Ultrasound Obstet. Gynecol. 10 (1997) 381–386

First-trimester screening for fetal aneuploidy: biochemistry and nuchal translucency

F. Orlandi, G. Damiani, T. W. Hallahan, D. A. Krantz* and J. N. Macri**

Prenatal Diagnosis Center, Cervello Hospital, Palermo, Italy; *Research Division, NTD Laboratories, Inc., Huntington Station, NY, USA

Key words: FREE BETA-HUMAN CHORIONIC GONADOTROPIN, PREGNANCY-ASSOCIATED PLASMA PROTEIN A, NUCHAL TRANSLUCENCY, DOWN'S SYNDROME, PRENATAL SCREENING

ABSTRACT

Maternal dried whole-blood specimens were collected prospectively from 2010 singleton pregnancies between 9 + 0 and 13 + 4 weeks that included 18 chromosomally abnormal pregnancies (11 Down's syndrome, four trisomy 18, two trisomy 13 and one triploidy). A subset of 744 pregnancies underwent ultrasound nuchal translucency measurement and included seven Down's syndrome, four trisomy 18, two trisomy 13 and one triploidy. Patients were evaluated for risk of Down's syndrome and trisomy 18 based on biochemistry (free β-human chorionic gonadotropin and pregnancy-associated plasma protein A), nuchal translucency and the combination of both. In prospective biochemical screening, false-positive rates for Down's syndrome and trisomy 18 were 5.1% (66/1297) and 1.9% (25/1297) in women < 35 years of age and 14.2% (99/695) and 1.6% (11/695) in women ≥ 35 years of age, respectively. The detection efficiency of aneuploidy was 6/6 (100%) in women < 35 years and 11/12 (92%) in women ≥ 35 years. Nuchal translucency measurement alone detected 57% (8/14) of cases of aneuploidy at a 5.8% (42/730) false-positive rate. Modelling with the age distribution of live births, a 5% false-positive rate resulted in Down's syndrome detection efficiency of 61% by biochemistry, 73% by nuchal translucency and 87% by combining both methods. The data in this study demonstrate that combined biochemical and ultrasound evaluation for Down's syndrome and other chromosomal abnormalities in the first trimester of pregnancy yield a detection capability that may exceed that of current second-trimester prenatal screening protocols. The potential for enhanced detection coupled to an earlier alert of fetal complications could represent a substantial advantage to both clinician and patient.

INTRODUCTION

Prenatal screening for Down's syndrome and other chromosomal abnormalities is conducted in the second trimester of pregnancy with a combination of maternal serum biochemical markers and maternal age. The most commonly used biochemical markers are α-fetoprotein, intact human chorionic gonadotropin (hCG), unconjugated estriol and free β-hCG. These analytes are used in various combinations in multi-marker screening approaches with the aim of optimizing the detection efficiency at the lowest possible false-positive rate.

Moving the window of maternal serum screening into the first trimester would represent a significant advantage to both clinician and patient. This option now appears promising based on data from retrospective reports[1,2]. Although most second-trimester biochemical markers in current use appear to be ineffective in first-trimester Down's syndrome screening[3–5], free β-hCG represents an important exception. A compilation of first-trimester retrospective reports demonstrated that maternal serum free β-hCG levels in cases of Down's syndrome approximated twice the normal level (2 multiples of the median (MoM)) in over 100 documented cases[6].

Measurement of maternal serum pregnancy-associated plasma protein-A (PAPP-A) may represent a further enhancement of first-trimester biochemical detection of Down's syndrome. Recent retrospective reports reveal first-trimester maternal serum median PAPP-A levels to be significantly reduced at less than 0.4 MoM in over 100 cases of Down's syndrome[6]. Importantly, there is a low correlation between free β-hCG and PAPP-A and therefore the two markers evaluated simultaneously may be expected to achieve an even higher degree of detection than either used independently.

Correspondence: Dr F. Orlandi, Centro Di Diagnosi Prenatale, Via Villareale, 35, 90141, Palermo, Italy

The use of biophysical modalities such as ultrasound markers for detection of Down's syndrome in the second trimester have met with limited success. In the first trimester, however, ultrasound evaluation of fetal nuchal translucency (NT) shows great promise. Detection of 30%[7] to 90%[8] of Down's syndrome cases have been reported with the use of this measurement alone.

We have established a collaborative effort to carry out prospective evaluation of biochemical and biophysical methods needed to screen for Down's syndrome in the first trimester. Initial results from this collaborative prospective project are presented here.

METHODS

Analytical specimens for this study were collected as maternal whole blood by a fingerstick lancet method. Maternal whole blood drops were dried onto no. 903 filter-paper collection devices (Schleicher and Schuell, Keene, NH) as previously reported[9]. Dried whole-blood specimens received in the laboratory were placed in a paper punch device (One punch Model II, IEM Screening Systems, Burbank, CA) and a 5.3-mm blood-impregnated disc was punched directly into a 96-well microtiter plate. Elution buffer was added to each well and blood components were allowed to elute from the punched filter paper discs for 1 h. Eluates were assayed by previously described in-house enzyme-linked immunosorbent assays modified to accept samples collected by the dried blood protocol[9].

Specimens from 2010 singleton pregnancies between 9 + 0 and 13 + 4 gestational weeks (ultrasound confirmed) were collected. Patients were recruited either through their private physician or through a genetic counselling program for women of advanced maternal age. The median maternal age was 32 years (range 15–46) in 1992 unaffected cases and 41.5 years (range 29–44) in 18 affected cases (11 Down's syndrome, four trisomy 18, two trisomy 13 and one triploidy).

NT measurement was performed prior to blood collection only when an ultrasonographer with certification from the Fetal Medicine Foundation (London, UK) was available. These ultrasonographers participate in an ongoing quality assessment program. NT measurement was performed on a subset of 744 patients including 14 affected cases (seven Down's syndrome, four trisomy 18, two trisomy 13, one triploidy). In this subgroup, the median age was 35 years (range 15–46) in unaffected and 42 years (range 29–44) in affected cases.

NT was measured by transabdominal ultrasound examination by means of a 3.75-MHz probe (Toshiba SSA 250A) or a 5-MHz probe (Acuson XP 10). The following previously described criteria[10] were used: (1) a good sagittal section of the fetus with sufficient magnification should be obtained; (2) care must be taken to distinguish between fetal skin and membranes, with observation of fetal movements; and (3) several measurements must be taken, with only the maximum thickness recorded by placement of calipers on the internal side of the lines delimiting the translucency in the 'on-to-on' position (Figure 1).

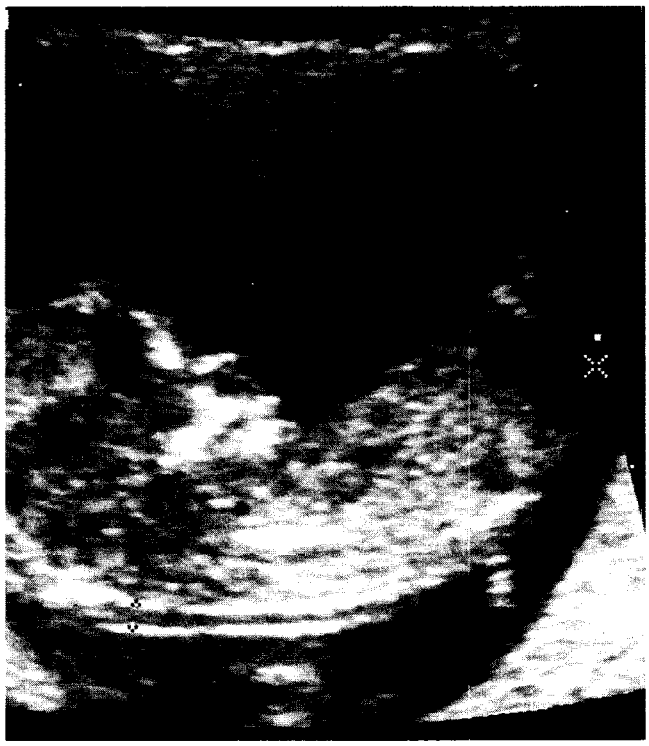

Figure 1 Measurement of nuchal translucency with calipers on the internal side of the lines delimiting the translucency in the 'on-to-on' position

Statistical analysis

Individual pregnancies were evaluated for risk of Down's syndrome and trisomy 18. As part of risk assessment, the free β-hCG and PAPP-A values of each patient specimen were divided by their respective gestational day-specific median level to determine the MoM for each analyte. Risks of Down's syndrome were calculated by multiplying the likelihood ratio (determined from bivariate log-Gaussian MoM distributions for free β-hCG and PAPP-A in Down's syndrome and unaffected pregnancies) by the patient's prior age-related risk. Throughout the course of the study, gestational day-specific medians and distribution parameters were updated. A Down's syndrome term risk of 1/380, equivalent to that of a 35-year-old gravida, was used as the cut-off level. An atypicality index based on Mahalanobis' squared distance with a cut-off level of 9.21 was used to identify patients who were not at increased risk for Down's syndrome but had extreme analyte levels (i.e. low free β-hCG and PAPP-A, high free β-hCG and PAPP-A, or low free β-hCG and high PAPP-A). Pregnancies were considered to be at increased risk for trisomy 18 when the atypicality index was greater than 9.21 and both free β-hCG and PAPP-A were less than 1.0 MoM.

NT measurements were reported to the nearest tenth of a millimeter. Gestational day-specific expected NT values were determined by a regression formula. For each pregnancy, a delta NT value was determined by subtracting the expected NT value from the observed NT value.

To assess the effectiveness of Down's syndrome screening with NT in combination with maternal age and the biochemical markers, a likelihood ratio for NT was

calculated for each patient. The risk of a chromosomal abnormality based on NT and age was calculated by multiplying the likelihood ratio by the patient's age-related risk of chromosomal abnormality. For the 744 patients with NT values, biochemical likelihood ratios for Down's syndrome and for trisomy 18 were then calculated based on the most recent distribution parameters. For the combination of biochemistry, NT and maternal age, both a Down's syndrome risk and a trisomy 18 risk were determined by multiplying the likelihood ratio based on the biochemistry by the likelihood ratio based on NT and then multiplying by the prior age-related risk of either Down's syndrome or trisomy 18, as appropriate. The cut-off risk for trisomy 18 screening in this analysis was set to 1/500 at term. Since the median age of patients in the study was greater than the median age in the general screening population, modelled false-positive rates and detection efficiency were determined from the observed likelihood ratios and the age distribution of live births in the USA to account for this age bias.

RESULTS

A summary of first-trimester prospective screening results incorporating maternal serum biochemistry and maternal age is illustrated in Figure 2. False-positive screening rates in women under 35 were 66/1297 (5.1%) and 25/1297 (1.9%) for Down's syndrome and trisomy 18, respectively. In women of 35 years and over, false-positive rates were 99/695 (14.2%) and 11/695 (1.6%) for Down's syndrome and trisomy 18, respectively. All 11 cases of Down's syndrome, all four cases of trisomy 18 and one of two cases of trisomy 13 were detected. One case of triploidy was atypical with high levels of both analytes (free β-hCG, 17.4 MoM; PAPP-A, 4.8 MoM). Overall, 6/6 (100%) and 11/12 (92%) aneuploidies were detected in younger and older women, respectively. All 11 Down's syndrome cases had elevated free β-hCG levels above 1.0 MoM and 10/11 Down's syndrome cases had depressed PAPP-A levels below 1.0 MoM.

Spearman rank correlation of delta NT with free β-hCG MoM, PAPP-A MoM, maternal age and maternal weight was −0.033 ($p = 0.3701$), 0.026 ($p = 0.4824$), 0.093 ($p = 0.0119$) and 0.122 ($p = 0.001$), respectively. Figure 3 illustrates the distribution of NT values by gestational age. Using a delta NT cut-off level of 1.0 mm, we observed a false-positive rate of 42/730 (5.8%) and a sensitivity of detection of all chromosomal anomalies of 8/14 (57%).

For the subgroup with NT measurement, observed false-positive rates and detection efficiency of Down's syndrome and trisomy 18 screening with three screening protocols (biochemistry, NT and the combination of NT and biochemistry) are shown in Table 1. Table 2 shows the detection efficiency of Down's syndrome and trisomy 18 at a fixed false-positive rate for all three screening protocols based on modelling for the age distribution of live births. With the use of a combined biochemical and NT screening protocol at a 5% false-positive rate, Down's syndrome detection efficiency was 87%. At an additional fixed 1% false-positive rate, the detection efficiency of trisomy 18 was 76%.

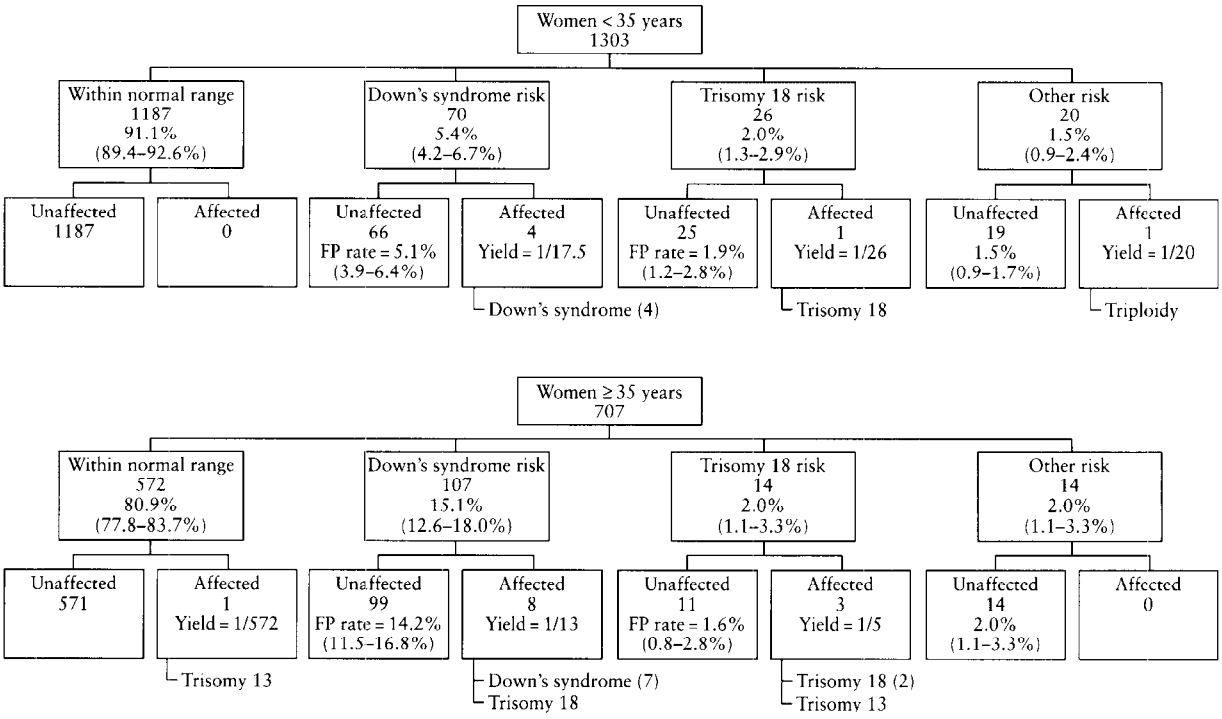

Figure 2 First-trimester prospective screening using biochemistry and maternal age. The cut-off level for Down's syndrome screening was 1 in 380 at term. The cut-off level for trisomy 18 screening was Mahalanobis' squared distance (MDS) > 9.21 and both free β-hCG and PAPP-A < 1.0 MoM. The cut-off level for other risk was MSD > 9.21 and either free β-hCG or PAPP-A ≥ 1.0 MoM. Numbers in parentheses indicate 95% confidence intervals. FP, false-positive

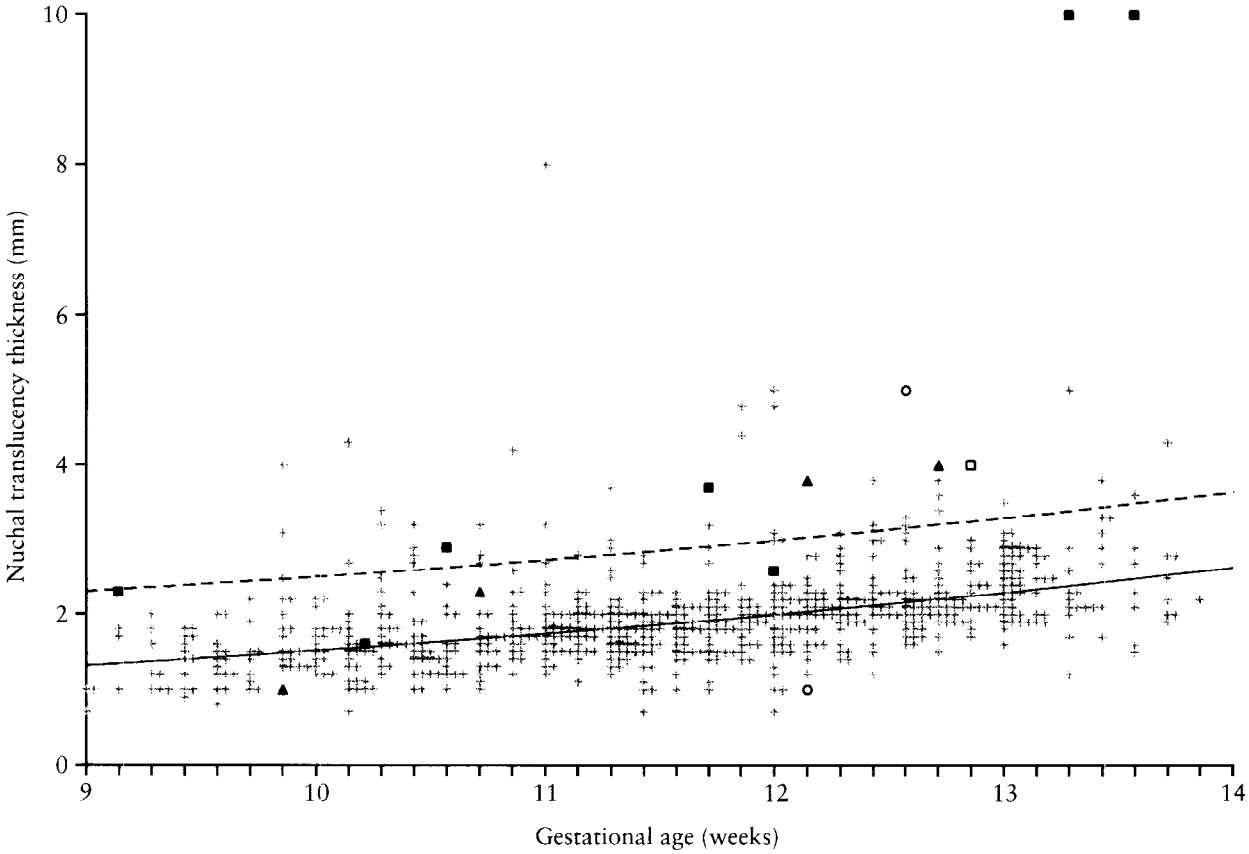

Figure 3 Distribution of nuchal translucency (NT) in unaffected cases (crosses) and those with Down's syndrome (filled squares), trisomy 18 (filled triangles), trisomy 13 (open circles) and triploidy (open squares). Solid line, expected; dashed line, delta NT = 1 mm

Table 1 Observed false-positive rate and sensitivity using different screening methods and maternal age in a subset of 744 patients undergoing nuchal translucency (NT) measurement (95% confidence intervals based on binomial distribution shown in parentheses)

Screening method	*Abnormality*	*False-positive rate (%)*	*Sensitivity (%)*
Biochemistry	Down's syndrome	9.2 (7.2–11.5)	100 (59–100)
Biochemistry	trisomy 18	2.0 (1.2–3.4)	75 (19–99)
Biochemistry	Down's syndrome or trisomy 18	11.2 (9.0–13.8)	91 (59–100)
NT	Down's syndrome or trisomy 18	14.1 (11.7–16.9)	82 (48–98)
Biochemistry and NT	Down's syndrome	4.7 (3.2–6.4)	86 (42–100)
Biochemistry and NT	trisomy 18	1.2 (0.6–2.3)	100 (40–100)
Biochemistry and NT	Down's syndrome or trisomy 18	5.8 (4.2–7.7)	91 (59–100)

In two cases of trisomy 13 and a single case of triploidy, one trisomy 13 and the triploidy would have been detected by all three screening protocols, but the second trisomy 13 would have been missed by all of the three screening protocols.

DISCUSSION

The combination of first trimester NT and biochemical markers (free β-hCG and PAPP-A) yielded a Down's syndrome detection efficiency in this study of 87% at a 5% false-positive rate. If these data are confirmed and sustained in expanded studies, the efficiency of first-trimester screening will surpass that observed with all common second-trimester multiple-marker screening protocols currently offered. Additionally, first-trimester screening can provide clinicians and patients with the substantial advantage of an early alert and 10–14-week scanning can allow for early detection of fetal structural anomalies.

Elevated free β-hCG and low PAPP-A levels are associated with an increased risk of Down's syndrome, and low free β-hCG and low PAPP-A levels are associated with an increased risk of trisomy 18. A third increased risk group was included in this study which identified patients with high PAPP-A and either low or high free β-hCG levels. Inclusion of this third risk group resulted in the detection of an additional case of triploidy and an increased number of false-positive results (1.5% of younger and 2.0% of older patients).

In this study, NT measurement without maternal age resulted in a detection efficiency of 58%, lower than the approximate 80% observed by some others[11–13]. We believe that, with added cases and data, further

Table 2 Modelled sensitivity of detection at fixed false-positive rates using observed likelihood ratios in a subset of 744 patients undergoing nuchal translucency (NT) measurement and the age distribution of live births in the USA

	Down's syndrome screening		*Trisomy 18 screening*	
Screening method	*False-positive rate (%)*	*Sensitivity (%)*	*False-positive rate (%)*	*Sensitivity (%)*
Biochemistry	5	61	1	63
NT	5	73	*	60
Biochemistry and NT	5	87	1	76

*No additional false-positive results, since the same criteria were used to detect both Down's syndrome and trisomy 18 in NT screening

improvement in NT discrimination will be realized. Further improvement may also be realized if the gestational age range is limited to a latter part of the first trimester. For example, in this study, limiting the gestational range to 10 + 3 – 14 weeks would have resulted in a detection of 73% instead of 57% of cases using a delta NT cut-off level of 1.0. Nonetheless, the combination of biochemistry and NT in this preliminary study resulted in a rate of Down's syndrome detection (87%) not achievable with current second-trimester Down's syndrome screening protocols.

No significant correlation between biochemistry and NT was observed in this study ($r = -0.033$ and 0.026 for free β-hCG MoM and PAPP-A MoM, respectively), indicating that the combination of the two screening modalities should improve screening sensitivity over what may be expected with either alone. Previous studies by Noble and colleagues[13], and Brizot and colleagues[14] have seen no correlation between free β-hCG and NT, but they did not evaluate PAPP-A. Additionally, an insignificant association was observed between NT and maternal age or maternal weight. Future studies which include diverse ethnic groups will reveal whether a need for correction is warranted.

When maternal age was added to NT, there was a significant increase in both the false-positive rate (5.8% to 14.1%) and the sensitivity (57% to 82%). This effect was due mainly to the fact that a significant number of patients with advanced maternal age were included in the study group. When the data were modelled to assess screening in the general pregnant population, the combination of maternal age and NT yielded a sensitivity of 73% at a 5% false-positive rate, better than that achieved with NT only. A study that contains patients with maternal ages more in line with the general screening population is needed to confirm these modelled results.

The combination of biochemical and ultrasound screening raises important logistic issues. The timely provision of laboratory and ultrasound services and the combination of results from these different analyses to produce an overall patient-specific risk will represent a significant challenge. Use of dried maternal blood spot technology may provide greater flexibility within the first-trimester screening process by allowing blood samples to be collected by different individuals (clinician, ultrasonographer, nurse, technician, or even patient). Sample collection may occur in the clinician's office, ultrasound suite, clinic or, ultimately, the patient's home. Examples of such in-home blood collection and testing (glucose and HIV) currently exist.

Expanded prospective studies will more precisely define the detection efficiency achievable through a combined biochemical and ultrasound screening protocol in the first trimester. If results remain as promising as those observed in this preliminary study, logistic issues and challenges will be met and shifting of the maternal serum screening window to an earlier stage of pregnancy may be realized. This earlier alert should result in significant advantages to both the patient and the clinician.

REFERENCES

1. Krantz, D. A., Larsen, J. W., Buchanan, P. D. and Macri, J. N. (1996). First-trimester Down syndrome screening: free beta human chorionic gonadotropin and pregnancy-associated plasma protein A. *Am. J. Obstet. Gynecol.*, **174**, 612–16
2. Macri, J. N., Spencer, K., Aitken, D., Garver, K., Buchanan, P. D., Muller, F. and Boue, A. (1993). First trimester free beta (hCG) screening for Down syndrome. *Prenat. Diagn.*, **13**, 557–62
3. Berry, E., Aitken, D. A., Crossley, J. A., Macri, J. N. and Connor, M. J. (1995). Analysis of maternal serum alpha-fetoprotein and free beta human chorionic gonadotropin in the first trimester: implications for Down's syndrome screening. *Prenat. Diagn.*, **15**, 555–65
4. Aitken, D. A., Mccaw, G., Crossley, J. A., Berry, E., Connor, J. M., Spencer, K. and Macri, J. N. (1993). First trimester biochemical screening for fetal chromosome abnormalities and neural tube defects. *Prenat. Diagn.*, **13**, 681–9
5. Wald, N. J., Kennard, A. and Hackshaw, A. K. (1995). First trimester serum screening for Down's syndrome. *Prenat. Diagn.*, **15**, 1227–40
6. Macintosh, McM. and Chard, T. (1993). Biochemical screening for Down's syndrome in the first trimester of pregnancy. *Fetal Matern. Med. Rev.*, 5, 181–90
7. Brambati, B., Cislaghi, C., Tului, L., Alberti, E., Amidani, M., Columbo, U. and Zuliani, G. (1995). First trimester Down's syndrome screening using nuchal translucency: a prospective study in patients undergoing chorionic villus sampling. *Ultrasound Obstet. Gynecol.*, **5**, 9–14
8. Szabo, J. and Gellen, J. (1990). Nuchal fluid accumulation in trisomy 21 detected by vaginography in first trimester. *Lancet*, **336**, 1133
9. Macri, J. N., Anderson, R. W., Krantz, D. A., Larsen, J. W. and Buchanan, P. D. (1996). Prenatal maternal dried blood screening with alpha-fetoprotein and free beta-human chorionic gonadotropin for open neural tube defect and Down syndrome. *Am. J. Obstet. Gynecol.*, **174**, 566–72
10. Nicolaides, K. H., Azar, G., Byrne, D., Mansur, C. and Marks, K. (1992). Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. *Br. Med. J.*, **304**, 867–9
11. Nicolaides, K. H., Brizot, M. L. and Snijders, R. J. M. (1994). Fetal nuchal translucency thickness: ultrasound screening for fetal trisomy in the first trimester of pregnancy. *Br. J. Obstet. Gynaecol.*, **101**, 782–6
12. Pandya, P. P., Snijders, R. J., Johnson, S. P., De-Lourdes-Brizot, M. and Nicolaides, K. H. (1995). Screening for fetal

trisomies by maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation. *Br. J. Obstet. Gynaecol.*, **102**, 957–62

13. Noble, P. L., Abraha, H. D., Snijders, R. J., Sherwood, R. and Nicolaides, K. H. (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**, 390–5
14. Brizot, M. L., Snijders, R. J. M., Butler, J., Bersinger, N. A. and Nicolaides, K. H. (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**, 127–32