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Editorial

Screening for chromosomal defects

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INTRODUCTION

Chromosomal abnormalities 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, chorionic villus sampling (CVS) or cordocentesis, is associated with a risk of miscarriage of about 1% and therefore these tests are carried out only in pregnancies considered to be at high-risk for chromosomal defects.

The methods of screening to identify the high-risk group are maternal age, ultrasound findings at 11–14 weeks and/or in the second trimester and maternal serum biochemical testing at 11–14 weeks and/or in the second trimester.

Performance of screening tests

The performance of each screening test, in terms of detection rate for trisomy 21 and false-positive rate, is summarized in Table 1 and will be discussed in the sections below.

Table 1 Detection rate for trisomy 21 and false-positive rate of screening tests

Screening test	*DR (%)*	*FPR (%)*
MA	30 (or 50)	5 (or 15)
MA + serum β-hCG and PAPP-A at 11–14 weeks	60	5
MA + fetal NT at 11–14 weeks	75 (or 70)	5 (or 2)
MA + fetal NT and NB at 11–14 weeks	90	5
MA + fetal NT and serum β-hCG and PAPP-A at 11–14 weeks	90 (or 80)	5 (or 2)
MA + fetal NT and NB and serum β-hCG and PAPP-A at 11–14 weeks	97 (or 95)	5 (or 2)
MA + serum biochemistry at 15–18 weeks	60–70	5
Ultrasound for fetal defects and markers at 16–23 weeks	75	10–15

β-hCG, beta-human chorionic gonadotropin; DR, detection rate; FPR, false-positive rate; MA, maternal age; NB, nasal bone; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A.

Calculation of individual patient-specific risk

Every woman has a risk that her fetus/baby has a chromosomal defect. In counseling women, we need to accept that decisions taken by health care planners based on arbitrary equations of the burdens of miscarriage to those of the birth of a chromosomally abnormal baby are contrary to the basic principle of informed consent. Our responsibility is to assess the risk of a pregnancy being affected using the most accurate method and to allow the parents to decide for themselves in favor or against invasive testing[1].

In order to calculate the individual risk it is necessary to take into account the background risk (which depends on maternal age and gestational age) and multiply this by a series of factors, which depend on the results of ultrasound findings and maternal serum biochemical tests carried out during the course of the pregnancy. Every time a test is carried out the background risk is multiplied by the test factor to calculate a new risk, which then becomes the background risk for the next test. This process is called sequential screening[2].

MATERNAL AGE AND GESTATION

The risk for many of the chromosomal defects increases with maternal age. Additionally, because fetuses with chromosomal defects are more likely to die *in utero* than normal fetuses, the risk decreases with gestation.

Estimates of the maternal age-related risk for trisomy 21 at birth are based on surveys with almost complete ascertainment of the affected patients. During the last decade, with the introduction of maternal serum biochemistry and ultrasound screening for chromosomal defects at different stages of pregnancy, it has become necessary to establish maternal and gestational age-specific risks for chromosomal defects. Such estimates were derived by comparing the birth prevalence of trisomy 21 to the prevalence in women undergoing second-trimester amniocentesis or first-trimester CVS (Table 2)[3]. The rates of fetal death in trisomy 21 between 12 weeks (when nuchal translucency (NT) screening is carried out) and term is 30% and between 16 weeks (when second-trimester serum biochemistry or ultrasound screening is carried out) and term is 20%[3].

The risk for trisomies 18 and 13 increases with maternal age and decreases with gestation; the rate of intrauterine lethality between 12 and 40 weeks is about 80% (Table 3)[4]. Turner syndrome is usually due to loss of the paternal X chromosome and, consequently, the frequency of conception of 45,X embryos, unlike that of trisomies, is unrelated to maternal age. The prevalence is about 1 in 1500 at 12 weeks, 1 in 3000 at 20 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 since the rate of intrauterine lethality is not higher than in chromosomally normal fetuses the overall prevalence (about 1 in 500) does not decrease with gestation. Polyploidy affects about 2% of recognized conceptions but it is highly lethal and thus very rarely observed in live births; the prevalence at 12 and 20 weeks is about 1 in 2000 and 1 in 250 000, respectively[4].

Table 2 Estimated risk for trisomy 21 (1/number given in the table) in relation to maternal age and gestation

	Gestation (weeks)			
MA (years)	*12*	*16*	*20*	*40*
20	898	1053	1175	1527
21	887	1040	1159	1507
22	872	1022	1140	1482
23	852	999	1114	1448
24	827	969	1081	1406
25	795	933	1040	1352
26	756	887	989	1286
27	710	832	928	1206
28	655	768	856	1113
29	593	695	776	1008
30	526	617	688	895
31	457	536	597	776
32	388	455	507	659
33	322	378	421	547
34	262	307	343	446
35	210	246	274	356
36	165	193	216	280
37	128	150	168	218
38	98	115	129	167
39	75	88	98	128
40	57	67	74	97
41	43	50	56	73
42	32	38	42	55

MA, maternal age.

Table 3 Estimated risk for trisomies 18 and 13 (1/number given in the table) in relation to maternal age and gestation

	Gestation (weeks)			
MA (years)	*12*	*16*	*20*	*40*
20	1886	2709	4897	18 013
25	1670	2399	4336	15 951
30	1105	1587	2869	10 554
31	959	1377	2490	9160
32	814	1169	2114	7775
33	676	971	1755	6458
34	550	790	1429	5256
35	440	632	1142	4202
36	346	497	899	3307
37	269	386	698	2569
38	207	297	537	1974
39	158	226	409	1505
40	119	171	310	1139
41	90	129	233	858
42	68	97	175	644

MA, maternal age.

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 countries there was an overall tendency for women to get pregnant at an older age, so that now about 15% of pregnant women are 35 years or older and this group contains about 50% of the total number of fetuses with trisomy 21.

PREVIOUS AFFECTED PREGNANCY

The risk for trisomies in women who have had a previous fetus or child with a trisomy is higher than the one expected on the basis of their age alone. In a study of 2054 women who had a previous pregnancy with trisomy 21 we found that the risk of recurrence in the subsequent pregnancy was 0.75% higher than the maternal and gestational age-related risk for trisomy 21 at the time of testing. Thus, for a woman aged 35 years who has had a previous baby with trisomy 21 the risk at 12 weeks of gestation increases from 1 in 249 (0.40%) to 1 in 87 (1.15%), and for a woman aged 25 years it increases from 1 in 946 (0.106%) to 1 in 117 (0.856%).

In 750 women who had a previous pregnancy with trisomy 18 the risk of recurrence of trisomy 18 in the subsequent pregnancy was also about 0.75% higher

than the maternal and gestational age-related risk for trisomy 18; the risk for trisomy 21 in these women was not increased. Therefore, the risk of recurrence is chromosomal abnormality specific.

THE 11–14-WEEK SCAN

In 1866, Langdon Down reported that the skin of individuals with trisomy 21 appears to be too large for their body[5]. In the 1990s it was realized that this excess skin could be visualized by ultrasonography as increased NT in the third month of intrauterine life[6]. Fetal NT at the 11–14-week scan has been combined with maternal age to provide an effective method of screening for trisomy 21; for an invasive testing rate of 5%, about 75% of trisomic pregnancies can be identified[7–20]. When maternal serum free-β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11–14 weeks are also taken into account, the detection rate of chromosomal defects is about 90%[21–25]. Recent studies suggest that at the 11–14-week scan the fetal nasal bone is not visible in about 60–70% of trisomy 21 fetuses and that examination of the nasal bone can be combined with fetal NT and maternal serum free β-hCG and PAPP-A to achieve a detection rate of more than 95%[26–30].

Fetal NT

The NT normally increases with gestation (crown–rump length, CRL). In a fetus with a given CRL, every NT measurement represents a factor that is multiplied by the background risk to calculate a new risk. The larger the NT, the higher the multiplying factor becomes and therefore the higher the new risk. In contrast, the smaller the NT measurement, the smaller the multiplying factor becomes and therefore the lower the new risk[12].

There are 14 prospective studies examining the implementation of NT measurement in screening for trisomy 21 (Table 4)[7–20]. Although different cut-offs were used for identifying the screen-positive group, with consequent differences in the false-positive and detection rates, all the studies reported high detection rates. The combined results on a total of 174 473 pregnancies, including 728 with trisomy 21, demonstrated a detection rate of 77% for a false-positive rate of 4.7%.

Fetal NT and maternal serum free β-hCG and PAPP-A

In trisomy 21 pregnancies at 11–14 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 detection rate of trisomy 21 by first-trimester biochemical screening is about 60% for a screen-positive rate of 5%[23].

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 individually. In a retrospective study we estimated that the detection rate for trisomy 21 by a combination of maternal age, fetal NT and maternal serum PAPP-A and free β-hCG would be about 90% for a screen-positive rate of 5% and these results were confirmed by the results of major prospective studies[23–25]. 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 min 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).

Chromosomal defects other than trisomy 21

Increased NT is also a marker of chromosomal abnormalities other than trisomy 21[12]. In addition to

Table 4 Studies examining the implementation of fetal nuchal translucency screening

Authors	n	*Gestation (weeks)*	*NT cut-off*	*FPR (%)*	*Trisomy 21 DR (%)*
Pandya *et al.* (1995)[7]	1763	10–14	≥ 2.5 mm	3.6	3/4 (75)
Szabo *et al.* (1995)[8]	3380	9–12	≥ 3.0 mm	1.6	28/31 (90)
Taipale *et al.* (1997)[9]	6939	10–14	≥ 3.0 mm	0.8	4/6 (67)
Hafner *et al.* (1998)[10]	4371	10–14	≥ 2.5 mm	1.7	4/7 (57)
Pajkrt *et al.* (1998)[11]	1547	10–14	≥ 3.0 mm	2.2	6/9 (67)
Snijders *et al.* (1998)[12]	96 127	10–14	≥ 95th centile	4.4	234/327 (72)
Economides *et al.* (1998)[13]	2281	11–14	≥ 99th centile	0.4	6/8 (75)
Schwarzler *et al.* (1999)[14]	4523	10–14	>2.5 mm	2.7	8/12 (67)
Theodoropoulos *et al.* (1998)[15]	3550	10–14	≥ 95th centile	2.3	10/11 (91)
Zoppi *et al.* (2001)[16]	12 311	10–14	≥ 95th centile	5.0	52/64 (81)
Gasiorek-Wiens *et al.* (2001)[17]	23 805	10–14	≥ 95th centile	8.0	174/210 (83)
Brizot *et al.* (2001)[18]	2996	10–14	≥ 95th centile	5.3	7/10 (70)
Audibert *et al.* (2001)[19]	4130	10–14	≥ 95th centile	4.3	9/12 (75)
Wayda *et al.* (2001)[20]	6750	10–12	≥ 2.5 mm	4.3	17/17 (100)
Totals	174 473			4.7	562/728 (77)

DR, detection rate; FPR, false-positive rate.

increased NT, there are other characteristic sonographic findings in these fetuses. In trisomy 18, there is early onset intrauterine growth restriction (IUGR), relative bradycardia and, in about 30% of cases, there is an associated exomphalos[31]. Trisomy 13 is characterized by fetal tachycardia (observed in about two-thirds of cases), early-onset IUGR and holoprosencephaly or exomphalos in about 30% of cases[32]. Turner syndrome is characterized by fetal tachycardia, observed in about 50% of cases, and early-onset IUGR[33]. In triploidy, there is early-onset asymmetrical IUGR, relative bradycardia, holoprosencephaly, exomphalos or posterior fossa cyst in about 40% of cases and molar changes in the placenta in about one-third of cases[34].

In trisomies 18 and 13, maternal serum free β-hCG and PAPP-A are decreased[35,36]. In cases of sex chromosomal anomalies, maternal serum free β-hCG is normal and PAPP-A is low[37]. In diandric triploidy, maternal serum free β-hCG is greatly increased, whereas PAPP-A is mildly decreased[38]. Digynic triploidy is associated with markedly decreased maternal serum free β-hCG and PAPP-A[38]. Screening by a combination of fetal NT and maternal serum PAPP-A and free β-hCG can identify about 90% of all these chromosomal abnormalities for a screen-positive rate of 1%.

Fetal nasal bone

At 11–14 weeks of gestation the nasal bone is not visible by ultrasonographic examination in about 60–70% of fetuses with trisomy 21 and in less than 1% of chromosomally normal fetuses[26–29]. In an extended study of 3788 pregnancies undergoing CVS at 11–14 weeks, the incidence of absent nasal bone was 2.8% in the 3358 chromosomally normal fetuses, in 67% of 242 fetuses with trisomy 21 and in 33% of the 188 with other chromosomal defects. However, in both the chromosomally normal and abnormal fetuses the incidence of absent nasal bone decreased with fetal CRL, increased with NT and was substantially higher in Afro-Caribbeans than in Caucasians. Consequently, in the calculation of likelihood ratios adjustments must be made for these confounding factors. For example, the likelihood ratio for trisomy 21 associated with absent nasal bone was 26 for Caucasians and only 7 for Afro-Caribbeans, it was 17 for CRLs of 45–54 mm and it increased to 44 for CRLs of 75–84 mm, and it was 34 for NT below the 95th centile and decreased to 5 for NT of > 5.5 mm[39].

Preliminary data suggest that screening for trisomy 21 at 11–14 weeks by a combination of the sonographic markers of nasal bone and NT and the biochemical markers of free β-hCG and PAPP-A could result in a detection rate of about 97% for a false-positive rate of 5%, or a detection of 95% for a false-positive rate of 2%[30].

NT followed by second-trimester biochemistry

In women having second-trimester biochemical testing following first-trimester NT screening (with or without first-trimester maternal serum biochemistry) the background risk needs to be adjusted to take into account the first-trimester screening results. Since first-trimester screening identifies almost 90% of trisomy 21 pregnancies, second-trimester biochemistry will identify – at best – 6% (60% of the residual 10%) of the affected pregnancies, with doubling of the overall invasive testing rate (from 5% to 10%). It is theoretically possible to use various statistical techniques to combine NT with different components of first- and second-trimester biochemical testing. One such hypothetical model has combined first-trimester NT and PAPP-A with second-trimester free β-hCG, estriol and inhibin A, claiming a potential sensitivity of 94% for a 5% false-positive rate[40]. Even if the assumptions made in this statistical technique are valid, it is unlikely that it will gain widespread clinical acceptability[41].

Two studies have reported on the impact of first-trimester screening by NT on second-trimester serum biochemical testing. In one study the proportion of affected pregnancies in the screen-positive group (positive predictive value) with screening by the double test in the second trimester was 1 in 40. After the introduction of screening by NT, 83% of trisomy 21 pregnancies were identified in the first trimester and the positive predictive value of biochemical screening decreased to 1 in 200[42]. In the second study, first-trimester screening by NT identified 71% of trisomy 21 pregnancies for a screen-positive rate of 2%, and the positive predictive value of second-trimester biochemical screening was only 1 in 150[43]. These data illustrate that in sequential screening it is essential that in the interpretation of results from a second screening test the results of the first screening test are taken into account.

Two studies reported on screening by a combination of fetal NT in the first trimester and maternal serum biochemistry in the second trimester. Schuchter *et al.* examined 9342 pregnancies and classified as screen-positive those with fetal NT $\geq$ 2.5 mm or those with estimated risk from biochemical testing of $\geq$ 1 in 250[44]. The screen-positive rate was 7.2% and the sensitivity for trisomy 21 was 94.7% (18/19 cases). Similarly, Audibert *et al.* examined 4130 pregnancies and classified as screen-positive those with fetal NT $\geq$ 3.0 mm or those with estimated risk from biochemical testing of $\geq$ 1 in 250[19]. The screen-positive rate was 5.0% and the sensitivity for trisomy 21 was 90% (9/10 cases). These results demonstrate that screening by a combination of maternal age, fetal NT and maternal serum PAPP-A and free β-hCG at 11–14 weeks or the triple test (β-hCG, α-fetoprotein (AFP), unconjugated estriol (uE3)) or quadruple test (β-hCG, AFP, uE3, inhibin A) in the second trimester can detect about 90% of trisomy 21 pregnancies for a screen-positive rate of 5%.

SECOND-TRIMESTER SERUM BIOCHEMISTRY

In 1984, a major advance in screening for chromosomal defects was made by Merkatz *et al.* who reported low levels of maternal serum AFP in trisomy 21

pregnancies[45]. Subsequently, altered maternal serum levels in affected pregnancies were reported for a series of other fetoplacental products including free β-hCG, inhibin A and uE3[46–48]. Screening by maternal age and various combinations of the fetoplacental products in maternal serum is associated with a detection rate of trisomy 21 of 60–70% for a false-positive rate of 5%[49]. However, an essential component of biochemical screening is accurate dating of the pregnancy by ultrasound, otherwise the detection rate is reduced by about 10%.

SECOND-TRIMESTER ULTRASOUND

In the first trimester, a common feature of many chromosomal defects is increased NT. In later pregnancy each chromosomal defect has its own syndromal pattern of abnormalities.

Phenotypic expression of chromosomal defects

Trisomy 21 is associated with a tendency for brachycephaly, mild ventriculomegaly, nasal hypoplasia, nuchal edema (or increased nuchal fold thickness), cardiac defects (mainly atrioventricular septal defects), duodenal atresia and echogenic bowel, mild hydronephrosis, shortening of the femur and more so of the humerus, sandal gap and clinodactyly or mid-phalanx hypoplasia of the fifth finger. Trisomy 18 is associated with strawberry-shaped head, choroid plexus cysts, absent corpus callosum, enlarged cisterna magna, facial cleft, micrognathia, nuchal edema, heart defects, diaphragmatic hernia, esophageal atresia, exomphalos, usually with bowel only in the sac, single umbilical artery, renal defects, echogenic bowel, myelomeningocele, growth restriction and shortening of the limbs, radial aplasia, overlapping fingers and talipes or rocker-bottom feet. In trisomy 13, common defects include holoprosencephaly and associated facial abnormalities, microcephaly, cardiac and renal abnormalities with often enlarged and echogenic kidneys, exomphalos and postaxial polydactyly. Triploidy where the extra set of chromosomes is paternally derived is associated with a molar placenta and the pregnancy rarely persists beyond 20 weeks. When there is a double maternal chromosome contribution the pregnancy may persist into the third trimester. The placenta is of normal consistency but thin and the fetus demonstrates severe asymmetrical growth restriction. Commonly there is mild ventriculomegaly, micrognathia, cardiac abnormalities, myelomeningocele, syndactyly, and 'hitch-hiker' toe deformity. The lethal type of Turner syndrome presents with large nuchal cystic hygromata, generalized edema, mild pleural effusions and ascites, cardiac abnormalities and horseshoe kidney, which are suspected by the ultrasonographic appearance of bilateral mild hydronephrosis.

Individual patient-specific risks based on ultrasound findings

The overall risk for chromosomal abnormalities increases with the total number of defects that are identified[50]. It is therefore recommended that when a defect/marker is detected at routine ultrasound examination, a thorough check is made for the other features of the chromosomal abnormality known to be associated with that marker, because the presence of additional defects increases the risk substantially.

In contrast, absence of any major or minor defects is associated with a reduction in the background risk. In the combined data from two leading centers of obstetric ultrasound in the USA there were no identifiable major defects or any of the following markers – increased nuchal fold thickness, echogenic bowel, echogenic intracardiac focus, mild hydronephrosis, short humerus or short femur – in 25.7% of the 350 fetuses with trisomy 21 and in 86.5% of the 9384 chromosomally normal fetuses[51,52]. Consequently, the likelihood ratio for trisomy 21 if there is no detectable defect or marker is 0.30 (95% CI 2.25–0.35).

A patient attending for amniocentesis at 16 weeks of gestation because she is 35 years old and considers her risk for trisomy 21 (1 in 246, see Table 2) to be sufficiently high to justify the 1 in 100 risk of miscarriage from an invasive test will inevitably have an ultrasound examination by the competent practitioner who is about to carry out the amniocentesis. If this scan demonstrates no major or minor defects the patient should be informed that her risk for trisomy 21 is actually reduced to 1 in 820 (which is equivalent to that of a 27-year-old) and she may well change her mind and avoid having an amniocentesis. The same is obviously true for a 31-year-old (background risk of 1 in 536) who after second-trimester biochemical testing is informed that she is now screen-positive and is offered an amniocentesis because her risk has increased to 1 in 200. However, the patient should also be informed that if an ultrasound examination shows no major defects or markers her risk can be reduced to 1 in 667 (which is equivalent to that of a 29-year-old) and she may well choose this option.

If the mid-trimester scan demonstrates major defects it is advisable to offer fetal karyotyping, even if these defects are apparently isolated. The prevalence of these defects is low and therefore the cost implications are small. If the defects 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 defect is potentially correctable by intrauterine or postnatal surgery, such as diaphragmatic hernia, it may be logical to exclude an underlying chromosomal abnormality – especially because, for many of these conditions, the associated chromosomal abnormality is trisomy 18 or 13.

Minor defects or markers are common and they are not usually associated with any handicap, unless there is an associated chromosomal abnormality. 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 abnormality,

Table 5 Incidence of major and minor defects or markers in the second-trimester scan in trisomy 21 and chromosomally normal fetuses in the combined data of two major series.[51,52] From these data the positive and negative likelihood ratios (with 95% CIs) for each marker can be calculated

	Trisomy 21 (%)	*Normal (%)*	*Positive LR (95% CI)*	*Negative LR (95% CI)*	*LR for isolated marker*
Nuchal fold	107/319 (33.5)	59/9331 (0.6)	53.05 (39.37–71.26)	0.67 (0.61–0.72)	9.8
Short humerus	102/305 (33.4)	136/9254 (1.5)	22.76 (18.04–28.56)	0.68 (0.62–0.73)	4.1
Short femur	132/319 (41.4)	486/9331 (5.2)	7.94 (6.77–9.25)	0.62 (0.56–0.67)	1.6
Hydronephrosis	56/319 (17.6)	242/9331 (2.6)	6.77 (5.16–8.80)	0.85 (5.16–8.80)	1.0
Echogenic focus	75/266 (28.2)	401/9119 (4.4)	6.41 (5.15–7.90)	0.75 (0.69–0.80)	1.1
Echogenic bowel	39/293 (13.3)	58/9227 (0.6)	21.17 (14.34–31.06)	0.87 (0.83–0.91)	3.0
Major defect	75/350 (21.4)	61/9384 (0.65)	32.96 (23.90–43.28)	0.79 (0.74–0.83)	5.2

LR, likelihood ratio.

rather than the arbitrary advice that invasive testing is recommended because the risk is 'high'. The estimated risk can be derived by multiplying the background risk (based on maternal age, gestational age, history of previously affected pregnancies and, where appropriate, the results of previous screening by NT and/or biochemistry in the current pregnancy) by the likelihood ratio of the specific defect.

The combined data from Nyberg *et al*. and Bromley *et al*. are summarized in Table 5[51,52]. The incidence of each marker in trisomy 21 pregnancies can be divided by their incidence in chromosomally normal pregnancies to derive the appropriate likelihood ratio. For example, an intracardiac echogenic focus is found in 28.2% of trisomy 21 fetuses and in 4.4% of chromosomally normal fetuses, resulting in a positive likelihood ratio of 6.41 (28.2/4.4) and a negative likelihood ratio of 0.75 (71.8/95.6). Consequently, the finding of an echogenic focus increases the background risk by a factor of 6.41, but at the same time absence of this marker should reduce the risk by 25%. The same logic applies to each one of the six markers in Table 5. Thus, in a 25-year-old woman undergoing an ultrasound scan at 20 weeks of gestation the background risk is about 1 in 1000. If the scan demonstrates an intracardiac echogenic focus, but the nuchal fold is not increased, the humerus and femur are not short and there is no hydronephrosis, hyperechogenic bowel or major defect, the combined likelihood ratio should be 1.1 ($6.41 \times 0.67 \times 0.68 \times 0.62 \times 0.85 \times 0.87 \times 0.79$) and consequently her risk remains at about 1 in 1000. The same is true if the only abnormal finding is mild hydronephrosis, which has a combined likelihood ratio of 1.0 ($6.77 \times 0.67 \times 0.68 \times 0.62 \times 0.75 \times 0.87 \times 0.79$). In contrast, if the fetus is found to have both an intracardiac echogenic focus and mild hydronephrosis but no other defects the combined likelihood ratio should be 8.42 ($6.41 \times 6.77 \times 0.67 \times 0.68 \times 0.62 \times 0.87 \times 0.79$) and consequently the risk is increased from 1 in 1000 to 1 in 119.

A recently described second-trimester ultrasound marker that is likely to have a major impact on screening for trisomy 21 is nasal bone hypoplasia, defined by a nasal bone that is not visible or with a length of less than 2.5 mm[53]. In 1046 singleton pregnancies undergoing amniocentesis for fetal karyotyping at 15–22 weeks, the nasal bone was hypoplastic in 21/34 (61.8%) fetuses with trisomy 21, in 12/982 (1.2%) chromosomally normal fetuses and in 1/30 (3.3%) fetuses with other chromosomal defects. In the chromosomally normal group, hypoplastic nasal bone was found in 0.5% of Caucasians and in 8.8% of Afro-Caribbeans. The likelihood ratio for trisomy 21 for hypoplastic nasal bone was 132.1 (95% CI 49.1–351.9) for Caucasians and 8.5 (95% CI 2.7–20.1) for Afro-Caribbeans and the respective values for present nasal bone were 0.39 (95% CI 0.24–0.58) and 0.27 (95% CI 0.05–0.77). It is premature to speculate on the precise detection rates that could be achieved in the second trimester by a combination of maternal age, serum biochemistry and ultrasound examination for the fetal nasal bone and other sonographic markers. Nevertheless, the findings of the study, that nasal hypoplasia is likely to be the single most sensitive and specific second-trimester marker of trisomy 21, indicate that examination of the nasal bone will inevitably be incorporated into a sonographic or combined screening program for trisomy 21.

There are no data on the interrelation between the second-trimester ultrasound markers and NT at 11–14 weeks or first- and second-trimester biochemistry. However, there is no obvious physiological reason for such an interrelation and it is therefore reasonable to assume that they are independent. Consequently, in estimating the risk in a pregnancy with a marker, it is logical to take into account the results of previous screening tests. For example, in a 39-year-old woman at 20 weeks of gestation (background risk for trisomy 21 of about 1 in 100), who had a 11–14-week assessment by fetal NT and serum free β-hCG and PAPP-A that resulted in a ten-fold reduction in risk (to about 1 in 1000), after the diagnosis of a short femur but no other abnormal findings at the 20-week scan (likelihood ratio of 1.6, see Table 5), the estimated new risk is 1 in 625.

There are some exceptions to this process of sequential screening, which assumes independence between the findings of different screening results. The findings of nuchal edema or a cardiac defect at the mid-trimester scan cannot be considered independently of NT screening at 11–14 weeks. Similarly, hyperechogenic bowel (which

may be due to intra-amniotic bleeding) and relative shortening of the femur (which may be due to placental insufficiency) may well be related to serum biochemistry (high free β-hCG and inhibin-A and low estriol may be markers of placental damage) and can therefore not be considered independently in estimating the risk for trisomy 21. For example, in a 20-year-old woman (background risk for trisomy 21 of 1 in 1175), with high free β-hCG and inhibin-A and low estriol at the 16-week serum testing resulting in a ten-fold increase in risk (to 1 in 118), the finding of hyperechogenic bowel at the 20-week scan should not lead to the erroneous conclusion of a further three-fold increase in risk (to 1 in 39). The coincidence of biochemical and sonographic features of placental insufficiency makes it very unlikely that the problem is trisomy 21 and should lead to increased monitoring for pre-eclampsia and growth restriction, rather than amniocentesis for fetal karyotyping.

CONCLUSIONS

In developed countries, there are approximately 100 000 deliveries per year per 10 000 000 of the population. The birth incidence of trisomy 21 is about 1 in 500, and therefore in such a population the total number of affected neonates is about 200.

A policy of screening on the basis of maternal age and offering an invasive test to all women aged 35 years or more would result in invasive testing in 15% of the pregnancies (15 000), with consequent miscarriage in 150, for the detection of 50% (100 of the 200) of the trisomy 21 fetuses. The practice of (1) subjecting all women aged 35 years or more to invasive testing; (2) in those under the age of 35 years, carrying out a series of additional sonographic and biochemical tests in the first and second trimesters; (3) interpreting the results of each screening test independently of each other and (4) performing an invasive test in all women with a screen-positive result, would potentially identify more than 95% (190 of the 200) of the trisomic fetuses, but this would be achieved by subjecting more than 40% of the population (40 000) to invasive testing and causing 400 miscarriages.

A more rational approach is to carry out a screening test at 11–14 weeks by combining maternal age with sonographic measurement of fetal NT and maternal serum measurement of free β-hCG and PAPP-A. In addition, the fetal profile can be examined for the presence or absence of the nasal bone. A detection rate of 95% can potentially be achieved with an invasive testing rate of about 2% (2000 pregnancies and 20 miscarriages). It would then be irrational, both in terms of logistics and economic cost, to subject the remaining 98 000 pregnancies to second-trimester serum biochemical testing with the objective of identifying about 60–70% of the remaining 10 cases of trisomy 21. Since all women should be offered a second-trimester ultrasound scan to identify major fetal abnormalities such as spina bifida and cardiac defects, the diagnosis of major and or minor defects, including nasal bone hypoplasia, will potentially lead to the detection of more than 70% of the remaining 10 cases of trisomy 21. This Editorial has demonstrated the methodology of calculating the likelihood ratio for trisomy 21 for some of the ultrasound markers and the process of sequential screening in the interpretation of results. I suspect that only nasal bone hypoplasia, nuchal edema and the presence of multiple other second-trimester sonographic markers will be associated with sufficiently high likelihood ratios to reverse a low background risk after first-trimester screening.

An alternative method of screening, for women not presenting in the first trimester, is by a combination of maternal age, serum biochemical testing and ultrasound scanning. The detection rate of such combined screening may also be more than 90% for a false-positive rate of less than 5%. However, extensive research is needed to establish accurate likelihood ratios for many of the ultrasound defects and their interdependence as well as their dependence on biochemical markers, gestational age, ethnic origin and other parental demographic characteristics.

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