

Noninvasive screening for prenatal genetic diagnosis*

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During the last two decades a number of methods of prenatal diagnosis have become available and have been used either in laboratory research or in routine genetic counselling. Despite the effectiveness of invasive sampling procedures in diagnosing genetic disorders, their use involves some risk. The advantage of noninvasive methods is that they provide an opportunity to make a genetic diagnosis without risk, and therefore are applicable for use in mass screening programmes. This article reviews three different approaches to noninvasive prenatal genetic diagnosis and offers conclusions and recommendations for their use. Maternal serum screening is a well-understood technique that should be universally offered to pregnant women, regardless of their risk status. Invasive tests can be used, as indicated, once serum testing results have been obtained. Although ultrasonography cannot be recommended for routine use, it can provide a useful adjunct to serum screening and deserves further investigation. Elaboration of fetal cells from maternal blood is a promising technique but can only be considered investigational on the basis of current research, and should not serve as the sole basis of clinical decision-making.

Introduction

Screening has been defined as the systematic application of a test or enquiry to identify individuals, among those who have not sought medical attention, who are at sufficient risk for a specific disorder to benefit from further investigation for direct preventive action (1). Screening should be performed with the informed consent of the subject, as regards both the invitation to screening and each stage of the screening and diagnostic process.

Screening procedures for pregnant women of advanced age have been available for over 25 years. First amniocentesis and more recently chorionic vil-

lus sampling have been offered to such women for detecting fetal chromosomal abnormalities. Approximately half of these women have accepted the procedure-related risks inherent with these invasive procedures. Because relatively few women under the age of 35 years are candidates for fetal screening, the birth prevalence of Down syndrome, for example, in the general population will decrease relatively little as a result of it (<20%), since only 10–20% of births occur to women who are 35 years of age or older.

Several different screening approaches for Down syndrome are possible. Second trimester maternal serum testing is a screening procedure that is already accepted in many countries. Ultrasonographic detection of organic anomalies associated with various forms of aneuploidy is an attractive possible alternative, given the widespread use of ultrasound for other obstetrical indications. Another strategy currently under clinical evaluation is the recovery and analysis of fetal cells in maternal blood.

Maternal serum screening

Since the late 1980s various maternal serum markers, principally α -fetoprotein (AFP) and human chorionic gonadotropin (hCG), have been used in combination with maternal alpha-fetoprotein to screen for Down syndrome. The detection rate (sensitivity) is approximately 60% over all age groups, ranging from >90% for women older than 35 years of age to <25% for those younger than 25 years of age (2, 3). This detection rate is

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achieved at an amniocentesis rate of about 5%. Serum screening between 15 and 22 weeks of gestation can identify more cases of Down syndrome in women who are at a risk high enough to justify an invasive procedure (e.g., amniocentesis) than can be identified using an age threshold alone (e.g., 35 years). In other words, for a given number of women identified with Down syndrome fetuses, fewer women need to undergo an invasive procedure when serum screening is used than when age-threshold maternal screening alone is used; thus, maternal serum screening for Down syndrome is more effective than simple age-threshold screening (4).

National practice in Down syndrome screening differs from country to country and reflects differences in clinical preferences and jurisprudence. For example, in the USA, the policy of offering an invasive procedure to women over the age of 35 years was recently reformulated.^a

Other countries, such as the United Kingdom, no longer routinely offer an invasive procedure to pregnant women older than a specific age, but rather pursue universal maternal serum screening. Nevertheless, some women may insist on having an invasive screening procedure outside the screening programme.

There is no consensus of opinion on whether the tested serum markers should be hCG and AFP or hCG, AFP, and unconjugated estriol (μE_3) ("triple test"). Most experience is based on the triple test, from which better screening performance can be expected (5).

The conclusions and recommendations shown below can be made.

- In countries that retain the age of 35 years as a threshold for offering amniocentesis or chorionic villus sampling, consideration should be given to offering all women the option of serum screening before they decide to have an invasive procedure. None the less, all pregnant women under the age of 35 years should be offered maternal serum screening (6).^a
- If women insist on invasive screening beyond that recommended in national policy, this request should be regarded as an individual matter and decided on a case-by-case basis.
- Serum screening for Down syndrome should be based on multiple serum markers (hCG, AFP, and μE_3) in addition to maternal age. Additional serum markers or alternative approaches should be used only if they can be justified in terms of increased sensitivity, specificity, or cost-effectiveness.

- Screening should be offered only as an "integrated" service: resources should be identified for coordination, patient counselling, provision of information, pregnancy management, professional education, and programme monitoring.

Screening before 15 weeks of gestation is still investigational. However, preliminary data with selected analytes (free β -hCG, AFP, and pregnancy-associated plasma protein-A (PAPP-A)) suggest that detection rates approximate those in the 15–22-week interval.

Ultrasonography

Clinically useful ultrasound has been available for about 30 years. During the past 20 years, improvements in technology and the availability of equipment have made the use of ultrasound an integral part of clinical care in pregnancy. Among the uses of obstetrical ultrasound are the following: identification of placenta previa; assessment of gestational age; identification of the number and position of fetuses; documentation of fetal life; assessment of amniotic fluid volume; and biophysical assessment of the fetus. Ultrasound has also been used to evaluate the uterus, uterine cervix, adnexa uteri, and fetal anatomy and has been instrumental in identifying malformations as early as 12 weeks of gestation (7). All this information is universally accepted as the benefits that should be obtained as part of the complete obstetrical ultrasound examination.

To date, the benefit of routine ultrasound screening of low-risk pregnant women remains controversial. Notably, the Routine Antenatal Diagnostic Imaging with Ultrasound Study (RADIUS) in the USA failed to demonstrate improvements in the morbidity or mortality of low-risk patients (8). Critics of the RADIUS study have pointed out its serious methodological limitations (9); however, the study demonstrated a significant difference in the detection of birth defects in patients undergoing routine screening compared with patients undergoing ultrasound for pre-established indications (10). Also, there was a difference in the detection rates of serious malformations in centres where fetal ultrasound examinations were performed by specialists compared with centres where they were performed by obstetricians or community-based radiologists (11).

Overall, there is general acceptance that ultrasound is beneficial in appropriately selected pregnant women, especially when there is an increased risk of structural malformations. More uncertain is the role of ultrasound in the detection of aneuploidy, although it is clear that some aneuploid fetuses may exhibit either structural changes or abnormalities of growth that are detectable by ultrasound.

^a *Down syndrome screening*. Washington, DC, American College of Obstetricians and Gynecologists Committee Opinion (ACOG 141, 1994).

Abnormal measurements of the femur and humerus in the first trimester were among the first ultrasound indices of aneuploidy to be proposed but have not gained wide acceptance because subsequent investigations have not reproduced the results of the initial studies. Unfortunately, investigators have used different cut-off values as indicative of aneuploidy, further confusing the issue. Other first trimester ultrasound findings in aneuploidy have been published. Aneuploid embryos tend to have smaller crown-rump lengths, but since substantial variability exists in measurements for fetuses with aneuploidy, this technique is not commonly used. Nicolaides et al. (12) first reported that nuchal translucency of ≥ 3 mm had 75% sensitivity for the identification of Down syndrome in the first trimester; however, Brambati et al. (13) found only an 18.6% prevalence of chromosomal abnormalities when nuchal translucency was ≥ 3 mm. Another proposal is to use Doppler flow studies to detect trisomy 21 (14) but standard criteria do not exist.

In the second trimester, an additional ultrasound marker of aneuploidy is occipital subcutaneous oedema, which if greater than 6 mm has been associated with an increased prevalence of trisomy 21 (15). Some investigators have confirmed this finding, but others were unable to document a relationship between nuchal thickening and Down syndrome (16). Some technical difficulties have been pointed out, such as the possibility of generating spurious signs of nuchal thickening by angling the ultrasonic transducer caudally instead of axially at the level of the cerebral hemispheres. Other proposed ultrasound markers in the second trimester, include hypoplasia of the middle phalanx of the fifth digit and clinodactyly. However, the size of the middle phalanx is quite small during the second trimester; thus detection on this basis may not be reliable. Because there is no fundamental doubt that various ultrasound measurements are indeed associated with aneuploidy, quantification of these relationships is awaited.

The other ultrasonographic approach to screening for aneuploidy is to search for structural malformations, e.g., a cardiac defect. Up to 50% of Down syndrome fetuses have a cardiac defect in the second trimester, but only 30% manifest such a defect at term (11). Similarly, up to 90% of trisomy 18 and trisomy 13 fetuses have been alleged to have cardiac defects. The RADIUS trial showed a very poor detection rate for cardiac malformations for examinations performed at nontertiary centres by general obstetric staff (10); other investigators, however, have demonstrated that approximately 33–63% of cardiac defects can be detected by a routine ultrasound scan (17, 18).

Nicolaides et al. were the first to report an association between choroid plexus cysts and trisomy 18 (19), a finding that has been confirmed subsequently (20, 21). Other findings associated with Down syndrome include pyelectasis, hyperechogenic bowel, enlarged cisterna magna, ventriculomegaly, and wide distance between the first and second toe. However, many reports show detection rates for aneuploidy that vary with the gestational age of the fetus and fetal sex. Some investigators have suggested that in the absence of anatomical defects amniocentesis would not be indicated; however, a difficulty with this recommendation is that an inability to identify heart defects may result in a failure to perform amniocentesis to detect trisomy 18.

Investigators have usually focused on only a single marker (e.g., pyelectasis, hyperechoic bowel, or abnormal nuchal fold) to detect Down syndrome with ultrasound. Ultrasonographic detection rates for aneuploidy might, nevertheless, be strengthened if examinations were carried out for a constellation of signs, since several signs occurring simultaneously would be a stronger indication of Down syndrome than any single sign. DeVore & Alfi (14) reported a sensitivity of 87% for trisomy 21 with ultrasound, with a false-positive rate of 11%.

As a result of the factors cited above, ultrasound markers alone will not at present replace maternal serum screening. However, as further research is carried out, ultrasound is likely to play an increasingly important role in detecting malformations in both low- as well as high-risk patients. In some areas of the world, ultrasound has already been implemented in routine early obstetrical screening.

Finally, one or more modalities of ultrasound screening may be used as an adjunct to conventional biochemical testing in appropriate instances. Such circumstances would include pregnancies of patients identified as "at risk" by other means, pregnancies in which structural malformations associated with aneuploidy have been identified, pregnancies in which further information is needed before eschewing invasive testing in women over the age of 35 years who show lower than expected aneuploidy risks by maternal serum screening, and pregnancies in which further information is needed on the need for invasive testing in women who, despite being aged below 35 years, show higher than expected aneuploidy risks by maternal serum screening.

Below are shown the conclusions and recommendations that were made on ultrasonography.

- Ultrasound is an integral part of fetal screening for Down syndrome, particularly in defining the gestational age associated with serum markers.

- Ultrasound is essential for identifying fetal structural malformations; however, the specific anomaly or anomalies that should be sought remain uncertain because their detection sensitivities for aneuploidy are not yet adequately defined.
- Routine ultrasound screening cannot currently be recommended because of unresolved questions concerning timing, equipment specifications, and the necessary operator experience and image quality.
- The inclusion of abnormal ultrasound findings and serum analyte risk assessment is an important project that should be given the highest priority.

Fetal cells in maternal blood

Obstetricians and pathologists have long known that fetal cells occasionally enter the maternal circulation, as exemplified by rhesus isoimmunization and amniotic fluid embolization. In 1969 Walknowska et al. (22) first reported recovering fetal cells from maternal blood on the basis of apparent 46, XY metaphases. In 1979 Herzenberg et al. (23) used flow-sorting technologies to recover cells positive for Y-chromatin from women whose fetus presumably inherited a paternal human leukocyte antigen (HLA) differing from that of its mother. However, there was no consensus that fetal cells were truly present in maternal blood until Lo et al. (24) used the polymerase chain reaction (PCR) to detect Y-chromosome sequences in the blood of women pregnant with male fetuses. Subsequently, the detection of single-gene (Mendelian) fetal mutations has been achieved through analysis of both sorted and unsorted maternal blood (25). PCR-based technology reveals that fetal cells are present as early as 35 days of gestation (26), and are consistently present by the end of the first trimester (27). The fetal cell type detected in these studies, however, is unclear.

Cell-enrichment techniques, followed by fluorescence *in-situ* hybridization (FISH) with chromosome-specific DNA probes, have demonstrated that fetal cells are present in maternal circulation during the first and second trimesters. Prenatal detection of fetal chromosomal abnormalities has been reported (28–33). For fetal cytogenetic diagnosis, selecting a specific fetal cell type is almost certainly necessary because the relative excess of maternal cells in the sample necessitates cell enrichment. Potential fetal cell types include nucleated red blood cells, trophoblasts, lymphocytes, and granulocytes. The greatest successes to date have been achieved with nucleated red blood cells; with such cells fetal trisomies were first detected in maternal blood using, e.g., density

gradient separations or magnetic activated-cell sorting with various positive and negative selection criteria. FISH analysis with chromosome-specific DNA probes was used to make a precise diagnosis.

Less experience has been gained with other fetal cell types. The presence of fetal lymphocytes in maternal blood has apparently been detected by PCR amplification of a paternally transmitted HLA allele; however, trisomies have not been detected (34). Perhaps a more attractive cell type for genetic diagnosis is the trophoblast. A consequence of placentation, trophoblasts are removed rapidly from maternal blood by the maternal lungs. Although trophoblast-specific monoclonal antibodies have proved difficult to generate, several groups have enriched maternal blood with trophoblast-specific antibodies and detected Mendelian disorders (25, 35). One group reported obtaining fetal karyotypes from trophoblasts (36).

Although recovery and analysis of fetal cells in maternal blood appears promising, key questions remain. The optimal fetal cell type for isolation, the frequency of fetal cells in maternal blood, the optimal timing during gestation for fetal cell isolation, and the likelihood of the persistence of cells after delivery all remain to be determined. As noted above, fetal cells of unknown type can be detected early in gestation (26) and are consistently present by 10 weeks of gestation (27). Although the number of fetal cells present increases during gestation, occurrence of fetal cells in maternal blood remains a rare event. Another concern is that fetal cells could persist for months if not years after delivery. The origin of such persistent fetal cells is clones established in the maternal haematopoietic system. The existence of such cells could result in an erroneous diagnosis in future pregnancies. Since lymphocytes are considered to be the cell type most likely to persist, erythroblasts or trophoblasts are the preferred cell type for fetal genetic diagnosis.

Recently, the U.S. National Institute of Child Health and Human Development funded four centres in an effort to recruit approximately 3400 women for a clinical evaluation of methods to isolate and analyse fetal cells in maternal blood. Simultaneously, information will be gathered on the attitudes, preferences, and behaviour of pregnant women towards such noninvasive techniques for prenatal diagnosis. The diagnostic accuracy of these studies will be compared with that of invasive techniques (e.g., amniocentesis and chorionic villus sampling). If the accuracy is found to be equivalent, fetal cell isolation from maternal blood could replace invasive procedures as a diagnostic test for fetal aneuploidy. Even if equivalent accuracy is not obtainable with current techniques, fetal cell isolation could still play an important role in prenatal screening for fetal genetic

disorders, either independently or in combination with other tests.

The following conclusions and recommendations were made.

- Fetal cells are present in maternal blood during the first and second trimester of pregnancy and offer the potential for early diagnosis of fetal genetic disorders.
- The optimal fetal cell type for isolation and analysis is not yet known. Both trophoblasts and erythroblasts are attractive candidates: the diagnosis of Mendelian disorders or Rh-status could be based on trophoblasts, but diagnosis of aneuploidy might be better accomplished using erythroblasts.
- The sensitivity, specificity, and positive and negative predictive value of screening based on this approach is unknown. Hence, isolating and analysing fetal cells from maternal blood must be regarded as an investigational technique for the detection of aneuploidy. Clinical decisions should not be based exclusively on the results of such investigations.

Résumé

Méthode de dépistage non invasive pour le diagnostic génétique prénatal

Un certain nombre de techniques de diagnostic prénatal qui ont fait leur apparition au cours des deux dernières décennies sont maintenant utilisées dans les laboratoires de recherche ou en routine pour le conseil génétique. Les méthodes invasives de prélèvement sont efficaces pour diagnostiquer les anomalies génétiques, mais leur emploi comporte quelques risques. Les méthodes non invasives ont l'avantage de permettre un diagnostic génétique sans risque, et par conséquent de pouvoir être appliquées dans les programmes de dépistage de masse. Cet article examine trois approches différentes et présente des conclusions et des recommandations concernant l'utilisation de ces méthodes. L'analyse systématique du sérum maternel est une technique bien connue qui devrait être proposée à toutes les femmes enceintes, quel que soit leur niveau de risque. Des méthodes invasives peuvent être utilisées, le cas échéant, une fois connus les résultats de l'analyse du sérum. L'échographie ne peut être recommandée comme examen de routine, mais elle peut constituer un complément utile de l'analyse du sérum et devrait faire l'objet d'études plus approfondies. L'isolement de cellules fœtales dans le sang maternel paraît prometteur, mais

dans l'état actuel des connaissances, il s'agit encore d'une technique de recherche qui ne peut à elle seule servir de base à une décision clinique.

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