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REVIEW

Screening for fetal aneuploidies at 11 to 13 weeks

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Effective screening for major aneuploidies can be provided in the first trimester of pregnancy. Screening by a combination of fetal nuchal translucency and maternal serum free-β-human chorionic gonadotrophin and pregnancy-associated plasma protein-A can identify about 90% of fetuses with trisomy 21 and other major aneuploidies for a false-positive rate of 5%. Improvement in the performance of first-trimester screening can be achieved by firstly, inclusion in the ultrasound examination assessment of the nasal bone and flow in the ductus venosus, hepatic artery and across the tricuspid valve, and secondly, carrying out the biochemical test at 9 to 10 weeks and the ultrasound scan at 12 weeks. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS: first-trimester screening; trisomy 21; aneuploidies; nuchal translucency; serum PAPP-A; serum free β-hCG

INTRODUCTION

Aneuploidies are major causes of perinatal death and childhood handicap. Consequently, the detection of chromosomal disorders constitutes the most frequent indication for invasive prenatal diagnosis. However, invasive testing, by amniocentesis or chorionic villus sampling (CVS), is associated with a risk of miscarriage, and therefore these tests are carried out only in pregnancies considered to be at high risk for aneuploidies.

In the 1970s, the main method of screening for aneuploidies was by maternal age and in the 1980s by maternal serum biochemistry and detailed ultrasonographic examination in the second trimester. In the 1990s, the emphasis shifted to the first trimester when it was realized that the great majority of fetuses with major aneuploidies can be identified by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free β-human chorionic gonadotrophin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A). In the last 10 years, several additional first-trimester sonographic markers have been described which improve the detection rate of aneuploidies and reduce the false-positive rate. The performance of the different methods of screening for trisomy 21 is summarized in Table 1.

SCREENING BY MATERNAL AGE

The risk for many aneuploidies increases with maternal age. Additionally, because aneuploid fetuses are more likely to die *in utero* than euploid fetuses, the risk decreases with gestation.

The rate of fetal death between 12 weeks (when first-trimester screening is performed) and term is about 30% for trisomy 21 and 80% for trisomies 18 and 13. (Hecht and Hook, 1994; Halliday *et al*., 1995; Snijders *et al*., 1994, 1995, 1999; Morris *et al*., 1999). In contrast, the rate of fetal death in euploid fetuses is only 1 to 2% and consequently the risk for trisomies decreases with gestation. The estimated risks for fetal trisomies 21, 18 and 13 for a woman aged 20 years at 12 weeks of gestation are about 1 in 1000, 1 in 2500 and 1 in 8000, respectively, and the risks of such woman delivering an affected baby at term are 1 in 1500, 1 in 18 000 and 1 in 42 000, respectively. The respective risks for these aneuploidies for a woman aged 35 years at 12 weeks of gestation are about 1 in 250, 1 in 600 and 1 in 1800, and the risks of delivering an affected baby at term are 1 in 350, 1 in 4000 and 1 in 10 000.

Turner syndrome is unrelated to maternal age and the prevalence is about 1 in 1500 at 12 weeks and 1 in 4000 at 40 weeks. For the other sex chromosome abnormalities (47,XXX, 47,XXY and 47,XYY), there is no significant change with maternal age, and because the rate of fetal death is not higher than in euploid fetuses the overall prevalence (about 1 in 500) does not decrease with gestation. Triploidy is unrelated to maternal age and the prevalence is about 1 in 2000 at 12 weeks, but it is rarely seen in live births because most affected fetuses die by 20 weeks.

In the early 1970s, about 5% of pregnant women were aged 35 years or more, and this group contained about 30% of the total number of fetuses with trisomy 21. Therefore, screening on the basis of maternal age, with a cut-off of 35 years to define the high-risk group, was associated with a 5% screen-positive rate (also referred to as false-positive rate, because the vast majority of fetuses in this group are normal) and a detection rate of 30%. In the subsequent years, in developed

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Table 1—Performance of different methods of screening for trisomy 21

Method of screening	Detection rate (%)	False-positive rate (%)
MA	30	5
First trimester		
MA + fetal NT	75–80	5
MA + serum free β-hCG and PAPP-A	60–70	5
MA + NT + free β-hCG and PAPP-A (combined test)	85–95	5
Combined test + nasal bone or tricuspid flow or ductus venosus flow	93–96	2.5
Second trimester		
MA + serum AFP, hCG (double test)	55–60	5
MA + serum AFP, free β-hCG (double test)	60–65	5
MA + serum AFP, hCG, uE3 (triple test)	60–65	5
MA + serum AFP, free β-hCG, uE3 (triple test)	65–70	5
MA + serum AFP, hCG, uE3, inhibin A (quadruple test)	65–70	5
MA + serum AFP, free β-hCG, uE3, inhibin A (quadruple test)	70–75	5
MA + NT + PAPP-A (11–13 weeks) + quadruple test	90–94	5

MA, maternal age; NT, nuchal translucency; β-hCG, β-human chorionic gonadotrophin; PAPP-A, pregnancy-associated plasma protein-A.

Table 2—Biochemical and sonographic features of trisomies 21, 18 and 13

	Euploid	Trisomy 21	Trisomy 18	Trisomy 13
NT mixture model				
CRL-independent distribution, %	5	95	70	85
Median CRL-independent NT, mm	2.0	3.4	5.5	4.0
Median serum free β-hCG, MoM	1.0	2.0	0.2	0.5
Median serum PAPP-A, MoM	1.0	0.5	0.2	0.3
Absent nasal bone, %	2.5	60	53	45
Tricuspid regurgitation, %	1.0	55	33	30
Ductus venosus reversed a-wave, %	3.0	66	58	55

NT, nuchal translucency; CRL, crown–rump length; β-hCG, β-human chorionic gonadotrophin; MoM, multiple of the median; PAPP-A, pregnancy-associated plasma protein-A.

countries there was an overall tendency for women to get pregnant at an older age, so that now about 20% of pregnant women are 35 years or older and this group contains about 50% of the total number of fetuses with trisomy 21.

MATERNAL SERUM BIOCHEMISTRY

Pregnancies with fetal aneuploidies are associated with altered maternal serum concentrations of various feto-placental products, including AFP, free β-hCG, inhibin A and unconjugated estriol (uE3) and PAPP-A (Merkatz *et al*., 1984; Canick *et al*., 1988; Macri *et al*., 1990; Van Lith *et al*., 1993; Brambati *et al*., 1993; Aitken *et al*., 1996).

In screening using maternal serum biochemical markers, the measured concentration of the markers is converted into a multiple of the median (MOM) of unaffected pregnancies at the same gestation. The Gaussian distributions of $\log_{10}$ (MoM) in trisomy 21 and unaffected pregnancies are then derived, and the ratio of the heights of the distributions at a particular MoM, which is the likelihood ratio for trisomy 21, is used to modify the *a priori* maternal age-related risk to derive the patient-specific risk.

Second trimester

Early attempts at incorporating maternal serum markers into screening for aneuploidies focused on the second trimester of pregnancy and demonstrated a substantial improvement in detection rates of trisomy 21, compared with screening by maternal age. At a false-positive rate of 5%, the detection rate improves from 30% in screening by maternal age alone to 60 to 65% by combining maternal age with serum AFP and free β-hCG (double test), 65 to 70% with the addition of uE_3 (triple test) and 70 to 75% with the addition of inhibin A (quadruple test) (Cuckle *et al*., 2005; Cuckle and Benn, 2009; Wald *et al*., 2003a, 2003b). If intact hCG rather than free β-hCG is used, the detection rates are reduced by about 5%.

First trimester

In the last decade biochemical testing has moved to the first trimester because when this is combined with the ultrasound marker of fetal NT thickness, the performance of screening is superior to second-trimester screening. In trisomy 21 pregnancies, the maternal serum concentration of free β-hCG is about twice as high and PAPP-A is reduced to half compared with euploid pregnancies (Table 2). The measured serum concentrations

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of these placental products are affected by maternal characteristics, including racial origin, weight, smoking and method of conception as well as the machine and reagents used for the analysis. Consequently, in the calculation of risk for aneuploidies using these products it is necessary to take into account the effects of these maternal variables in defining MoMs before comparing affected and unaffected pregnancies (Kagan *et al*., 2008a). In euploid pregnancies, the average adjusted value for both free β-hCG and PAPP-A is 1.0 MoM at all gestations, whereas in trisomy 21 the average free β-hCG is 2.0 MoM and the average PAPP-A is 0.5 MoM and they both increase with gestation.

In screening for trisomy 21 by maternal age and serum free β-hCG and PAPP-A, the detection rate is about 65% for a false-positive rate of 5%. The performance is better at 9 to 10 weeks than at 13 weeks because the difference in PAPP-A between trisomic and euploid pregnancies is greater in earlier gestations (Cuckle and van Lith, 1999; Spencer *et al*., 2003a; Kagan *et al*., 2008a; Wright *et al*., 2010). Although the difference in free β-hCG between trisomic and euploid pregnancies increases with gestation, the magnitude of the difference is smaller than that of the opposite relation of PAPP-A.

In trisomies 18 and 13, maternal serum free β-hCG and PAPP-A are decreased (Tul *et al*., 1999; Spencer *et al*., 2000a). In cases of sex chromosomal anomalies, maternal serum free β-hCG is normal and PAPP-A is low (Spencer *et al*., 2000b). In diandric triploidy, maternal serum free β-hCG is greatly increased, whereas PAPP-A is mildly decreased (Spencer *et al*., 2000c). Digynic triploidy is associated with markedly decreased maternal serum free β-hCG and PAPP-A.

A new biochemical marker of aneuploidies is ADAM12 (A disintegrin and metalloprotease) because in trisomic pregnancies maternal serum levels during the first trimester are lower than in euploid pregnancies (Christiansen *et al*., 2010). However, it is unlikely that this marker will improve screening, because the reduction is small and there is a significant association between ADAM12 and the traditional biochemical markers of free β-hCG and PAPP-A (Poon *et al*., 2009).

SCREENING BY FETAL NT THICKNESS

In 1866, Langdon Down reported that in individuals with trisomy 21 (the condition that came to bear his name), the skin appears to be too large for their body (Langdon Down, 1866). In the 1990s, it was realized that this excess skin may be the consequence of excessive accumulation of subcutaneous fluid behind the fetal neck which could be visualized by ultrasonography as increased NT in the third month of intrauterine life (Nicolaides *et al*., 1992a).

Extensive research in the last 20 years has established that the measurement of fetal NT thickness provides effective and early screening for trisomy 21 and other major aneuploidies (Snijders *et al*., 1998; Wald *et al*., 2003a; Nicolaides, 2004; Malone *et al*., 2005). Furthermore, high NT is associated with cardiac defects and a wide range of other fetal malformations and genetic syndromes (Hyett *et al*., 1996a; Souka *et al*., 1998, 2005).

The optimal gestational age for measurement of fetal NT is 11 to 13 weeks and 6 days. The minimum fetal crown–rump length (CRL) should be 45 mm and the maximum 84 mm. The lower limit is selected to allow the sonographic diagnosis of many major fetal abnormalities, which would have otherwise been missed, and the upper limit is such to provide women with affected fetuses the option of an earlier and safer form of termination. Fetal NT can be measured either by transabdominal or transvaginal sonography and the results are similar.

The ability to achieve a reliable measurement of NT is dependent on appropriate training of sonographers, adherence to a standard ultrasound technique in order to achieve uniformity of results among different operators. The magnification of the image should be high so that only the fetal head and upper thorax are included in the picture. A good sagittal section of the fetus in the neutral position should be obtained, and the maximum thickness of the subcutaneous translucency between the skin and the soft tissue overlying the cervical spine should be measured. The mid-sagittal view of the fetal face is defined by the presence of the echogenic tip of the nose and rectangular shape of the palate anteriorly, the translucent diencephalon in the center and the nuchal membrane posteriorly (Plasencia *et al*., 2007). Deviations from the exact midline plane result in non-visualization of the tip of the nose and visibility of the zygomatic process of the maxilla.

There are three elements in the assessment of NT that can introduce operator bias and either under- or overestimation of the measurement and consequent increase in the variability of measurements. Firstly, the selection of the exact place behind the fetal neck containing the maximum vertical distance between the nuchal membrane and the edge of the soft tissue overlying the cervical spine because the two lines are not usually parallel, secondly, the selection of the appropriate gain to reduce the thickness of the lines and thirdly, accurate placement of the calipers on the two lines. In order to avoid these problems, a semi-automated method of measuring NT thickness has been developed which has the potential to substantially reduce between- and within-operator variability in measurements of NT from a given image (Moratalla *et al*., 2010).

Fetal NT increases with CRL and therefore it is essential to take gestation into account when determining whether a given NT thickness is increased. There are essentially two approaches to quantifying the deviation of NT from the normal median. One approach is to subtract the normal median from the NT measurement and to produce a deviation in millimeters referred to as delta NT (Pandya *et al*., 1995a; Spencer *et al*., 2003b). The other approach is to divide NT by the normal median to produce a MoM value (Nicolaides *et al*., 1998). In the calculation of patient-specific risks for trisomy 21, the *a priori* maternal age-related risk is multiplied by the likelihood ratio for a measured NT, which is the ratio of the heights of distributions of measurements in trisomy 21 and unaffected pregnancies. Recently, a new approach

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has been proposed for quantifying the deviation in the measured NT from the normal. This is based on the observation that in both aneuploid and euploid pregnancies, fetal NT follows two distributions, one which is CRL dependent and another which is CRL independent (Wright *et al*., 2008). In this mixture model, the distribution in which NT increases with CRL is observed in about 95% of euploid fetuses, 5% with trisomy 21, 30% with trisomy 18, 15% with trisomy 13 and 10% with Turner syndrome. The median CRL-independent NT was 2.0 mm for the euploid group and 3.4, 5.5, 4.0 and 7.8 mm for trisomies 21, 18, 13 and Turner syndrome, respectively.

Several prospective interventional studies in hundreds of thousands of pregnancies have demonstrated that firstly, fetal NT is successfully measured in more than 99% of cases, secondly, the risk of chromosomal abnormalities increases with both maternal age and fetal NT thickness and thirdly, in pregnancies with low fetal NT the maternal age-related risk is decreased. For a 5% false-positive rate, fetal NT screening identifies 75 to 80% of fetuses with trisomy 21 and other major aneuploidies (Nicolaides, 2004).

SCREENING BY FETAL NT THICKNESS AND SERUM BIOCHEMISTRY

There is no significant association between fetal NT and maternal serum free β-hCG or PAPP-A in either trisomy 21 or euploid pregnancies, and therefore the ultrasononographic and biochemical markers can be combined to provide more effective screening than either method individually (Brizot *et al*., 1994, 1995; Noble *et al*., 1995; Spencer *et al*., 1999).

Several prospective interventional studies in many thousands of pregnancies have demonstrated that for a 5% false-positive rate, the first-trimester combined test identifies about 90% of trisomy 21 pregnancies (Krantz *et al*., 2000; Bindra *et al*., 2002; Schuchter *et al*., 2002; Spencer *et al*., 2003c; Wapner *et al*., 2003; Nicolaides *et al*., 2005; Ekelund *et al*., 2008; Kagan *et al*., 2009a; Leung *et al*., 2009).

Timing of ultrasound and blood testing within the first trimester

One option in first-trimester combined screening for trisomy 21 is to perform biochemical and ultrasonographic testing as well as to counsel women in one-stop clinics for assessment of risk (OSCAR) (Bindra *et al*., 2002; Spencer *et al*., 2000d). This has been made possible by the introduction of biochemical analyzers which provide automated, precise and reproducible measurements within 30 min of obtaining a blood sample. The ideal gestation for OSCAR is 12 weeks because the aim of the first-trimester scan is not just to screen for trisomy 21 but also to diagnose an increasing number of fetal malformations, and in this respect the ability to visualize fetal anatomy is best at 12 weeks (Souka *et al*., 2004). The detection rate of trisomy 21 with OSCAR at 12 weeks is about 90% at a false-positive rate of 5%.

An alternative strategy for first-trimester combined screening is for biochemical testing and ultrasound scanning to be carried out in two separate visits, with the first done at 9 to 10 weeks and the second at 12 weeks (Borrell *et al*., 2004; Kagan, 2008b; Kirkegaard *et al*., 2008; Wright *et al*., 2010). It has been estimated that this approach would improve the detection rate from 90% to 93 to 94%. A third option would be to perform the scan at 12 weeks and optimize the performance of biochemical testing by measuring PAPP-A at 9 weeks and free β-hCG at the time of the scan at 12 weeks or even later with an estimated detection rate of 95%. The cost and patient acceptability of the alternative policies of first trimester testing will depend on the existing infrastructure of antenatal care. The potential advantage of two- or three-stage screening in terms of detection rate may be eroded by the likely increased non-compliance with the additional steps.

Additional first-trimester sonographic markers

In addition to NT, other highly sensitive and specific first-trimester sonographic markers of trisomy 21 are absence of the nasal bone, increased impedance to flow in the ductus venosus and tricuspid regurgitation (Table 2). Absence of the nasal bone, reversed a-wave in the ductus venosus and tricuspid regurgitation are observed in about 60, 66 and 55% of fetuses with trisomy 21 and in 2.5, 3.0 and 1.0%, respectively, of euploid fetuses. (Matias *et al*., 1998; Cicero *et al*., 2001, 2006; Huggon *et al*., 2003; Nicolaides, 2004; Faiola *et al*., 2005; Falcon *et al*., 2006; Kagan *et al*., 2009b, 2009c; Maiz *et al*., 2009).

Assessment of each of these ultrasound markers can be incorporated into first-trimester combined screening by maternal age, fetal NT and serum free β-hCG and PAPP-A resulting in improvement of the performance of screening with an increase in detection rate to 93 to 96% and a decrease in false-positive rate to 2.5% (Kagan *et al*., 2009b, 2009c; Maiz *et al*., 2009). A similar performance of screening can be achieved by a contingent policy in which first-stage screening by maternal age, fetal NT and serum free β-hCG and PAPP-A is offered to all cases. Patients with a risk of 1 in 50 or more are considered to be screen positive and those with a risk of less than 1 in 1000 are screen negative. Patients with the intermediate risk of 1 in 51 to 1 in 1000, which constitutes about 15% of the total population, have second-stage screening with nasal bone, ductus venosus or tricuspid blood flow which modifies their first-stage risk. If the adjusted risk is 1 in 100 or more, the patients are considered to be screen positive and those with a risk of less than 1 in 100 are screen negative.

A recently described first-trimester sonographic marker of trisomy 21 is increased flow in the fetal hepatic artery (Bilardo *et al*., 2010; Zvanca *et al*., 2011). This marker is also likely to find an application in the

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assessment of the intermediate risk group after first-stage combined screening.

Individual risk-orientated two-stage screening for trisomy 21 is compatible with the basic principles of clinical practice in all fields of medicine. For example, in the great majority of patients presenting with abdominal pain, the correct diagnosis of the presence or absence of a serious problem is reached after history taking and clinical examination. In a minority of cases, a series of further investigations of increasing sophistication may be necessary before the correct diagnosis is made.

Selective use of ultrasound or biochemistry within the first trimester

The best performance of first-trimester screening is achieved by a combination of maternal age, serum biochemical testing and multiple sonographic markers. At a risk cut-off of 1 in 100, the detection rate of trisomy 21 is about 95% at a false-positive rate of 2.5%. This performance of screening is achieved by either a policy in which biochemical testing is undertaken in all cases or by a contingent policy in which first-stage screening is based on maternal age, fetal NT and either tricuspid or ductus venosus flow, and biochemical testing is then performed in only those with an intermediate risk, which constitute about 20% of the total (Kagan *et al*., 2010a).

An alternative first-trimester contingent screening policy consists of maternal serum biochemistry in all pregnancies followed by fetal NT only in those with an intermediate risk after biochemical testing. Studies examining the potential performance of such policy have estimated that the detection rates and false-positive rates would be 80 to 90% and 4 to 6%, respectively, and measurement of fetal NT would be necessary in only 20 to 40% of cases (Christiansen *et al*., 2002; Wright *et al*., 2004; Vadiveloo *et al*., 2009; Kagan *et al*., 2010a; Sahota *et al*., 2010). The advantage of biochemical testing as a first-stage policy relies on its apparent simplicity. However, interpretation of biochemical results necessitates accurate ultrasonographic measurement of fetal CRL and therefore an ultrasound examination cannot be avoided. In the studies estimating the potential performance of contingent screening, the fetal CRL was measured by appropriately trained sonographers during assessment of fetal NT. It would be wrong to assume that the motivation of sonographers and the accuracy in measuring CRL would remain as high if the scans were carried out purely for measurement of CRL and not examining the fetus.

Major advantages of choosing ultrasound assessment rather than biochemical testing as a first-stage policy are that firstly, there is a substantial reduction in the cost of screening because measurement of maternal serum free β-hCG and PAPP-A is undertaken in only 20% rather than all pregnancies, secondly, the fetal anatomy can be examined leading to early diagnosis of many major abnormalities in all pregnancies rather than in just the subgroup with positive first-stage screening results, thirdly, the Doppler studies can be carried out in the same ultrasound examination as for measurement of fetal NT and fourthly, reversed a-wave in the ductus venosus or tricuspid regurgitation are not only useful in screening for trisomy 21 and other major aneuploidies, but also they can identify pregnancies at increased risk for cardiac defects and adverse pregnancy outcome. The disadvantage is that Doppler assessment of tricuspid and ductus venosus flow can be time consuming and requires appropriately trained sonographers.

First-trimester screening followed by second-trimester scan

In the second trimester scan, each chromosomal defect has its own syndromal pattern of detectable abnormalities (Nicolaides *et al*., 1992b; Nicolaides, 1996). For example, trisomy 21 is associated with cardiac defects, duodenal atresia, nasal hypoplasia, increased nuchal fold and prenasal thickness, intracardiac echogenic foci, and echogenic bowel, mild hydronephrosis, shortening of the femur and more so of the humerus, sandal gap and mid-phalanx hypoplasia of the fifth finger. In trisomy 18, common findings include strawberry-shaped head, choroid plexus cysts, absent corpus callosum, enlarged cisterna magna, facial cleft, micrognathia, nuchal edema, heart defects, diaphragmatic hernia, esophageal atresia, exomphalos, single umbilical artery, renal abnormalities, echogenic bowel, myelomeningocoele, growth restriction and shortening of the limbs, radial aplasia, overlapping fingers and talipes or rocker bottom feet. Trisomy 13 is associated with holoprosencephaly, microcephaly, facial abnormalities, cardiac abnormalities, enlarged and echogenic kidneys, exomphalos and post axial polydactyly.

If the second-trimester scan demonstrates major abnormalities, it is advisable to offer fetal karyotyping, even if these abnormalities are apparently isolated. The prevalence of such abnormalities is low and therefore the cost implications are small. If the abnormalities are either lethal or they are associated with severe handicap, such as holoprosencephaly, fetal karyotyping constitutes one of a series of investigations to determine the possible cause and thus the risk of recurrence. If the abnormality is potentially correctable by intrauterine or postnatal surgery, such as diaphragmatic hernia, it may be logical to exclude an underlying chromosomal defect—especially because, for many of these conditions, the usual defect is trisomy 18 or 13.

Minor fetal abnormalities or soft markers are common and they are not usually associated with any handicap, unless there is an underlying chromosomal defect. Routine karyotyping of all pregnancies with these markers would have major implications, both in terms of miscarriage and in economic costs. It is best to base counseling on an individual estimated risk for a chromosomal defect, rather than the arbitrary advice that invasive testing is recommended because the risk is 'high'. The individual risk can be derived by multiplying the *a priori* risk (based on the results of previous screening by NT and/or biochemistry in the current pregnancy) by the likelihood ratio of the specific abnormality or marker (Benacerraf *et al*., 1992; Vintzileos and Egan,

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1995, Vintzileos *et al*., 1996; Bahado-Singh *et al*., 1998; Nyberg *et al*., 2001; Smith-Bindman *et al*., 2001; Bromley *et al*., 2002; Nicolaides, 2003). It has been estimated that the second-trimester scan can improve the detection rate of trisomy 21 achieved by first-trimester combined screening by about 6% for an additional 1.2% false-positive rate (Krantz *et al*., 2007).

First-trimester screening followed by second-trimester biochemical testing

Three mathematical models have been proposed for the additional use of second-trimester biochemical testing with the aim of improving first-trimester combined screening. Firstly, the integrated test in which all patients have first-trimester NT and PAPP-A and second-trimester AFP, uE3, free β-hCG and inhibin, and the combined results are given on completion of this process so that high-risk patients have second-trimester amniocentesis (Wald *et al*., 1999). Secondly, step-wise sequential screening, in which all patients have first-trimester NT and serum PAPP-A and free β-hCG, and high-risk patients are offered CVS, whereas low- or intermediate-risk patients have second-trimester AFP, uE3, free β-hCG and inhibin, and if the combined risk from first- and second-trimester testing becomes high, the patients have second-trimester amniocentesis. Thirdly, contingent screening, which is similar to step-wise sequential screening, but second-trimester biochemical testing is performed only in those with an intermediate risk after first-trimester screening (Wright *et al*., 2004).

The estimated performance of the three approaches is similar with a detection rate of 90 to 94% at a false-positive rate of 5%. The advantages of the contingent approach are that firstly, second trimester testing is avoided in 75 to 80% of patients and secondly, the diagnosis of about 60% of fetuses with aneuploidies is made in the first trimester (Wright *et al*., 2004, Benn *et al*., 2005; Cuckle *et al*., 2008).

The disadvantages of this across trimesters approach to screening are firstly, the performance of screening is poorer that with an integrated first-trimester approach incorporating the new sonographic markers, secondly, reassurance of parents with a low-risk result is delayed by several weeks, thirdly, many women with an affected pregnancy are deprived of the option of safer first-trimester termination of pregnancy and fourthly, many women who do not complete the two-stage test are essentially deprived of screening.

Screening for aneuploidies other than trisomy 21

A beneficial consequence of screening for trisomy 21 is the early diagnosis of trisomies 18 and 13, which are the second and third most common chromosomal abnormalities. At 11 to 13 weeks, the relative prevalences of trisomies 18 and 13 to trisomy 21 are one to three and one to seven, respectively. All three trisomies are associated with increased maternal age, increased fetal NT and decreased maternal serum PAPP-A, but in trisomy 21 serum free β-hCG is increased, whereas in trisomies 18 and 13 this is decreased. In addition, trisomy 13, unlike trisomies 21 and 18, is associated with fetal tachycardia, with the heart rate being above the 95th centile of euploid fetuses in 85% of fetuses with trisomy 13 (Hyett *et al*., 1996b; Liao *et al*., 2000; Papageorghiou *et al*., 2006).

Use of the algorithm for trisomy 21 identifies about 75% of fetuses with trisomies 18 and 13. The combined use of the algorithm for trisomy 21 with specific algorithms for trisomies 18 and 13 improves the detection of these aneuploidies to 95% with a small increase in false-positive rate by about 0.1% (Kagan *et al*., 2008c). Another beneficial consequence of the use of the combined three algorithms is the early identification of about 85% of fetuses with triploidy (Kagan *et al*., 2008d).

In addition to the measurement of fetal NT, the 11 to 13 weeks scan can identify many major defects, such as holoprosencephaly, exomphalos and megacystis found in about 1 in 1300, 1 in 400 and 1 in 1600 fetuses, respectively. Aneuploidies, mainly trisomies 18 and 13, are observed in about 65% of fetuses with holoprosencephaly, 55% with exomphalos and 30% with megacystis (Kagan *et al*., 2010b). At 11 to 13 weeks absence of the nasal bone, abnormal flow in the ductus venosus and tricuspid regurgitation are observed in about 50, 55 and 30%, respectively, of fetuses with trisomies 18 and 13 (Kagan *et al*., 2009b, 2009c; Maiz *et al*., 2009).

SCREENING IN TWIN PREGNANCIES

In twin pregnancies, effective screening for chromosomal abnormalities is provided by a combination of maternal age and fetal NT thickness (Pandya *et al*., 1995b; Sebire *et al*., 1996a, 1996b; Maymon *et al*., 2001). The performance of screening can be improved by the addition of maternal serum biochemistry, but appropriate adjustments are needed for chorionicity (Sepulveda, *et al*., 1996). In dichorionic twins at 11 to 13 weeks, the levels of maternal serum free β-hCG and PAPP-A are about twice as high as in singleton pregnancies, but in monochorionic twins the levels are lower than in dichorionic twins (Spencer and Nicolaides, 2000, 2003; Spencer *et al*., 2008; Linskens *et al*., 2009).

In dichorionic twins, patient-specific risks for trisomy 21 are calculated for each fetus based on maternal age and fetal NT, and the detection rate (75–80%) and false-positive rate (5% per fetus or 10% per pregnancy) are similar to those in singleton pregnancies (Sebire *et al*., 1996a). In the calculation of risk for trisomies, it has been assumed that in each pregnancy the measurements of NT for CRL between the two fetuses were independent of each other. However, recent evidence indicates that in euploid dichorionic twins, the measurements of NT in each twin pair are correlated and this correlation is not a simple reflection of the common effect of sonographers (Wøjdemann *et al*., 2006; Cuckle and Maymon, 2010; Wright *et al*., 2011). In screening in twins it is

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therefore necessary to take this correlation into account because it has a substantial impact on the estimated patient-specific risk for trisomies. First-trimester screening allows the possibility of earlier and therefore safer selective fetocide in cases where one fetus is euploid and the other is abnormal (Sebire *et al*., 1996b). An important advantage of screening by fetal NT is that when there is discordance for a chromosomal abnormality, the presence of a sonographically detectable marker helps to ensure the correct identification of the abnormal twin should the parents choose selective termination.

In monochorionic twin pregnancies, the false-positive rate of NT screening is higher than in dichorionic twins, because increased NT in at least one of the fetuses is an early manifestation of twin-to-twin-transfusion syndrome, as well as a marker of chromosomal abnormalities (Sebire *et al*., 1997, 2000; Kagan *et al*., 2007). In the calculation of risk of trisomy 21, the NT of both fetuses should be measured and the average of the two should be considered (Vandecruys *et al*., 2005).

CONCLUSION

Effective screening for all major aneuploidies can be achieved in the first trimester of pregnancy with a detection rate of about 95% and a false-positive rate of less than 3%.

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REFERENCES

Aitken DA, Wallace EM, Crossley JA, *et al*. 1996. Dimeric inhibin A as a marker for Down's syndrome in early pregnancy. *N Engl J Med* **334**: 1231–1236.

Bahado-Singh R, Deren O, Oz U, *et al*. 1998. An alternative for women initially declining genetic amniocentesis: individual Down syndrome odds on the basis of maternal age and multiple ultrasonographic markers. *Am J Obstet Gynecol* **179**: 514–519.

Benacerraf BR, Neuberg D, Bromley B, Frigoletto FD, Jr. 1992. Sonographic scoring index for prenatal detection of chromosomal abnormalities. *J Ultrasound Med* **11**: 449–458.

Benn P, Wright D, Cuckle H. 2005. Practical strategies in contingent sequential screening for Down syndrome. *Prenat Diagn* **25**: 645–652.

Bilardo CM, Timmerman E, Robles de Medina PG, Clur SA. 2010. Increased hepatic artery flow in first trimester fetuses: an ominous sign. *Ultrasound Obstet Gynecol*. [Epub ahead of print]. DOI: 10.1002/uog.7766.

Bindra R, Heath V, Liao A, Spencer K, Nicolaides KH. 2002. One stop clinic for assessment of risk for trisomy 21 at 11–14 weeks: a prospective study of 15,030 pregnancies. *Ultrasound Obstet Gynecol* **20**: 219–225.

Borrell A, Casals E, Fortuny A, *et al*. 2004. First-trimester screening for trisomy 21 combining biochemistry and ultrasound at individually optimal gestational ages. An interventional study. *Prenat Diagn* **24**: 541–545.

Brambati B, Macintosh MCM, Teisner B, *et al*. 1993. Low maternal serum level of pregnancy associated plasma protein (PAPP-A) in the first trimester in association with abnormal fetal karyotype. *BJOG* **100**: 324–326.

Brizot ML, Snijders RJM, Bersinger NA, Kuhn P, Nicolaides KH. 1994. Maternal serum pregnancy associated placental protein A and fetal nuchal translucency thickness for the prediction of fetal trisomies in early pregnancy. *Obstet Gynecol* **84**: 918–922.

Brizot ML, Snijders RJM, Butler J, Bersinger NA, Nicolaides KH. 1995. Maternal serum hCG and fetal nuchal translucency thickness for the prediction of fetal trisomies in the first trimester of pregnancy. *Br J Obstet Gynaecol* **102**: 1227–1232.

Bromley B, Lieberman E, Shipp TD, Benacerraf BR. 2002. The genetic sonogram. A method of risk assessment for Down syndrome in the second trimester. *J Ultrasound Med* **21**: 1087–1096.

Canick J, Knight GJ, Palomaki GE, *et al*. 1988. Low second trimester maternal serum unconjugated oestriol in pregnancies with Down's syndrome. *BJOG* **95**: 330–333.

Christiansen M, Olesen Larsen S. 2002. An increase in cost-effectiveness of first trimester maternal screening programmes for fetal chromosome anomalies is obtained by contingent testing. *Prenat Diagn* **22**: 482–486.

Christiansen M, Pihl K, Hedley PL, *et al*. 2010. ADAM 12 may be used to reduce the false positive rate of first trimester combined screening for Down syndrome. *Prenat Diagn* **30**: 110–114.

Cicero S, Curcio P, Papageorghiou A, Sonek J, Nicolaides KH. 2001. Absence of nasal bone in fetuses with Trisomy 21 at 11–14 weeks of gestation: an observational study. *Lancet* **358**: 1665–1667.

Cicero S, Avgidou K, Rembouskos G, Kagan KO, Nicolaides KH. 2006. Nasal bone in first-trimester screening for trisomy 21. *Am J Obstet Gynecol* **195**: 109–114.

Cuckle H, Benn P. 2009. Multianalyte maternal serum screening for chromosomal defects. In *Genetic Disorders and the Fetus: Diagnosis, Prevention and Treatment* (6th edn), Milunsky A (ed). Johns Hopkins University: Baltimore.

Cuckle H, Maymon R. 2010. Down syndrome risk calculation for a twin fetus taking account of the nuchal translucency in the co-twin. *Prenat Diagn* **30**: 827–833.

Cuckle HS, van Lith JMM. 1999. Appropriate biochemical parameters in first-trimester screening for Down syndrome. *Prenat Diagn* **19**: 505–512.

Cuckle H, Benn P, Wright D. 2005. Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. *Semin Perinatol* **29**: 252–257.

Cuckle HS, Malone FD, Wright D, *et al*. 2008. Contingent screening for Down syndrome—results from the FaSTER trial. *Prenat Diagn* **28**: 89–94.

Ekelund CK, Jørgensen FS, Petersen OB, Sundberg K, Tabor A; Danish Fetal Medicine Research Group. 2008. Impact of a new national screening policy for Down's syndrome in Denmark: population based cohort study. *BMJ* **337**: DOI:10.1136/bmj.a2547.

Faiola S, Tsoi E, Huggon IC, Allan LD, Nicolaides KH. 2005. Likelihood ratio for trisomy 21 in fetuses with tricuspid regurgitation at the 11 to 13 + 6-week scan. *Ultrasound Obstet Gynecol* **26**: 22–27.

Falcon O, Faiola S, Huggon I, Allan L, Nicolaides KH. 2006. Fetal tricuspid regurgitation at the 11 + 0 to 13 + 6-week scan: association with chromosomal defects and reproducibility of the method. *Ultrasound Obstet Gynecol* **27**: 609–612.

Halliday JL, Watson LF, Lumley J, Danks DM, Sheffield LJ. 1995. New estimates of Down syndrome risks at chorionic villus sampling, amniocentesis, and livebirth in women of advanced maternal age from a uniquely defined population. *Prenat Diagn* **15**: 455–465.

Hecht CA, Hook EB. 1994. The imprecision in rates of Down syndrome by 1-year maternal age intervals: a critical analysis of rates used in biochemical screening. *Prenat Diagn* **14**: 729–738.

Huggon IC, DeFigueiredo DB, Allan LD. 2003. Tricuspid regurgitation in the diagnosis of chromosomal anomalies in the fetus at 11–14 weeks of gestation. *Heart* **89**: 1071–1073.

Hyett J, Moscoso G, Papapanagiotou G, Perdu M, Nicolaides KH. 1996a. Abnormatlities of the heart and great arteries in chromosomally normal fetuses with increased nuchal translucency thickness at 11–13 weeks of gestation. *Ultrasound Obstet Gynaecol* **7**: 245–250.

Hyett JA, Noble PL, Snijders RJ, Montenegro N, Nicolaides KH. 1996b. Fetal heart rate in trisomy 21 and other chromosomal abnormalities at 10–14 weeks of gestation. *Ultrasound Obstet Gynecol* **7**: 239–244.

Kagan KO, Gazzoni A, Sepulveda-Gonzalez G, Sotiriadis A, Nicolaides KH. 2007. Discordance in nuchal translucency thickness in the prediction of severe twin-to-twin transfusion syndrome. *Ultrasound Obstet Gynecol* **29**: 527–532.

Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. 2008a. First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. *Ultrasound Obstet Gynecol* **31**: 493–502.

Kagan KO, Wright D, Baker A, Sahota D, Nicolaides KH. 2008b. Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* **31**: 618–624.

Kagan KO, Wright D, Valencia C, Maiz N, Nicolaides KH. 2008c. Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free β-hCG and pregnancy-associated plasma protein-A. *Hum Reprod* **23**: 1968–1975.

Kagan KO, Anderson JM, Anwandter G, Neksasova K, Nicolaides KH. 2008d. Screening for triploidy by the risk algorithms for trisomies 21, 18 and 13 at 11 weeks to 13 weeks and 6 days of gestation. *Prenat Diagn* **28**: 1209–1213.

Kagan KO, Etchegaray A, Zhou Y, Wright D, Nicolaides KH. 2009a. Prospective validation of first-trimester combined screening for trisomy 21. *Ultrasound Obstet Gynecol* **34**: 14–18.

Kagan KO, Cicero S, Staboulidou I, Wright D, Nicolaides KH. 2009b. Fetal nasal bone in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation. *Ultrasound Obstet Gynecol* **33**: 259–264.

Kagan KO, Valencia C, Livanos P, Wright D, Nicolaides KH. 2009c. Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at $11+0$–$13+6$ weeks of gestation. *Ultrasound Obstet Gynecol* **33**: 18–22.

Kagan KO, Staboulidou I, Cruz J, Wright D, Nicolaides KH. 2010a. Two-stage first-trimester screening for trisomy 21 by ultrasound assessment and biochemical testing. *Ultrasound Obstet Gynecol* **36**: 542–547.

Kagan KO, Staboulidou I, Syngelaki A, Cruz J, Nicolaides KH. 2010b. The 11–13 weeks scan: diagnosis and outcome of holoprosencephaly, exomphalos and megacystis. *Ultrasound Obstet Gynecol* **36**: 10–14.

Kirkegaard I, Petersen OB, Uldbjerg N, Tørring N. 2008. Improved performance of first-trimester combined screening for trisomy 21 with the double test taken before a gestational age of 10 weeks. *Prenat Diagn* **28**: 839–844.

Krantz DA, Hallahan TW, Orlandi F, *et al*. 2000. First-trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. *Obstet Gynecol* **96**: 207–213.

Krantz DA, Hallahan TW, Macri VJ, Macri JN. 2007. Genetic sonography after first-trimester Down syndrome screening. *Ultrasound Obstet Gynecol* **29**: 666–670.

Langdon Down J. 1866. Observations on an ethnic classification of idiots. *Lond Hosp Rep* **3**: 259–262.

Leung TY, Chan LW, Law LW, *et al*. 2009. First trimester combined screening for Trisomy 21 in Hong Kong: outcome of the first 10,000 cases. *J Matern Fetal Neonatal Med* **22**: 300–304.

Liao AW, Snijders R, Geerts L, Spencer K, Nicolaides KH. 2000. Fetal heart rate in chromosomally abnormal fetuses. *Ultrasound Obstet Gynecol* **16**: 610–613.

Linskens IH, Spreeuwenberg MD, Blankenstein MA, van Vugt JM. 2009. Early first-trimester free beta-hCG and PAPP-A serum distributions in monochorionic and dichorionic twins. *Prenat Diagn* **29**: 74–78.

Macri JN, Kasturi RV, Krantz DA, *et al*. 1990. Maternal serum Down syndrome screening: free beta protein is a more effective marker than human chorionic gonadotrophin. *Am J Obstet Gynecol* **163**: 1248–1253.

Maiz N, Valencia C, Kagan KO, Wright D, Nicolaides KH. 2009. Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11–13 weeks of gestation. *Ultrasound Obstet Gynecol* **33**: 512–517.

Malone FD, Canick JA, Ball RH, *et al*. First- and Second-Trimester Evaluation of Risk (FASTER) Research Consortium. 2005. First-trimester or second-trimester screening, or both, for Down's syndrome. *N Engl J Med* **353**: 2001–2011.

Matias A, Gomes C, Flack N, Montenegro N, Nicolaides KH. 1998. Screening for chromosomal abnormalities at 10–14 weeks: the role of ductus venosus blood flow. *Ultrasound Obstet Gynecol* **12**: 380–384.

Maymon R, Jauniaux E, Holmes A, *et al*. 2001. Nuchal translucency measurement and pregnancy outcome after assisted conception versus spontaneously conceived twins. *Hum Reprod* **16**: 1999–2004.

Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. 1984.. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. *Am J Obstet Gynecol* **148**: 886–894.

Moratalla J, Pintoffl K, Minekawa R, *et al*. 2010. Semi-automated system for the measurement of nuchal translucency thickness. *Ultrasound Obstet Gynecol* **36**: 412–416.

Morris JK, Wald NJ, Watt HC. 1999. Fetal loss in Down syndrome pregnancies. *Prenat Diagn* **19**: 142–145.

Nicolaides KH. 1996. *Ultrasound Markers for Fetal Chromosomal Defects.* Parthenon Publishing: Carnforth, UK.

Nicolaides KH. 2003. Screening for chromosomal defects. *Ultrasound Obstet Gynecol* **21**: 313–321.

Nicolaides KH. 2004. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *Am J Obstet Gynecol* **191**: 45–67.

Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. 1992a. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. *Br Med J* **304**: 867–869.

Nicolaides KH, Snijders RJM, Gosden RJM, Berry C, Campbell S. 1992b. Ultrasonographically detectable markers of fetal chromosomal abnormalities. *Lancet* **340**: 704–707.

Nicolaides KH, Snijders RJ, Cuckle HS. 1998. Correct estimation of parameters for ultrasound nuchal translucency screening. *Prenat Diagn* **18**: 519–523.

Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. 2005. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol* **25**: 221–226.

Noble PL, Abraha HD, Snijders RJ, Sherwood R, Nicolaides KH. 1995. Screening for fetal trisomy 21 in the first trimester of pregnancy: maternal serum free beta-hCG and fetal nuchal translucency thickness. *Ultrasound Obstet Gynecol* **6**: 390–395.

Nyberg DA, Souter VL, El-Bastawissi A, *et al*. 2001. Isolated sonographic markers for detection of fetal Down syndrome in the second trimester of pregnancy. *J Ultrasound Med* **20**: 1053–1063.

Pandya PP, Snijders RJM, Johnson SJ, Brizot M, Nicolaides KH. 1995a. Screening for fetal trisomies by maternal age and fetal nuchal translucency thickness at 10 to 14 weeks of gestation. *BJOG* **102**: 957–962.

Pandya PP, Hilbert F, Snijders RJ, Nicolaides KH. 1995b. Nuchal translucency thickness and crown-rump length in twin pregnancies with chromosomally abnormal fetuses. *J Ultrasound Med* **14**: 565–568.

Papageorghiou AT, Avgidou K, Spencer K, Nix B, Nicolaides KH. 2006. Sonographic screening for trisomy 13 at 11 to 13(6) weeks of gestation. *Am J Obstet Gynecol* **194**: 397–401.

Plasencia W, Dagklis T, Sotiriadis A, Borenstein M, Nicolaides KH. 2007. Frontomaxillary facial angle at $11+0$ to $13+6$ weeks' gestation-reproducibility of measurements. *Ultrasound Obstet Gynecol* **29**: 18–21.

Poon LC, Chelemen T, Minekawa R, Frisova V, Nicolaides KH. 2009. Maternal serum ADAM12 (A disintegrin and metalloprotease) in chromosomally abnormal pregnancy at 11–13 weeks. *Am J Obstet Gynecol* **200**: 508.e1–6.

Sahota DS, Leung TY, Chan LW, *et al*. 2010. Comparison of first-trimester contingent screening strategies for Down syndrome. *Ultrasound Obstet Gynecol* **35**: 286–291.

Schuchter K, Hafner E, Stangl G, *et al*. 2002. The first trimester 'combined test' for the detection of Down syndrome pregnancies in 4939 unselected pregnancies. *Prenat Diagn* **22**: 211–215.

Sebire NJ, Snijders RJM, Hughes K, Sepulveda W, Nicolaides KH. 1996a. Screening for trisomy 21 in twin pregnancies by maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation. *BJOG* **103**: 999–1003.

Sebire NJ, Noble PL, Psarra A, Papapanagiotou G, Nicolaides KH. 1996b. Fetal karyotyping in twin pregnancies: selection of technique by measurement of fetal nuchal translucency. *BJOG* **103**: 887–890.

Sebire NJ, Hughes K, D'Ercole C, Souka A, Nicolaides KH. 1997. Increased fetal nuchal translucency at 10–14 weeks as a predictor of severe twin-to-twin transfusion syndrome. *Ultrasound Obstet Gynecol* **10**: 86–89.

Sebire NJ, Souka A, Skentou H, Geerts L, Nicolaides KH. 2000. Early prediction of severe twin-to-twin transfusion syndrome. *Hum Reprod* **15**: 2008–2010.

Sepulveda W, Sebire NJ, Hughes K, Odibo A, Nicolaides KH. 1996. The lambda sign at 10–14 weeks of gestation as a predictor of chorionicity in twin pregnancies. *Ultrasound Obstet Gynecol* **7**: 421–423.

Smith-Bindman R, Hosmer W, Feldstein V, Deeks J, Goldberg J. 2001. Second-trimester ultrasound to detect fetuses with Down syndrome a meta-analysis. *JAMA* **285**: 1044–1055.

Snijders RJM, Holzgreve W, Cuckle H, Nicolaides KH. 1994. Maternal age-specific risks for trisomies at 9–14 weeks' gestation. *Prenat Diagn* **14**: 543–552.

Snijders RJM, Sebire NJ, Cuckle H, Nicolaides KH. 1995. Maternal age and gestational age-specific risks for chromosomal defects. *Fetal Diagn Ther* **10**: 356–367.

Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. Fetal Medicine Foundation First Trimester Screening Group. 1998. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. *Lancet* **352**: 343–346.

Snijders RJM, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. 1999. Maternal age and gestation- specific risk for trisomy 21. *Ultrasound Obstet Gynecol* **13**: 167–170.

Souka AP, Snidjers RJM, Novakov A, Soares W, Nicolaides KH. 1998. Defects and syndromes in chromosomally normal fetuses with increased nuchal translucency thickness at 10–14 weeks of gestation. *Ultrasound Obstet Gynecol* **11**: 391–400.

Souka AP, Pilalis A, Kavalakis Y, *et al*. 2004. Assessment of fetal anatomy at the 11–13-week ultrasound examination. *Ultrasound Obstet Gynecol* **24**: 730–734.

Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. 2005. Increased nuchal translucency with normal karyotype. *Am J Obstet Gynecol* **192**: 1005–1021.

Spencer K, Nicolaides KH. 2000. First trimester prenatal diagnosis of trisomy 21 in discordant twins using fetal nuchal translucency thickness and maternal serum free beta-hCG and PAPP-A. *Prenat Diagn* **20**: 683–684.

Spencer K, Nicolaides KH. 2003. Screening for trisomy 21 in twins using first trimester ultrasound and maternal serum biochemistry in a one-stop clinic: a review of three years experience. *BJOG* **110**: 276–280.

Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. 1999. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* **13**: 231–237.

Spencer K, Ong C, Skentou H, Liao AW, Nicolaides KH. 2000a. Screening for trisomy 13 by fetal nuchal translucency and maternal serum free beta hCG and PAPP-A at 10–14 weeks of gestation. *Prenat Diagn* **20**: 411–416.

Spencer K, Tul N, Nicolaides KH. 2000b. Maternal serum free beta hCG and PAPP-A in fetal sex chromsome defects in the first trimester. *Prenat Diagn* **20**: 390–394.

Spencer K, Liao A, Skentou H, Cicero S, Nicolaides KH. 2000c. Screening for Triploidy by fetal nuchal translucency and maternal serum free β-hCG and PAPP-A at 10–14 weeks of gestation. *Prenat Diagn* **20**: 495–499.

Spencer K, Spencer CE, Power M, Moakes A, Nicolaides KH. 2000d. One stop clinic for assessment of risk for fetal anomalies; a report of the first year of prospective screening for chromosomal anomalies in the first trimester. *BJOG* **107**: 1271–1275.

Spencer K, Crossley JA, Aitken DA, *et al*. 2003a.. The effect of temporal variation in biochemical markers of trisomy 21 across the first and second trimesters of pregnancy on the estimation of individual patient specific risks and detection rates for Down's syndrome. *Ann Clin Biochem* **40**: 219–231.

Spencer K, Bindra R, Nix ABJ, Heath V, Nicolaides KH. 2003b. Delta- NT or NT MoM: which is the most appropriate method for calculating accurate patient-specific risks for trisomy 21 in the first trimester?. *Ultrasound Obstet Gynecol* **22**: 142–148.

Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. 2003c. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one stop clinic: a review of three years prospective experience. *Br J Obstet Gynaecol* **110**: 281–286.

Spencer K, Kagan KO, Nicolaides KH. 2008. Screening for trisomy 21 in twin pregnancies in the first trimester: an update of the impact of chorionicity on maternal serum markers. *Prenat Diagn* **28**: 49–52.

Tul N, Spencer K, Noble P, Chan C, Nicolaides KH. 1999. Screening for trisomy 18 by fetal nuchal translucency and maternal serum free beta hCG and PAPP-A at 10–14 weeks of gestation. *Prenat Diagn* **19**: 1035–1042.

Vadiveloo T, Crossley JA, Aitken DA. 2009. First-trimester contingent screening for Down syndrome can reduce the number of nuchal translucency measurements required. *Prenat Diagn* **29**: 79–82.

Vandecruys H, Faiola S, Auer M, Sebire N, Nicolaides KH. 2005. Screening for trisomy 21 in monochorionic twins by measurement of fetal nuchal translucency thickness. *Ultrasound Obstet Gynecol* **25**: 551–553.

Van Lith JM, Pratt JJ, Beekhuis JR, Mantingh A. 1993. Second trimester maternal serum immuno-reactive inhibin as a marker for fetal Down's syndrome. *Prenat Diagn* **12**: 801–806.

Vintzileos AM, Egan JF. 1995. Adjusting the risk for trisomy 21 on the basis of second-trimester ultrasonography. *Am J Obstet Gynecol* **172**: 837–844.

Vintzileos AM, Campbell WA, Rodis JF, *et al*. 1996. The use of second-trimester genetic sonogram in guiding clinical management of patients at increased risk for fetal trisomy 21. *Obstet Gynecol* **87**: 948–952.

Wald NJ, Watt HC, Hackshaw AK. 1999.. Integrated screening for Down's syndrome on the basis of tests performed during the first and second trimesters. *N Engl J Med* **341**: 461–467.

Wald NJ, Rodeck C, Hackshaw AK, *et al*. SURUSS Research Group. 2003a. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *Health Technol Assess* **7**: 1–88.

Wald NJ, Huttly WJ, Hackshaw AK. 2003b. Antenatal screening for Down's syndrome with the quadruple test. *Lancet* **361**: 835–836.

Wapner R, Thom E, Simpson JL, *et al*. 2003. First trimester maternal serum biochemistry and fetal nuchal translucency screening (BUN) study group. First trimester screening for trisomies 21 and 18. *N Engl J Med* **349**: 1405–1413.

Wright D, Bradbury I, Benn P, Cuckle H, Ritchie K. 2004. Contingent screening for Down syndrome is an efficient alternative to non-disclosure sequential screening. *Prenat Diagn* **24**: 762–766.

Wright D, Kagan KO, Molina FS, Gazzoni A, Nicolaides KH. 2008. A mixture model of nuchal translucency thickness in screening for chromosomal defects. *Ultrasound Obstet Gynecol* **31**: 376–383.

Wright D, Spencer K, Kagan KO, *et al*. 2010. First-trimester combined screening for trisomy 21 at 7–14 weeks gestation. *Ultrasound Obstet Gynecol* **36**: 404–411.

Wright D, Syngelaki A, Staboulidou I, Cruz JJ, Nicolaides KH. 2011. Screening for trisomies in dichorionic twins by measurement of fetal nuchal translucency thickness according to the mixture model. *Prenat Diagn* **31**(1): 16–21.

Wøjdemann KR, Larsen SO, Shalmi AC, *et al*. 2006. Nuchal translucency measurements are highly correlated in both mono- and dichorionic twin pairs. *Prenat Diagn* **26**: 218–220.

Zvanca M, Gielchinsky Y, Abdeljawad F, Bilardo K, Nicolaides KH. 2011. Hepatic artery Doppler in trisomy 21 and euploid fetuses at 11–13 weeks. *Prenat Diagn* **31**(1): 22–27.