

# Screening for Down syndrome using first-trimester ultrasound and second-trimester maternal serum markers in a low-risk population: a prospective longitudinal study

F. AUDIBERT, M. DOMMERGUES, C. BENATTAR\*, J. TAIEB\*, J.-C. THALABARD† and R. FRYDMAN

Departments of Obstetrics and Gynecology and \*Biochemistry, Hopital Antoine Beclere, Université Paris XI and †Biostatistique – Informatique Médicale et Médecine de la Reproduction, Hopital Necker, Université Paris V, Paris, France

**KEYWORDS:** Combined risk, Down syndrome, Nuchal translucency, Serum screening

## ABSTRACT

**Objectives** To compare nuchal translucency and second-trimester maternal serum measurements as alternative methods of antenatal screening for Down syndrome in a low-risk population and to evaluate the consequence of combining the results in the estimation of risk.

**Design** In a consecutive series of 4130 women aged less than 38 years with a singleton pregnancy, we examined both the detection rate of Down syndrome by nuchal translucency measurement at 10–14 weeks and maternal serum screening by human chorionic gonadotrophin and alpha-fetoprotein at 14–18 weeks. Women with a nuchal translucency measurement of  $\geq 3$  mm and women with a maternal serum screening-derived risk  $\geq 1/250$  were recommended to have amniocentesis. A second-trimester detailed ultrasound scan was also performed in all women. The outcome of all pregnancies was recorded prospectively and the detection rate and false-positive rate of different screening strategies were retrospectively analyzed.

**Results** Out of the 4130 pregnancies that were followed (mean maternal age, 30.1 years), 12 cases of Down syndrome were observed (0.28%), all detected prenatally. Seven of 12 cases had a nuchal translucency measurement of  $\geq 3$  mm (58%), and six out of 10 cases with available maternal serum screening had a calculated risk of  $\geq 1/250$  (60%). Four of the five Down syndrome cases with a nuchal translucency measurement of  $< 3$  mm were detected by subsequent maternal serum screening. At a threshold giving 5% of positive tests, the sensitivity of nuchal translucency, maternal serum screening and combined risk screening were 75%, 60% and 90%, respectively.

**Conclusions** In screening for Down syndrome, an approach which combines the results from first-trimester nuchal translucency and second-trimester biochemistry is effective and

increases the detection rate compared to the use of any single test. However, this strategy is likely to raise the false-positive rate and the interpretation of maternal serum screening-derived risk should be combined with the first-trimester nuchal translucency measurement.

## INTRODUCTION

In the past 10 years, alternative methods of screening for Down syndrome (trisomy 21) have been studied. Second-trimester biochemical screening<sup>1,2</sup> uses a combination of two or three serum markers (human chorionic gonadotrophin (hCG), alpha-fetoprotein, estriol) at 14–18 weeks of gestation, whilst ultrasound screening<sup>3–7</sup> is based upon nuchal translucency (NT) measurement at 12 weeks (10–14 weeks). The detection rate of second-trimester biochemical screening ranges from 40% to 70% with a 5% false-positive rate, depending on the combination of markers analyzed<sup>2</sup>. The sensitivity of first-trimester ultrasound screening ranges from 30% to 85%<sup>4,5,7–9</sup>. However, studies analyzing both methods in the same pregnancies strongly suggest that the implementation of a NT screening program reduces the positive predictive value of maternal serum screening (MSS)<sup>10,11</sup>. Thus the sequential use of both tests is likely to raise the false-positive rate, i.e. the number of women to whom an invasive procedure is recommended. This underscores the need for re-evaluating the calculation of the risk when both tests are applied. The aim of our study was to compare different strategies of screening for the detection of Down syndrome in a low-risk population.

## PATIENTS AND METHODS

From May 1994 to December 1997, prenatal screening for Down syndrome was routinely offered to women who booked

Correspondence: Dr F. Audibert, Department of Obstetrics and Gynecology, Hopital Antoine Beclere, 92140 Clamart, France (e-mail: francois.audibert@abc.ap-hop-paris.fr)

Received 21-3-00, Revised 20-3-01, Accepted 24-4-01

at our institution. The screening included a first-trimester sonographic scan and second-trimester MSS. All women received information on the screening program and written consent was obtained in all cases. The local ethical committee approved the study. The first-trimester scan was planned at 12–13 weeks and performed by one of eight physicians or six midwives specially trained in first-trimester ultrasound. When a transabdominal scan could not provide a complete survey, the transvaginal route was used. Nuchal translucency was measured in the sagittal plane as the maximum thickness of the sonolucent zone between the fetal skin and the soft tissue overlying the cervical spine or the occipital bone. The fetuses with NT of  $\geq 3$  mm were examined by at least two observers. Cystic hygroma was defined as a sonolucency in soft tissues of the occipital region, consisting of two cavities separated by a midline septum. For the purpose of this study, cystic hygroma was classified as increased nuchal thickness. Maternal serum screening was performed by measurement of alpha-fetoprotein and total human chorionic gonadotrophin (Amerlite, Ortho Clinical Diagnostics France, Issy-les-Moulineaux, France) between 14 and 17<sup>+6</sup> weeks, with a cut-off risk of 1/250 (Prenata Software, Ortho Clinical Diagnostics). All women with an ongoing pregnancy underwent a detailed second-trimester ultrasound scan between 20 and 24 weeks. Partial results for the 1554 first pregnancies have been reported previously<sup>12</sup>.

Karyotyping by amniocentesis was recommended for increased NT ( $\geq 3$  mm), positive MSS, or suggestive abnormalities at second-trimester ultrasound. Although second-trimester amniocentesis was the technique of choice, chorionic villus sampling (CVS) was usually offered to patients with cystic hygroma, which is associated with a poor outcome and a high risk of aneuploidy<sup>5</sup>. For the majority of women, serum markers were also studied in cases where NT indicated karyotyping to permit comparison of the two screening methods. Maternal serum was obtained before amniocentesis. This was of course not possible in women who requested termination of pregnancy by suction before 14 weeks, in particular in cases where CVS was performed before week 14. Fetal postmortem examination was performed following each termination of pregnancy, spontaneous abortion or intrauterine demise. Every newborn was examined by a pediatrician and karyotyping was performed in newborns suspected of having any type of chromosomal abnormality. Women lost to follow up were excluded from the final analysis. Women above 38 years were not included in this analysis because karyotyping is routinely proposed in France after age 38. Data on pregnancies and deliveries were prospectively entered in a database.

Although the threshold initially used for clinical decision was 3 mm, we studied the distribution of NT in our population. Nuchal translucency was plotted against crown–rump length and a regression analysis was performed on the observed medians. Our results were similar to those of Snijders *et al.*<sup>13</sup> Using estimates of the parameters required for risk calculation previously published by Nicolaides *et al.*<sup>14</sup>, we calculated the NT-derived likelihood ratio for each patient.

The detection rate and false-positive rate of the following strategies were analyzed: (i) karyotyping when NT  $\geq 3$  mm; (ii) NT  $\geq 95$ th percentile; (iii) NT-derived risk  $\geq 1/250$ ; (iv) MSS-derived risk  $\geq 1/250$ .

The independence between NT and serum markers was tested by a correlation test on the joined distributions of the log-transformed variables expressed in MoMs, giving a correlation coefficient of  $-0.02$  and  $-0.0075$  for hCG and alpha-fetoprotein, respectively.

Finally, in order to provide a risk evaluation combining NT and MSS, we multiplied the age and MSS-derived risk by the likelihood ratio derived from NT:

Combined risk =

Age and MSS-derived risk  $\times$  NT-derived likelihood ratio.

## RESULTS

Between May 1994 and December 1997, a total of 5245 consecutive women with a viable pregnancy were scanned in the first trimester. One thousand and eighty women were excluded from the study for the following reasons: twin pregnancy ( $n = 161$ ), CRL  $< 38$  mm or  $> 84$  mm ( $n = 303$ ), NT not measured or not recorded ( $n = 219$ ), or maternal age above 38 ( $n = 397$ ). Thus 4165 singleton pregnancies with a crown–rump length of between 38 and 84 mm in whom NT measurements were performed were initially included in the study. The outcome of 35 pregnancies (0.8%) could not be ascertained because women changed their address and/or hospital and were lost to follow up. Although no ultrasound abnormalities were found in this group, they were excluded from the study. Thus, a total of 4130 pregnancies were finally analyzed. Of these, maternal serum biochemistry was studied in 3790 pregnancies, including 65 cases with positive NT screening (10 cases of trisomy 21). Serum screening was not performed in 340 women who declined the test or who underwent spontaneous abortion or termination of pregnancy before 14 weeks. In 37 women the pregnancy resulted in either spontaneous abortion ( $n = 21$ ), or intrauterine fetal demise ( $n = 16$ ). In addition, pregnancy was terminated in 39 cases (22 aneuploidies, 2 chromosomal structural abnormalities, 15 multiple abnormalities with normal karyotype).

The mean age of the study population was 30.1 years (range, 16–37 years). Three thousand five hundred and forty-eight women were less than 35 years old (86%) and 582 women were aged between 35 and 37 years (14%). The distribution of the population according to crown–rump length is shown in Table 1, along with the number of NTs  $> 3$  mm. Figure 1 shows NT measurement percentiles according to crown–rump length calculated from our population.

Overall, prenatal karyotyping was performed in 315/4130 women (7.6%). Table 2 shows the number of karyotypings

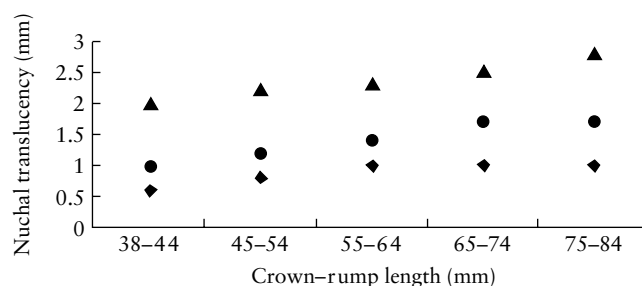


Figure 1 Nuchal translucency measurement related to crown–rump length showing 5th (◆), 50th (●) and 95th (▲) percentiles.

performed according to the screening criteria, and chromosomal anomalies observed in each group. All significant chromosomal defects were diagnosed prenatally.

Eighty-three women had a NT of  $\geq 3$  mm (2.0%), of whom 59 requested antenatal karyotyping (71%). Among 130 women with abnormal serum biochemistry (Risk  $\geq 1/250$ , representing 3.4% of the women screened), 62 had amniocentesis (48%). All women with increased NT and abnormal serum markers underwent karyotyping ( $n = 4$ , of whom two had a fetus with Down syndrome).

Among 3599 women at low risk for both tests (NT measurement  $< 3$  mm and a MSS-derived risk  $< 1/250$ ), a total of 169 karyotypings were performed (4.7%). Indications for amniocentesis were parental anxiety ( $n = 46$ ), history of chromosomal defects or parental translocation ( $n = 23$ ), abnormal second-trimester scan ( $n = 79$ ) (including short femur, pyelectasis, choroid plexus cysts, hyperechogenic bowel, intrauterine growth restriction, omphalocele, cleft lip and palate), maternal seroconversion for toxoplasmosis or cytomegalovirus ( $n = 22$ ) because the parents requested karyotyping in addition to infection screening. In this group only one trisomy 21 was found; this was a 37-year-old woman with a 2.8-mm nuchal thickness who was counseled by her physician to have amniocentesis. In addition, one trisomy 18 and one trisomy 9 were found; both fetuses had multiple defects at the second-trimester scan.

Among the 4130 women, we identified 32 chromosomal abnormalities, including 23 aneuploidies (12 trisomy 21, 6 trisomy 18, 1 trisomy 13, 1 trisomy 9, 2 cases of 45,X0, 1 case of 69,XXX), and nine structural abnormalities (1 unbalanced translocation, 4 balanced translocations, 4 inversions). Details of the cases of trisomy 21 and other clinically significant chromosomal defects are shown in Tables 3 and 4. The incidence of Down syndrome was 12/4130 (0.29%). Table 5 compares the performances of different Down syndrome screening strategies. Nine out of 10 cases would have been detected by the calculation of the 'combined risk'. The sensitivity for the detection of Down syndrome in our population at an arbitrarily fixed 5% false-positive rate was 60%, 75% and 90% for MSS, NT, and combined screening, respectively (Table 6).

## DISCUSSION

Antenatal screening for trisomy 21 represents a difficult issue for both women and physicians because confirmation of diagnosis requires an invasive procedure, whose indication may result from several screening tests. Among these tests, NT measurement and MSS, used separately or together, are becoming part of routine antenatal care in many countries. Few studies have compared the performance of nuchal thickness measurement and second-trimester MSS

**Table 1** Distribution of nuchal translucency and crown-rump length

CRL (mm)	n	Nuchal translucency			NT $\geq 3$ mm (n (%))
		5th percentile (mm)	50th percentile (mm)	95th percentile (mm)	
38-44	82	0.6	1	2	0
45-54	601	0.8	1.2	2.2	11 (1.8%)
55-64	1552	1	1.4	2.3	32 (2.0%)
65-74	1379	1	1.7	2.5	21 (1.5%)
75-84	516	1	1.7	2.8	19 (3.7%)
Total	4130	1	1.5	2.5	83 (2%)

CRL, crown-rump length; NT, nuchal translucency.

**Table 2** Observed number of antenatal karyotypings and chromosomal abnormalities by screening criteria

Maternal age (years)	NT (mm)	Maternal serum screening (risk)	Antenatal karyotyping (n (%))	Chromosomal abnormalities (n (details))
< 35 (n = 3548)	< 3 mm (n = 3484)	Risk $< 1/250$	116/3130 (3.7%)	n = 3 (T18, T9, unbal. trans.)
		Risk $\geq 1/250$	34/74 (46%)	n = 3 (T21, T21, 45X0)
		N/D	16/280 (5.7%)	n = 0
35-37 (n = 582)	$\geq 3$ mm (n = 64)	Risk $< 1/250$	27/47 (57%)	n = 5 (T21, T21, T18, T18, 45X0)
		Risk $\geq 1/250$	2/2 (100%)	n = 1 (T21)
		N/D	15/15 (100%)	n = 5 (T21, T18, T18, T13, 69XXX)
$\geq 3$ mm (n = 19)	< 3 mm (n = 563)	Risk $< 1/250$	53/469 (11%)	n = 2 (T21, T18)
		Risk $\geq 1/250$	24/52 (46%)	n = 2 (T21, T21)
		N/D	13/42 (31%)	n = 0
$\geq 3$ mm (n = 19)	$\geq 3$ mm (n = 19)	Risk $< 1/250$	10/14 (71%)	n = 1 (T21)
		Risk $\geq 1/250$	2/2 (100%)	n = 1 (T21)
		N/D	3/3 (100%)	n = 1 (T21)

Balanced translocations and inversions are not shown. N/D, maternal serum screening not performed; T, trisomy; unbal. trans., unbalanced translocation.

**Table 3** Maternal age, crown–rump length, nuchal translucency, comparison of risk derived from nuchal translucency, maternal serum screening and both, and outcome in the 12 pregnancies affected by Down syndrome

Maternal age (years)	CRL (mm)	NT (mm)	NT ≥ 95th percentile	NT-derived risk	MSS-derived risk	Combined risk	Outcome
36	51	1.6	No	1 : 700	1 : 16	1 : 38	TOP
31	70	1.9	No	1 : 3000	1 : 65	1 : 232	TOP
37	70	2	No	1 : 640	1 : 75	1 : 202	TOP
31	72	2.8	Yes	1 : 401	1 : 96	1 : 45	TOP
37	62	2.8	Yes	1 : 75	1 : 410 000†	< 1 : 10 000	TOP
34	55	3.4	Yes	1 : 26	1 : 240	1 : 20	TOP
36	60	4.3	Yes	1 : 6	1 : 81	1 : 2	TOP
33	64	4.5	Yes	1 : 9	1 : 479	1 : 11	TOP
27	46	5	Yes	1 : 14	1 : 371	1 : 6	TOP
35	69	5.2	Yes	1 : 6	1 : 520	1 : 12	TOP
25	69	6*	Yes	1 : 17	—	—	TOP
35	70	11*	Yes	1 : 6	—	—	TOP

Combined risk = (NT-derived risk/Background risk) × MSS-derived risk. CRL, crown–rump length; NT, nuchal translucency; MSS-derived risk, risk derived from maternal age and serum screening; TOP, termination of pregnancy. \*Cases with cystic hygroma; karyotyping performed by chorionic villus sampling; MSS not performed. †hCG = 0.28 multiples of the median (MoM) and alpha-fetoprotein = 4.25 MoM.

**Table 4** Maternal age, crown–rump length, nuchal translucency, karyotype and outcome of 12 pregnancies affected by chromosomal defects other than Down syndrome

Maternal age (years)	CRL (mm)	NT (mm)	Indication for karyotyping	Karyotype	Outcome
17	70	2	Hydrops at 17 weeks	45,X0	TOP
27	66	10	Cystic hygroma	45,X0	TOP
34	48	9	NT	69,XXX	TOP
34	57	3.7	NT, holoprosencephaly	Trisomy 13	TOP
35	53	1	Cardiac defect at 20 weeks	Trisomy 18	TOP
29	51	6	NT	Trisomy 18	TOP
24	63	3.2	NT	Trisomy 18	TOP
19	48	2	Multiple defects at 20 weeks	Trisomy 18	TOP
33	45	5	NT	Trisomy 18	TOP
34	60	8	NT	Trisomy 18	TOP
27	57	1	Paternal translocation	47,XX + der (22)	TOP
32	60	1.9	Multiple defects at 20 weeks	Trisomy 9	TOP

CRL, crown–rump length; NT, nuchal translucency; TOP, termination of pregnancy.

**Table 5** Screen-positive rate, sensitivity, and positive predictive value for Down syndrome of different modes of screening

Mode of screening	Screen-positive rate (n (%))	Sensitivity for Down syndrome (n (%))	PPV for Down syndrome (% (proportion))
NT ≥ 3 mm	83/4130 (2.0)	7/12 (58)	8.4 (1 : 12)
NT risk ≥ 1/250	186/4130 (4.5)	8/12 (67)	3.9 (1 : 25)
NT ≥ 95th percentile	210/4130 (5.0)	9/12 (75)	4.3 (1 : 23)
MSS ≥ 1/250	130/3790 (3.4)	6/10 (60)	4.6 (1 : 22)
NT ≥ 3 or MSS ≥ 1/250	191/3790 (5.0)	9/10 (90)	4.7 (1 : 21)
Combined risk ≥ 1/250	106/3790 (2.8)	9/10 (90)	8.5 (1 : 12)

PPV, positive predictive values; NT, nuchal translucency; MSS, risk estimated by maternal age and second-trimester serum markers.

**Table 6** Comparison of detection rates of Down syndrome for a 5% fetal karyotyping rate

	Cut-off level for a 5% karyotyping rate	Observed number /screen positive (n)	Sensitivity (% (95% CI))
Nuchal translucency	2.5 mm	9/210	75.0 (42.8–94.5)
Maternal serum screening	1/334	6/189	60.0 (26.2–87.8)
Combined risk	1/500	9/190	90.0 (55.5–99.7)

95% CI, 95% confidence interval.

for the detection of trisomy 21 in the same population. Thilaganathan *et al.*<sup>10</sup> performed second-trimester MSS in 1904 women who had had earlier nuchal thickness screening, including only one NT screen-positive patient. The authors concluded that the implementation of a nuchal thickness screening program will significantly reduce the positive predictive value of second-trimester maternal serum testing if both tests are performed sequentially. Kadir and Economides<sup>11</sup> reached the same conclusion when analyzing the positive predictive value of biochemical screening, which fell from 5% to 0.45% after the introduction of NT screening. However, in this study, MSS was not performed in NT screen-positive patients. Recently Wald *et al.* proposed an 'integrated screening' by first- and second-trimester tests<sup>15</sup>. Based on an analysis of published data, they concluded that this method would lead to the detection of more cases of Down syndrome with a much lower false positive-rate than current tests.

Our results suggest that, as a single test, NT screening compares favorably with MSS (75% vs. 60% detection rate for a 5% invasive testing rate), although the overlap of confidence intervals does not allow a firm conclusion. Even if detection rates were similar with both methods, we believe that if a single test has to be done, ultrasound screening offers significant advantages over MSS, by providing other important information including confirmation of embryo viability, accurate dating of pregnancy, early diagnosis of multiple pregnancies (and identification of chorionicity), and detection of major structural abnormalities<sup>16</sup>. However, sonographic screening requires specific training, and both methods require strict audit and quality control, which are easier to obtain with biological measurement than with sonographic screening.

In this study we initially decided to use a single cut off for NT of 3 mm, regardless of maternal age and gestational age. This threshold has also been used in other large studies<sup>9</sup>. Since this study was initiated, a method to calculate the risk based on a combination of maternal age, NT and gestational age (determined by crown-rump length) has been described<sup>6,13</sup> and the software for calculation of this risk is available from The Fetal Medicine Foundation<sup>13</sup>. The correction for gestational age or crown-rump length is justified by the normal increase of NT thickness between 10 and 14 weeks, as observed in our series. However, the results obtained with a single threshold may be of interest to clinicians who do not have the software to calculate the risk. Furthermore, a study by Pajkrt *et al.* demonstrated that correction of NT measurements for differences due to gestational age, either by using the 'delta-value' or multiples of the median (MoM), did not improve the detection rate or improve the false-positive rate<sup>17</sup>.

We have shown that the sequential use of NT screening and MSS allows a very high detection rate of trisomy 21; 11 out of 12 cases in our series were detected by at least one of the two tests at the chosen thresholds. However, the best way to combine these tests would be to use an algorithm calculating a risk based on maternal age, NT (related to crown-rump length), and serum markers. To our knowledge, such an algorithm inclusive of second-trimester markers is not available. The number of subjects in this study does not allow building this algorithm confidently, but pooling results from similar

studies would provide a sufficient amount of data to do so. Another option is to estimate the combined risk by multiplying the MSS risk by the likelihood ratio derived from NT. This raises the issue of the independence between NT and serum markers. Although we found no correlation, our calculation does not take into account the cases in which serum markers were not tested based on abnormal NT values. Further studies are needed on larger data sets to clarify this issue.

One of the advantages of ultrasound screening over second-trimester MSS is that it is performed earlier in pregnancy. However, the use of sequential screening using first-trimester ultrasound and second-trimester biochemistry has the significant drawback of delaying the decision of invasive testing and eventual termination of pregnancy. Several recent studies pointed out the importance of first-trimester serum maternal markers<sup>18-20</sup>. A preliminary assessment of first-trimester screening using NT, free  $\beta$ -hCG, and pregnancy-associated plasma protein-A (PAPP-A) measurement with maternal age yielded an estimated detection rate of 80% for a 5% false-positive rate<sup>19</sup>. In another retrospective study, Spencer *et al.* found that this protocol could detect 89% of Down syndrome cases at a fixed false-positive rate of 5%<sup>20</sup>. If these results are confirmed in further prospective studies, this combination may provide the method of choice in antenatal screening for Down syndrome, and we are currently conducting a study comparing this strategy to second-trimester serum screening. However, second-trimester screening is widely used and is the only recommended method of screening in many countries including ours. Therefore a risk assessment formula including second-trimester serum screening along with the other parameters will be useful as long as this type of test continues to be used.

An important issue in Down syndrome screening is the rate of spontaneous fetal loss after the time of screening. Approximately 30% of fetuses affected by trisomy 21 die spontaneously between 12 weeks of gestation and term, of which one third are between 12 and 16 weeks<sup>21</sup>. Thus, effective first-trimester screening may in some instances result in unnecessary termination of pregnancy.

Despite our aim to keep the invasive testing rate as low as possible, 7.6% of the screened women had antenatal karyotyping, in addition to karyotyping performed on the basis of a maternal age of above 38 years. Surprisingly, in women at low risk for maternal age, NT measurement and MSS, a significant number of amniocenteses was performed (4.6%). Almost half of these tests were indicated by an abnormal second-trimester scan. We feel that our experience would now allow us to avoid a significant number of amniocenteses in women with an isolated ultrasound anomaly, such as short femur or isolated pyelectasis. Other authors have also reported the importance of combining the results of MSS and second-trimester ultrasound<sup>22,23</sup> in order to decrease the amniocentesis rate.

Finally our data suggest that the various methods of screening for Down syndrome should be combined, leading to an improved detection rate while keeping the false-positive rate as low as possible. Before a validated algorithm is available, the calculation of the 'combined risk' is a reasonable option.

## ACKNOWLEDGMENTS

We thank Mrs Catherine Champagne for her help in collecting data and Professor Victor Gomel for his helpful advice in the preparation of the manuscript.

## REFERENCES

- 1 Wald N, Kennard A, Densem J, Cuckle H, Chard T, Butler L. Antenatal maternal serum screening for Down's syndrome: results of a demonstration project. *BMJ* 1992; 305: 391-4
- 2 Spencer K, Carpenter P. Prospective study of prenatal screening for Down's syndrome with free beta human chorionic gonadotrophin. *BMJ* 1993; 307: 764-9
- 3 Nicolaides K, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. *BMJ* 1992; 304: 867-9
- 4 Nicolaides K, Brizot M, Snijders R. Fetal nuchal translucency: ultrasound screening for fetal trisomy in the first trimester of pregnancy. *Br J Obstet Gynaecol* 1994; 101: 782-6
- 5 Pandya P, Kondylis A, Hilbert L, Snijders R, Nicolaides K. Chromosomal defects and outcome in 1015 fetuses with increased nuchal translucency. *Ultrasound Obstet Gynecol* 1995; 5: 15-9
- 6 Pandya P, Snijders R, Johnson S, De Lourdes Brizot M, Nicolaides K. Screening for fetal trisomies by maternal age and fetal nuchal translucency thickness at 10-14 weeks of gestation. *Br J Obstet Gynaecol* 1995; 102: 957-62
- 7 Snijders R, Johnson S, Sebire N, Noble P, Nicolaides K. First-trimester ultrasound screening for chromosomal defects. *Ultrasound Obstet Gynecol* 1996; 7: 216-26
- 8 Kornman L, Morssink L, Beekhuis J, De Wolf B, Heringa M, Mantingh A. Nuchal translucency cannot be used as a screening test for chromosomal abnormalities in the first trimester of pregnancy in a routine ultrasound practice. *Prenat Diagn* 1996; 16: 797-805
- 9 Taipale P, Hiilesmaa V, Salonen R, Ylöstalo P. Increased nuchal translucency as a marker for fetal chromosomal defects. *N Engl J Med* 1997; 337: 1654-8
- 10 Thilaganathan B, Slack A, Wathen N. Effect of first trimester nuchal translucency on second-trimester maternal serum biochemical screening for Down's syndrome. *Ultrasound Obstet Gynecol* 1997; 10: 261-4
- 11 Kadir R, Economides D. The effect of nuchal translucency measurement on second-trimester biochemical screening for Down's syndrome. *Ultrasound Obstet Gynecol* 1997; 9: 244-7
- 12 Benattar C, Audibert F, Taieb J, Ville Y, Roberto A, Lindenbaum A, Frydman R. Efficiency of ultrasound and biochemical markers for Down's syndrome risk screening. *Fetal Diagn Ther* 1999; 14: 112-7
- 13 Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH. 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* 1998; 351: 343-6
- 14 Nicolaides KH, Snijders RJM, Cuckle HS. Correct estimation of parameters for ultrasound nuchal translucency screening. *Prenat Diagn* 1998; 18: 519-23
- 15 Wald NJ, Watt HC, Hackshaw AK. Integrated screening for Down's syndrome based on tests performed during the first and second trimesters. *N Engl J Med* 1999; 341: 461-7
- 16 Economides DL, Braithwaite JM. First trimester ultrasonographic diagnosis of fetal structural abnormalities in a low risk population. *Br J Obstet Gynaecol* 1998; 105: 53-7
- 17 Pajkrt E, Mol BWJ, van Lith JMM, Bleker OP, Bilardo CM. Screening for Down's syndrome by fetal nuchal translucency measurement in a high-risk population. *Ultrasound Obstet Gynecol* 1998; 12: 156-62
- 18 Haddow JE, Palomaki GE, Knight GJ, Williams J, Miller WA, Johnson A. Screening of maternal serum for fetal Down's syndrome in the first trimester. *N Engl J Med* 1998; 338: 955-61
- 19 Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. *J Med Screening* 1997; 4: 181-246
- 20 Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10-14 weeks using fetal nuchal translucency, maternal serum free  $\beta$ -human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 1999; 13: 231-7
- 21 Snijders RJM, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age and gestation specific risk for trisomy 21. *Ultrasound Obstet Gynecol* 1999; 13: 167-70
- 22 Nyberg DA, Luthy DA, Cheng EY, Sheley RG, Resta RG, Williams MA. Role of prenatal ultrasonography in women with positive screen for Down syndrome on the basis of maternal serum markers. *Am J Obstet Gynecol* 1995; 173: 1030-5
- 23 Bahado-Singh RO, Oz AU, Kovanci E, Deren O, Copel J, Baumgarten A, Mahoney J. New Down syndrome screening algorithm: Ultrasonographic biometry and multiple serum markers combined with maternal age. *Am J Obstet Gynecol* 1998; 179: 1627-31