Non Invasive Prenatal Diagnosis

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The possibility of obtaining material for fetal molecular analysis without the need of invasive procedures has been a goal of prenatal diagnosis for many years and now is becoming a reality by the demonstration of the presence of circulating cell-free fetal nucleic acids in maternal plasma. These signals are already been used in clinical practice, in the management of X-linked conditions by the determination of fetal sex or in Red cell all oimmunisation by prediction of fetal D status, where the sensitivity of this technique enables highly accurate results, therefore reducing the number of invasive procedures needed. This approach has also been used for diagnosis of genetic disorders or in obstetric complications where the level of free fetal DNA may be a marker for placental abnormalities.

The possibility of non-invasive access to fetal genetic material has been a goal of fetal medicine for many years.

The approaches used have been based mainly on

- The detection of fetal nucleated cells in maternal blood.
- Isolation of fetal trophoblastic cellular elements shed into the uterine cavity and the endocervical canal.
- The analysis of fetal genetic material present in maternal plasma.

In this review we attempted to summarise the current state of detection of fetal nucleated cells in maternal plasma, the use of trophoblastic cellular elements for prenatal diagnosis, and the use of free fetal nucleic acids in maternal plasma. The recent research and success in the use of free fetal DNA in the prediction of D blood grouping, fetal sexing, and identification of some single gene disorders are discussed in more detail, together with the current research into its use in obstetric complications and also the use of fetal mRNA for clinical applications.

Fetal Cells in Maternal Blood

The fact that fetal cells are present (albeit in low number) in maternal blood has been known for many years, but the many attempts and approaches used to isolate these cells in sufficient quantities from the maternal blood to make a fetal diagnosis have proven disappointing. One of the main reasons for the lack of success of this approach is the fact that fetal cells constitute a tiny proportion of the total maternal blood cell population and much work remains to be done before this could be a practical technology for consistent recovery and assay[1].

Trophoblastic Cells in Cervical Samples

An alternative approach to the detection of nucleated fetal cells in maternal plasma is the detection of trophoblastic cells from the lower part of the uterus or cervical mucus by means of irrigation or aspiration. Using this approach, trophoblastic cellular elements retrieved in transcervical cell samples have been successfully used for prenatal diagnosis of fetal Rh (D) phenotypes and some single gene defects, such as those causing thalassemia and sickle cell anemia[2]. However there are some problems that remain to be solved before this technique could be used for prenatal diagnosis on a clinical basis. One is the marked variation in the proportion of cells retrieved from the endocervical canal which are of fetal origin as opposed to maternal origin, with the percentage varying in different reports between 4 to 80 percent. This difference depends mainly on the method used to retrieve the cells (transcervical irrigation or cervical mucus aspiration) and operator variability[3]. A second problem is that this technique is still at least minimally invasive. Nevertheless the patients that have had this procedure did not find it especially uncomfortable and reported that it was comparable to having a cervical smear taken[3]. However, if the same information can be obtained from maternal blood then that would obviously be preferable. On the other hand if the percentage of free fetal DNA needed for tests has to be higher than what can be achieved from maternal blood, then a transcervical washing approach may be useful.

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Free Fetal Nucleic Acids

In the last decade the presence of circulating cell-free fetal nucleic acids in maternal plasma has been demonstrated[4], and this phenomenon has rapidly been applied to patient care. The origin of these molecules in maternal plasma is not a resolved issue but there is strong evidence supporting the idea that the placenta is the major source of free fetal DNA[3] probably due to the release of trophoblastic cells nucleic acids after apoptosis into the maternal circulation.

The prolonged persistence of fetal cells in maternal blood and bone marrow6 raised the concern that cell-free fetal DNA might persist in the maternal plasma from previous pregnancies. Nevertheless, the current evidence strongly suggests that the circulating fetal DNA is rapidly eliminated from the peripheral blood within hours of delivery7-11 and also that the half life of free DNA is very short and therefore there must be a continuous source of the material in pregnancy. Moreover, the absence of false-positive results related to persistent fetal DNA from a previous pregnancy in studies where free fetal DNA was used to assess fetal rhesus status and sex determination for clinical management is another strong evidence that previous pregnancy does not seem to be a problem[12,13].

Another recent step forward in the research on this field has been the detection of cell-free fetal mRNA in maternal plasma. As with DNA, the placenta seems to be the most important source of this material because of the correlation of the fetal mRNA and placental protein levels in maternal blood[14]. The detection of fetal nucleic acids in maternal plasma is not an easy technical task because of the relatively low percentage of these molecules compared with the maternally originated nucleic acids present in the maternal blood stream. Very sensitive real time PCR technology needs to be used to detect only a few copies of the target DNA or RNA in a sample.

Prediction of Fetal Rhesus Phenotype in Maternal Plasma

Prenatal determination of fetal D blood group in pregnancies at risk of haemolytic disease of the fetus and newborn (HDFN) is an important task[15] because of the fetal and neonatal morbidity and mortality caused by the maternal alloantibodies directed against paternally inherited antigens on fetal red cells[16]. The previous approach for the prediction of fetal blood group was the use of fetal material from amniotic fluid. However, the procedure-related risk of fetal loss[17] and also the risk of feto-maternal haemorrhage[18] which is in turn associated with increased incidence of maternal immunisation[19] made this approach less than ideal.

There is sufficient cell-free fetal DNA in the plasma of pregnant women for determination of fetal RhD genotype[20] which allows fetal D typing using a non invasive approach. This is now available world-wide as a clinical service[13,2,1] and we have recently demonstrated 100% accuracy with this technique in 137 pregnancies[15]. The recent developments of this technique have now significantly reduced the number of invasive procedures carried out for fetal D grouping in our unit[21]. We are confident that soon this will be used for fetal blood grouping in all rhesus negative pregnant women routinely at booking in order to administer anti-D only to those women with a D-positive fetus. Testing for other blood group antigens, such as Kell, Rhc, RhE, or other alloimmune conditions such as neonatal alloimmune thrombocytopenia, using the same technique, is already proving successful but still in experimental stages[22].

Prediction of Fetal Sex in Maternal Plasma

The detection of Y-chromosome specific sequences in maternal cell-free DNA4, has been reproducible in many laboratories, and the identification of fetal gender has been successful as early as 14 days post conception (Soothill, unpublished data) and is nearly 100% accurate in the late first trimester[13,23,24]. These results clearly have a huge impact in clinical practice for the management of X-linked genetic disorders. It is also possible to determine the sex very early in pregnancy, well before CVS would be undertaken, to guide fetal therapy in cases at risk of 21-hydroxylase deficiency[24-26]. In case of X-linked conditions, invasive tests such as CVS may now only be required to test whether a male fetus is affected by the disease or not, when the fetus is already shown to be male by free fetal DNA. This means CVS can be avoided when the fetus is known to be female at an early gestational age.

New Clinical Applications

Genetic Disorders

Circulating nucleic acids are increasingly being used for non-invasive prenatal diagnosis of certain genetic disorders. The diagnoses of autosomal dominant disorders such as myotonic dystrophy[27] and Huntington disease[28], or single gene mutations such as achondroplasia[29], are examples of the wide range of problems where this has been achieved. In cases of autosomal

recessive disorders, such as cystic fibrosis (CF), prenatal diagnosis currently requires a CVS. Since there are many different mutations that can cause this particular disease, the diagnosis of an unaffected fetus may be made by exclusion of the presence of the paternally inherited mutation signal in maternal plasma. In that way, invasive prenatal diagnosis could be limited to those pregnancies which have inherited the paternal mutation and so have a potentially affected fetus[30]. Another common autosomal recessive single-gene disorder is β-thalassaemia and this has been excluded by testing for the paternally transmitted mutation[31].

In cases of aneuploidy, one obvious goal is the non-invasive diagnosis of Down's syndrome. However since chromosome 21 signals are always present in all pregnancies, this requires the much harder task of trying to test quantitatively for an increased volume of chromosome 21 signals compared to those of other chromosomes. That may be easily possible when there is pure or very high concentration of the fetal DNA (PCR rapid diagnosis test) but not in the proportion of fetal DNA actually present in maternal blood. Current research is aiming to increase the proportion of fetal to maternal DNA in vitro and both chemical and size separation approaches are being explored[32,33].

Obstetric Complications

Pathological processes in the placenta, such as preeclampsia, fetal growth restriction or fetomaternal hemorrhage seem to increase apoptosis of trophoblast cells[34,35]. Therefore, higher levels of free fetal DNA are found in preeclamptic patients[36,37] or in cases of fetal growth restriction[38]. This measurement may serve as a marker for placental abnormalities and it is even possible that quantification of fetal DNA might be helpful in predicting pre-eclampsia[39] as its concentration was found to be increased before the disease onset[40]. The same concept may apply to cases of fetal growth restriction with a correlation between increasing fetal DNA values and the severity of the disease.

Fetal RNA in Maternal Plasma

The demonstration of fetal-derived, male-specific mRNA in plasma of pregnant women with male fetuses has been the most recent step forward in this field[41]. As with DNA, the placenta seems to be the main source of fetal mRNA and this may be used as markers for the studying and monitoring of pregnancy disorders related to placental pathology[42,43]. The Circulating mRNA seems to provide information regarding gene expression patterns in the placenta that could be altered in some pathology. For example, the concentration of maternal plasma CRH mRNA is increased in pregnancies complicated with preeclampsia when compared to normal pregnancies[44] and may also be correlated with the severity of the disease[45]

Conclusion

A new phase of non-invasive prenatal diagnosis has just started. With surprising speed, the field moved from initial identification of free fetal DNA in maternal blood to clinical application in the NHS in less than 10 years. The implications for the future are being explored within an FP6 European Network of Excellence grant (http://www2.warwick.ac.uk/fac/sci/bio/safe/) and we are confident this will have application both in routine obstetric care and specialised prenatal diagnosis as described in this text.

References

1. Jackson L. Fetal cells and DNA in maternal blood. *Prenat Diagn* 2003; **23**: 837-46.
2. Adinolfi M, Sherlock J. Fetal cells in transcervical samples at an early stage of gestation. *J Hum Genet* 2001; **46**: 99-104.
3. Daryani Y., Barker G., Penna L., et al. Transcervical sampling as a means of detection of fetal cells during the first trimester of pregnancy. *Am J Obstet Gynecol* 2000; **183**: 752-4.
4. Lo Y., Corbetta N., Chamberlain P., et al. Presence of fetal DNA in maternal plasma and serum. *Lancet* 1997; **350**: 485-7.
5. Wataganara T., Bianchi D. Fetal cell-free nucleic acids in the maternal circulation: new clinical applications. *Ann N Y Acad Sci* 2004; **1022**: 90-9.
6. Bianchi D., Zickwolf G., Weil G., et al. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci* 1996; **93**: 705-8.
7. Lo Y., Zhanf J., Leung T., et al. Rapid clearance of fetal DNA from maternal plasma. *Am J Hum Genet* 1999; **64**: 218-24.
8. Johnson-Hopson C., Artlett C. Evidence against the long-term persistence of fetal DNA in maternal plasma after pregnancy. *Hum Genet* 2002; **111**: 575.
9. Smid M., Galbiati S., Vassallo A., et al. No evidence of fetal DNA persistence in maternal plasma after pregnancy. *Hum Genet* 2003; **112**: 617-8.
10. Kolialexi A., Tsangaris G., Antsaklis A., et al. Rapid clearance of fetal cells from maternal circulation after delivery. *Ann N Y Acad Sci* 2004; **1022**: 113-8.
11. Benachi A., Steffann J., Gautier E., et al. Fetal DNA in maternal serum: does it persist after pregnancy? *Hum Genet* 2003; **113**: 76-9.
12. Costa J., Benachi A., Gautier E., et al. First trimester fetal sex determination in maternal serum using real-time PCR. *Gynecol Obstet Fertil* 2002; **30**: 953-7.
13. Finning K., Martin P., Soothill P., et al. Prediction of fetal D status from maternal plasma:

introduction of a new non invasive fetal RHD genotyping service. *Transfusion* 2002; **42**: 1079–85.
14. Ng EK, Tsui N., Lau T., et al. MRNA of placental origin is readily detectable in maternal plasma. *Proc Natl Acad Sci USA* 2003; **100**: 4748-53
15. Daniels G., Finning K., Martin P., et al. Fetal blood group genotyping from DNA from maternal plasma: an important advance in the management and prevention of haemolytic disease of the fetus and newborn. *Vox Sang* 2004; **87**: 225-32.
16. National Institute for Clinical Excellence. Technology appraisal guidance 41. Guidance on the use of routine antenatal anti-D prophylaxis for RhD-negative women. *London. NICE*. 2002.
17. Nanal R., Kyle P., Soothill P. A classification of pregnancy losses after invasive prenatal diagnostic procedures: an approach to allow comparison of units with a different case mix. *Prenat Diagn* 2003; **23**: 488-92
18. Tabor A., Bang J., Nørgaard-Pedersen B. Feto-maternal haemorrhage associated with genetic amniocentesis: results of a randomized trial. *Br J Obstet Gynecol* 1987; **94**: 528-34
19. Murray J., Karp L., Williamson R., et al. Rh isoimmunization related to amniocentesis. *Am J Med Genet* 1983; **16**: 527-34.
20. Lo YMD. Fetal RhD genotyping from maternal plasma. *Ann Med* 1999; **31**: 308-12
21. Finning K., Martin P., Daniels G. A clinical service in the UK to predict fetal Rh (Rhesus) D blood group using free fetal DNA in maternal plasma. *Ann N Y Acad Sci* 2004; **1022**: 119-23.
22. Van der Schoot C., Tax G., Rijnders R., et al. Prenatal typing of Rh and Kell blood group system antigens: the edge of a watershed. *Transfus Med Rev* 2003; **17**: 31-44.
23. Lo YM, Tein M., Lau T., et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for non invasive prenatal diagnosis. *Am J Hum Genet* 1998; **62**: 768-75.
24. Rijnders R., van der Schoot C., Bossers B., et al. Fetal sex determination from maternal plasma in pregnancies at risk for congenital adrenal hyperplasia. *Obstet Gynecol* 2001; **98**: 374-8.
25. Bartha J., Finning K., Soothill P. Fetal sex determination from maternal blood at 6 weeks of gestation when at risk for 21-hydroxylase deficiency. *Obstet Gynecol* 2003; **101**: 1135-6
26. Costa J., Benachi A., Gautier E New strategy for prenatal diagnosis of X-linked disorders. *N Engl J Med* 2002; **346**: 1502.
27. Amicucci P., Gennarelli M, Novelli G, et al. Prenatal diagnosis of myotonic dystrophy using fetal DNA obtained from maternal plasma. *Clin Chem* 2000; **46**: 301-2.
28. Gonzalez-Gonzalez MC, Trujillo MJ, Rodriguez de Alba M, Ramos C. Early Huntington disease prenatal diagnosis by maternal semiquantitative fluorescent-PCR. *Neurology* 2003; **60**: 1214-5.
29. Saito H., Sekizawa A., Morimoto T., et al. Prenatal DNA diagnosis of a single-gene disorder from maternal plasma. *Lancet* 2000; **356**: 1170.
30. Gonzalez-Gonzalez M., Garcia-Hoyos M., Trujillo M., et al. Prenatal detection of a cystic fibrosis mutation in fetal DNA from maternal plasma. *Prenat Diagn* 2002; **22**: 946-8.
31. Chiu R., Lau T., Leung T., et al. Prenatal exclusion of beta thalassaemia major by examination of maternal plasma. *Lancet* 2002; **360**: 998-1000.
32. Li Y., Zimmermann B., Rusterholz C., et al. Size separation of circulatory DNA in maternal plasma permits ready detection of fetal DNA polymorphisms. *Clin Chem* 2004; **50**: 1002-11
33. Dhallan R., Au WC, Mattagajasingh S., et al. Methods to increase the percentage of free fetal DNA recovered from the maternal circulation. *JAMA* 2004; **291**: 1114-9
34. Tjoa M., Oudejans C., van Vugt J., et al. Markers for presymptomatic prediction of preeclampsia and intrauterine growth restriction. *Hypertens Pregnancy* 2004; **23**: 171-89
35. Ishihara N., Matsuo H., Murakoshi H., et al. Increased apoptosis in the syncytiotrophoblast in human term placentas complicated by either preeclampsia or intrauterine growth retardation. *Am J Obstet Gynecol* 2002; **186**: 158-66
36. Zhong X., Laivuori H., Livingston J., et al. Elevation of both maternal and fetal extracellular circulating deoxyribonucleic acid concentrations in the plasma of pregnant women with preeclampsia. *Am J Obstet Gynecol* 2001; **184**: 414–19
37. Lo Y., Leung T., Tein M., et al. Quantitative abnormalities of fetal DNA in maternal serum in preeclampsia. *Clin Chem* 1999; **45:** 184–88.
38. Smid M., Vassallo A., Lagona F., et al. Quantitative analysis of fetal DNA in maternal plasma in pathological conditions associated with placental abnormalities. *Ann N Y Acad Sci* 2001; **945**: 132-7
39. Leung T., Zhang J., Lau T., et al. Increased maternal plasma fetal DNA concentrations in women who eventually develop preeclampsia. *Clin Chem* 2001; **47:** 137–39.
40. Cotter A., Martin C., O'leary J., et al. Increased fetal DNA in the maternal circulation in early pregnancy is associated with an increased risk of preeclampsia. *Am J Obstet Gynecol* 2004; **191**: 515-20
41. Poon L., Leung T., Lau T., et al. Presence of fetal RNA in maternal plasma. *Clin Chem* 2000; **46**: 1832-34.
42. Lo Y., Chiu R. The biology and diagnostic applications of plasma RNA. *Ann N Y Acad Sci* 2004; **1022**: 135-9.
43. Tsui N., Chim S., Chiu R., et al. Systematic micro-array based identification of placental mRNA in maternal plasma: towards non-invasive prenatal gene expression profiling. *J Med Genet* 2004; **41**: 461-7
44. Ng EK, Leung T., Tsui N., et al. The concentration of circulating corticotropin-releasing hormone mRNA in maternal plasma is increased in preeclampsia. *Clin Chem* 2003; **49**: 727-31.
45. Farina A., Chan C., Chiu R., et al. Circulating corticotropin-releasing hormone mRNA in maternal plasma: relationship with gestational age and severity of preeclampsia. *Clin Chem* 2004; **50**: 1851-4.