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Biochemical serum markers for Down syndrome screening.

Review

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SUMMARY.

Down syndrome is caused by an extra chromosome 21 and is the most common autosomal chromosome aberration in which affected individuals survive beyond infancy. Down syndrome results in mental retardation and several congenital malformations.

The overall prevalence of this disease varies from 1:40 to 1:900 births. The risk of carrying a Down syndrome fetus depends on various factors, and it increases almost exponentially with maternal age.

The methods established for detecting the disease are based on testing the fetus, either directly, by invasive techniques, or indirectly, based upon indices of relative fetal development. Maternal serum screening is a non-invasive method. It relies on the measurements and quantification of multiple biochemical markers such as: alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), free β -hCG, unconjugated estriol, and inhibin-A. These

concentrations increase or decrease depending on whether they belong to a fetus with this anomaly or not.

We review the main characteristics of this disease, as well as the methods of detection used, and the biochemical serum markers for Down syndrome screening with a higher reliability and *detectability*. (*Rev Biomed 2005; 16:259-271*)

Key words: Down syndrome, AFP, hCG, free β -hCG, unconjugated estriol, inhibin-A.

RESUMEN.

Marcadores bioquímicos séricos para el tamizaje del síndrome de Down.

El síndrome de Down es un desorden que implica una copia extra del cromosoma 21 y es la principal aneuploidía autosómica, en la cual los individuos afectados sobreviven más allá de la infancia. Se caracteriza por el retraso mental y varias malformaciones congénitas.

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La prevalencia de esta enfermedad varía desde 1:40 hasta 1:900 nacimientos. El riesgo de la embarazada de portar un feto con síndrome de Down depende de varios factores, incrementándose éste a medida que aumenta la edad materna.

Los métodos utilizados para detectar esta enfermedad se basan en pruebas realizadas al feto mediante técnicas invasivas o la medición de índices del desarrollo fetal mediante técnicas no invasivas. Los más empleados son la determinación y cuantificación en suero de varios marcadores bioquímicos tales como: la alfafetoproteína (AFP), la hormona gonadotropina coriónica humana (hCG), la subunidad b libre de la hCG, la inhibina-A y el estriol no conjugado, cuyas concentraciones aumentan o disminuyen dependiendo de si corresponden o no a un feto con esta anomalía.

En este artículo se revisan las características principales de esta enfermedad, los métodos de diagnóstico empleados y los marcadores bioquímicos presentes en el suero que ofrecen mayor confiabilidad y detectabilidad empleados en el tamizaje del síndrome de Down.

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Palabras clave: síndrome de Down, alfafetoproteína, hCG, b-hCG libre, inhibina A, estriol no conjugado.

INTRODUCTION.

Down Syndrome (DS), or Trisomy 21, is the most common serious autosomal chromosome aberration in which affected individuals survive beyond infancy (1-3). It is the most frequent form of mental retardation and is characterized by well-defined and distinctive phenotypic features and natural history. Down children have a widely recognized characteristic appearance. The head may be smaller than normal and abnormally shaped. Prominent facial features include a flattened nose, protruding tongue, and upward slanting eyes (Mongolian slant). The hands are short and broad with short fingers and often have a single palmar crease. Retardation of normal growth and

development is typical, and most affected children never reach average adult height. The average mental age achieved is that of an 8 year old (2, 3). The severity of the syndrome includes congenital cardiac malformation, immune system disorders, gastro-intestinal malformation such as esophageal and duodenal atresia, and slow physical development (3).

Human cells normally have 46 chromosomes which can be arranged in 23 pairs. These cells divide into two ways: mitosis and meiosis. Many errors can occur during cell division. An error in cell development results in forty-seven chromosomes rather than the usual forty-six. In meiosis, the pairs of chromosomes are supposed to split up and go to different spots in the dividing cell; this event is called "disjunction." However, occasionally one pair does not divide, and the whole pair goes to one spot. This means that in the resulting cells, one will have 24 chromosomes and the other will have 22 chromosomes. This accident is called "nondisjunction." If a sperm or egg with an abnormal number of chromosomes merges with a normal mate, the resulting fertilized egg will have an abnormal number of chromosomes. In DS, 95% of all cases are caused by this event: a cell has two 21st chromosomes instead of one, so the resulting fertilized egg has three 21st chromosomes. Hence the scientific name, trisomy 21. Roughly four percent have translocation, where the extra chromosome twenty-one is broken off and becomes attached to another chromosome. About one percent has mosaicism. These people have a mixture of cell lines, some of which have a normal set of chromosomes and others have a trisomy 21 (4, 5). In addition, DS gives rise to a variety of traits with variable expressivity and penetrance (6).

The overall birth prevalence of DS is approximately 1 per 900 births (2, 5).

The 21st chromosome and Down syndrome.

Chromosomes are thread-like structures composed of DNA and other proteins. They are present in every cell of the body and carry the

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genetic information needed for that cell to develop. The chromosomes are the holders of the genes. In DS, the presence of an extra set of genes leads to over-expression of the involved genes, leading to an increased production of certain products (1). For most genes, their over-expression has little effect due to the body's regulation mechanisms of genes and their products. But the genes that cause DS appear to be exceptions. On trisomy 21, it has been found that only a small portion of the 21st chromosome actually needs to be triplicated to get the effects seen in this serious disorder (5). This is called the Down syndrome critical region. The 21st chromosome may actually hold 200 to 250 genes (being the smallest chromosome in the body in terms of total number of genes); but it has been estimated that only 20 to 50 genes may eventually be included in the Down syndrome critical region (5).

Genes that may have input into DS include:

- Superoxide Dismutase (SOD1).
- COLGA1- over-expression may be the cause of heart defects.
- ETS2- over-expression may be the cause of skeletal abnormalities and/or leukaemia.
- CAF1A- over-expression may be the cause of detrimental DNA synthesis.
- Cystathione Beta Synthase (CBS)- disrupts metabolism and DNA repair.
- DYRK- over-expression may be the cause of mental retardation.
- CRYA1- over-expression may be the cause of cataracts.
- GART- over-expression may be the cause of disrupted DNA synthesis and repair.
- IFNAR- over-expression may interfere with the immune system as well as with other systems.

Other genes that are also suspects include APP, GLUR5, S100B, TAM, PFKL, and a few others.

Prenatal Screening for fetal Down's Syndrome.

It has been estimated that the cost to detect

each case of fetal Down syndrome is much lower than the average cost of health care, education, and residential costs for such an individual. At present there is no screening test available which is capable of specifically detecting parental predisposition for offspring with DS. The established methods of detection are therefore based on testing the fetus, either directly by invasive techniques or indirectly based on indices of relative fetal development.

Amniocentesis is an invasive technique in which a sample of the amniotic fluid is removed from the uterus. This method is associated with approximately 0.5-1 % increase in the risk of spontaneous abortion (7). It may be performed between 14-16 weeks of gestation. Chorionic villus sampling (CVS) is another invasive procedure performed in the first trimester (between 10-12 weeks of gestation), where a small piece of chorionic villus for chromosomal analysis is withdrawn. CVS results approximately in a 1.5% increased risk of spontaneous fetal losses through 28 weeks of gestation (8, 9).

Both CVS and amniocentesis may be used as prenatal diagnostic tests for Down syndrome and can conclusively determine its presence in the fetus with a high degree of reliability. The risk associated with invasive diagnostic procedures and the costs of the analyses preclude the adoption of these methods for mass screening of prenatal women (9).

The risk of conceiving a fetus with DS is significantly related to maternal age. This risk increases almost exponentially with age.

Down syndrome screening has been offered to pregnant women since the early 1980s. The first screening programs for DS relied upon amniocentesis offered only to women of 35 years of age or older, thus being a high risk population screening approach. However, using this approach only about 20 - 30% of all Down syndrome pregnancies could be detected, because the majority of these children are born from women under 35 years old. In spite of the higher risk of having DS babies in older mothers, about 80% of these babies are born from young women. By using the maternal

age of 35 years or older as the criteria for prenatal screening, most of the cases that occur in women under 35 years old remain undetected.

Maternal serum screening is a non-invasive method. The objective of this screening is to reduce the proportion of women who have to undergo invasive diagnostic testing and, at the same time, increase the proportion of affected fetuses detected (10, 11). It relies upon the measurement of multiple biochemical markers (AFP, hCG, Ue3, free β -hCG, inhibin A, and pregnancy-associated plasma protein A (PAPP-A)), the calculation of the risk factor based on the parameters measured, and the mother's age at the time of conception (12-18).

Screening procedures vary from country to country. Controversy exists about the number of markers used and which combination yields the highest efficacy. Parental counselling is essential to insure informed consent for further investigation and for any termination of pregnancy that may result.

Several factors influence the accuracy of screening tests, including gestation dating method, maternal weight, number of fetuses, etc.

Biochemical markers in maternal serum for Down syndrome screening.

The sensitivity and specificity of prenatal screening for DS have improved in recent years with the identification of new biochemical markers in maternal serum. Since the observation that serum levels of alpha-fetoprotein (AFP) were reduced in women with fetuses affected by chromosomal abnormalities, numerous other fetoplacental markers in maternal serum have been found to have altered levels in pregnancies with fetuses affected by aneuploidy (12).

The most useful markers for prenatal screening in the first trimester are the free β -subunit of human chorionic gonadotropin (hCG), and pregnancy-associated plasma protein-A (PAPP-A) (16-18, 20, 21). Serum concentrations of the free β -subunit of hCG are higher than average, and PAPP-A concentrations are lower. In the second trimester intact human chorionic gonadotropin (13,16-19),

alpha-fetoprotein (12,16-22), inhibin-A (15-19), and unconjugated estriol are used (14,18,19,21,22). Measurement of these serum markers has been proposed as a means of identifying pregnant women of all ages who are likely to have a Down syndrome fetus. Women found to be at high risk would be offered confirmatory testing by karyotyping tissue obtained by amniocentesis or CVS.

The serum levels of all these markers overlap in affected and unaffected populations, however, and so the odds that a particular value is associated with an affected pregnancy are used to modify the a priori risk specific to maternal age.

A very important consideration in the screening is the age of the fetus. The correct analysis of the different components depends on knowing the gestational age precisely. The best way to determine this is by ultrasound (8, 23-25).

Once the blood test results are determined, a risk factor is calculated based on the "normal" blood test values for the testing laboratory. The average of normal is called the "population median". Test results are sometimes reported to doctors as "Multiples of the Median" (MoM) (12, 22).

The most effective approach to screening is to use combinations of markers (taking into account correlations between markers), and most protocols use AFP and either hCG or its β subunit, with or without unconjugated estriol (15,16, 23, 24).

Examples of related tests: Maternal Serum Double Serum (maternal serum AFP, hCG), Maternal Serum Quadruple Screen (maternal serum AFP, hCG, unconjugated estriol, inhibin-A), and Maternal Serum Triple Screen (Maternal Serum AFP, hCG, unconjugated estriol) (21-25). These tests are carried out in the second trimester of pregnancy.

In multiple marker screening, the "bottom line" maternal risk calculation for DS must start from accurate patient personal information in order for the interpretation to be valid. Each piece of information about the patient and each of the laboratory analyte levels has equal weight in the algorithm used to calculate the DS risk (26, 27).

Biochemical markers for Down syndrome.

The Foundation for Blood Research and the College of American Pathologists recommend that the patient report should include:

- 1- The gestational age (GA).
- 2- How the GA was calculated (ultrasound, physical exam, etc).
- 3- Patient's age at delivery.
- 4- The age-related Down syndrome risk.
- 5- The patient's weight, race, diabetic status.
- 6- The multiple of the median (MoM) values for each analyte.
- 7- And the Down syndrome risk based on the above.

One limitation of multiple marker screening is that Down syndrome risk for pregnancies with multiple fetuses cannot be assessed (22, 28).

AFP.

Alpha-fetoprotein is a glycoprotein with a molecular weight of 68 000 Daltons produced by the fetal yolk sac and fetal liver (29,30). AFP has approximately 4% carbohydrate moiety represented by one oligosaccharide residue (31). The protein moiety has been completely determined, consisting of one polypeptide chain of 590 a.a. arranged in three well-defined domains (32).

A small portion of this substance eventually passes into the mother's bloodstream. Fetal plasma concentration increases to a maximum (approximately 3.0-4.0 g/L) between 13-14 weeks of gestation (17-19, 21,27,29,33). Maternal serum levels peak at about 30 weeks (about 250 mg/L). After birth, maternal and infant AFP rapidly decline.

Elevated levels of AFP can be found in certain conditions such as: spina bifida, anencephaly (failure of brain and skull development), fetal death, abdominal wall defects, twin gestation, or inaccurate dating of pregnancy (19, 21).

In 1984 Merkatz and co-workers reported that maternal serum AFP (MSAFP) in the second trimester from pregnancies affected with fetal trisomy 21 was lower (AFP < 0.7 MoM) than in normal pregnancies (12).

It was also demonstrated that the decrease in

MSAFP was independent of maternal age, making prenatal screening for fetal Down syndrome possible in women younger than 35 years. The report of Merkatz and co-workers was confirmed by other studies (18-22, 27, 33-35). The levels of MSAFP in Down syndrome pregnancies are about 72% of the normal values for weeks 14-21. It is obvious, by examining the distribution of MSAFP levels found in Down syndrome versus unaffected pregnancies, that no level of MSAFP will clearly separate affected from unaffected pregnancies.

Although AFP screening represents major advances, it can still identify only 20-30% of the DS cases in younger women (11,27,34,36).

The AFP finding stimulated a search for other maternal serum markers for which concentrations might be altered in cases of fetal DS. Recent studies suggest that by adding other types of measurements in maternal serum, specifically, hCG or β -hCG, and unconjugated estriol, the accuracy of the serum test can be appreciably increased (16, 23, 27, 36, 37). These results can also be combined with maternal age to allow a more reliable estimation of the risk of DS. This newer procedure is known as the Triple test or tri-screen or AFP Plus.

Even with a normal tri-screen and ultrasound, there is still the possibility of having a baby with DS (38). Amniocentesis or chorionic villus sampling are the only 100% accurate tests for DS, as well as other chromosomal problems.

hCG.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone composed of two non-covalently linked glycosylated polypeptide chains, α (92 aminoacids) and β (145 aminoacids) (39). The α -subunit of hCG is similar to that of pituitary and placental gonadotropins, which includes luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH) (40, 41). The β -subunit, however, is unique and distinguishes hCG from these other hormones.

hCG is produced by the trophoblast cells of the placenta. hCG production starts at an early stage of development, just a few days after conception,

before implantation in the uterus. hCG enters the maternal circulation almost immediately after implantation of the embryo (blastocyst) on about day 21 of the menstrual cycle (10, 11, 13).

High maternal serum levels of hCG with low levels of MSAFP have been associated with an increased risk of carrying a Down syndrome fetus. Studies have reported elevated second trimester hCG levels varying from 2.04 to 2.5 MoM or greater. A geometric mean MoM for Down syndrome pregnancies determined from the results of 18 studies, comprising a total of 559 DS cases, was 2.03 (10). Other investigators examined hCG levels in 77 Down syndrome pregnancies and, using maternal age and hCG levels, estimated a 60% detection rate at a false positive rate of 6.7% (16,17,27,28,36). At a cut-off risk of 1/380 Kevin Spencer *et al.* found a 57.9% detection rate at a false positive rate of 8.5% (42).

Other studies showed that the usefulness of free β -hCG is superior to that of total hCG in maternal serum screening (43, 44). In 2000 Hallahan *et al.* analysed 63 cases of DS and 400 unaffected control pregnancies between 10 and 13 weeks of gestation to compare free β -hCG versus intact hCG in first trimester Down syndrome screening. Free β -hCG combined with maternal age detected 45% of Down syndrome pregnancies at a 5% false positive rate. Intact hCG combined with maternal age demonstrated detection efficiency comparable to maternal age alone (35% versus 32%) (45). This study demonstrated that free β -hCG was actually a better marker than intact hCG.

Free β -hCG.

The free β -subunit of hCG is present in serum throughout pregnancy. Reports of amounts of free β -subunit in second trimester serum, however, vary widely. Free β -hCG subunit concentrations average 0.5% to 4% of total hCG levels (46, 47, 48). The possible causes of variations in free β -subunit values include hCG dissociation and effects of nicks in free β -hCG on immunoreactivity.

In 1995, Eldar-Geva *et al.* showed that, although the production of each subunit's hCG

messenger RNA is increased in Down syndrome pregnancies, β -subunit production is more markedly increased (49). This finding suggests that the free β -hCG subunit might be superior to intact hCG for Down syndrome detection. Various authors have reported that the effectiveness of free β -hCG methodologies is superior to that of total hCG in maternal serum screening (43-45).

Ultrasound is a powerful diagnostic tool, but its accuracy lies in the skill and experience of the practitioner, and therefore the accuracy of ultrasound analysis can vary. Some studies suggest that the risk for Down syndrome may be reduced when an ultrasound is deemed normal after knowledgeable interpretation by an experienced practitioner. Due to the inconsistencies that currently exist in the training and interpretation of ultrasound, the American College of Obstetricians and Gynecologists recommends that ultrasound screening for Down syndrome be limited to specialized centers (23, 24, 50).

An ultrasound finding that shows an increase in the size of the normal, clear area behind the baby's neck (nuchal translucency) early in pregnancy is associated with an increased incidence of Down syndrome. Researchers believe that nuchal translucency may reflect accumulation of lymph fluid (25, 26).

In 2000, Spencer *et al.* showed that the detection of affected pregnancies can be improved to over 80% with ultrasound measurement of fetal nuchal thickness or translucency, with or without measurements of free β -hCG, and a new marker, pregnancy-associated plasma protein-A (PAPP-A), in the mother's blood during the first trimester (51). Studies have reported elevated first trimester β -hCG levels from 2.45 MoM or greater (34).

Studies indicate a wide variation (30% to 86%) in the accuracy of nuchal translucency as a predictor of DS. This range may result from differences in expertise and techniques for measuring nuchal translucency. In addition, there is no consensus on the definition of what measurement constitutes an increased nuchal translucency (25, 26, 52, 53).

Biochemical markers for Down syndrome.

In 2001, Yaron *et al.* and Audibert *et al.*, evaluated for first-trimester biochemical screening for DS, PAPP-A, and free β -hCG in conjunction with nuchal translucency measurements, and it was estimated to achieve a DS detection rate of 80% to 85% at a 5% false-positive rate (52, 53).

In 2004, Platt *et al.* demonstrated that a sequential screening program that provides patients with first-trimester results and offers the option for early invasive testing or additional serum screening in the second trimester, can detect 98% of trisomy 21-affected pregnancies (54).

Unconjugated estriol (uE3).

Estriol is a hormone produced by the placenta from precursors provided by the fetal adrenal glands and liver, and it increases steadily throughout pregnancy (10, 14). Estriol diffuses from the placenta into the maternal blood where it can be measured as unconjugated uE3. The levels of uE3 in normal pregnancies increase from about 4 nmol/L at 15 weeks of gestation to about 40 nmol/L at delivery (19, 23). Second trimester maternal serum uE3 levels are decreased in trisomy 21 and trisomy 18 (19, 23, 27, 28, 36, 44).

Unconjugated estriol was initially thought to add perhaps 5% detection efficiency in second trimester Down syndrome screening protocols. In 1992 Spencer *et al.* found a 45.7% detection rate at a false positive rate of 9.1%, using maternal age and uE3 levels (55). In 1993 Crossley *et al.* found a 53% detection rate at false positive of 5%, using the triple test (AFP, hCG, and uE3) (56). Many investigations have been unable to reproduce these results indicating that uE3 does not add to the detection of Down syndrome and may increase the false positive rate (20-22).

In 2002 Benn *et al.* showed the quadruple test (Maternal serum AFP, hCG, uE3, inhibin-A) had a sensitivity of 81.5% and false-positive rate of 6.9% (positive predictive value: 1 in 42). The combination of the quadruple test with the ultrasound measurement of fetal nuchal translucency may achieve 90% sensitivity and a 3.1% false-positive rate (positive predictive value: 1 in 18) (23). In

2004 Stenhouse *et al.* reported similar findings, a 93% (14/15) detection rate for Down syndrome at a false-positive rate of 5.9%, and for all chromosome abnormalities 96% (25/26) at an overall false-positive rate of 6.3% (57).

Inhibin-A.

Inhibin is a glycoprotein hormone produced in both the ovaries and the testes, under the major regulation of FSH (58). It is a dimeric molecule consisting of α and β subunits, both found in several molecular forms (59, 60). The major form has a size of 32 kDa and consists of an α subunit of 18 kDa and a β subunit of 14 kDa (60). Concentrations of inhibin-A in peripheral serum gradually decreased from 1.76 ± 0.15 μ g/L in week 8 of gestation to 0.86 ± 0.12 μ g/L in week 16 (15, 59, 60). The concentrations remained low during the second trimester but increased markedly during the third trimester, reaching a maximal value of 5.68 ± 0.86 μ g/L in week 36 (59, 61, 62).

Maternal serum levels of inhibin-A in the second trimester of pregnancy are twice as high in pregnancies affected by DS as in unaffected ones. Inhibin-A is a marker for Down syndrome as effective as hCG, yet provides information that hCG and the other markers do not (23, 27, 28, 63).

Inhibin-A is used with three other analytes and maternal age to characterize more accurately Down syndrome risk (15, 18, 21). Screening programs can use inhibin-A either to increase the DS detection rate while maintaining the screen-positive rate, or to decrease the screen-positive rate while maintaining the detection rate.

In 1996, Wallace *et al.* obtained a higher prediction rate (75% at a 5% false-positive rate) using the triple test (AFP, β -hCG, and inhibin-A) in the first trimester screening (60). Reinier *et al.* (1997) and Lambert *et al.* (1998) confirmed these results in the second trimester of pregnancy (15, 64, 65). In 2002, Benn *et al.* and Wald *et al.* showed that the combination of the quadruple test with nuchal fold and long bone measurements can increase the detection rate to 90%, and false-

positive rate at 3.1% (positive predictive value: 1 in 18) (23, 66).

Pregnancy-associated plasma protein-A (PAPP-A).

Pregnancy-associated plasma protein A is a large glycoprotein (MW 720,000 daltons). Its biological function is mostly unknown, although recent research has demonstrated granulosa cells as a source of PAPP-A in ovaries, and suggested that PAPP-A is a marker of ovarian follicle selection and corpus luteum formation (67). During pregnancy PAPP-A levels rise all the way to term. Reports in the early 1990s suggested that PAPP-A is reduced in pregnancies with trisomic fetuses. The deviation from normality decreases with advancing gestation (68, 69). The latter finding is compatible with reports stating that in the second trimester there is no significant difference in maternal serum PAPP-A between pregnancies with trisomy 21 and controls (70). A meta-analysis stated that median maternal serum PAPP-A level in DS pregnancies is 0.35 MoM, 0.40 MoM, and 0.62 MoM at gestational weeks 6–8, 9–11, and 12–14, respectively, and 0.94 MoM thereafter. The estimated DS detection rate for a 5% false positive rate was 52% for PAPP-A alone (71).

Brizot *et al.* studied the possible causes for the decrease of PAPP-A in trisomic pregnancies. They investigated the relationship between placental messenger-RNA expression and the concentration of PAPP-A in both placental tissue and maternal serum in normal and trisomic pregnancies. The maternal serum concentration of PAPP-A in the trisomic group of pregnancies was significantly lower than in the normal controls. However, there were no significant differences in PAPP-A mRNA expression or PAPP-A protein concentration in the placental tissues. There was no significant association between the level of placental mRNA and maternal serum PAPP-A concentrations in the normal or trisomic pregnancies. These findings suggest that the decrease in maternal serum PAPP-A in trisomic pregnancies is due to alterations in

post-translational events such as protein stability, alterations in the release mechanism of the protein, impaired protein transport across the placenta or modified serum stability of PAPP-A (72).

The integrated method, including maternal age, NT measurement, and PAPP-A in the first trimester, and AFP, hCG, uE3, and inhibin A in the second trimester, yielded very good results, with detection rates of 94% and 85%, with false positive rates of 5% and 1%, respectively (73). Two retrospective integrated studies with 321 DS cases and 15 DS cases gave detection rates of 90% and 93% with false positive rates of 5% and 5.9%, respectively (56, 74).

CONCLUSIONS.

Down syndrome screening has been offered to pregnant women since the early 1980s. Protocols have changed as research confirmed improvements that resulted in higher detection rates and lower false-positive rates. Screening protocols that include ultrasound measurement of nuchal translucency and biochemical testing in the first and second trimester are now available. First-trimester screening is an option if there are adequate ultrasound, diagnostic, and counselling services available. Regional variation in the availability of these services may limit the implementation of first-trimester screening. Combining screening tests for Down syndrome from both trimesters as an integrated test offers the highest detection rate with the lowest false-positive rate (table 1). Timing, detection rate, false-positive rates, and personal factors influence the decision women make regarding screening versus diagnostic testing.

The integrated test, combining first-trimester sonographic and biochemical markers (free β -hCG + PAPP-A) with second-trimester markers (maternal serum alpha-fetoprotein, hCG, and uE3), provides a single estimate of patients with DS risk, and may yield a DS detection rate of 95% at a 5% false-positive rate. Many researchers have recently come up with detection rates over 90% during the first trimester using a combination of free β -hCG,

Table 1
Screening strategies for Down syndrome.

First trimester screening (10 to 14 weeks):	
• Maternal age	32 %
• Nuchal translucency measurement (by ultrasound)	74 %
• First trimester double test (PAPP-A, β -hCG)	63 %
• First trimester combined test (nuchal translucency, PAPP-A, β -hCG)	>86 %
Second trimester screening (15 to 19 weeks):	
• Maternal age	32 %
• Second trimester double test (AFP, HCG)	60 %
• Triple test (AFP, HCG, uE3)	68 %
• Quadruple test (AFP, HCG, uE3, inhibin A)	79 %
• Integrated test (first trimester: nuchal translucency, PAPP-A and β -hCG; second trimester: quadruple test)	95 %
Prenatal diagnosis:	
Amniocentesis (14-16 weeks)	100 %
Chorionic villus sampling (10-12 weeks)	100 %
FISH	100 %

maternal serum alpha-fetoprotein, unconjugated estriol (uE3), pregnancy-associated plasma protein-A, and nuchal translucency with ultrasound (21, 52, 53, 75).

The use of new biochemical markers can improve screening sensitivity for fetal Down syndrome. Hyperglycosylated human chorionic gonadotropin (hCG) is a promising marker that can be measured in urine or serum in the first or second trimester. In 2004 Palomaki *et al.* showed that the false-positive rate could be reduced by substituting hyperglycosylated human chorionic gonadotropin for hCG measurements (from 5.6 to 2.6 for the triple test), or by adding hyperglycosylated human chorionic gonadotropin measurements to existing combinations (from 3.3 to 2.0 for the quadruple test) at a 75% detection rate (76). Urinary β -core hCG, a breakdown product of hCG, has shown to be elevated in affected pregnancies. When combined with maternal age, predicted detection rates range from 41-80% (77).

Prenatal multiple marker screening is a valuable tool to assess the risk of Down syndrome in a given pregnancy, but the accuracy of any approach depends on accurate pregnancy dating and patient information as well as the reliability of the screening parameters utilized by a particular laboratory. Again, the definitive diagnosis of Down syndrome

in the second trimester is made by chromosome analysis of amniocentesis, and the multiple marker screening should not be mistaken for a diagnostic test. As stated, a limitation of multiple marker screening is that DS risk for pregnancies with multiple fetuses cannot be assessed.

The future for DS diagnosis is in the development of sensitive and specific non-invasive tests, for example: Fetal cells or DNA in maternal circulation and transcervical cell sampling. Fetal DNA has been found to be increased in maternal blood when the fetus has trisomy 21, possibly due to accelerated apoptosis of fetal cells, although there is a considerable degree of overlap with euploid fetuses. The most successful type of fetal cell recoverable from maternal blood is the nucleated red blood cell. Given the rarity of these cells in the maternal blood, at approximately 1-2 fetal cells per 10 million maternal cells, sophisticated techniques must be used for their analysis. Fluorescent in-situ hybridisation (FISH), magnetic activated cell sorting (MACS) or polymerase chain reaction (PCR) techniques to identify the extra chromosome 21 are employed. FISH is a method used to identify specific parts of a chromosome. For example, if you suspect that there has been a translocation in a chromosome, you can use a probe that spans the site of breakage/translocation. However, its

widespread use is not financially feasible at this point in time (78, 79).

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