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Combined ultrasound and biochemical screening for Down's Syndrome in the first trimester: a Scottish multicentre study

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Objective To evaluate the use of ultrasound measurements of fetal nuchal translucency (NT) obtained in a routine antenatal clinic setting in combination with appropriate biochemical markers as a first trimester screening test for Down's Syndrome.

Design Multicentre observational study.

Setting Fifteen Scottish maternity units.

Population Pregnant women ($n = 17{,}229$) attending routine antenatal clinics at 10–14 weeks of gestation.

Methods NT measurements were attempted in all women along with the measurement of maternal serum free beta human chorionic gonadotrophin (FβhCG) and pregnancy-associated plasma protein-A (PAPP-A). All results were converted to multiples of the appropriate gestational median (MoM) and using a statistical model the risk of an affected pregnancy was derived. No results were given to participating women but all were offered routine second trimester biochemical screening. All cases of Down's Syndrome within the study group were ascertained and the detection rate for each marker was estimated.

Main outcome measures Success rate of obtaining NT measurements and overall effectiveness of ultrasound and biochemical markers individually and in combination for the detection of Down's Syndrome pregnancies.

Results NT measurements were obtained in 72.9% of women and blood samples in 98.4%. Forty-five cases of Down's Syndrome were ascertained (2.6/1000). NT measurements were obtained in 37 cases (median NT 1.65 MoM), blood samples in 42 cases and both NT and blood in 34 cases. In combination with the *a priori* maternal age risk, observed detection rates at a 5% false positive rate were 20/37 (54%) for NT, 23/42 (55%) for FβhCG and PAPP-A and 28/34 (82%) for a combination of NT, FβhCG and PAPP-A using a cutoff risk of 1:250. The effect of failing to obtain NT measurements in all cases reduces the overall detection rate to 62% (i.e. 28/45) if the entire series of affected pregnancies within the study group is considered.

Conclusions NT in combination with appropriate serum markers has the potential to detect over 80% of Down's Syndrome fetuses in early pregnancy. However, NT measurement is highly operator-dependent. It requires training, external quality control and adequate time to allow accurate measurement, otherwise suboptimal performance will result.

INTRODUCTION

Screening for Down's Syndrome in the second trimester by analysis of feto-placental markers in maternal blood is well established. The most commonly used combinations of two or three markers detect around 65–70% of affected pregnancies at a 5% false positive rate[1–3]. This approach improves detection rates over those achievable by maternal age alone and provides a method of restricting invasive diagnostic testing to a predetermined proportion (usually 5%) of women with the highest risks of having an affected pregnancy. It has, however, two obvious disadvantages: around 30% of the chromosomally abnormal pregnancies are missed and testing is carried out at a relatively advanced stage resulting in the late termination of affected pregnancies when parents opt for this course of action.

Serum marker studies in the first trimester have identified free beta human chorionic gonadotrophin (FβhCG) and pregnancy-associated plasma protein-A (PAPP-A) as the most discriminatory markers with PAPP-A proving more effective at earlier gestations[4–6]. Retrospective studies of this marker combination with maternal age have reported between 55% and 68% detection of Down's Syndrome pregnancies at a 5% false positive rate[6–9], but there is evidence to suggest that first trimester biochemical screening fails to match the performance of the established second trimester screening protocols[6].

A more sensitive first trimester marker for Down's Syndrome appears to be the ultrasound measurement of

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fetal nuchal translucency (NT) which is generally increased in aneuploid fetuses[10]. A review of the performance of NT measurements in both selected and routine screening populations estimated mean detection rates for Down's Syndrome of 77% and 62%, respectively, at corresponding false positive rates of 6.0% and 4.0% but with wide variation in each population group[11]. Subsequently, in a large multicentre study co-ordinated by the Fetal Medicine Foundation (FMF)[12], 22 centres in the UK contributed data on NT measurements in 96,127 women including 326 cases of Down's Syndrome. Based on NT measurements and maternal age, a detection rate of 82.2% was estimated at an 8.3% false positive rate demonstrating the potential of NT screening. Other studies, however, have failed to match this performance and the usefulness of NT measurement as a screening test in unselected pregnancies has been questioned[13–15].

Several attempts have been made to assess the combined performance of ultrasound NT and serum marker measurements in the first trimester. Spencer *et al.*[16] retrospectively analysed FβhCG and PAPP-A in stored serum from 210 Down's Syndrome pregnancies and when these data were combined with NT measurements in the same series of pregnancies[12,17] a detection rate of 89% at a 5% false positive rate was obtained — close to the modelled detection rates of 80–87% predicted for the combined test in other studies[18,19]. Similar results have been reported in other smaller retrospective studies of combined ultrasound and biochemical (CUB) screening[20], but there is relatively little information on the prospective performance of such screening in the general pregnant population. Here we report the results of a large multicentre, prospective, non-intervention study of CUB screening for Down's Syndrome in an unselected population in a routine antenatal clinic setting.

METHODS

Of the 25 maternity units approached with details of the proposed study, 15 agreed to participate and began recruiting women after Local and Multicentre Ethics approval had been obtained. Reasons given by non-participating units for not joining the study included lack of time and/or staff to carry out the ultrasound scanning, lack of ultrasound machines with adequate resolution or a large proportion of patients booking in the community.

Specific training in the protocol for NT measurement[21] was provided for nominated staff at each hospital by the FMF (Professor K. Nicolaides). Attendance at practical and theoretical training was made a condition before staff could contribute data to the study. A total of 65 ultrasound operators (55% radiographers, 20% midwives and 25% obstetricians or radiologists) received training before commencement or during the course of the study.

Batches of patient information leaflets and consent forms were sent to participating units for distribution to women along with their booking appointment notification. When women attended the antenatal clinic, those whose gestations were estimated to be within the 10–14 week gestation window were invited to participate in the study and if they agreed, asked to sign the consent form. Women were informed that no results would be given from the study and management of their pregnancy would not be altered through participation. All were offered routine second trimester biochemical screening. Although the study was intended to be non-interventional, three of the larger units already had a policy of offering chorionic villus sampling (CVS) if a 'large' (locally defined) NT measurement was noted during a first trimester scan. Continuation of these policies during the study resulted in a number of pregnancies being lost to the longitudinal aspect of the study.

For consenting women, fetal maturity was assessed by ultrasound measurement of crown–rump length (CRL) and/or biparietal diameter (BPD) and where these fell within the limits defined for the study (CRL 31–94 mm, BPD < 30 mm) three separate transabdominal NT measurements were attempted as an additional measurement during the routine booking scan session following a published protocol[21]. Transvaginal scanning in cases of awkward fetal position or maternal obesity or return appointments for a repeat NT scan at a later session did not form part of the study protocol.

Completed data forms from each patient, accompanied by a clotted blood sample, were sent to the co-ordinating centre where, immediately on receipt, serum was separated from the clot by centrifugation, an aliquot used for serum marker analysis and the remainder stored at -20°C. Patient details, pregnancy information and NT measurements on each data form were entered into a specially compiled database set up for the study.

All serum samples were assayed for FβhCG and PAPP-A using the *Kryptor* random access immunoassay analyser (Brahms, Germany, formerly supplied by CIS UK). All results were entered into the appropriate patient record in the database but final analysis of the data was not carried out until the end of the study when the last women recruited had delivered. A subset of serum samples (*c.* 2000), which included all affected pregnancies, was also analysed for FβhCG and PAPP-A levels using the alternative *Delfia* immunoassay methods (Perkin-Elmer, UK) for comparison.

NT measurements (in millimetres) were plotted against the appropriate CRL (in millimetres) and a median NT calculated at each CRL increment. The medians at each CRL millimetre were smoothed by regression and individual NT measurements were converted to a multiple of the appropriate regressed NT median (MoM). The distribution parameters of NT measurements in MoM (medians, means, standard deviations) were established and the fit to Gaussian distributions in unaffected and Down's Syndrome pregnancies was confirmed. Median levels for maternal serum FβhCG and PAPP-A in unaffected pregnancies were calculated at each individual CRL measurement between

20 (equivalent to 61 days of gestation) and 94 mm (equivalent to 104 days of gestation) and smoothed by regression. FβhCG and PAPP-A levels in affected and unaffected pregnancies were converted to multiples of the appropriate regressed median (MoM) level for the corresponding CRL and the parameters of the distribution of results in MoM (medians, means, standard deviations) and goodness of fit to Gaussian distributions estimated.

RESULTS

A total of 17,229 women were recruited to the study over a two-year period. The maternal age distribution profile of the study group closely matched that of the normal screening population (median maternal age 29.9 *vs* 29.1 years and 15.4% *vs* 12.8% ≥35 years, respectively) (Fig. 1).

NT measurements

An example of the typical variation in NT measurements found for individual operators over a three-month period is shown for one of the participating hospitals (Fig. 2). Using this format, a summary of NT measurements was compiled for each individual operator on a three-monthly basis and sent back to the participating hospital. A comparison of NT measurements obtained between 13 hospitals is shown in Fig. 3. A random selection of ultrasound images from each operator was also sent to the FMF (Dr V. Soutar) for review. Overall, assessment of NT distribution data and images suggested that NT measurements were being performed to an acceptable standard. No hospital achieved 100% success rate in obtaining NT measurements from patients. The average success rate was 72.9% for at least one measurement and only 51.9% for the 'gold standard' of three measurements. The median gestation when women had an NT scan was 12 weeks and 3 days (range between hospitals: 11 weeks and 0 day to 12 weeks and 6 days) by ultrasound and there was a clear association between success of obtaining an NT measurement and the stage of pregnancy (Fig. 4). The best performance was seen during the 11th, 12th and 13th weeks of gestation. An analysis of the reasons given on the request form for failing to obtain an NT measurement showed that 'fetal position' was cited most frequently (16.7%) with maternal size accounting for only 2% of the failure rate. Generally, there was not enough time within the structure of the average routine antenatal clinic appointment to allow persistent attempts at NT measurement to be made in difficult cases.

Interpretation of NT measurements depends upon an accurate knowledge of the gestation. In addition to collecting information on the date of the last menstrual period, ultrasound operators were requested to measure and record on the form both CRL and BPD so that NT measurements could be assessed against one or both measures of fetal size

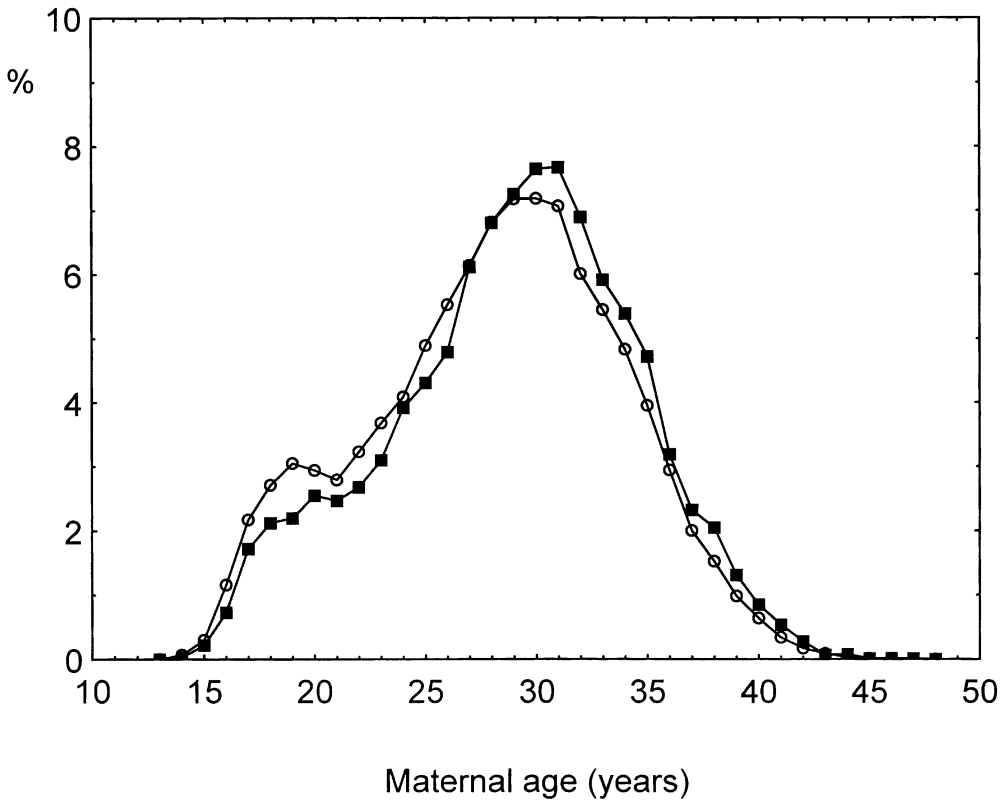

Fig. 1. Distribution of pregnancies by maternal age in the study group (■——■) and routine screened population (○—○).

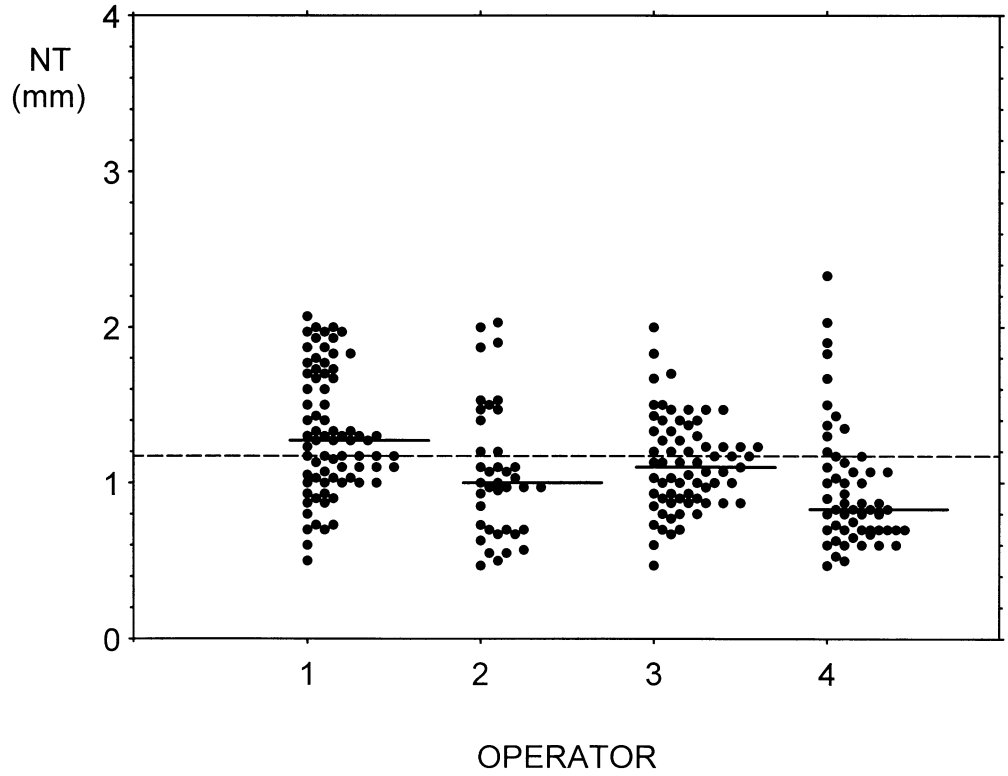

Fig. 2. Inter-operator variation in NT measurements in one hospital over a three-month period. The dashed line represents the overall three-month median and the solid lines the median for each operator.

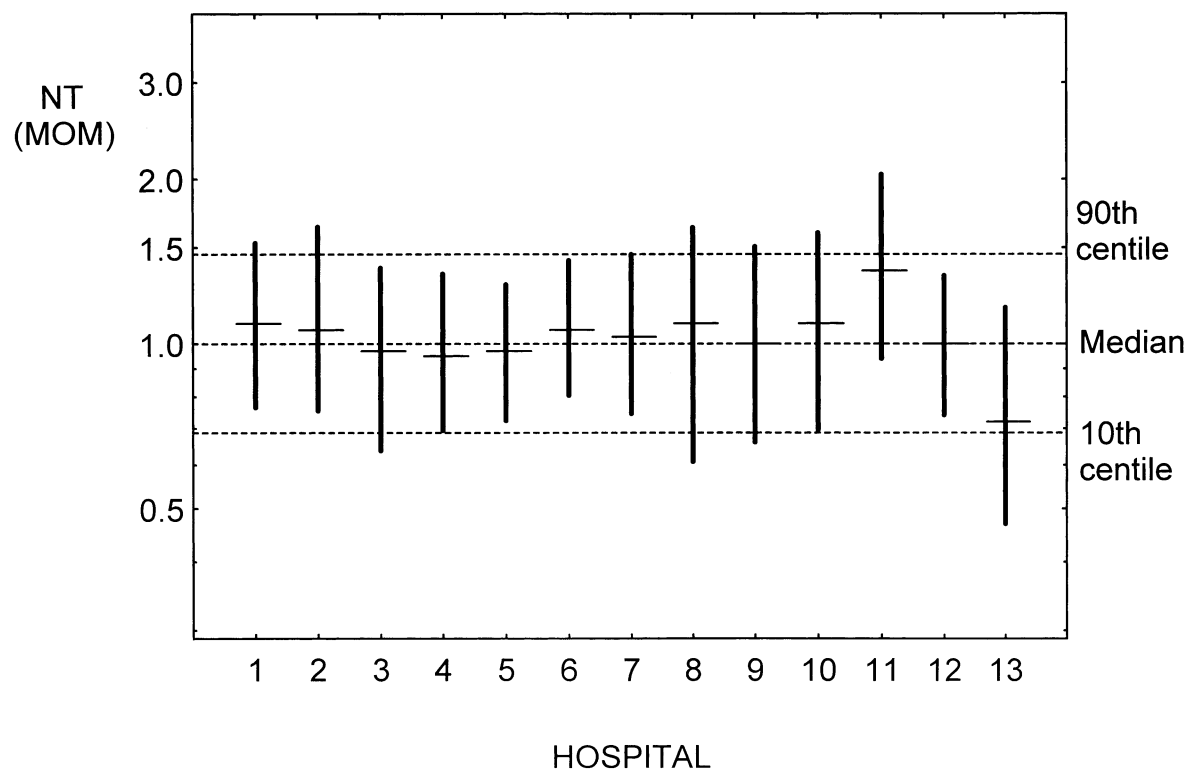

Fig. 3. Range of NT measurements (in MoM) between hospitals.

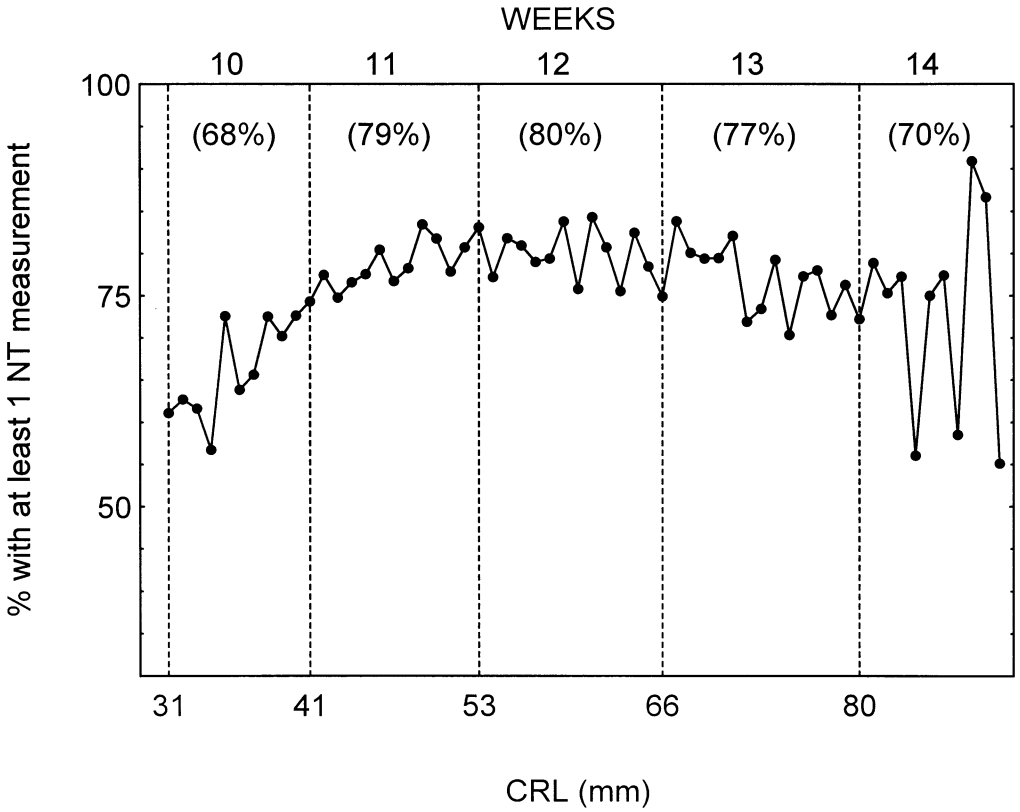

Fig. 4. Variation with gestation of success of obtaining at least one NT measurement.

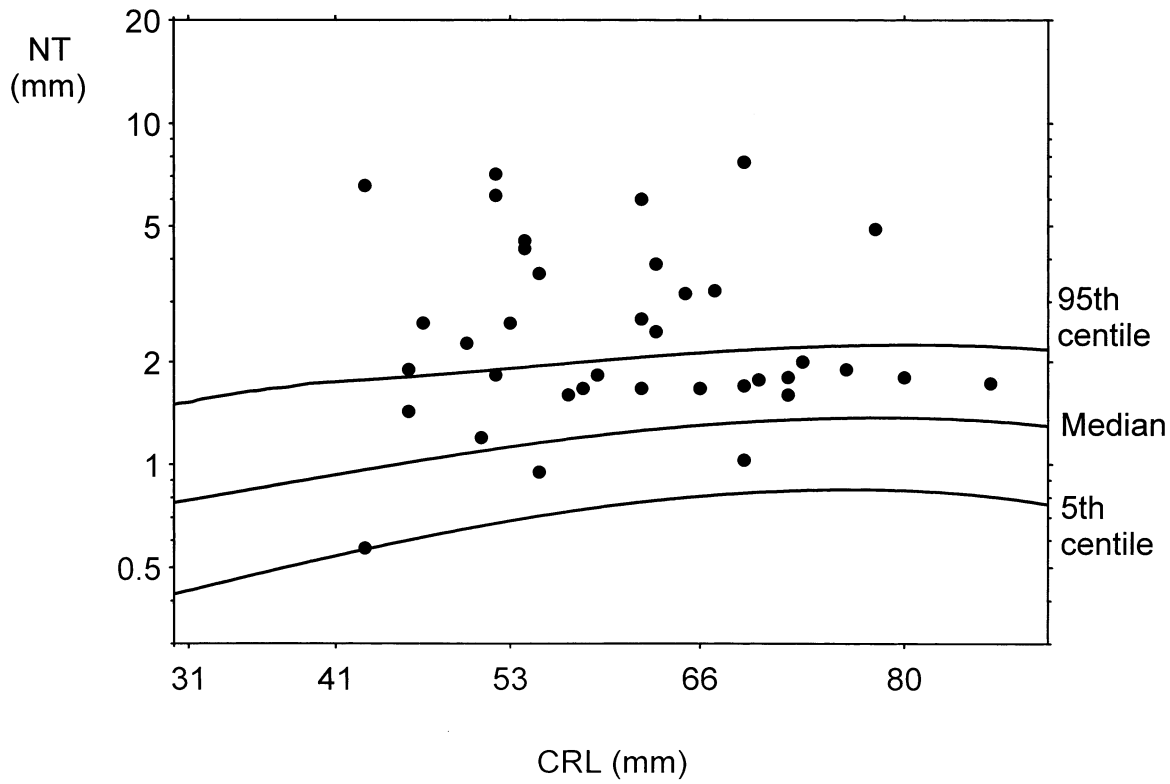

Fig. 5. Distribution of NT measurements (in mm) in 37 Down's Syndrome pregnancies.

Table 1. Parameters of the distributions of NT measurements and FβhCG and PAPP-A concentrations (in MoM) in Down's Syndrome and unaffected pregnancies. The means and standard deviations (SD) are of the $\log_{10}$ distributions.

	Median	Mean	SD
NT (MoM)			
Down's Syndrome	1.65	0.217	0.285
Unaffected	1.00	−0.002	0.128
FβhCG (MoM)			
Down's Syndrome	1.58	0.199	0.274
Unaffected	1.00	0.00	0.269
PAPP-A (MoM)			
Down's Syndrome	0.54	−0.268	0.317
Unaffected	1.00	0.00	0.225

as well as fetal age. CRL measurements were obtained in 83.7% of pregnancies, BPD measurement in 46.2% and both CRL and BPD in 34.7%. There was a strong correlation between CRL and BPD measurements during the 10–14 week gestation window such that CRL = BPD × 3. This conversion factor allowed interpretation of an NT measurement to be made against either measurement of fetal maturity with acceptable accuracy within this narrow gestational window. In normal pregnancies, there was a clear trend of increasing NT size with advancing gestation, which peaked at a maximum regressed median NT size of 1.40 mm at a CRL of 78 mm (equivalent to 13 weeks and 6 days of gestation). Conversion of NT measurements to MoM and $\log_{10}$ transformation gave a reasonable fit to a Gaussian distribution.

The outcome of all pregnancies was followed up and 45 cases of Down's Syndrome were ascertained (2.6/1000). Nine (20%) were diagnosed at first trimester CVS (advanced maternal age or large NT), 23 (51%) at second trimester amniocentesis (high risk on routine biochemical screening or advanced maternal age) and 13 (29%) at birth (unscreened, low risk on screening or declined diagnostic testing). NT measurements were obtained in 37 and blood samples in 42 of the 45 cases. The median maternal age of the women with Down's Syndrome pregnancies was 33.6 years and 37.8% of the affected pregnancies were in women aged 35 years and over. This number and distribution of Down's Syndrome pregnancies is close to the proportions expected in a population with the age structure of the study group. Figure 5 shows the distribution of NT measurements in mm in the 37 cases of Down's Syndrome where measurements were obtained. There is a substantial overlap of NT measurements in normal and affected pregnancies. The median NT size in this series of Down's Syndrome cases was 1.65 MoM. The parameters of the distribution of NT results (in MoM) are presented in Table 1. Figure 6 shows a probability plot of the distribution of NT MoM in normal

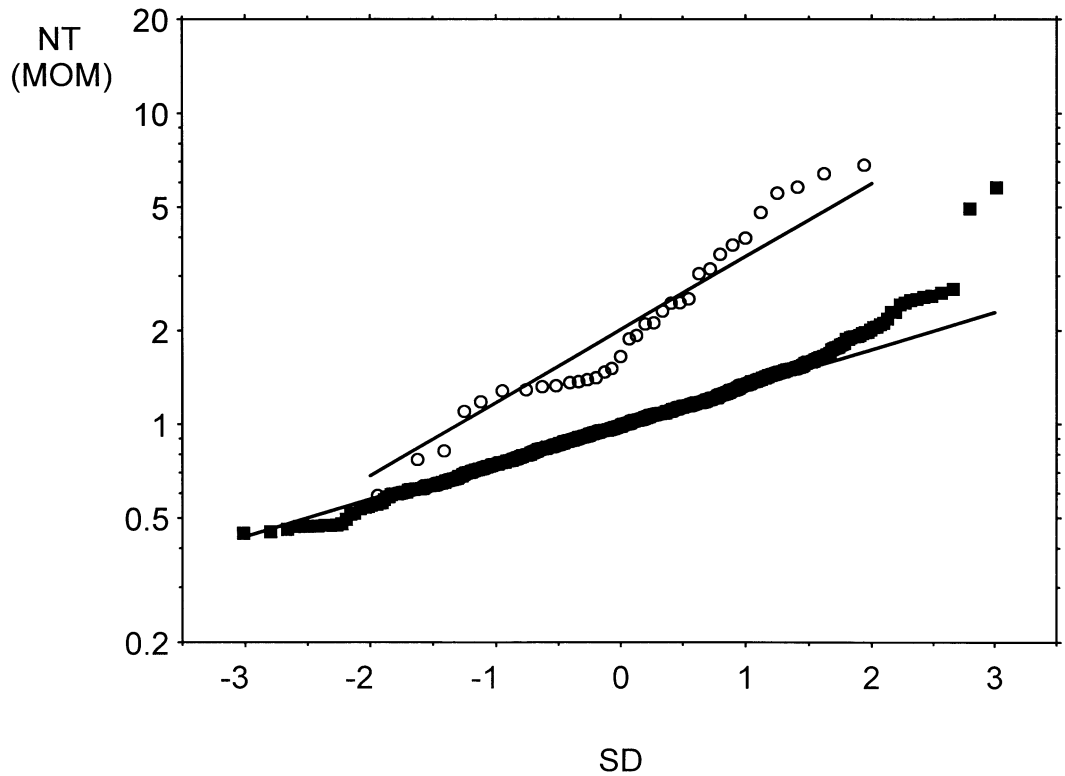

Fig. 6. Probability plot of NT measurements (in MoM) in Down's Syndrome (○) and unaffected pregnancies (■). The lines represent the corresponding probability distributions derived from published data[17].

Table 2. Correlations (r values) between first trimester screening markers in Down's Syndrome and unaffected pregnancies.

		log FβhCG (MoM)	log PAPP-A (MoM)
Unaffected	log NT (MoM)	−0.04	0.02
Down's Syndrome	log NT (MoM)	0.15	−0.24
Unaffected	log FβhCG (MoM)	–	0.18
Down's Syndrome	log FβhCG (MoM)	–	0.23

and Down's Syndrome pregnancies showing a comparison of the fit to $\log_{10}$ Gaussian distributions obtained in another large published series[16,17].

NT measurements were also assessed against a number of maternal and pregnancy variables including maternal weight, smoking status and multiple pregnancy. Apart from gestation, none of these co-variables was found to affect NT measurements (data not shown).

Biochemical measurements

In normal pregnancies, FβhCG levels fall with advancing gestation across 9–14 weeks and PAPP-A levels rise. When results are converted to MoM, the data for each marker fit $\log_{10}$ Gaussian distributions. There was a strong correlation between the serum marker results obtained by the *Kryptor* and *Delfia* methods (for $\log_{10}$ FβhCG, $r = 0.88$; for $\log_{10}$ PAPP-A, $r = 0.90$). The parameters of the distributions are presented in Table 1. In the Down's Syndrome pregnancies, FβhCG levels were generally increased (median MoM 1.58) and PAPP-A levels were reduced (median MoM 0.54). Both markers show an inverse correlation with maternal weight and women who smoked had a 15% reduction in their PAPP-A levels compared with non-smokers. FβhCG and PAPP-A levels in twin pregnancies ($n = 98$) were approximately twice the levels found in singleton pregnancies (FβhCG 2.02 MoM, PAPP-A 2.04 MoM). There was no significant correlation between the $\log_{10}$ distributions of either biochemical marker or NT measurements (in MoM) and only a small correlation between FβhCG MoM and PAPP-A MoM (Table 2).

Predictive value of ultrasound and biochemical markers

As the NT data obtained during the CUB screening study are a reasonable fit to the distributions established on a much larger series of unaffected and Down's Syndrome pregnancies[16,17] (Fig. 6), we used these published parameters to derive a risk for each individual NT measurement. NT in combination with maternal age risks detected 20/37 (54%) (95% confidence interval [CI] 37–71%) of the Down's Syndrome pregnancies at a 5% false positive rate. Similarly, using the published parameters from the same study[16] for FβhCG and PAPP-A, the serum markers in combination with maternal age detected 23/42 (55%) (95% CI 39–70%) of the Down's Syndrome pregnancies at a 5% false positive rate. Thirty-four of the 45 cases had both NT and biochemical measurements and when these were combined with maternal age to provide a risk or odds ratio that a particular pregnancy is affected by Down's Syndrome, 28/34 (82%) (95% CI 65–93%) of affected pregnancies were identified for a 5% follow up rate using a threshold risk at term of 1:250.

In total, 30 of the 45 affected pregnancies had routine second trimester biochemical screening for Down's Syndrome carried out in one of three Scottish laboratories. The median maternal age of this subgroup of affected pregnancies was 33.8 years. Using each laboratories' definition of screen positive, 22/30 (73%) (95% CI 54–88%) of these cases were classified as being at high risk based on the markers α-fetoprotein, total human chorionic gonadotrophin and maternal age at an average false positive rate of 6.0%.

DISCUSSION

This study targeted unselected women from the general pregnant population attending routine antenatal clinics in the first trimester. The most striking finding is the relatively low level of success achieved in obtaining NT measurements under these conditions. Since in clinical practice this would be a failure of the screening process, the potential detection rate of over 80% observed for those cases where both ultrasound and biochemical marker information were available was not achieved. If the entire series of affected pregnancies within the study group is considered, the detection rate was 28/45 (62%). Our analysis has shown that failure to obtain an NT measurement was due principally to time pressure in the antenatal clinic and it seems clear that if NT measurements are to become a routine component of screening tests, around 10–15 minutes of scanning time will have to be allocated to each patient. However, we also noted during the study that within a busy antenatal clinic, there was a tendency for ultrasonographers not to persist in difficult cases as it was 'only a study' and results were not being reported to women. There is good reason to believe that this performance would improve in routine practice provided that adequate time is allowed for the NT scan: a pilot of CUB screening in our centre based on a restructured antenatal clinic appointment system and reporting of results to women has achieved an overall 99.8% success rate for NT measurements in over 2000 pregnancies screened, although this required a return visit by 9% of women for a further attempt at NT measurement.

Where data were obtained, NT and age combined would have detected 54% (95% CI 37–71%) of the Down's Syndrome cases at a 5% follow up rate—somewhat lower than the rates reported in some other studies[11,12] and consistent with the lower median NT MoM found in this study. While this may have been due to chance in our relatively small series of affected pregnancies, it could

reflect some systematic difference in the measurement of NT. However, a review of the NT measurements and images obtained in this study and the distribution parameters of NT in unaffected pregnancies compared with those of the large FMF study[12] suggest that measurements, when obtained, were done to a satisfactory standard.

With regard to the biochemical markers, the median FβhCG level (1.58 MoM) in the Down's Syndrome cases was also less markedly elevated than the levels reported in a number of other studies in the first trimester[22]. It is known that the magnitude of the change in serum marker levels in Down's Syndrome pregnancies varies with gestation and lower FβhCG levels might be expected if samples are collected early in the first trimester[16]. However, inspection of the distribution of Down's Syndrome cases with gestation (Fig. 5) shows that this is not the case in this study (median gestation 12 weeks and 4 days) and the more modest elevation in FβhCG levels found here is presumably due to chance.

Nevertheless, when NT measurements were combined with the FβhCG and PAPP-A results, a substantial increase in detection (to 82%) was observed. This emphasises the value of combining biophysical and biochemical measurements to optimise detection. This detection rate exceeds that reported for two- and three-marker biochemical screening protocols used routinely in the second trimester and falls within the ranges reported for CUB screening in other, smaller prospective series[23,24]. There are also two reports of CUB screening in prospective practice. In one study from the USA, detection of 91% of Down's Syndrome pregnancies at a 7.8% false positive rate based NT, FβhCG and PAPP-A analyses in over 5000 women has been reported[25] while in the UK, Spencer *et al.*[26] reported 86% detection for Down's Syndrome and 95% detection for all aneuploidies at a 6.8% false positive rate by screening over 4000 women in one centre. In addition to the improvement in sensitivity over second trimester screening, CUB screening also has the potential to deliver a screening result to pregnant women as much as 4 weeks earlier. A comparison of the performance of various first and second trimester screening modalities, including CUB screening, has recently been reported[27].

Earlier published studies of NT in trisomic pregnancies used a fixed cutoff (typically 2.5 or 3.0 mm) to define a high risk group. This is inefficient as a fixed cutoff represents a different centile at different gestations. It is clear from this and other studies that NT measurements in normal pregnancies increase with advancing gestation up to 14 weeks. The best solution is to convert all NT measurements to MoM and use the overlapping Gaussian distributions to derive a risk or probability that can be combined with other risk factors (maternal age and biochemical markers) to give a more accurate estimate of risk in individual pregnancies[18]. In this way, a small NT measurement will contribute to a reduction in risk just as a large NT measurement will increase the risk and therefore it is important that a precise and accurate NT measurement is obtained in every pregnancy. To maintain such a high standard in routine practice, participation in an external

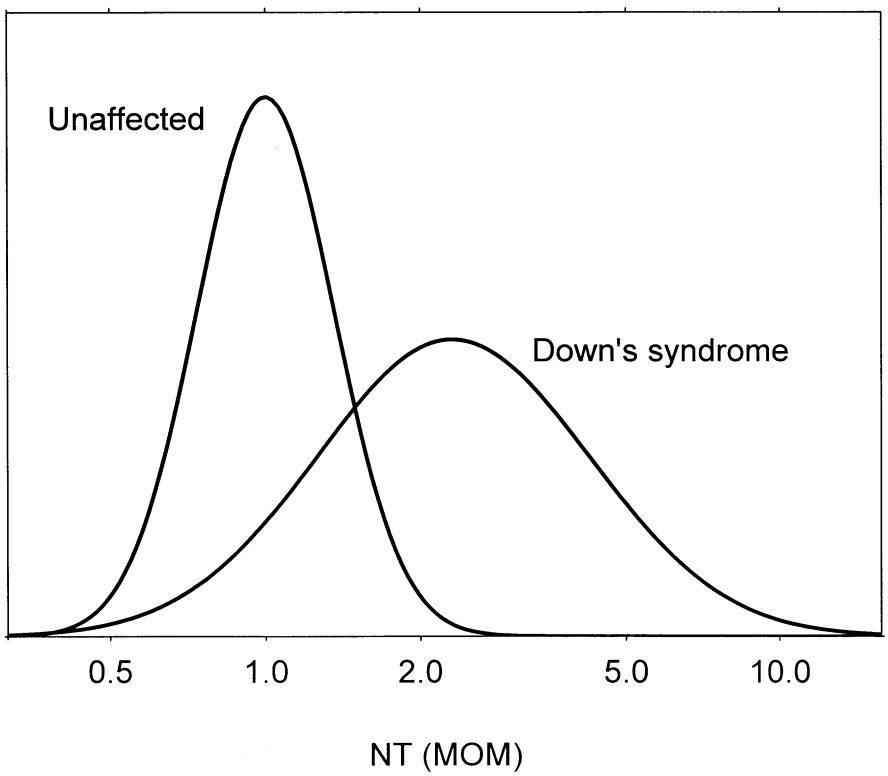

Fig. 7. Distribution of NT measurements (in MoM) in Down's Syndrome and unaffected pregnancies derived from published data[17].

quality control scheme based on the statistical analysis of NT measurements from individual operators (Fig. 2)[28] and on analysis of images[29] will be required. It is also clear that NT measurements are not increased in all cases of Down's Syndrome and that there is a substantial overlap of the measurements in normal and affected pregnancies[17]. Analysis of the distribution data (Fig. 7) shows that very large odds ratios in favour of an affected pregnancy are derived when NT measurements are above about 3.0 MoM while for NT measurements below 0.8 MoM, likelihood ratios begin to increase again as a function of the changing slopes of the left hand tails of the distributions. Thus, a lower truncation limit of 0.8 MoM should be used in all calculations of risk[30].

We conclude that moving screening for Down's Syndrome to the first trimester based on the combined use of biophysical and biochemical markers will have some advantages for pregnant women in terms of improved sensitivity and earlier detection of affected pregnancies. Before this can become routine however, several practical difficulties identified in our study need to be addressed. Paramount among these is the need to allocate sufficient time per scan to maximise the likelihood of a successful NT measurement at the first clinic visit. This may require a reorganisation of the flow of women through the antenatal clinic and the need to accommodate a return visit or a transvaginal scan in those cases where an initial attempt is unsuccessful. Also, early pregnancy scanning in peripheral clinics is often performed with ultrasound equipment of lower resolution than that required for accurate NT measurement. In order to provide a uniform service for all women requesting screening, such equipment and logistical problems would require review.

Acknowledgements

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References

1. Crossley JA, Aitken DA, Berry E, Connor JM. Impact of a regional screening programme using maternal serum AFP and hCG on the birth incidence of Down's syndrome in the west of Scotland. *J Med Screen* 1994;**1**:180–183.
2. Goodburn SF, Yates JR, Raggatt PR, et al. Second-trimester maternal serum screening using alpha-fetoprotein, human chorionic gonadotrophin and unconjugated oestriol: experience of a regional programme. *Prenat Diagn* 1994;**14**:391–402.
3. Spencer K. Second trimester prenatal screening for Down's syndrome using alpha-fetoprotein and free beta hCG: a seven year review. *Br J Obstet Gynaecol* 1999;**106**:1287–1293.
4. Spencer K, Macri JN, Aitken DA, Connor JM. Free beta hCG as a first trimester marker of fetal trisomy. *Lancet* 1992;**339**:1480.
5. Spencer K, Aitken DA, Crossley JA, et al. First trimester biochemistry screening for trisomy 21: the role of free beta hCG, alpha fetoprotein and pregnancy associated plasma protein A. *Ann Clin Biochem* 1994; **31**:447–454.
6. Berry E, Aitken DA, Crossley JA, Macri JN, Connor JM. Screening for Down's syndrome: changes in marker levels and detection rates between first and second trimester. *Br J Obstet Gynaecol* 1997;**104**: 811–817.
7. Wald NJ, George L, Smith D, Densem JW, Petterson K, on behalf of the international Prenatal Screening Research Group. Serum screening for Down's syndrome between 8 and 14 weeks of pregnancy. *Br J Obstet Gynaecol* 1996;**103**:407–412.
8. Krantz DA, Larsen JW, Buchanan PD, Macri JN. First-trimester Down syndrome screening: free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A. *Am J Obstet Gynecol* 1996;**174**:612–616.
9. Forest JC, Masse J, Moutquin JM. Screening for Down syndrome during first trimester: a prospective study using free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A. *Clin Biochem* 1997;**30**:333–338.
10. Nicolaides KH, Brizot ML, Snijders RJM. Fetal nuchal translucency: ultrasound screening for fetal trisomy in the first trimester of pregnancy. *Br J Obstet Gynaecol* 1994;**101**:782–786.
11. Chitty LS, Pandya PP. Ultrasound screening for fetal abnormalities in the first trimester. *Prenat Diagn* 1997;**17**:1269–1281.
12. 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**:343–346.
13. Roberts LJ, Bewley S, Mackinson AM, Rodeck CH. First trimester fetal nuchal translucency: problems with screening the general population 1. *Br J Obstet Gynaecol* 1995;**102**:381–385.
14. Bewley S, Roberts LJ, Mackinson AM, Rodeck CH. First trimester fetal nuchal translucency: problems with screening the general population 2. *Br J Obstet Gynaecol* 1995;**102**:386–388.
15. Kornman LH, Morssink LP, Beekhuis JR, De Wolf BT, Heringa MP, 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.
16. 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 β-human chorionic gonadotrophin and pregnancy-associated plasma protein A. *Ultrasound Obstet Gynecol* 1999;**13**:231–237.
17. Nicolaides KH, Snijders RJM, Cuckle HS. Correct estimation of parameters for ultrasound nuchal translucency screening. *Prenat Diagn* 1998;**18**:519–521.
18. Wald NJ, Hackshaw AK. Combining ultrasound and biochemistry in first-trimester screening for Down's Syndrome. *Prenat Diagn* 1997; **17**:821–829.
19. Cuckle HS, van Lith JMM. Appropriate biochemical parameters in first-trimester screening for Down Syndrome. *Prenat Diagn* 1999; **19**:505–512.
20. De Graaf IM, Pajkrt E, Bilardo CM, Leschot NJ, Cuckle HS, Van Lith JMM. Early pregnancy screening of fetal aneuploidy with serum markers and nuchal translucency. *Prenat Diagn* 1999;**19**: 458–462.
21. Pandya PP, Altman DG, Brizot ML, Nicolaides KH. Repeatability of

measurement of fetal nuchal translucency thickness. *Ultrasound Obstet Gynecol* 1995;**5**:334–337.
22. Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. *J Med Screen* 1997;**4**:181–246.
23. Orlandi F, Damiani G, Hallahan TW, Krantz DA, Macri JN. First-trimester screening for fetal aneuploidy: biochemistry and nuchal translucency. *Ultrasound Obstet Gynecol* 1997;**10**:381–386.
24. De Biasio P, Siccardi M, Volpe G, Famularo L, Santi F, Canini S. First-trimester screening for Down syndrome using nuchal translucency measurements with free β-hCG and PAPP-A between 10 and 13 weeks of pregnancy—the combined test. *Prenat Diagn* 1999;**19**: 360–363.
25. Krantz DA, Hallahan TW, Orlandi F, Buchanan P, Larsen JW, Macri JN. First-trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. *Obstet Gynecol* 2000; **96**:207–213.
26. Spencer K, Spencer CE, Power M, Moakes A, Nicolaides KH. 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* 2000;**107**:1271–1275.
27. Gilbert RE, Augood C, Gupta R, et al. Screening for Down's syndrome: effects, safety and cost effectiveness of first and second trimester strategies. *BMJ* 2001;**323**:1–6.
28. Crossley JA, Aitken DA, Cameron AD, Connor JM. Quality assessment of ultrasound nuchal translucency measurements. *Eur J Ultrasound* 2000;**11**:S19–S20.
29. Herman A, Maymon R, Dreazen E, Caspi E, Bukovsky I, Weinraub Z. Nuchal translucency audit: a novel image-scoring method. *Ultrasound Obstet Gynaecol* 1998;**12**:398–403.
30. Crossley JA, Aitken DA. Deriving risks of Down's syndrome from nuchal translucency measurements. *Ultrasound Obstet Gynecol* 1999; **14**:438.

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