

Screening for Down Syndrome

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Down syndrome is the commonest chromosomal defect with a live birth incidence of 1/670. It is associated with a spectrum of mental and physical handicap and in view, some couples will elect to terminate the pregnancy.

It is important to differentiate between screening and diagnostic testing.

Screening will give an estimated risk of affection, there is no such thing as a "no-risk" result

Invasive testing i.e chorionic villus sampling (CVS) or amniocentesis can be offered to high risk pregnancies by screening to confirm affection. They are associated with a risk of miscarriage; therefore one important feature of a screening test is the low risk of loss of a karyotypically normal fetus.

1st trimester screening

A number of maternal serum markers have been investigated in order to determine their potential use, between the 10th and 14th week of pregnancy, in cases of singleton pregnancies. Some of these markers are also used in second-trimester prenatal Down syndrome screening¹. The results of assays of these serum markers, which are expressed in multiples of the median (MoM), not in absolute values, are used to calculate a likelihood ratio. The ratio is then multiplied by the prevalence of Down syndrome for the mother's age to arrive at an estimate of the individual risk, expressed as 1/N, for each woman. The median is the value observed for serum markers in unaffected pregnancies of the same gestational age in the reference population.

Two markers seem to offer the most accuracy during the first trimester: PAPP-A and the free β -subunit of chorionic gonadotropin (β -hCG), both of which are measured between the 8th and 13th week of pregnancy, the ideal time being before the 12th week².

2nd trimester screening

Alpha fetoprotein is a fetal specific protein produced initially by the fetal yolk sac and subsequently by the fetal liver.

Alpha fetoprotein is lower in pregnancies affected by trisomy 21 with a pooled median multiples of the median (MOM) of 0.75.

Intact hCG and free β -HCG are the most discriminatory for trisomy 21 compared with normal pregnancies with a MOM of 2.06.

Adding other markers, such as inhibin-A, measured between 10 and 14 weeks gestation, does not seem to improve the performance of Down syndrome screening³. In this report, I have limited myself to reviewing studies that included PAPP-A and free β -subunit hCG measurements. β -hCG values are almost twice as high (1.8 MoM) and PAPP-A values 50% lower (0.4 MoM) in Down syndrome fetuses compared to the values observed in unaffected singleton pregnancies. The efficacy of the PAPP-A measurement decreases after the 14th week, its median value being 0.9 MoM at that point and 1 MoM between the 17th and 19th week⁴.

The combination of these markers and maternal age yields a detection rate of 62%, with 5% false-positive results¹. Other markers and different combinations have been investigated, but the results were even less accurate⁵. Down syndrome screening based on serum markers seems to have low sensitivity in twin pregnancies, although this has been investigated very little.

The detection rate observed by combining β -hCG and PAPP-A measurements and maternal age is between 56 and 67% (5% false-positive results). The estimated detection rate for a given population, based on a model using calculated efficacy data and the risk according to maternal age, is between 49 and 79%. These rates are relatively uniform, based on observations in various studies, and they compare with the performance obtained with two markers measured during the second trimester, although the efficacy is lower compared to the second-trimester triple and quadruple markers⁶.

Comparable results were obtained by Cuckle and Vanlith by combining, in a meta-analysis, the results of 44 studies that had examined the performance of prenatal screening with different markers between the 9th and 11th week. The detection rate obtained with PAPP-A (18 studies) and β -hCG (17 studies) combined was 64.6%, with 5% false positives⁷.

However, a certain number of affected pregnancies end spontaneously between the first and second trimester. It is estimated that 43% of Down syndrome pregnancies end spontaneously in abortion or stillbirth between the chorionic biopsy

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(first trimester) and term. The figure is 23% between the amniocentesis (second trimester) and term⁸.

Given these spontaneous fetal losses, the detection rate has to be at least 8.3% higher in order for the efficacy of first-trimester screening to be superior to that O* second-trimester screening⁹. Berry et al. compared the proportion of pregnancies considered to be at high risk upon first-trimester screening with those at high risk identified during the second trimester.¹⁰ They compared the first-trimester measurements of free β -hCG and PAPP-A and the second-trimester measurements of AFP and hCG for 45 Down syndrome pregnancies. Upon combining the risk obtained with that associated with maternal age, they detected, during the first trimester, 27 of the 45 cases (60%). Second-trimester screening detected 39 of the 45 cases, or 12 additional cases (87%)¹⁰.

Most of the published studies of first-trimester maternal serum screening were retrospective and involved high-risk women. Although the efficacy of first trimester prenatal screening is relatively well established, its effectiveness has not been investigated. Certain factors make it difficult to calculate the detection rate and to assess its impact on the decrease in the prevalence of Down syndrome¹¹.

First, it is possible that the detection rate is overestimated, since, when calculating it, spontaneous losses of Down syndrome fetuses are not always included in the denominator. This applies to second-trimester screening as well. Second, since most pregnancies diagnosed with aneuploidy were terminated voluntarily, the relationship between first-trimester screening and spontaneous pregnancy terminations cannot be evaluated. If spontaneous abortions occur mainly during pregnancies considered to be at high risk, screening will have little impact on the birth prevalence of Down syndrome. Furthermore, if this assumption is correct, many pregnant women will be confronted with the choice of voluntarily terminating their pregnancy, even though it could terminate spontaneously. The repercussions of this have not been assessed. The same overestimation bias is present when the outcome of the pregnancy is not known in all cases and when certain cases of aneuploidy may not be included in the denominator for test performance calculations¹¹.

Estimating the risk with second-trimester maternal serum markers requires certain adjustments for maternal weight and smoking, parity, fetal sex, the presence of type 1 diabetes, if applicable, and the mother's ethnic origin; the effect of these factors on the calculation of second-trimester marker MoM is well documented, which

is not the case for the first trimester markers. However, in practice, the said adjustments are not always made. Spencer et al. examined the influence of ethnic origin in a population of 5,422 Caucasian women, 752 Afro-Caribbean women and 170 Asian women following first trimester marker screening. The serum marker levels were significantly different between the groups, but the impact or the correction on the detection rate was relatively modest, namely, a 1.4% increase¹².

Maternal weight and smoking also have a significant influence on first-trimester marker levels, while gravidity, parity and fetal sex seem to have little influence¹³. A recent exploratory study revealed a significant correlation, during subsequent pregnancies, between the results of first-trimester biochemical marker screening. In a woman who is screen-positive for Down syndrome during her first pregnancy, the probability of having the same result during subsequent pregnancies is 1.5 to 2 times higher. This correlation had previously been observed during second-trimester screening. However, the same exploratory study showed that there is no such correlation between different pregnancies study showed that there is no such correlation between different pregnancies in a given woman with regard to nuchal translucency¹².

Table 1. Factors affecting serum biochemical markers.

Variable	Serum Marker
Weight	Inversely proportional
IDDM	Decrease Urinary E ₃ and inhibin A total/free hCG unchanged AFP? significant effect
Recent Bleeding during pregnancy	Increase AFP
Afrocaribbean race	Increase AFP +increase hCG
Smoking	Decrease total /decrease free β hCG

First-trimester biochemical marker measurement can also detect 63% of cases of trisomy 18¹⁵, but it does not detect open neural tube defects. The serum hCG, β -hCG and PAPP-A concentrations were 0.31, 0.22 and 0.30 MoM, respectively, in pregnancies with trisomy 18 fetuses¹⁴. The spontaneous abortion rate is 83% between the first

trimester and term for trisomy 18 or 13 (Patau's syndrome)¹⁵.

Nuchal Translucency and Maternal Serum Biochemistry

Trisomic pregnancies are associated with altered maternal serum concern ratios of various fetoplacental products, including AFP, free β -hCG, uE3, inhibin A and PAPP-A. Screening in the second trimester by maternal age and various combinations of free β -hCG, AFP, uE3 and inhibin A can identify 50-75% of trisomy 21 pregnancies for a false positive rate of 5%. Screening in the first trimester by a combination of maternal age and serum free β -hCG and PAPP-A identifies about 60% of affected pregnancies for a false positive rate of 5%. However, an essential component of biochemical screening is accurate dating of the pregnancy by ultrasound; otherwise the detection rate is reduced by about 10%¹⁶.

Fetal NT and maternal serum testing in the first-trimester

In trisomy 21 pregnancies at 12 weeks, the maternal serum concentration of free β -hCG (about 2 MoM) is higher than in chromosomally normal fetuses whereas PAPP-A is lower (about 0.5 MoM). The difference in maternal serum free β -HCG between normal and trisomy 21 pregnancies increases with gestation. These temporal variations in marker levels, their interrelation and their association with maternal weight should be taken into account when developing risk algorithms in order to produce accurate patient-specific risks¹⁸.

There is no significant association between fetal NT and maternal serum free β -hCG or PAPP-A in either trisomy 21 or chromosomally normal pregnancies and therefore the ultrasonographic and biochemical markers can be combined to provide more effective screening than either method individually¹⁸. Six prospective screening studies have confirmed the feasibility and effectiveness of combining fetal NT and maternal serum free β -hCG and PAPP-A. In the combined data on a total of 38,804 pregnancies, including 182 with trisomy 21, the detection rate for trisomy 21 at a 5% false positive rate was 86%¹⁸.

In trisomies 18 and 13 maternal serum free β -hCG and PAPP-A are decreased. In cases of sex chromosomal anomalies maternal serum free β -hCG is normal and PAPP-A is low. In paternally derived triploidy maternal serum free β -hCG is greatly increased, whereas PAPP-A is mildly decreased maternal serum free β -hCG and PAPP-A. Screening by a combination of fetal NT and maternal serum PAPP-A and free β -hCG can

Table 2. Ultrasound anomalies of trisomy 21

Structural anomalies	Soft markers
Cystic hygroma	Increased NT
AV - septal defect	Short femur/humerus
VSD	Echogenic bowel
Duodenal atresia	Echogenic intracardiac focus
Ventriculomegaly	
Exomphalos	Renal pyelectasis
Hydrothorax	Hypoplasia of middle phalanx of 5th finger
	Sandal gap

identify about 90% of all these chromosomal abnormalities for a screen positive rate of 1%, in addition to the 5% necessary in screening for trisomy 21. An important development in biochemical analysis is the introduction of a new technique (random access; immunoassay analyzer using time-resolved-amplified-cryptate-emission), which provides automated, precise and reproducible measurements within 30 minutes of obtaining a blood sample, this has made it possible to combine biochemical and ultrasonographic testing as well as to counsel in one-stop clinics for early assessment of fetal risk (OSCAR)^{20,17}.

Ultrasound standards

The Ultrasound Standards Working Group has derived the following specific ultrasound standards from the above antenatal screening standards:

- All pregnant women must have an early dating scan when undergoing Down syndrome screening. Ideally this should be:
 - Before serum screening
 - Ideally between eight weeks and 12 weeks and six days for most accurate dating.
 - Ideally between 11 weeks and 13 weeks and six days if nuchal translucency (NT) is to be measured.
- There should be a regionally or locally agreed written policy and protocol that adheres to national standards and defines the purpose of the early dating scan including the possibility of detecting an abnormality.
- Written information must be given and discussed with all pregnant women prior to the screening procedure, to enable them to make an informed choice.
- Gestational age assessment is by measurement

of crown-rump length (CRL) before 13 weeks and head circumference (HC) or biparital diameter (BPD) after 13 weeks.

5. Techniques and biometric charts used for fetal measurements must meet nationally agreed standards:
 - CRL, HC, BPD as advised by the British Medical Ultrasound Society.
 - NT to be developed.
6. Any health professional undertaking an ultrasound scan must have an accredited certificate in obstetric ultrasound or equivalent.
7. Equipment standards must be in place for the specification, maintenance schedule and upgrading of scanning equipment.
8. There should be continuous assessment and monitoring of the quality of the ultrasound screening programme, which includes operator performance and patient satisfaction with the service.
9. There should be an identified professional lead in each maternity ultrasound or radiology unit who is accountable for service quality and responsible for local processes of dealing with poor performance and system failures.
10. Measurements and results of ultrasound scans should be recorded in the women's pregnancy health record and in the ultrasound clinical information system or written record.
11. All health professionals performing ultrasound scans should attend an appropriate communication/counseling course.

There is general consensus among most experts in maternal- fetal medicine that earlier diagnosis is better as long as it remains safe. Three recent studies (2 of which have been released) demonstrate that first-trimester screening is better or at least equal to second-trimester screening for the detection of Down syndrome. There are two national Institute of Health studies. First Trimester Maternal Serum Biochemistry and Fetal Nuchal Translucency Screening (BUN) Study Group and the first and the second Trimester evaluation of risk for Aneuploidy study group (FASTER).

The FASTER trial has not yet been published but is available in abstract form from many recent meetings. Hopefully, the long-awaited results will be published later in 2005. A third study comes from the UK, entitled the serum, Urine, and Ultrasound screening study (SURUSS)²⁰.

The BUN study was conducted at 12 prenatal diagnostic centers in North America, and screening was completed in 8514 patients with singleton pregnancies. Patients of any age with a singleton pregnancy between 74 and 97 days gestation were offered prenatal diagnosis using the blood tests for pregnancy-associate plasma protein A(PAPP-A)

and free beta human chorionic gonadotropin (HCG) along with nuchal translucency. The results revealed 78.7% of cases of Down syndrome with a false-positive rate of 5%. This first-trimester combined screening compares favorably with the second-trimester Quad test, which samples 4 analyses, including AFD, estriol, HCG, and inhibin-A.

SURUSS took place at 24 prenatal centers in the UK and 1 in Austria. . It evaluated 47,053 pregnancies and found a detection rate of 93% for Down syndrome with a false-positive rate of 5%. The study found that first-trimester screening was as effective as second-trimester screening but not as good as combined (integrated) screening.

Table 3. Types of test combinations to screen for Down syndrome

Combined test	First trimester test based on combining NT measurement with free β -human chorionic gonadotrophin (β -hCG), pregnancy associated plasma protein A (PAPP-A) and maternal age.
Integrated test	The integration of measurements performed during the first and second trimester of pregnancy into a single test result (integrated test is qualified as integration of NT and PAPP-A measurements in the first trimester with the quadruple test markers in the second).
Quadruple test	Second trimester test based on the measurement of A-fetoprotein, unconjugated estriol, free β -hCG (or total hCG) and inhibin A, together with maternal age.
Serum integrated test	A variant of the integrated test using serum markers only (PAPP-A in the first trimester and the quadruple test markers in the second trimester).

Although this integrated test does have some benefits, the problem is that a patient needs to still wait to second trimester to get her results.

The FASTER trial involved 33,557 patients undergoing integrated first- and second-trimester testing. The results of FASTER are still awaiting final publication this year. Abstracts from previous meetings indicated that the sensitivity of screening for Down syndrome was nearly 90% with a 5% false-positive rate, which is similar to the results of the SURUSS trial.

One interesting result that FASTER did release was the safety of amniocentesis in the tertiary care

setting. Obstetricians usually quote patients a procedure loss rate of 1/200 (0.5%), although many of these studies were done before the use of ultrasound guidance. FASTER demonstrated a loss rate of 1/667 (0.15%), which is much lower than patients are quoted. The safety of amniocentesis changes the entire paradigm of prenatal diagnosis, as the age of 35 has been deemed to be the time when the risk of Down syndrome is greater than the risk of loss from amniocentesis.

Now, what if women could undergo a definitive test that would not have any miscarriage risk (i.e., even lower than the 1/667 from the FASTER trial)? For many years, the National Institute of Health has been funding studies to look for fetal cells in maternal circulation, hoping that this could lead to prenatal diagnosis. Unfortunately, this has not panned out, yet. However, an even more exciting possibility exists! Apparently, fetal trophoblasts can be recovered from cervical secretions. Biocept Laboratories, San Diego, California, has a product called *preCEED*TM (Prenatal Cell Enrichment and Extraction Diagnostic), which creates an enriched specimen of fetal trophoblasts from endocervical mucus and then analyzes these cells. Prenatal diagnosis could then be used by standard fluorescence in situ hybridization (FISH) technology.

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