory. The first centrifugation was performed at low speed (10 minutes at the speed of 2.500 x g at 4 °C), followed by „purification" of plasma with a centrifuge at a high speed (10 minutes at 15.500 x g at 4 °C). Aliquots of such adjusted plasma were deeply frozen (-80 °C) ready to be shipped to the Women & Infants Hospital in Providence. Overall, the study examined 4,664 pregnant women who have an increased risk of the presence of some of the observed developmental defects. Our laboratory contributed 255 samples. Detailed evaluation of the study is described in the publication – by Palomaki et al. (4). At the end of 2011 the results were used when the first commercial test for Down syndrome (MaterniT21™) by a U.S. company called Sequenom (http://www.sequenom.com/home), based on the detection of free fetal DNA, was launched.

In February 2012, the test was supplemented by the detection of trisomies of chromosome 13 and 18 (5, MaterniT21™ PLUS). These tests became the first contributions to a non-invasive prenatal testing (NIPT) of the most common chromosomal aberrations. The principle of this test comes from the fact that each fragment of free DNA can be assigned to a specific chromosome. If we focus for example on chromosome 21 and find a higher rate of fragments corresponding to this chromosome, we can conclude that this is a pregnancy with a trisomy of chromosome 21.The evaluation process is quite time consuming and financially demanding and in this respect, it can not be compared to traditional methods, which are used for present types of screening. The SCMM Laboratory, the test provider, indicates that the sensitivity of the test is 99.1% (T21), 99.9% (T18) or 91.7% (T13). Specificity of determination is then 99.9% (T21), 99.6% (T18) or 99.7% (T13) (5). This is the only test that can identify aneuploidies in multiple (gemini) pregnancies (6). In 2011, further research were published which showed that the presence of fragments of free fetal nucleic acids in maternal blood circulation can be used for detection of the most common chromosomal aberrations. In March 2012 the Senhert et al. (7) and Bianchi et al. (8) validation studies led to the release of the Verify Test™ Prenatal test of the US Verinata Health (http://www.verinata.com/). In June 2012 the Harmony™ Prenatal test of Ariosa diagnostics (http://www.ariosadx.com/) based on the Sparks et al. (9) and Norton et al. (10) validation studies was launched. The results of this work showed that using of massive parallel sequencing can detect nearly 100% of the searched chromosomal aberrations, while the number of samples which were impossible to be evaluated (very low level of free fetal DNA) was negligible. On the other hand, it should be noted that the work involved testing of a high-risk population but for general use a comparative study of a low-risk population must be carried out. It is also necessary to mention that there is not a definite consensus on an involvement of the test in fetal aneuploidy screening program. We are waiting for the evaluation of professional bodies.

References

1. Lo YM, et al. Presence of fetal DNA in maternal plasma and serum. Lancet. 1997;350(9076):485-487
2. Ehrich M, Deciu C, Zwiefelhofer T, et al. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. Am J Obstet Gynecol. 2011;204:205.e1-11
3. Wright C. Cell-free fetal nucleic acids for non-invasive prenatal diagnosis. Report of the UK Expert Working Group. CHG Foundation. Jan 2009
4. Palomaki GE, et al. DNA sequencing of maternal plasma to detect Down syndrome: An international clinical validation study. Genetics in Medicine. 2011;13(11):913-920
5. Palomaki GE, et al. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. Genetics in Medicine. 2012;14(3):296-305
6. Canick JA, et al. DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. Prenatal Diagnosis. 2012;32:730-734
7. Senhert AJ, et al. Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. Clinical Chemistry. 2011;57(7):1042-1049
8. Bianchi DW, et al. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstetrics & Gynecology. 2012;119(5):890-901
9. Sparks AB, et al. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. AM J Obstetrics & Gynecology. 2012;206(4):319.e1-9
10. Norton ME, 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 Obstetrics & Gynecology. 2012;207(2):137.e1-8