

Noninvasive Prenatal Diagnosis from Maternal Blood: Finally Available after 20 Years of Research

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ABSTRACT

Since all prenatal invasive procedures, such as amniocentesis and chorionic villus sampling carry a small risk for the pregnant woman and a risk to induce the loss of a pregnancy of up to 1%, there have been efforts now for at least a quarter of a century to develop a noninvasive method from the blood of pregnant women. First there was a considerable effort to isolate fetal cells from maternal circulation, and these techniques were carefully evaluated in a NIH-sponsored study of a few US American centers and ours in Basel/Switzerland. It turned out; however, that interphase fluorescence to identify fetal aneuploidies from these isolated cells was not reliable enough for clinical use. The breakthrough came with the recognition of the group by D Lo et al; who showed for the first time that cell-free fetal DNA in maternal plasma and serum can be used reliably for prenatal diagnosis. One of the first successful applications was the detection of the fetal Rhesus factor around 11 weeks of gestation in pregnancies of Rhesus-negative mothers. The Sequenom Company in San Diego, USA, which acquired the patent of D Lo et al on the use of cell free DNA and ours on size separation of fetal vs maternal DNA subsequently showed in large series that the noninvasive prenatal diagnosis of fetal trisomy 21 from maternal blood by massive parallel sequencing has an accuracy around 99%, and currently up to 100,000 cases have been investigated already in different laboratories. Also the noninvasive prenatal diagnosis of trisomies 18 and 13 is possible, and an increasing amount of single gene anomalies will be diagnosable in the future noninvasively. The whole development of noninvasive prenatal diagnosis is apposite example that long-term research pays-off to bring a concept from the first steps finally into clinical use.

Keyword: Noninvasive prenatal diagnosis, Cell-free fetal DNA, Chromosomal anomalies, Single gene disorders.

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INTRODUCTION

Ever since amniocentesis was introduced in the 70s and chorionic villus biopsy in the 80s there was the dream to have an alternative method which could obtain the same results noninvasively, with no risk for the mother and especially not for the unborn child. This long-lasting hope became now a reality, and recently the noninvasive prenatal diagnosis (NIPD) from the blood of pregnant women could be offered routinely, first to detect trisomy 21, but also for other genetic conditions in the meantime.

Since the prenatal diagnosis of untreatable aneuploidies or other genetic anomalies can lead to the decision of pregnant women to ask for a termination of pregnancy, all different forms of prenatal diagnosis, whether invasive or noninvasive, have been the focus of ethical discussions from the beginning. In this context, however, we have to remind ourselves of the fact that already in the 80s a study by Ferguson-Smith et al on 52,965 patients with amniocentesis based on the so-called 'age-indication' at that time showed that 97.7% of all these investigations had normal results thus taking away special anxieties from these pregnant women.¹ The fundamental ethical dilemma of prenatal diagnosis is that the autonomy of the pregnant woman and the couple is important and needs protection; on the other hand the life of an unborn child also needs protection. The compromise in this basically unsolvable dilemma usually is that a careful counseling has to proceed any decision regarding continuation of a pregnancy after prenatal diagnosis, and that termination of pregnancy is only allowed after certain conditions are met. The risk of an amniocentesis and chorionic villus biopsy to cause loss of the pregnancy is up to 1%, and this severe burden was the reason for the long-lasting research to develop a method for NIPD, which lasted more than 30 years.

History of Research

The German pathologist Schmorl was the first to describe cells from the fetal side of the placenta (trophoblast) in the blood of women who suffered from pre-eclampsia, and more than 100 years later our group could show that in pre-eclampsia there is indeed an increased amount of fetal cells and cell-free fetal DNA in the blood of pregnant women.^{2,3} Originally, in the research to achieve a reliable method for NIPD intact fetal cells were the target,⁴ but for the trophoblast cells no good antibody is available, fetal white blood cells are too rare early in gestation, and nucleated red blood cells, although they are present much more frequently in the fetal compared to the maternal blood, are not good candidates for fluorescence *in situ* hybridization (FISH) any more due to their degree of degeneration even before they lose their nucleus. The NIH-sponsored so called-NIFTY study showed that using fetal erythroblasts for FISH did not have a sufficient sensitivity and specificity for routine use in prenatal diagnosis.⁵

The breakthrough toward clinical application came with the idea of Denis Lo who was then in Oxford/England and is now back in Hong Kong/China that the observation of cell-free DNA in the fluid of plants by Anker and Stroun in Geneva could be transferred to the detection of cell free-fetal DNA in maternal blood.⁶ Later also the group of Quake reported promising results using 'Digital size selection and relative mutation dosage'.⁷ The Rhesus factor was the first genetic trait which could be detected reliably by our and other groups⁸ with an accuracy of around 99%. In the meantime mutations for the Kell factor, beta-thalassemia, achondroplasia, alpha-thalassemia, Tay-Sachs, mucoviscidosis and other genetic anomalies have been detected by NIPD,⁹ and techniques, such as MALDI-TOF mass spectrometry¹⁰ and spectral karyotyping¹¹ have been used.

For the detection of trisomy 21 the 'massive parallel sequencing' technology with so-called 'next generation sequencers' has been successful, because in the maternal blood 10% of the DNA-fragments are fetal. This technique became commercially available as MaterniT21 PLUSTM test by the company Sequenom from San Diego which holds the patent of D Lo et al on the use of cell-free DNA and ours on size separation. The technique is also offered in Germany by the Company LifeCodexx which got a license from Sequenom to offer the test after proper quality control. On the market also other companies such as Verinata, Ariosa and Natera appeared as well as the Beijing Genomics Institute¹² which all use next generation sequencers for the NIPD. The group of D Lo could already show in 2011 in a study on 232 probands and 86 cases of trisomy 21 a sensitivity of 100% and a specificity of 97.9%.¹³ Subsequently; three bigger studies were published all confirming a sensitivity of 100%.¹⁴⁻¹⁶

Ehrich et al from the Sequenom Center for Molecular Medicine examined 480 samples including 39 trisomy 21 cases with 4.8% cases without result and a specificity of 99.7%.

Bianchi et al together with the Verinata group examined 532 samples including 89 trisomy 21 cases with 5.8% cases without result and a specificity of 100%

Ashor et al together with Ariosa Diagnostics examined over 400 samples including 50 trisomy 21 cases with 6.6% cases without result and a specificity of 100%.

The currently largest series was published by Palomaki et al¹⁷ together with the Sequenom Center for Molecular Medicine in 2011 on 1,696 samples and 212 trisomy 21 cases. In this study there were only 0.8% cases without result, and a sensitivity of 99.1% and a specificity of 99.9% were found. The same group conducted the so-called Women's and Infants Hospital of Rhode Island (WIHR)

Brown University Study with 4,664 cases, and the results regarding trisomies 21, 18 and 13 were just published¹⁸ with sensitivities and specificities for trisomies 18 and 13 of 99.9 and 99.6 and 91.7 and 99.7%, respectively.

CONCLUSION

D Lo and his group and the patent-holding company Sequenom as well as their license holders LifeCodexx in Germany were the first to be able offering NIPD for trisomy 21 in a clinical setting routinely. NIPD like any invasive method for prenatal diagnosis, however, should only be offered out of the context of appropriate and skilled prenatal counseling.¹⁹ The development in this area is fast, and it is therefore impossible to predict how the 'noninvasive prenatal diagnosis in 2020'²⁰ will look like. Lyn S Chitty et al²¹ have asked the question: 'Noninvasive prenatal testing for aneuploidy-ready for prime time?' which from my point of view can be answered with a 'yes', but under the condition that the quality control in this area remains high and proper follow-up is recorded from all cases. Even a 'Noninvasive prenatal measurement of the fetal genome' was published already,²² and Diana Bianchi in a recent editorial in Nature²³ used the title 'From prenatal genomic diagnosis to fetal personalized medicine'. It is rewarding at least for the researchers in the field who have worked on NIPD for more than 30 years with a lot of frustrations in between that this dream finally became a reality in practice for the benefit of women and couples. This rewarding experience also illustrates well once again that sometimes with big research tasks it is worthwhile to stick to the challenge for decades before the success becomes a reality.

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