CPD Paper 2

Screening for Chromosomal Anomalies: First or Second Trimester, Biochemical or Ultrasound?

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Abstract

Prenatal diagnosis pf chromosomal abnormalities can be accurately made by cytogenetic studies of samples obtained from invasive procedures, such as amniocentesis or chorionic villus sampling. Because these procedures are associated with a risk of miscarriage, the common approach is to perform non-invasive test to define an individual woman's risk of having a chromosomal abnormal pregnancy. Screening for chromosomal abnormalities has developed over the last decade. Prenatal screening can be performed in the late first trimester, the early second trimester or in both. Screening test can be carried out biochemically, ultrasonographically or by both modalities. A major goal of screening test is to achieve maximum accuracy and minimum harm at low cost. The integrated test currently meets best those criteria.

***Ann Acad Med Singapore* 2003; 32:583-9**

Key words: Down's syndrome, Integrated test, Prenatal screening

Introduction

A chromosome abnormality contributes significantly to fetal loss during pregnancy, and perinatal morbidity and mortality. The contribution of chromosomal abnormalities to fetal loss decreases as pregnancy progresses; an estimated 50% of first-trimester spontaneous abortions are due to chromosomal abnormalities. Prenatal screening for aneuploidy (in particular, Down's syndrome) can be undertaken based on maternal age, maternal serum biochemistry, fetal ultrasound or a combination of all 3. Down's syndrome is a frequent (1 in 700) form of mental and physical disability, and prenatal screening has been mainly focused on the detection of trisomy 21. Other chromosomal anomalies, such as trisomies 13 and 18, are clinically less important because they occur less frequently and are often lethal. However, when screening for trisomy 21, other chromosomal anomalies may also be identified.

Prenatal screening for chromosomal abnormalities can be performed in the late first trimester (10 to 14 weeks), the early second trimester (15 to 22 weeks) or in both.[1,2] The introduction of second-trimester biochemical screening was a milestone in the process of antenatal detection of Down's syndrome in a low-risk population.[3,4] The model for assessing a combined risk for Down's syndrome published over a decade ago by Cuckle and colleagues[5] is still valid today and has been expanded to incorporate additional markers.[6]

Subsequently, a first-trimester ultrasound screening test, the measurement of nuchal translucency (NT) thickness, was introduced.[7-10] The recent trend is to generate a calculated risk based on mathematical models that combine maternal-related background risk, NT thickness and crown rump length.[8]

Both first-trimester ultrasound and second-trimester biochemical screening tests are associated with a similar false-positive rate of about 5%[8,10-12] for a detection rate of 50% to 70%.[10,12-14] However, some studies reported that the introduction of first-trimester screening led to decreased efficacy of second-trimester screening.[15,16] Wald et al[2] presented the potential benefit of integrating first- and second-trimester screening test, reaching a detection rate of 85% for a screen-positive rate of 1%.

Screening by Maternal Age

The risk for many of the chromosomal defects increases with maternal age. In 1933, Penrose reported the association between advanced maternal age and birth of a child with Down's syndrome.[17] This has since been strengthened by

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many other reports.[5,18-20] In the early 1980s, antenatal screening for Down's syndrome relied on identifying women above a specific age (cut off age, between 35 and 37 years) and an amniocentesis was usually carried out at about 16 to 18 weeks of pregnancy. The use of maternal age alone does not appear to be an effective screening test, and the traditional estimate that 30% of Down's syndrome cases can be detected using maternal age alone has been recently challenged by Howe et al.[21]

Early Second-trimester Biochemical Screening

Second-trimester serum screening for chromosomal abnormalities is carried out between 15 and 22 weeks of pregnancy. In 1984, an association between fetal chromosomal abnormalities and low maternal serum alpha-fetoprotein (AFP) was noted.[3,5] In 1987, levels of maternal serum free beta human chorionic gonadotrophin (β-hCG) were shown to be about twice as high in Down's syndrome pregnancies as in unaffected pregnancies.[22] This discovery formed the basis of the double test, AFP with either total or β-hCG combined with maternal age, which remained the most widely used method of screening.[22] Later reports showed that the hCG level was raised in Down's syndrome pregnancies and unconjugated oestriol (uE_3) was reduced.[6,23,24] These serum markers formed the basis of the triple test for screening (AFP, uE_3 and total hCG with maternal age) introduced in 1988.[12] The quadruple test calculates the risk of a Down's syndrome term pregnancy from maternal age at term and the concentration of 4 markers in maternal serum (AFP, uE_3, total or free β-hCG and inhibin-A).[25,26] The function of inhibin-A during pregnancy has not been established, but it has been associated with spontaneous fetal loss and Down's syndrome. Maternal serum levels of inhibin increase during the first trimester and decline after about 10 weeks; it remains stable during the second trimester and then rises again to a peak at term.[26,27]

The detection rates for a 5% false-positive rate for all methods were 30%, 58%, 68% and 78% for age alone, double, triple and quadruple tests, respectively. Wald et al[27] confirmed that, in the second trimester, the quadruple test is sufficiently more effective than the double or triple test and that it should be the test of choice in screening for Down's syndrome. The Serum, Urine and Ultrasound Screening Study (SURUSS) agreed with the results from the other studies.[28]

Late First-trimester Biochemical Screening

The advent of chorionic villus sampling has increased the demand for early prenatal diagnosis. This has stimulated the search for biochemical markers for screening in the first trimester (between 10 and 14 weeks) of pregnancy. Moving the test to earlier in the pregnancy has some advantages: earlier reassurance and, if necessary, therapeutic abortions before fetal movements are felt. One disadvantage is that neural tube defect detection would require either a separate AFP test after 15 weeks or reliance on the ultrasound anomaly scan at 18 to 20 weeks. Another disadvantage is that earlier screening preferentially identifies those chromosomally abnormal pregnancies that are destined to miscarry. Approximately 30% of affected fetuses die between 12 weeks of gestation and term.[29] Thus, women are unnecessarily forced to decide to terminate a pregnancy that is going to miscarry.

In 1986, the association between low maternal serum AFP and fetal aneuploidy in the first trimester was reported.[30] Other serum markers have also been studied, such as pregnancy-associated plasma protein-A (PAPP-A), Schwangerschafts protein 1 or pregnancy-specific β_1, cancer antigen 125, free β-hCG, free alpha hCG and inhibin-A.[31,32] Other markers that have been investigated included eosinophil major basic protein p43 and isoferritin p43.[33,34]

Brambati et al[35] first recognised the potential value of measuring maternal serum PAPP-A in screening for fetal aneuploidy in the first trimester. Several studies have confirmed that PAPP-A is low (about 60%) in first-trimester pregnancies affected by Down's syndrome.[36-38] PAPP-A is produced by the placental trophoblast and its function is largely unknown.[39] There are data to suggest that in women with threatened abortion, PAPP-A is a good predictor of fetal demise.[40] Low concentrations of PAPP-A may preferentially identify Down's syndrome pregnancies with the highest risk for fetal death. Low PAPP-A levels are also associated with ectopic pregnancy, hydatidiform mole and Cornelia de Lange syndrome.

Total hCG appeared to be useful for screening performed after 11 weeks of gestation. Free β-hCG is substantially elevated (about 80% higher) in affected pregnancies at 8 to 10 weeks of gestation.[1]

Of all these markers, PAPP-A and free β-hCG appear to be the most effective first trimester biochemical markers in screening for Down's syndrome pregnancies. Both markers, when combined with maternal age, can achieve a detection rate of approximately 67% for a 5% false-positive rate.[41]

Maternal Urine Markers

Maternal urine markers have also been evaluated in the first and second trimesters. Free β-hCG, β-core hCG or total hCG and total estriol have shown a relationship, but the data indicated insufficient differences between affected and unaffected pregnancies.[41-43] Currently, the focus is on hyperglycosylated hCG or invasive trophoblastic antigen (ITA), which is elevated in affected pregnancies.[44] Urine ITA is the only useful urinary marker and is best in the second trimester. When coupled with the quadruple test at

an 85% detection rate, the false-positive rate decreased from 6.2% to 4.2%; when coupled with the integrated test, the false-positive rate decreased to 0.7%.[28]

First-trimester Ultrasound Markers

Improvements in ultrasound technologies have enabled the use of ultrasound markers, which can aid in screening fetuses with chromosomal anomalies.

Nuchal Translucency

The most important ultrasound marker in first-trimester screening for chromosomal abnormalities is the measurement of NT thickness between 10 and 14 weeks of gestation. In the 1990s, Szabo and Gellen[7] realised the potential of measuring NT thickness, which is the excess skin of individuals with Down's syndrome. Subsequently, Nicolaides et al[45] reviewed measurements of fetal NT thickness in predominantly high-risk pregnancies. NT measures the subcutaneous fluid-filled space between the back of the spine and the skin in the fetal neck. Having measured the NT, one must take into account the fact that NT increases with gestational age at about 17% a week. Using NT measurement and maternal age alone, it was estimated that 73% of affected pregnancies could be identified with a 5% false-positive rate. Furthermore, increased NT is attributed to aortic isthmus narrowing, cardiovascular defects which cause overperfusion of the head and neck, or abnormal/delayed development of the lymphatic system.[46,47] Increased NT measurement may also be associated with miscarriage.[48,49]

Nasal Bone

Cicero and colleagues[50] have described a new ultrasound marker, the nasal bone, which was absent in about 70% of fetuses with trisomy 21 and in 0.5% of chromosomally normal fetuses at 10 to 14 weeks of gestation. It was estimated that screening for trisomy 21 by using a combination of maternal age, fetal NT and examination of the nasal bone could increase the detection rate to 85%, whilst lowering the false-positive rate to 1%.[50] The latest findings from Cicero et al[51] suggest that inclusion of the nasal bone yielded a 90% detection rate with a reduction in the false-positive rate from 5% to 0.5%. Alternatively, for a 5% false-positive rate, the detection rate could increase to 97%. Further work is needed to assess the reliability of this marker in a large population and in other ethnic groups.

Combined Test

In the late first trimester, combining the measurement of fetal NT thickness with maternal serum biochemical markers and maternal age was first suggested by Wald and Hackshaw.[52] It is referred to as the combined test.

The use of maternal age, fetal NT thickness and maternal serum free β-hCG and PAPP-A has been shown, both retrospectively and prospectively, that for a false-positive rate of 5%, the detection rate of trisomy 21 is about 90%.[41,53,54] This screening test also detects 90% of other chromosomal anomalies, including trisomy 13, trisomy 18, Turner's syndrome and triploidy.[55-59]

Integrated Test

Recently, a combination of maternal age, NT and first- and second-trimester biochemical markers has been proposed as a highly effective test that could achieve a detection rate of 85% for a false-positive rate of 1.2%.[2] Known as the integrated test, it consists of 2 steps. First, measurements of NT thickness and PAPPA in the late first trimester (about 12 weeks) are taken. Second, the quadruple test is performed in the early second trimester (about 15 weeks). A single risk figure is then obtained. A useful variant of the integrated test, if NT measurement is not available or reliable, is the use of a serum integrated test (using only PAPP-A in the late first trimester) and the quadruple test in the early second trimester. At a detection

TABLE I: SCREENING TEST OF CHOICE: EFFICACY, SAFETY AND COST

Test	Efficacy			Safety	Cost
	DR (%)	FPR (%)	OAPR	Number of procedure-related fetal losses per 100,000 pregnancies	£ per woman screened to achieve an 85% DR*
Integrated test	90	2.8	1:14	20	30
Serum integrated test	88	3.4	1:20	29	29
Quadruple test	84	5.7	1:30	41	28
Combined	83	5.0	1:27	36	30

DR: detection rate; FPR: false-positive rate; OAPR: odds of being affected given a positive screening result

* Costs include diagnosis and termination of pregnancy where applicable

Data extracted from the SURUSS report[28]

rate of 85%, the false-positive rate for the serum integrated test is 2.7%.[28] The integrated test has several advantages. Besides being safe and efficacious, it allows women more time for decision-making. It also allows affected pregnancies that were going to miscarry to do that, rather than making those women go through the anguish of terminating what was a wanted pregnancy. Finally, it has a much better positive predictive value and, therefore, fewer amniocentesis and fewer losses of normal fetuses (Table I).

Future Directions in Chromosomal Screening

The identification of fetal nucleated cells in the maternal circulation is a potentially non-invasive prenatal diagnosis.[60] However, the small number of these fetal cells in maternal blood is a major obstacle to the routine use of this technique. More recently, free fetal DNA in maternal serum has been identified as a screening method.[61,62] Recent data indicate that the detection rate for Down's syndrome was 43% for a false-positive rate of 5.6% but this may change[63] with improvements in technology.

Conclusion

Antenatal screening for Down's syndrome, other than by maternal age alone, has changed significantly since 1991, but there is a wide variation in the methods and markers used. The ongoing debate is when a test should be carried and how, either biochemically, ultrasonographically, or both.

Currently, most antenatal screening takes place in the second trimester using biochemical markers. For women in the second trimester of pregnancy, the quadruple test is the test of choice.[27,28]

The most effective method of screening for chromosomal abnormalities in the first trimester is achieved by the combination of maternal age, fetal NT thickness and maternal serum free β-hCG and PAPP-A.[28,41,53,54]

As a single test, NT screening compares favourably with the maternal serum test (a detection rate of 78% versus 60% for a false-positive rate of 5%). On the other hand, one of the main conclusions from the SURUSS study was that NT is a poor screening test for Down's syndrome, either on its own or with maternal age alone.[28] Ultrasound screening offers significant advantages over maternal serum screening. These include confirmation of embryo viability, accurate assessment of gestational age, early diagnosis of multiple pregnancies and identification of chorionicity, and the detection of major structural abnormalities, major defects of the heart and great arteries, as well as a wide range of skeletal dysplasias and genetic syndromes. In addition, it measures NT thickness in assessing the risk for Down's syndrome.[8] However, the use of ultrasound in routine screening still faces problems with reliability and quality control.

A major goal of screening tests is to achieve maximum accuracy (high detection rate) and minimum harm (low false-positive rate) at a low cost. The integrated test best meets these criteria (Table I) and is closely linked with the best and most widely available diagnostic test, i.e., amniocentesis. With rapid methods for chromosomal diagnosis using polymerase chain reaction or fluorescent in-situ hybridisation, results are available in the early second trimester before fetal movements are felt. Both medical and surgical termination of pregnancy should be made available as, given the choice, most women prefer one or the other.

An achievable goal in chromosomal anomaly screening is a level of efficiency in which amniocentesis and chorionic villus sampling are no longer used as initial or primary diagnostic tools, but are only offered to confirm and define precisely the chromosomal anomaly.

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QUESTIONS

1.

	Down's syndrome	
Screening test	Present	Absent
Positive	TP	FP
Negative	FN	TN

FN: false negative; FP: false positive; TN: true negative; TP: true positive

The following statements are true:

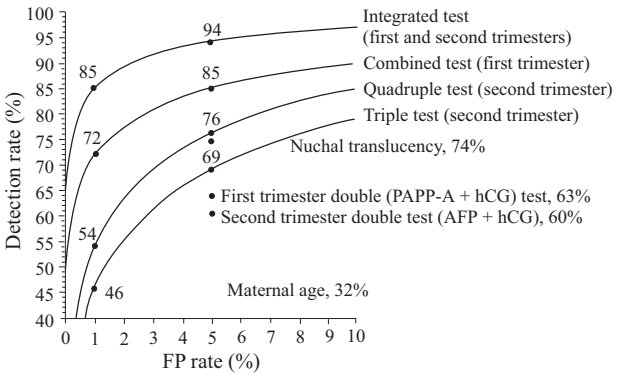
a) Detection rate or sensitivity = TP/(TP + FN)
b) Positive predictive value = TP/(TP + FP)
c) FP rate = FP/(FP + TN)
d) Screen positive rate = (TP + FP)/ (TP + FN + FP + TN)
e) Specificity = TN/(TN + FP) = 1 – FP rate

2. The following statements are true of the 2 x 2 table below.

		Incidence of Down's syndrome deliveries at term	Non-Down's syndrome delivery
Mother's age ≥35 years	4903	31.5	4872
Mother's age <35 years	24,457	26.0	24,431
Total	29,360	57.5	29,303

a) FP rate = FP/(FP + TN) = 4872/29,303 = 16.6%
b) The screen positive rate = 4903/29,360 = 16.7%
c) The sensitivity or detection rate = 31.5/57.5 = 55%
d) The incidence of Down's syndrome pregnancies at term in the absence of intervention would be 57.5/ 29,360 or 1 in 511 deliveries.
e) A receiver operating curve (ROC) with the sensitivity on the y-axis and (1- specificity)/or FP rate on the x-axis can be constructed by varying the cut-off age (or risk) used.

3. The following is true of the ROCs shown below:



a) The ROCs are based on mathematical models and assume a 100% uptake in the screening test for the entire maternal population in the UK, and adjust for differing loss rates of Down's syndrome from the first and second trimesters to term.
b) The 5% FP rate was chosen to keep the amniocentesis rate the same as that using an age-based approach.
c) The ROCs show that for a 5% FP rate, the detection rate for Down's syndrome is 85% using the combined first trimester test (NT , PAPP-A and free beta-hCG), 74% using NT alone and 69% using the second trimester triple test.

d) The ROCs would be different for differing maternal age population structures.
e) A higher cut off risk used (such as 1:250) would have a lower FP rate compared to a lower cut-off risk (such as 1:370) on any ROC.

4. The following statements are true of Down's syndrome screening:
   a) In order to compare the efficacy of first trimester screening versus second trimester screening, the intrauterine lethality rate of Down's syndrome pregnancies between the first and second trimesters must be accounted for.
   b) Approximately 55% to 60% of Down's syndrome pregnancies are lost between 10 weeks of gestation and delivery through spontaneous miscarriage.
   c) Approximately 23% to 30% of Down's syndrome pregnancies are lost between 16 weeks of gestation and delivery.
   d) Earlier screening requires earlier diagnosis by CVS, which carries a higher procedural-associated loss rate compared to amniocentesis.
   e) In counselling for Down's syndrome, the point-estimate risk of Down's syndrome must be balanced against the risk of loss of a normal pregnancy from the intended karyotyping procedure offered.

5. The following statements are true of Down's syndrome screening:
   a) The screening performance of serum and/or NT screening is more efficient than that of screening based on maternal age alone.
   b) The slope of the ROC represents the efficiency of any particular screening methodology.
   c) The various population-based serum screening policies would detect more Down's syndrome pregnancies than an age-related screening policy for the same number of amniocentesis performed.
   d) The number of Down's syndrome pregnancies missed in older mothers by serum screening is more than compensated for by the Down's syndrome pregnancies detected in younger mothers, who would otherwise not be screened using age alone (for the same number of amniocentesis performed).
   e) Second trimester ultrasonography can be used to modify the risk obtained by various first or second Down's syndrome screening tests.