Antenatal Screening

Antenatal Screening for Down's Syndrome

The Combined Test

Information for Health Professionals

The Wolfson Institute of Preventive Medicine
Barts and The London School of Medicine and Dentistry

Antenatal Screening

SUMMARY

- The purpose of screening is to identify women with an increased risk of having a pregnancy with Down's syndrome so that they can be offered a diagnostic test.

- The combined test is a method of screening involving the measurement of two substances in the maternal serum (serum markers) and an ultrasound scan measurement. The serum markers are Pregnancy Associated Plasma Protein-A **(PAPP-A)** and the free ß-subunit of human chorionic gonadotrophin **(free ß-hCG)**. The ultrasound marker is the nuchal translucency **(NT)**.

- The three markers are used together with the woman's age to estimate the risk of having a pregnancy with Down's syndrome. Women with a risk of 1 in 150 or greater are interpreted as screen-positive for Down's syndrome and offered a diagnostic test, usually a CVS. About 1 in 40 of all women screened will fall into the screen-positive group, and about 1 in 10 women with screen-positive results will have an affected pregnancy.

- The combined test identifies 8 - 9 out of 10 cases of Down's syndrome.

- Measurements used as part of the combined test can also identify pregnancies at high risk of Edwards' syndrome (trisomy 18). The test identifies about 6 out of 10 caes of Edwards' syndrome.

CONTENTS

DOWN'S SYNDROME (TRISOMY 21)

Down's syndrome is the most common cause of severe learning disability in children. It arises from an extra copy of chromosome 21 in the cells of the fetus. In the absence of antenatal screening, about 1 in 500 babies born would be affected.

People with Down's syndrome have varying degrees of learning disability, but most often the disability is severe. Some people will lead semi-independent lives while others will be completely dependent. About 40% of Down's syndrome pregnancies will miscarry between 11 weeks and term, but nine out of ten affected babies who reach term will survive their first year. About 40% of babies with Down's syndrome are born with a serious heart defect. The average life expectancy of a person with Down's syndrome is now about 60 years, although most will develop pathological changes in the brain associated with Alzheimer's disease after the age of 40.

EDWARDS' SYNDROME (TRISOMY 18)

Edwards' syndrome is a rare and usually fatal abnormality which is caused by the presence of an extra chromosome number 18 in the cells of the developing baby. In the absence of screening about 1 in every 7,000 babies born would be affected.

TIMING OF THE COMBINED TEST

The test is best performed at 11 weeks of pregnancy, but anytime between 10 and 13 completed weeks is acceptable. An ultrasound scan is performed to estimate the gestational age of the pregnancy and to obtain the nuchal translucency (NT) measurement. A 10ml sample of clotted blood is taken at about the time of the ultrasound scan to measure Pregnancy Associated Plasma Protein-A (PAPP-A) and the free ß-subunit of human chorionic gonadotrophin (free ß-hCG).

INTERPRETATION OF THE COMBINED TEST

The test categorises women into two groups: screen-positive with a high risk of having an affected pregnancy and screen-negative with a lower risk of having an affected pregnancy.

Screen-positive
A woman is screen-positive if the risk of having a pregnancy with Down's syndrome based on the maternal age, the levels of PAPP-A, free β-hCG and the NT measurement, is estimated to be 1 in 150 or greater. About 1 in 40 women screened will be in this group.

Screen-negative
A screen-negative result means that the risk of a pregnancy with Down's syndrome is below the specified risk cut-off. A screen-negative result does not exclude the possibility of an affected pregnancy.

The combined test can identify pregnancies at high risk of Edwards' syndrome (trisomy 18). In cases where the risk is high this is reported.

ACTION FOLLOWING A SCREEN-POSITIVE RESULT

If the result is screen-positive for Down's syndrome the women concerned are offered a diagnostic chorionic villus sampling (CVS) or amniocentesis.

REPORTING OF RESULTS

The screening results are usually ready within 48 hours of receipt of the blood sample and will be sent to the antenatal clinic or doctor who ordered the test. Screen-positive results are telephoned and faxed directly to the doctor or antenatal clinic.

PERFORMANCE OF THE TEST

The screening performance of a test is usually defined in terms of the detection rate (also called 'sensitivity'), false positive rate and the odds of being affected given a positive result (OAPR) which is the ratio of true positives to false positives. The detection rate (DR) is the proportion of affected pregnancies with screen-positive results and the false positive rate (FPR) is the proportion of unaffected pregnancies with screen-positive results.

Down's syndrome
DR = 84% for a 2.2% FPR
OAPR = 1:9 (i.e. among women with a screen-positive result for Down's syndrome one will have an affected pregnancy for every 9 that do not).

CALCULATION OF THE RISK OF DOWN'S SYNDROME

Maternal Age
The risk of having a pregnancy with Down's syndrome increases with maternal age as shown in Table 1 opposite. The maternal age-specific risk is the background risk of Down's syndrome that is used to calculate a woman's screening result based on the measurement of the screening markers.

Table 1

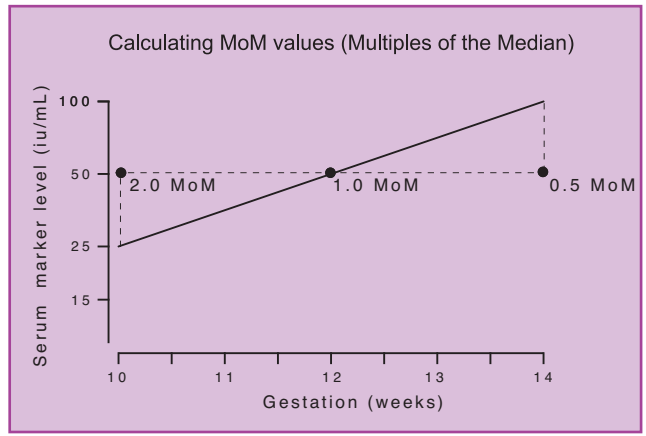
Maternal age at EDD*	Risk of Down's syndrome+	Maternal age at EDD*	Risk of Down's syndrome+	Maternal age at EDD*	Risk of Down's syndrome+
20	1:1450	30	1:940	40	1:85
21	1:1450	31	1:820	41	1:70
22	1:1450	32	1:700	42	1:55
23	1:1400	33	1:570	43	1:45
24	1:1400	34	1:460	44	1:40
25	1:1350	35	1:350	45	1:35
26	1:1350	36	1:270	46	1:30
27	1:1200	37	1:200	47	1:30
28	1:1150	38	1:150	48	1:30
29	1:1050	39	1:110	49	1:25

*EDD = expected date of delivery
+Ratio of affected to unaffected pregnancies

Morris et al (2003)

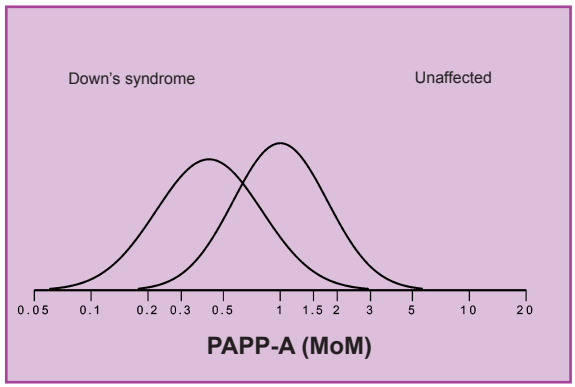
The markers

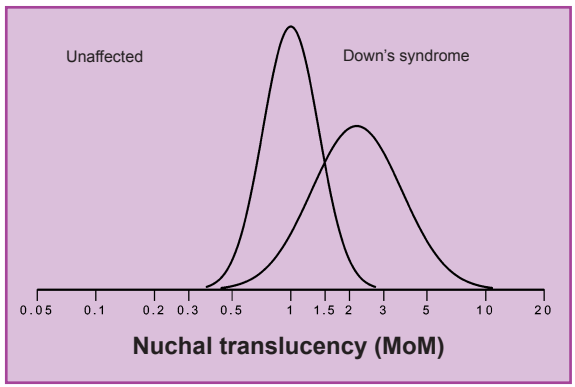
The first trimester maternal serum PAPP-A level is, on average, low in Down's syndrome pregnancies (about half that of unaffected pregnancies), the free β-hCG level is, on average, high (about double that of unaffected pregnancies) and the NT measurement is, on average, high (about double that of unaffected pregnancies).

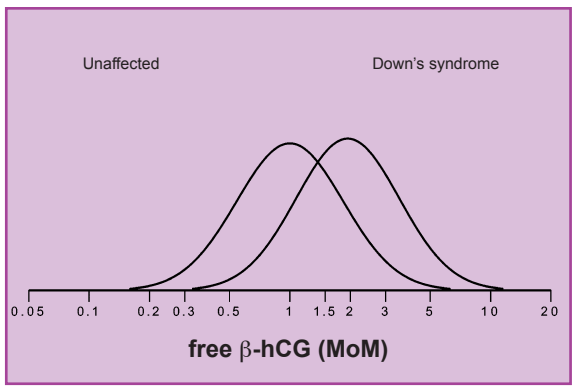


The concentrations of the three markers vary with gestational age (PAPP-A and NT increase, free β-hCG decreases). To take account of this variation, the concentration of each marker is expressed as a multiple of the median for unaffected pregnancies of the same gestational age (MoM).

The figure above illustrates the concept. A hypothetical marker has a median level of 25 iu/mL at 10 weeks, 50 iu/mL at 12 weeks and







100 iu/mL at 14 weeks. If a woman were found to have a level of 50 iu/mL at 10 weeks her level would be twice the median (50/25) or 2.0 MoM. Similarly if the level were 50 iu/mL at 14 weeks this would be half the median (50/100) or 0.5 MoM.

Risk of Down's syndrome in relation to marker levels

The graphs on this page show the overlapping relative frequency distributions of PAPP-A, NT and free β-hCG in Down's syndrome pregnancies and unaffected pregnancies. The points of intersection are the values at which the risk of Down's syndrome is the same as the background risk in the population. From these graphs it can be seen that PAPP-A values below 0.64 MoM and NT and free β-hCG values above 1.46 MoM and 1.38 MoM respectively will tend to increase the risk of Down's syndrome above the background risk while values in the opposite directions will tend to decrease the risk.

FACTORS AFFECTING THE TEST

Maternal weight, ethnic group and smoking

- Serum marker levels tend to be decreased in heavier women, and increased in lighter women.
- PAPP-A levels tend to be about 60% higher in Afro-Caribbean women than in Caucasian women.
- PAPP-A levels tend to be about 20% lower in women who smoke.

Appropriate adjustments of the MoM values are made for these factors.

Twins

The serum marker levels are raised in twin pregnancies. Adjustments are made to take account of this.

Screening in twin pregnancies poses a difficulty because of the possibility that one fetus may be affected and the other may not. Because of the presence of two fetuses the CVS and amniocentesis are slightly more complex procedures in a twin pregnancy. If one twin is found to be affected and the other unaffected, selective feticide can be offered. This procedure poses a substantial risk to the unaffected twin. The presence of a twin pregnancy may therefore be seen by some women as a reason to avoid screening.

Previous affected pregnancies

If a previous pregnancy with Down's syndrome is reported, the result will be classified as 'screen-positive' regardless of the level of the screening markers so that further testing can be discussed with the woman. A risk is calculated which takes account of a woman's previous pregnancy with Down's syndrome. The woman's age at the time of her previous pregnancy with Down's syndrome affects the recurrence risk and this is taken into account in the risk interpretation.

Taking account of screening in a previous pregnancy

If a woman has been screened for Down's syndrome in a previous pregnancy the levels of the screening markers in that pregnancy can be used to adjust the marker levels in the current pregnancy. This is useful because markers used in screening tend to 'track' between pregnancy (e.g. a free ß-hCG level that is high in one pregnancy tends to be high in a subsequent pregnancy). So a woman with a false positive result in one pregnancy is likely to have a false positive result again in a subsequent pregnancy. Adjusting marker levels for those in a previous pregnancy can help avoid this problem of false-positives recurring in different pregnancies.

DIAGNOSTIC TESTS

Chorionic Villus Sampling (CVS)

A CVS is performed at about 11 to 14 weeks of pregnancy. Under ultrasound guidance and using a local anaesthetic, a sample of placental tissue is collected either using a needle inserted through the abdominal wall or with a fine forceps passed through the cervix.

Cells from the sample can be used to diagnose Down's syndrome. There is a small risk of miscarriage (about 1%) associated with the procedure and in about 1% of cases it is not possible to obtain a conclusive result in which case an amniocentesis is required.

Amniocentesis

An amniocentesis is performed at about 15 to 16 weeks of pregnancy. Under ultrasound guidance a sample of amniotic fluid is collected using a needle inserted through the abdominal wall. Cells from the sample can be used to diagnose Down's syndrome. The risk of miscarriage is similar to that associated with a CVS (about 1%).

Down's syndrome, trisomies 13 and 18 and sometimes sex chromosomes can be diagnosed using a technique called quantitative fluorescence polymerase chain reaction (QF-PCR). This provides a rapid diagnosis of these conditions, usually within 48 hours. To diagnose other conditions the cells must grow before they are examined and so the final results can take up to 10 days (CVS) or 2 - 3 weeks (Amniocentesis).

EFFECT OF MATERNAL AGE ON SCREENING PERFORMANCE

An older woman is more likely to have a screen-positive result than a younger woman as she starts with a higher age-specific risk of Down's syndrome. For this reason, the test is more likely to detect a Down's syndrome pregnancy in an older woman than in a younger woman. Table 2 below shows, using a 1 in 150 cut-off, according to age, the probability of a screen-positive result and the proportion of Down's syndrome pregnancies detected. Whatever the woman's age, the best estimate of her risk of having an affected pregnancy is obtained using her age in conjunction with her marker values.

Table 2

Maternal age group (years)	Probability of a screen-positive result	Proportion of Down's syndrome pregnancies detected (%)
Under 25	1 in 120	71
25-29	1 in 100	72
30-34	1 in 50	78
35-39	1 in 20	86
40-44	1 in 7	93
45 and over	1 in 4	95
All	1 in 40	84

(early mid trimester estimates, test performed at 11 completed weeks of pregnancy)

COMPARISON WITH OTHER DOWN'S SYNDROME SCREENING TESTS

Table 3 below shows the estimated detection rate (DR) and odds of being affected given a positive result (OAPR) for various Down's syndrome screening methods using a 3% fixed false positive rate (FPR) and, for the integrated test, also using a 1% false positive rate. The estimates are based on a large UK study (Wald et al 2003) and apply to the early second trimester of pregnancy. They are corroborated by results from other studies.

Table 3

Method of screening	FPR(%)	DR (%)	OAPR
Maternal age alone	3	25	1:45
Triple test (AFP, uE_3 , free ß-hCG)	3	71	1:15
Quadruple test (AFP, uE_3 , free ß-hCG, inhibin)	3	78	1:14
Combined test (Nuchal translucency [NT], free ß-hCG. PAPP-A at 11 weeks)	3	86	1:12
Serum Integrated test (PAPP-A at 11 weeks and Quadruple markers at 14-22 weeks)	3	83	1:13
Integrated test (NT and PAPP-A at 11 weeks and Quadruple markers at 14-22 weeks)	3	94	1:11
	1	89	1:4

(Gestational age estimated by ultrasound scan and marker levels adjusted for maternal weight)
NB All tests include maternal age

POINTS TO REMEMBER WHEN DISCUSSING THE SCREENING TEST WITH A WOMAN CONSIDERING WHETHER TO BE SCREENED

- Obtain an explicit decision on whether to be screened.
- Assess her knowledge of Down's syndrome and whether more information is needed.
- Satisfy yourself that she understands that the test does not give a definitive answer – it divides women into a higher risk group (screen-positive) and a lower risk group (screen-negative). For Down's syndrome the result is screen-positive if the risk is 1 in 150 or greater.
- Explain that about 1 in 40 women screened will have a screen-positive result for Down's syndrome and they will be offered a CVS or an amniocentesis, both of which carry a risk of miscarriage. Most women with a screen-positive result will **not** have affected pregnancies.
- Check that she knows that the test will not detect all pregnancies with Down's syndrome.
- Explain that in the few pregnancies in which Down's syndrome is diagnosed, the woman will be offered a termination of pregnancy.

Women should have the opportunity to have time to consider whether to be screened, and discuss this with others before making a decision. While screening cannot provide complete reassurance and will cause anxiety, particularly if the screening test is positive, it provides the opportunity of finding out whether the pregnancy is affected with Down's syndrome. If women do not want this information while pregnant screening is best avoided.

OTHER SCREENING APPROACHES

Patients should know that other tests such as the integrated test provide better screening performance. The Integrated test is a more effective test because it has a higher detection rate for a lower false positive rate (see table 3). This means that with the integrated test fewer women need to have an invasive diagnostic procedure and more Down's syndrome pregnancies can be detected. The integrated test avoids the dilemma of choosing a separate screening test in the second trimester with the confusion of receiving two different risk estimates in the same pregnancy. It also permits screening for open neural tube defects based on early second trimester alpha-feto protein (AFP) measurement.

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The Wolfson Institute of Preventive Medicine has played a leading role in the discovery, development and implementation of antenatal screening methods. It is committed to improving the efficacy and safety of screening. We use information collected as part of our screening programme, including measurements on stored blood samples, to audit our screening programme and ensure that it is meeting our expected quality standards. Such information may also be used to help discover and validate new tests that improve the quality of screening services.

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