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Prenatal screening and diagnosis

Noninvasive prenatal screening tests are available to identify fetuses at increased risk of chromosome abnormalities such as trisomy 21. The identification of fetuses at increased risk of these conditions allows women to then be offered diagnostic testing.

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There are requirements to be met before instituting any screening test. These requirements include:

- the disease or condition being screened for must have a relatively high prevalence; there is a significant burden of disease (morbidity or mortality in affected individuals, high financial and social costs)
- diagnostic tests are readily available and easy to perform and process
- diagnostic tests are not excessively expensive to perform
- diagnostic test results are reliable, and
- treatments are available for the disease or condition being screened for and are not expensive.[1]

Prenatal screening is voluntary. Patients should receive counselling about what a screening program can offer (including both false-positive and false-negative detection rates), the availability of the various options for screening, and the benefits and risks when invasive testing is undertaken. Patients also need to be aware of the time taken for some test results to become available (up to three weeks for some tests that require the culture of fetal cells before testing can commence), and that sometimes cell cultures fail.[2] They need to understand the difference between a screening test and a diagnostic test.

Patients found to be at high risk of having fetuses with chromosome abnormalities benefit from detailed counselling, and the benefits of referral to a genetic counselling service can not be over emphasised.

Testing can be categorised into two groups: noninvasive screening and invasive diagnostic testing.

Screening: noninvasive testing

Several noninvasive screening options are available during the antenatal period (Table 1).[3]

The morphology ultrasound scan at between 18 and 20 weeks' gestation is not considered an

IN SUMMARY

- **Prenatal screening is voluntary. Patients should receive counselling about what a screening program can offer.**
- **Several noninvasive screening options, including nuchal translucency screening and serum analyte testing, are available during the antenatal period.**
- **Invasive diagnostic tests include chorionic villus sampling, amniocentesis and fetal blood sampling.**
- **Maternal age remains a very important predictor of fetal abnormalities.**
- **First-trimester screening provides a greater accuracy of results than the second-trimester quadruple test.**

appropriate primary screening test for chromosome abnormalities. However, it is a primary screening test for structural abnormalities, but further discussion is beyond the scope of this article.

Serum analyte testing (or maternal serum screening)

Serum levels of certain markers are sampled and interpreted in the setting of maternal age-related risk. The mechanism for these altered markers is unclear, but one hypothesis suggests that the placental physiology changes when poorly functioning fetal tissue is present. As the pregnancy progresses different markers are measured and provide accurate screening tools for fetal anomalies (see Table 1).

Nuchal translucency screening

In 80% of fetuses with Down syndrome there is an increase in lymphatic fluid accumulation under the skin of the fetal neck (Figure), which gives rise to an increased nuchal translucency or thickness.[4] Other conditions are also associated with an increased nuchal translucency, as listed in Table 2. Therefore, a detailed fetal morphology assessment and serum analyte testing are combined in the screening process.

Diagnostic: invasive testing

Invasive diagnostic tests include chorionic villus sampling (CVS), amniocentesis and fetal blood sampling.

Chorionic villus sampling

CVS obtains small pieces of placenta for chromosome or DNA analysis and is generally undertaken between 11 and 13 weeks' gestation. More DNA is obtained from CVS than with amniocentesis, which leads to a quicker turn around time of results.

Placental samples are obtained under ultrasound guidance using a transabdominal or transcervical approach. Rapid karyotyping can be performed in 48 hours by examining metaphase cytotrophoblastic cells, but there is a 0.1% chance of a false-positive result. Therefore, long-term cultures are performed at the same time. The mesenchymal cells that are grown in long-term cultures are more closely related to the fetus than the trophoblast cells because they are from a more recently separated lineage of the extraembryonic mesoderm.[5]

CVS is more usually conducted via the transabdominal route. The transcervical route is rarely used; contraindications to this route are cervical infection and technical difficulties in entering the cervix and uterine cavity. A markedly retroverted uterus or a fetus obstructing access to a posterior placenta can make the abdominal approach difficult. However, the abdominal approach to CVS has fewer complications and a lower fetal loss rate than the cervical approach but the difference between the two methods is small (4.1 *v.* 4.0).[6] Failure to obtain a placental

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Prenatal screening tests allow clinicians to 'look into their crystal balls' and identify fetuses at increased risk of certain chromosomal abnormalities. However, these tests have limitations that patients should be made aware of.

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Table 1. Summary of noninvasive screening tests available antenatally[3]*

Test name	Type of test	Gestational age when test recommended
Combined first-trimester screening		
• Serum analyte testing	Two blood tests for beta human chorionic gonadotrophin (HCG) and pregnancy-associated plasma protein-A (PAPP-A) without ultrasound. Two tests are performed to increase the specificity	Ideally between 9 and 12 weeks
• Nuchal translucency assessment	Ultrasound scan to measure nuchal thickness. Results assessed in conjunction with those from the serum analyte testing	Between 11 weeks and 13 weeks and 6 days (best practice). Recommended as standard for all pregnant women
Second-trimester screening		
• Serum analyte testing	Either: – triple testing (alpha fetoprotein, free beta HCG or total HCG, and unconjugated oestriol) or – quadruple testing (as above plus inhibin A)	Between 15 and 17 weeks. Used as a fall back test when combined ultrasound and serum analyte testing has not occurred due to a woman presenting for care after 14 weeks' gestation

* Tests are listed chronologically.

sample with either a transabdominal or transcervical approach to CVS is less than 1%.[6]

Complications of CVS include fetal damage or fetal loss (1 to 2% with transabdominal CVS and 2 to 2.5% with transcervical CVS), frank bleeding (<6%), isoimmunisation (anti-D gamma globulin is given to rhesus-negative patients), infection (<0.5%) and uncertain results.[6] The reasons for delaying CVS until 11 weeks' gestation is to reduce the risk of transverse limb abnormalities, which are thought to be due to damage to the placenta leading to disruption of arteries supporting the development of embryonic limbs.

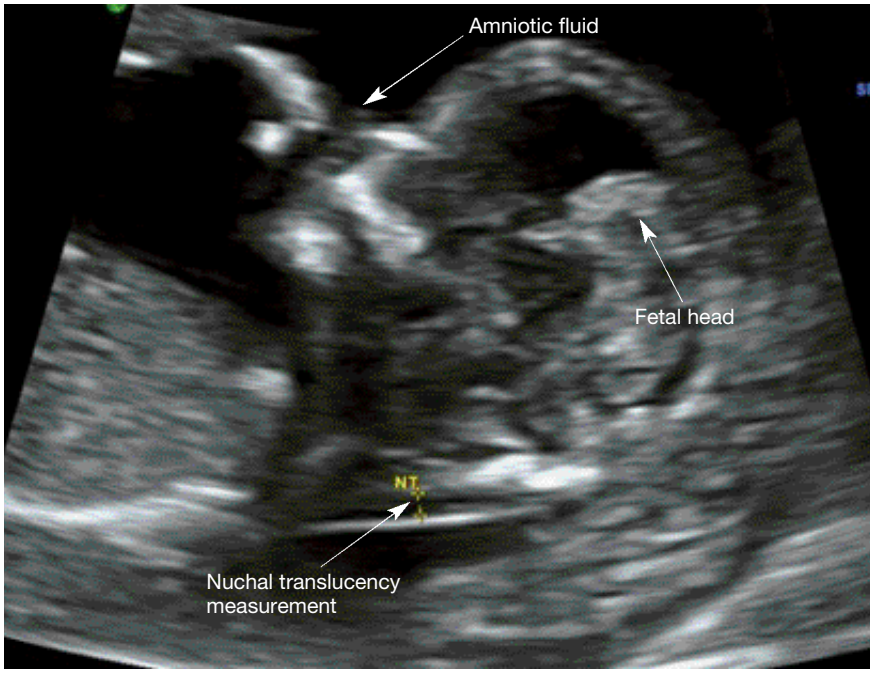

Figure. Ultrasound scan showing measurement of nuchal thickness.

Long-term placental culture of CVS samples give a false-positive result of rare trisomies in 1 to 2% of cases. This risk is twice as high using the faster direct method of metaphase testing and increases with advancing maternal age.[7,8] The false-negative rate with CVS is 0.03%.

Amniocentesis

Amniocentesis withdraws amniotic fluid from the amniotic sac. Most of the cells floating in the amniotic fluid are morphologically and biochemically classified as epithelioid, fibroblastoid and amniotic fluid-specific cells. Only 3 to 4% of floating cells are capable of being cultured and culture failure occurs in 0.1% of samples.[5]

Amniocentesis is technically possible after 11 weeks' gestation, but for genetic studies is performed between 15 and 17 weeks' gestation. Using ultrasound guidance, about 15 mL of amniotic fluid is obtained at amniocentesis. Complications of the procedure include membrane rupture (1.7%), which usually seals itself within a week. Direct fetal injury is

rare, but indirect fetal injury resulting in orthopaedic malformations and respiratory problems in the newborn have been reported on a few occasions.[7] Transmission of infection (hepatitis C, cytomegalovirus, toxoplasmosis and HIV) from mother to fetus has been described in case reports. Fetal loss occurs in less than 1% of cases.[9]

It should be appreciated that the interpretation of miscarriage rates between different studies requires knowledge of the selection criteria of each study. Definitions of miscarriage vary from 20 to 28 weeks' gestation as do the choice of control group. (In Australia, a miscarriage is defined as up to 20 weeks, the World Health Organization defines it as up to 22 weeks and in the late 1960s it was equivalent to the time before fetal viability and that was 28 weeks' gestation.) An increase in spontaneous miscarriage rate after amniocentesis compared with controls was found in all studies but did not reach statistical significance.[7,9] The rate of pregnancy loss related to the procedure ranges from 0.06 to 1%, with most losses occurring in the four weeks after amniocentesis.[10] This excess pregnancy loss rate is operator independent, but there appears to be an increased loss when amniocentesis is performed after 18 weeks' gestation.[11]

On some occasions amniocentesis may be performed in the third trimester and is successful in 99% of cases. Maternal complications from this procedure are rare (<0.001).[2] Testing for fetal lung maturity and measuring the sphingomyelin to lecithin ratio using amniocentesis are rarely performed these days. In addition, amniocentesis is the method of choice for diagnosing fetal infection acquired from the mother.[12] Such infections include toxoplasmosis, rubella, cytomegalovirus, varicella, parvovirus and congenital syphilis. The area of testing for fetal infections using amniotic fluid obtained at amniocentesis is complex and is beyond the scope of this article.

Fluorescent *in situ* hybridisation

Fluorescent *in situ* hybridisation (FISH) is the application of fluorescently labelled DNA molecules to metaphase chromosomes and interphase nuclei for the detection of chromosome abnormalities and alterations. The aneuploidy FISH analysis is performed on interphase nuclei. A result is considered to be interpretable if hybridisation is consistent in at least 70% of cells examined. Results are available in 24 to 48 hours from receipt of the sample.

The specimens required for FISH are 15 to 20 mg of chorionic villi in sterile media (from CVS) or 15 mL of amniotic fluid (from amniocentesis). Chromosome-specific DNA probes, with different colours for different chromosome pairs, are used to detect chromosome defects. The usual array of probes used for detecting numerical abnormalities of chromosomes are for chromosomes 21, 18, 13, X and Y. FISH testing is performed in addition to the cell culture with both samples being obtained at the same time because FISH results are not considered to be final until the karyotyping from the cultures are completed.

Fetal blood sampling

Fetal blood sampling is most useful for cytogenetic diagnosis in the second and third trimesters when karyotype results are required in a few days, for the diagnosis of fetal hyperthyroidism and hypothyroidism, and in the diagnosis and management of fetal thrombocytopenia. Fetal blood sampling can be achieved by intrahepatic blood sampling and occasionally by umbilical cordocentesis.

Colour Doppler mapping helps to locate the ideal spot for collecting fetal blood, which is preferentially collected from the larger and lower pressure umbilical vein thereby reducing the risk of haemorrhage. The intrahepatic approach is aimed at the intrahepatic site of the umbilical vein. The benefits include not needing to confirm the fetal origin of the sample, lower risk of fetomaternal haemorrhage and, compared with cordocentesis, less streaming (the larger fetal cells would tend to be in the lateral column of blood flow and so could be missed when blood is sampled from the centre of the flow) from the sampling site.[13] Cardiocentesis has a high rate of fetal loss (5%), and is rarely used.[14]

Complications associated with fetal blood sampling include bleeding,[15] cord haematoma (in 17% of cases in one series),[16] transient bradycardia (3 to 12%)[15] and infection (chorioamnionitis in less than 1% of cases).[16] The failure rate of fetal blood sampling is 9% for cordocentesis

Table 2. Causes of an increased nuchal translucency measurement between 11 and 14 weeks' gestation[23]

Chromosomal abnormalities
Cardiac abnormalities
Diaphragmatic hernia
Exomphalos
Skeletal dysplasias
Noonan's syndrome*
Myotonic dystrophy
Spinal muscle atrophy
Smith-Lemli-Opitz syndrome†
Congenital adrenal hyperplasia
Fetal akinesia deformation sequence‡

*Noonan's syndrome is an autosomal dominant condition affecting males and females equally. Incidence is one in 1000 to 2500. Features include short stature, congenital heart defects, indented chest, characteristic facial features, learning problems and impaired blood clotting.

†Smith-Lemli-Opitz syndrome is an autosomal recessive condition affecting males and females equally. Incidence is one in 20,000 to 60,000. Features include multiple congenital abnormalities, mental retardation, dysmorphic facial features, microcephaly, syndactyly between second and third toes, learning and behavioural disabilities, and defective cholesterol synthesis.

‡Fetal akinesia deformation sequence is a rare autosomal recessive condition. Features include intrauterine growth restriction, decreased or absent fetal movements, congenital limb contractures, pulmonary hypoplasia, craniofacial abnormalities and polyhydramnios.

continued

and 5% for intrahepatic vein blood sampling.[13] Maternal complications are unusual. When a fetus is viable, fetal blood sampling is performed in proximity to an operating room in case an emergency caesarean section is required.

Reliability of screening test results

Maternal age is highly predictive of fetal abnormalities. Women aged between 15 and 25 years have a one in 1500 risk of a fetal abnormality and this rate rises almost linearly to a risk of one in 10 at age 45 years.[17] However, given that 49% of pregnancies leading to the birth of a child with Down syndrome occur before 35 years of maternal age, age is no longer used as a criterion to determine who will undergo further testing.[18]

When combined with this maternal age-related risk, nuchal translucency screening detects up to 80% of cases of Down syndrome with a false-positive rate of 5%.[19] The detection rate can be increased to almost 90% when combined with first-trimester biochemical screening (the combined first-trimester test) and almost 95% when followed by second-trimester biochemical screening.[20,21]

For those patients who present in the second trimester and undergo the quadruple test (Table 1), a detection rate of up to 83% is obtained with a 5% false-positive rate. This reiterates the point that first-trimester screening provides a greater accuracy of results.[22]

Who to screen and when

The previous recommendations to screen women aged 35 years and over by nuchal translucency and analyte screening or by second-trimester analyte screening does not recognise the risk for women under 35 years of age. The Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) recommends all pregnant women should be made aware of the availability of prenatal screening tests as early as possible in pregnancy. This allows the women to be offered diagnostic testing options if indicated during screening and therefore make an informed decision of whether to terminate or continue with the pregnancy.[3]

During the first trimester of singleton pregnancies, the RANZCOG recommends the combined first-trimester screening, which comprises an ultrasound scan to measure nuchal translucency (at between 11 weeks and 13 weeks and six days' gestation) and a blood test to measure serum pregnancy-associated plasma protein-A (PAPP-A) and free beta human chorionic gonadotrophin (HCG) levels (nine weeks to 13 weeks and six days).[3] In multiple pregnancies, an ultrasound scan to measure nuchal translucency is the preferred method of screening because the biochemical test becomes increasingly inaccurate with increased fetal numbers.

During the second trimester of singleton and multiple pregnancies when nuchal translucency testing has not been performed, the RANZCOG recommends the quadruple test, which measures levels of alpha fetoprotein, free (or total) beta HCG, unconjugated oestriol and inhibin A. If the laboratory is not equipped to conduct the quadruple test, then the triple test (alpha fetoprotein, free or total beta HCG, and unconjugated oestriol) is recommended. An increased risk is defined as being more than one in 300, as determined by the combined first-trimester screening, or more than one in 250, as determined by the quadruple test. Women found to be at increased risk should be offered definitive diagnostic testing with amniocentesis along with appropriate counselling.[3]

Anencephaly can be diagnosed on an ultrasound scan at between 11 weeks and 13 weeks and six days' gestation, and spina bifida can be defined at the 18 to 20 week morphology scan. The morphology scan is not recommended as a screening tool for trisomy 21 or 18. Women with serum alpha fetoprotein levels of more than 2.0 multiples of the median (MoM) are defined as being at increased risk for a neural tube defect and need to be referred for a diagnostic ultrasound scan in a tertiary centre.[3]

Detection of structural abnormalities

The detection of fetal structural abnormalities depends on the gestational age of the fetus. As mentioned previously, gross abnormalities such as anencephaly may be found at 11 weeks' gestation but small spinal neural tube defects are not detected until the routine morphology scan at 18 to 20 weeks' gestation. Deficient limb growth may not be apparent until the third trimester. In addition to detecting defects in the fetal skeleton, abnormalities of organ development are usually apparent by 18 to 20 weeks' gestation. MT

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A list of references is available on request to the editorial office.

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