EDITORIAL

Fetal RhD typing with free DNA in maternal plasma

Kenneth J. Moise, Jr, MD*

University of North Carolina School of Medicine, Chapel Hill, NC

The finding of free fetal DNA (ffDNA) in the plasma of pregnant women was first reported by Lo et al in 1997. Considerable research has aided our understanding of this phenomenon. The source of the fetal DNA is probably the result of apoptosis of villous trophoblasts, either in situ or after they have entered the maternal circulation. The breakdown of intact fetal cells in the maternal circulation may also contribute to ffDNA. Total cell-free DNA is higher in maternal serum than plasma; however, similar levels of ffDNA are detected in both. FfDNA is demonstrable as early as 32 days of gestation and increases to comprise 3% of the total DNA pool in maternal plasma in the second trimester and increases to 6% in the late third trimester. After delivery by cesarean section, the mean half-life of ffDNA is 16 minutes, with no detectable levels by 2 hours after delivery in virtually all patients. In the case of a heterozygous paternal genotype, pregnant women who are alloimmunized to the RhD red cell antigen are at risk to carry an affected fetus in only 50% of cases. Because, in the past, a method for the determination of the RhD status of the fetus was not available, pregnancies were followed routinely with serial amniocenteses for ΔOD₄₅₀. In the mid 1980s, fetal blood sampling gained widespread acceptance for the determination of the RhD status of the fetus with the use of serologic testing. Although the procedure was associated with a 1% risk for fetal loss, pregnancies in which the fetus was determined to be RhD-negative were no longer subject to unnecessary multiple amniocenteses. In 1993, Bennett et al reported the use of amniotic fluid DNA analysis to perform fetal RhD typing, thereby eliminating the need for fetal blood sampling. Five years later, preliminary work on RhD typing of the fetus with ffDNA in maternal plasma was described.

In this month’s journal, Gautier et al report a large prospective series of 285 patients who were examined at a prenatal diagnostic center in France and who underwent fetal RhD typing with ffDNA in maternal serum. Confirmation of fetal blood type was available in 95% of cases through DNA typing by amniocentesis and/or neonatal serologic testing. In 102 patients who carried an RhD-negative fetus and 170 patients who carried an RhD-positive fetus, Gautier et al noted a diagnostic accuracy of 100%. The authors identify 2 important issues that must be considered in the use of ffDNA for RhD typing of the fetus.

In the first instance, the ffDNA may provide a false-positive result. In this scenario, the fetus would be found to be RhD-positive when in reality it was RhD-negative. This could lead to unnecessary interventions in the case of an alloimmunized pregnancy. In most circumstances, an RhD-positive ffDNA result indicates that the fetus is RhD-positive because maternal plasma (from the RhD-negative patient) should not contain RhD genetic sequences. The one exception to this situation is when an RhD gene that is present in the mother is not

* Reprint requests: Kenneth J. Moise, MD, University of North Carolina School of Medicine, 214 MacNider Bldg, CB #7516, Chapel Hill, NC 27599-7516.
E-mail: kmoisejr@med.unc.edu

0002-9378/S - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ajog.2005.01.005
expressed in her phenotype (ie, the patient is determined to be RhD-negative through serologic testing). This has been reported because of the presence of the RhD pseudogene or the RhD-CE-D3 gene in up to 50% of black pregnant patients and other RhD gene variations that are not expressed in 12% of Japanese individuals.9 In their study, Gautier et al8 used primers that targeted exon 10 of the RhD gene. This technique will not detect the abnormal exon 4 of the RhD pseudogene. It is therefore likely that the presence of the RhD pseudogene was the explanation for the usually high levels of RhD sequences that were present in the maternal serum of 2 patients in their series. Because ethnicity cannot be determined reliably for all patients, fDNA testing should be undertaken with polymerase chain reaction primers that target multiple exons of the RhD gene.

The second concern with the use of fDNA for fetal RhD typing is the issue of a false-negative diagnosis. This scenario has more grave implications for fetal management because an RhD-positive fetus may be misdiagnosed as being RhD-negative and appropriate prenatal interventions would be omitted. The most likely explanation for this error is the lack of amplification of the fetal DNA in the overwhelming background of maternal DNA in the plasma sample. Gautier et al8 added tracer mouse DNA to their assay as an internal control to indicate that the DNA amplification was successful. However, this technique does not confirm that fetal DNA was amplified specifically. The authors state in their discussion that they have not encountered a false-negative result to date. Some laboratories use the presence of the SRY gene (on the Y chromosome) to confirm the presence of fetal DNA in cases of a male fetus. This would seem to represent a more reliable control to prove the amplification of fetal DNA. In the case of a female fetus, maternal DNA polymorphisms in leukocytes from the buffy coat of the maternal blood sample can be used to verify the presence of fetal DNA.

The finding of unique polymorphisms (inherited by the fetus from the partner) that were not seen in the maternal white cells indicates that fetal DNA had therefore likely been amplified and that an RhD-negative result accurately reflects the fetal RhD type. In as many as 4% of cases, however, this type of analysis may not be informative.10 FfDNA testing should then be repeated on a new maternal sample. Alternatively, amniocentesis can be undertaken to confirm the fetal RhD type before a decision is made on a course of nonsurveillance for the fetus.

Unfortunately, laboratories in the United States are lagging behind our British and European counterparts. FfDNA for fetal RhD typing is not yet available in the United States but is used clinically on a routine basis in countries such as the United Kingdom.10 The current report by Gautier et al8 contributes to a growing body of evidence that fDNA for fetal RhD typing is now ready for prime time.

What is the future for testing with fDNA in maternal plasma? Clearly, assays will be developed for other red-cell antigens that are involved in severe hemolytic disease of the fetus/newborn, such as c, E and K. Fetal typing for HPA-1 (PlA1) and other platelet antigens that are associated with alloimmune thrombocytopenia purpura is plausible. Routine fetal typing in all RhD-negative, unsensitized pregnant women could eliminate the need for antenatal Rhesus immune globulin in cases of an RhD-negative fetus. Such maternal testing that uses automated technology and real-time polymerase chain reaction has been estimated to cost less than one third that of Rhesus immune globulin. Studies of routine screening of all RhD-negative women in pregnancy with fDNA are ongoing in the Netherlands and France and have been recommended by the National Institute of Clinical Excellence in the United Kingdom.8, 10 The report by Gautier et al8 opens the door to other future applications for noninvasive prenatal diagnosis. Larrabee et al11 have reported that microarray technology can be applied to fDNA in amniotic fluid. These investigators found that increased hybridization was present for most markers on chromosome 21 in cases of fetal trisomy 21. The eventual application of such exciting new technology to screen for fetal trisomies with fDNA in maternal plasma may relegate such invasive techniques as chorion villus biopsy and amniocentesis to the historic annals of obstetrics.

References
