PRENATAL DIAGNOSIS
Prenat Diagn 2003; **23**: 306–310.
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/pd.588

Integrated ultrasound and biochemical screening for trisomy 21 using fetal nuchal translucency, absent fetal nasal bone, free β-hCG and PAPP-A at 11 to 14 weeks

Simona Cicero[1], Renu Bindra[1], Georgios Rembouskos[1], Kevin Spencer[2]* and Kypros H. Nicolaides[1]

[1]*Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, Denmark Hill, London, UK*
[2]*Endocrine Unit, Clinical Biochemistry Department, Harold Wood Hospital, Gubbins Lane, Romford, UK*

Background Screening for trisomy 21 by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free β-hCG and pregnancy-associated plasma protein-A (PAPP-A) at 11 to 14 weeks of gestation is associated with a detection rate of 90% for a false-positive rate of 5%. Recent evidence suggests that in about 70% of fetuses with trisomy 21, the nasal bone is not visible at the 11th- to 14th-week scan (Cicero *et al*., 2001). The aim of this study was to examine whether fetal NT thickness and the level of maternal serum biochemical markers is independent of the presence or absence of the nasal bone, and to estimate the performance of a screening test that integrates the two sonographic and the two biochemical markers.

Methods This was a retrospective case-control study comprising 100 trisomy 21 and 400 chromosomally normal singleton pregnancies at 11 to 14 weeks of gestation. Ultrasound examination was carried out for measurement of fetal NT and assessment of the presence or absence of the fetal nasal bone. Maternal serum free β-hCG and PAPP-A were measured using the Kryptor rapid random-access immunoassay analyser (Brahms Diagnostica GmbH, Berlin). The distribution of fetal NT, maternal serum free β-hCG and PAPP-A in trisomy 21 fetuses with absent and present nasal bone was examined.

Results The nasal bone was absent in 69 and present in 31 of the trisomy 21 fetuses. There were no significant differences in median maternal age, median gestational age, NT delta, free β-hCG MoM and PAPP-A MoM in trisomy 21 fetuses with and without a visible nasal bone. For a false-positive rate of 5%, it was estimated that screening with the four markers in combination with maternal age would be associated with a detection rate of 97%. For a false-positive rate of 0.5%, the detection rate was 90.5%.

Conclusions An integrated sonographic and biochemical test at 11 to 14 weeks can potentially identify about 90% of trisomy 21 fetuses for a false-positive rate of 0.5%. Copyright © 2003 John Wiley & Sons, Ltd.

KEY WORDS: prenatal screening; chromosomal anomalies; Down syndrome; OSCAR; nuchal translucency; nasal bone; free β-hCG; PAPP-A

INTRODUCTION

The most effective method of screening for trisomy 21 is by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free β-hCG and pregnancy-associated plasma protein-A (PAPP-A) at 11 to 14 weeks of gestation. It has been shown prospectively that for a false-positive rate of 5%, the detection rate of trisomy 21 by this method is about 90%, which is superior to the 30% achieved by maternal age alone or the 65% by maternal age and second-trimester serum biochemistry (Wald *et al*., 1998; Spencer *et al*., 2000; Bindra *et al*., 2002; Spencer *et al*., 2003).

More recently, a new ultrasound marker has been described in which the nasal bone at 11 to 14 weeks was found to be absent in about 70% of fetuses with trisomy 21 and in 0.5% of chromosomally normal fetuses. Furthermore, in trisomy 21 fetuses NT thickness was not significantly different between those with and those without a nasal bone. It was estimated that screening for trisomy 21 by a combination of maternal age, fetal NT and examination of the nasal bone could increase the detection rate to 85% whilst decreasing the false-positive rate to 1% (Cicero *et al*., 2001).

The aim of this study was to examine whether the level of maternal serum biochemical markers was independent of the presence or absence of nasal bone, and to estimate the performance of a screening test that integrates the two sonographic and the two biochemical markers.

METHODS

The study population comprised 100 trisomy 21 and 400 chromosomally normal singleton pregnancies at 11 to 14 weeks of gestation. All fetuses had been found to have possible chromosomal defects after screening with a combination of maternal age and fetal nuchal translucency. Of these, 38 cases with trisomy 21 and 148

*Correspondence to: Dr Kevin Spencer, Clinical Biochemistry Department, Harold Wood Hospital, Gubbins Lane, Romford, UK. E-mail: KevinSpencer1@aol.com

Received: 8 October 2002
Revised: 17 December 2002
Accepted: 21 December 2002

unaffected cases had been part of a previous observational study of the absence of nasal bone in fetuses with aneuploidy (Cicero *et al*., 2001). In all cases, chorionic villous sampling (CVS) for fetal karyotyping was carried out at the request of the parents after first-trimester screening. Ultrasound examination was carried out for measurement of fetal crown-rump length (CRL) and NT and for a prospective assessment of the presence or absence of the fetal nasal bone as previously described (Snijders *et al*., 1998; Cicero *et al*., 2001). Immediately before CVS, maternal blood samples were collected and the serum was aliquoted and stored at −20°Celsius prior to analysis. Serum free β-hCG and PAPP-A were measured retrospectively (with samples blinded to the assayist) with the Brahms Kryptor rapid random-access immunoassay analyser (Brahms GmbH, Henningsdorf, Berlin) using time-resolved amplified cryptate emission technology (TRACE). The analytical performance of this system has been described before (Spencer *et al*., 1999). Each case of trisomy 21 was matched for gestational age and maternal age with four unaffected cases investigated at the same time. Table 1 summarises the demographic characteristics of the two groups.

Statistical analysis

Delta NT was calculated for each case using the median values established from a previous large study (Snijders *et al*., 1998). All biochemical marker measurements were converted to multiples of the median values (MoM) using the median values derived from previous studies of unaffected pregnancies with gestational age calculated in weeks and days from the fetal crown-rump length (Snijders *et al*., 1998; Spencer *et al*., 1999). MoM values for biochemical markers were calculated with correction for maternal weight using the reciprocal weight-correction procedure of Neveux *et al*. (1996). Statistical analysis of data was performed with Analyse-It (Smart Software, Leeds, UK), a statistical software add-in for Microsoft Excel.

In order to simulate the performance of population screening using the four markers (fetal NT, maternal serum free β-hCG and PAPP-A, absent fetal nasal bone) in combination with maternal age, we used the maternity age distribution of England and Wales (ONS, 2000) and standard statistical modelling techniques (Royston & Thompson, 1992). We used the distribution parameters and inter-relationships for the first 3 markers from a well-described large population of trisomy 21 cases and from a large unaffected population (Nicolaides *et al*., 1998; Spencer *et al*., 1999), and the frequency of absent nasal bone in the affected and unaffected populations from the cases included in the study by Cicero *et al*. (2001) and the additional 72 new cases in this study. A series of 15 000 delta NT values and MoMs for free β-hCG and PAPP-A for the unaffected pregnancy group and for the trisomy 21 group were selected at random from the Gaussian distribution of each marker in each pregnancy group. These marker values were then used to calculate an NT and biochemistry likelihood ratio for each population group. A computerised random-number generator was then used to assign the same proportion of fetuses with absent nasal bone found in the combined series in this and a previous study (Cicero *et al*., 2001) to the trisomy 21 pregnancy group and the unaffected pregnancy group, and it converted them to the appropriate likelihood ratio for the presence or absence of the nasal bone. The nasal bone likelihood ratio was then multiplied by the delta NT and biochemistry likelihood ratio to provide an integrated likelihood ratio for all four markers. The integrated likelihood ratios for the 2 pregnancy populations were then used together with the age-related risk of trisomy 21 at 12 weeks (Snijders *et al*., 1999) to calculate the expected detection rate of affected pregnancies at various false-positive rates in a population with the maternal age distribution of pregnancies in England and Wales (ONS, 2000).

Table 1—Demographic characteristics of trisomy 21 and chromosomally normal pregnancies

	Trisomy 21	Normal
Number	100	400
Absent nasal bone	69 (69%)	1 (0.25%)
Median (range) maternal age (years)	38.6 (24.6–45.4)	38.3 (19.6–47.4)
Median (range) fetal CRL (mm)	64.4 (49–83)	65.0 (45–84)
Median (range) gestation (weeks)	12.67 (11.3–14)	12.71 (11–14)
Median (range) maternal weight (Kg)	63.6 (42–99)	62.0 (37–115)
Caucasian (%)	96%	94%
Non-cigarette smoker (%)	96%	92%
Male fetus (%)	51%	52%

RESULTS

Absent nasal bone was found in 50 out of 72 (69%) of the newly reported cases with trisomy 21 and in 1 out of 251 (0.4%) of the newly reported control cases. A combination of these results from this series and from those previously reported (Cicero *et al*., 2001) showed an incidence of absent nasal bone in 93/131 (71%) of trisomy 21 pregnancies and in 4/851 (0.5%) of unaffected pregnancies. This frequency was used in further modelling exercises.

The median delta NT, and free β-hCG and PAPP-A MoM in all cases of trisomy 21 in this study were 2.28, 2.44 and 0.42, respectively, compared with 0.01, 1.06 and 0.98 in the unaffected population. These levels are similar to those reported in a much larger series (Spencer *et al*., 1999). The Mann–Whitney U test was used to calculate the significance of any difference between the median maternal age, median gestational age, median delta NT, median free β-hCG MoM and PAPP-A MoM in trisomy 21 fetuses with and without a visible nasal bone. There were no statistical differences between the two groups (Table 2) and the individual results are plotted graphically in Figures 1, 2 and 3. Since the

Table 2—Median marker MoMs in trisomy 21 pregnancies in the presence or absence of fetal nasal bone

	Absent nasal bone (n = 69)	Present nasal bone (n = 31)	Probability of difference
Median maternal age (years)	38.9	37.9	0.5761
Median gestational age (weeks)	12.65	12.71	0.8428
Median delta NT	2.40	1.75	0.2038
Median free β-hCG MoM	1.98	1.54	0.090
Median PAPP-A MoM	0.40	0.52	0.078

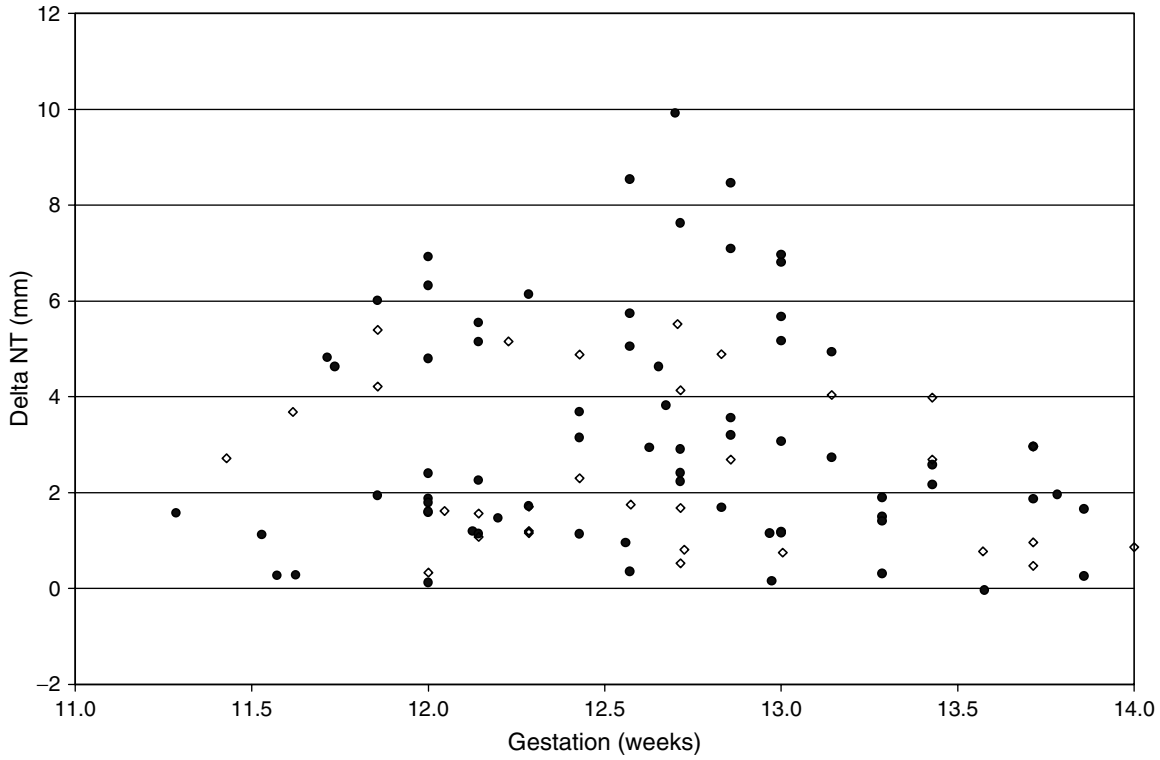

Figure 1—Delta NT in cases of trisomy 21 in which the fetal nasal bone was absent (●) or present (◇)

markers free β-hCG and PAPP-A are known to fit a Gaussian distribution after log transformation in both unaffected and trisomy 21 pregnancies (Spencer *et al*., 1999), further tests of significance between those fetuses with and without nasal bone were carried out using t-tests of unequal variance on the $\log_{10}$ transformed MoMs. Each marker showed no significant difference ($p > 0.05$) in MoM between the two groups. Having established that the presence or absence of the fetal nasal bone in cases affected by trisomy 21 is not correlated with altered levels of the other three markers, we investigated the potential detection and false-positive rates that would be achieved by combining all four markers with maternal age (Table 3).

DISCUSSION

The findings of this study confirm that firstly, in about 70% of fetuses with trisomy 21 the nasal bone is not visible by ultrasonographic examination at 11 to 14 weeks of gestation (Cicero *et al*., 2001), and secondly, trisomy 21 is associated with increased fetal NT (Nicolaides *et al*., 1992), elevated levels of maternal serum free β-hCG (Spencer *et al*., 1992) and reduced levels of maternal serum PAPP-A (Brambati *et al*., 1991). Furthermore, the data demonstrate that in trisomy 21 maternal age, fetal NT thickness (as delta NT) and the levels of maternal serum free β-hCG and PAPP-A are independent of the presence or absence of the nasal bone. Consequently, maternal age and the ultrasonographic markers of NT and nasal bone and the biochemical markers of free β-hCG and PAPP-A can be combined in the calculation of risk for trisomy 21.

With the advent of rapid immunoassays, it has become possible to provide pre-test counselling, biochemical testing of the mother, ultrasound examination of the fetus and post-test counselling of a combined risk estimate, all within a one-hour visit to a multidisciplinary one-stop clinic for assessment of risk (OSCAR) for fetal anomalies (Spencer, 1998; Spencer *et al*., 2000; Bindra *et al*., 2002; Spencer *et al*., 2003). One-stop clinics have developed over the past decade in several clinical areas,

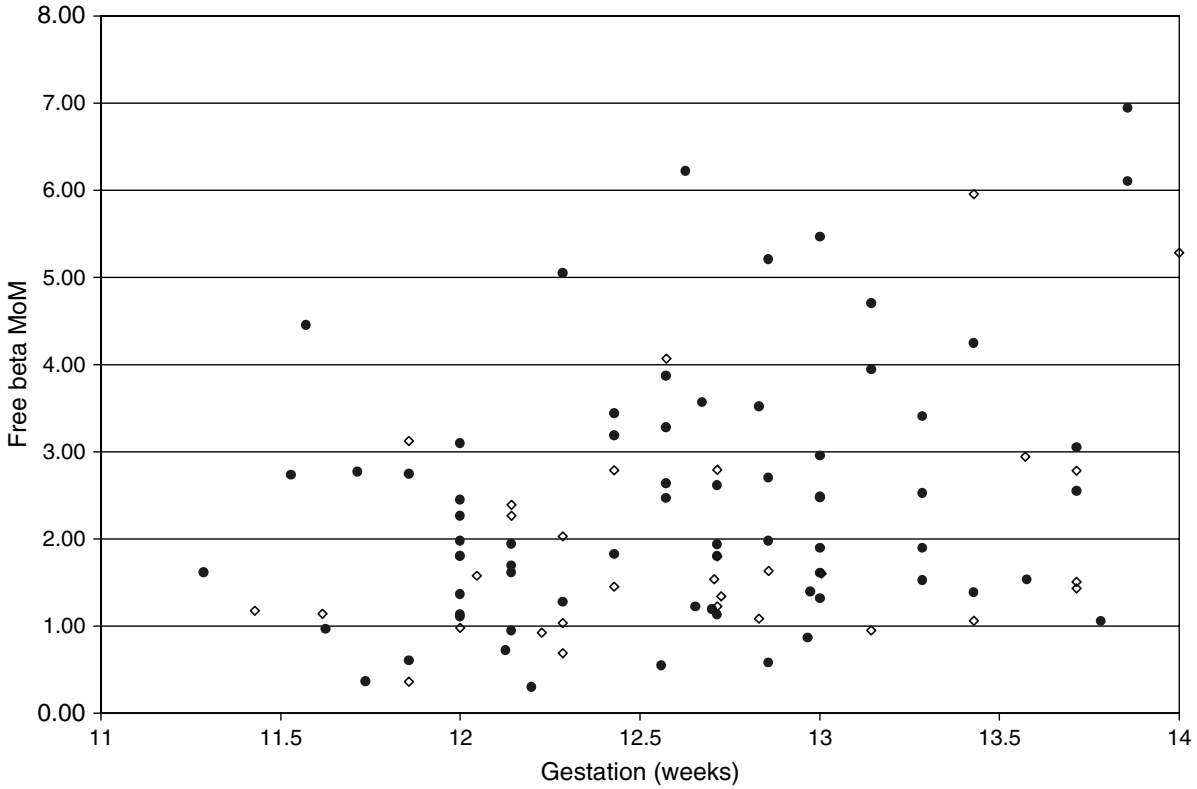

Figure 2—Free β-hCG MoM in cases of trisomy 21 in which the fetal nasal bone was absent (●) or present (◇)

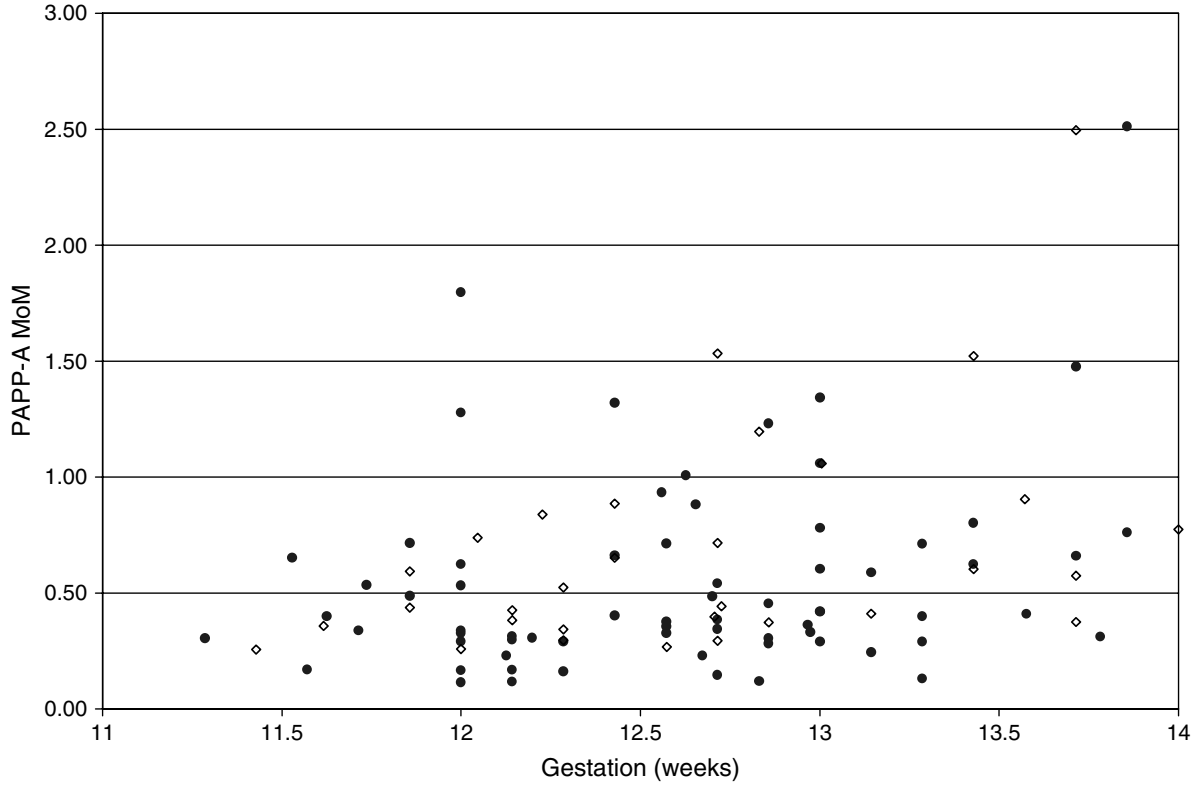

Figure 3—PAPP-A MoM in cases of trisomy 21 in which the fetal nasal bone was absent (●) or present (◇)

Table 3—Modelled detection rates for different fixed false-positive rates using various marker combinations with maternal age

	NT and free β-hCG and PAPP-A	NT and free β-hCG and PAPP-A and nasal bone
False-positive rate (%)	Detection rate (%)	Detection rate (%)
0.5	69.5	90.5
1.0	75.2	93.5
2.0	80.4	94.8
3.0	83.8	95.7
4.0	86.2	96.5
5.0	88.9	97.1

ranging from breast cancer screening to cardiovascular risk clinics and one-stop surgical clinics. These services all have in common the integration of a range of clinical and diagnostic services that allow for a better use of clinical time and improved diagnostic efficiency (Spencer, 2002). They aim to maximise patient satisfaction by reducing the number of patient visits and minimise patient travel costs, anxiety and stress. In the context of prenatal screening for chromosomal anomalies, the integration of counselling, ultrasound, biochemistry, midwifery and obstetrics in a one-stop clinic does seem to be acceptable to women, and while offering maximum utilisation of hospital outpatient resources, it provides a high diagnostic efficiency and potentially allows for a more informed choice. Recent prospective studies have demonstrated that screening for trisomy 21 in an OSCAR setting by a combination of maternal age, fetal NT and maternal serum free β-hCG and PAPP-A at 11 to 14 weeks is associated with a detection rate of 90% for a false-positive rate of 5% (Spencer *et al*., 2000; Bindra *et al*., 2002; Spencer *et al*., 2003). The findings of the current study suggest that inclusion of the nasal bone could retain a 90% detection rate with a simultaneous ten-fold reduction in the false-positive rate from 5% to 0.5% (Table 3). Alternatively, for a 5% false-positive rate, the detection rate could increase to 97%.

This study has demonstrated the potential performance of integrated first-trimester ultrasonographic and biochemical screening in a multidisciplinary OSCAR setting. The true performance of the test now needs to be assessed prospectively. In such prospective studies, it is imperative that the sonographers examining fetal NT and nasal bone receive appropriate training and certification of their competence in such scans (http://www.fetalmedicine.com/), and that measurements of free β-hCG and PAPP-A are carried out using accurate and reproducible assays in centres with external quality assessment in place.

REFERENCES

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.

Brambati B, Lanzani A, Tului L. 1991. Ultrasound and biochemical assessment of first trimester pregnancy. In *The Embryo: Normal and Abnormal Development and Growth*, Chapman M, Grudzinskas JG, Chard T (eds). Springer-Verlag: New York; 181–194.

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

Neveux LM, Palomaki GE, Larivee DA, Knight GJ, Haddow JE. 1996. Refinements in managing maternal weight adjustment for prenatal screening results. *Prenat Diagn* **16**: 1115–1119.

Nicolaides KH, Azar G, Bryne D, Mansur C, Marks K. 1992. Fetal nuchal translucency: ultrasound screening for chromosomal defects in the first trimester of pregnancy. *BMJ* **304**: 867–869.

Nicolaides KH, Snijders RJM, Cuckle HS. 1998. Correct estimation of parameters for ultrasound nuchal translucency screening. *Prenat Diagn* **18**: 519–521.

Office for National Statistics. 2000. *Birth Statistics, Series FM1, No. 28*. Stationery Office: London.

Royston P, Thompson SG. 1992. Model based screening for risk with application to Down's syndrome. *Stat Med* **11**: 257–268.

Snijders RMJ, Noble P, Sebire N, Souka A, Nicolaides KH. 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* **351**: 343–346.

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

Spencer K, Macri JN, Aitken DA, Connor JM. 1992. Free β-hCG as a first trimester marker of fetal trisomy. *Lancet* **339**: 1480.

Spencer K. 1998. Near patient testing and Down's syndrome screening. *Proc UKNEQAS* **3**: 130.

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, Spencer CE, Power M, Moakes A, Nicolaides KH. 2000. 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. *Br J Obstet Gynaecol* **107**: 1271–1275.

Spencer K. 2002. Point of care screening for chromosomal anomalies in the first trimester of pregnancy. *Clin Chem* **48**: 403–404.

Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. 2003. 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* (in press).

Wald NJ, Kennard A, Hackshaw A, McGuire A. 1998. Antenatal screening for Down's syndrome. *Health Technol Assess* **2**: 1–112.