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First-trimester screening for Down syndrome in France combining fetal nuchal translucency measurement and biochemical markers

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Background In France, there is a strictly regulated National Screening Programme for Down syndrome, based on second-trimester maternal serum markers. A prospective study of nuchal translucency together with retrospective evaluation of maternal serum markers was carried out to inform decisions on whether to move the programme to the first trimester.

Methods Between January 1998 and June 2001, all women who presented for their prenatal care at 12 participating maternity units were, regardless of age, invited to provide a blood sample and to attend for an NT scan at 11 to 13 weeks. The results were used to derive Gaussian distribution parameters. Detection and false-positive rates were computed in two ways: statistical modelling and directly. The cut-off risk was 1 in 250 at term.

Results A total of 5694 women with singleton pregnancies were screened including 26 with Down syndrome and 24 with other aneuploidies. The model-predicted detection and false-positive rates for combined ultrasound and serum screening were 81 and 4.5% compared to 64 and 6.0% for ultrasound alone. The directly observed rates were 73 and 4.7%, compared to 62 and 5.0% respectively.

Conclusion In France, first-trimester screening with nuchal translucency and maternal serum markers is likely to achieve a high screening efficiency. This has important implications for the national screening policy.

KEY WORDS: trisomy 21; aneuploidy; nuchal translucency; first trimester; screening

INTRODUCTION

Prenatal screening for Down syndrome has a similar efficiency using either multiple maternal serum markers tested in the first or second trimester of pregnancy, or ultrasound nuchal translucency (NT) measured at 11 to 13 weeks gestation (Cuckle, 2001). In 1997, a strictly regulated National Screening Programme was established in France, based on second-trimester maternal serum markers alone (Muller *et al*., 2002). Currently, about 80% of pregnant women participate in the programme but about half also have NT screening; first-trimester maternal serum screening is not currently allowed. Since the NT scan and the serum test are carried out sequentially and interpreted independently, the proportion of women undergoing amniocentesis is very high, leading to unnecessary fetal losses.

The screening efficiency of combining the ultrasound and serum tests is much higher than using them either alone or both sequentially (Cuckle, 2001). Thus, it would be more efficient to move to the first trimester of pregnancy the serum tests carried out as part of the National Screening Programme and combine the results with concurrent NT measurements. We therefore carried out a multi-centre non-intervention study to estimate the likely benefits of first-trimester screening using maternal serum pregnancy associated plasma protein (PAPP)-A and free β-human chorionic gonadotrophin (hCG) with or without α-fetoprotein (AFP), and NT.

METHODS

Nine centres serving 12 maternity units and 12 ultrasound departments collaborated in the study. Between January 1998 and June 2001, all women who presented for their prenatal care at participating maternity units were, regardless of age, invited to provide a blood sample and to attend for an NT scan at 11 to 13 weeks gestation. In compliance with the local ethics committee requirements, each woman completed an informed-consent form. A total of 5694 women with singleton pregnancies were included in the study.

Most women had a dating scan at the time of presentation that could be used to schedule the NT scan appointment. For this study, no special arrangements

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concerning NT training and audit were made. However, of the 60 sonographers, 2 received training at the UK Foetal Medicine Foundation, 30 were trained by an FMF-trained sonographer, 8 received specific training in France and 20 were self-taught. Some women whose appointments were only based on menstrual dates were found at the time of the NT scan to be under 11 weeks and a return appointment was scheduled before 14 weeks. NT measurements on 211 women (3.7%) who did not return or who were found to be 14 weeks or more were excluded from the analysis. The NT was regarded as too small for precise measurement in 82 women, and the value was assumed to be under 0.5 mm in the analysis.

Blood samples were centrifuged and serum was stored at −20 °C. Samples were subsequently retrieved from storage in batches and retrospectively tested for PAPP-A, free β-hCG and AFP using time-resolved fluorescent assay (Perkin-Elmer Life Sciences, Turku, Finland). Each laboratory participated in the national external quality control scheme organised at Chambery Hospital.

The first-trimester serum marker levels were not used clinically, but the NT results were. When the NT measurement was regarded as high—usually exceeding 3 mm—women were offered invasive prenatal diagnosis. Those not scanned or with NT considered to be normal were offered second-trimester screening.

For the current analysis, all marker levels, NT as well as serum, were expressed as multiples of the normal gestation-specific median (MoMs). Gestational age was calculated from the crown-rump length (CRL) measurement made at the same time as the NT using a standard chart (Robinson and Fleming, 1975). Normal medians were estimated by regression of the median level for each half week of gestation on the median gestation, in days, weighted by the number of women at that gestation. Maternal weight was available in 4963 pregnancies (87%) and to adjust the serum marker MoM values for weight, each was divided by the expected weight-specific MoM obtained by inverse regression analysis (Neveux *et al*., 1996).

As part of the second-trimester French National Screening Programme, the pregnancy outcome is sought for all women. Using data collected for the programme, we know of 26 Down syndrome cases in the study population, of whom 9 were detected because of a high NT, 1 had prenatal diagnosis because of a parental balanced 14 to 21 translocation and 1 because of maternal age, 14 were detected following second-trimester screening, and 1 affected birth was observed. On the basis of the maternal age distribution, 11 births would have been expected using a standard birth-frequency equation (Cuckle *et al*., 1987); assuming a 45% early fetal-loss rate (Cuckle, 1999); this number is consistent with the 26 observed cases. NT was available for all cases. There were 24 cases of aneuploidy other than Down syndrome: 9 had Turner syndrome, 6 had Edwards syndrome, 3 had Patau syndrome, 2 had triploidy, 1 had Klinefelter syndrome, 1 had 47 XYY, 1 was with trisomy 7 mosaicism and 1 was with an additional abnormal marker 15q11.

The results were used to estimate Gaussian distribution parameters of $\log_{10}$ MoM for unaffected pregnancies. The mean was estimated from the log median value. To avoid the undue influence of occasional outliers, the standard deviation was calculated from the 10th to 90th centile range divided by 2.563. Correlation coefficients were obtained directly after excluding outlying values exceeding three standard deviations from the mean. For Down syndrome pregnancies, the mean was estimated by the observed median but the standard deviations and correlation coefficients for the serum markers were obtained by meta-analysis and tailored to the unaffected population (Cuckle and Van Lith, 1999). The Down syndrome standard deviation for NT was obtained from the large Fetal Medicine Foundation (FMF) study (Nicolaides *et al*., 1998), uncorrected for viability bias, and tailored to the unaffected population in the same way as the serum markers. There is no correlation between the serum markers and NT within unaffected or Down syndrome pregnancies.

The parameters were used to make two estimates of detection and false-positive rates (FPRs), one using statistical modelling and the other using direct modelling. For the indirect approach, standard statistical modelling techniques were used (Royston and Thompson, 1992) and the maternal age distribution was that of the study population. For the direct approach, the term *risk of Down syndrome* was retrospectively estimated for each woman using different marker combinations.

RESULTS

Table 1 shows the observed standard deviation for each marker and the correlation coefficient between markers in unaffected pregnancies. The table also shows the estimated parameters for Down syndrome, tailored to the observed unaffected distributions. The observed median levels in the Down syndrome pregnancies for maternal serum PAPP-A, free β-hCG and AFP were 0.43, 1.88 and 0.92 MoM. These compare well with values obtained by meta-analysis of 0.62, 1.98 and 0.79 MoM respectively (Cuckle and Van Lith, 1999); for PAPP-A, the mean was estimated from the average of the gestation-specific means weighted for the number of affected pregnancies at each completed week. The observed median for NT was 1.92 MoM, compared with 2.27 MoM in the FMF study (Nicolaides *et al*., 1998).

Table 2 shows the model-estimated detection and false-positive rates, using a 1 in 250 term cut-off, together with the retrospectively observed rates. On the basis of the model, biochemical markers alone would achieve a higher detection rate (DR) than NT alone with a slightly higher FPR; and the combination of both modalities would both increase the DR and reduce the false-positive rate. The direct results also yielded a higher detection rate for biochemistry than ultrasound, albeit with a large increase in the false-positive rate. Combining modalities considerably reduced the false-positive rate with a small increase in detection.

When a fixed 5% false-positive rate was used, the modelled and directly observed detection rates were as

Table 1—Standard deviations and correlation coefficients of $\log_{10}$ MoM: observed values in unaffected pregnancies and estimated values in Down syndrome

Parameter	Unaffected pregnancies (no.)	Down syndrome
Standard deviation		
PAPP-A	0.267 (5636)	0.336
Free β-hCG	0.269 (5634)	0.273
AFP	0.194 (5280)	0.217
NT	0.158 (5605)	0.262
Correlation coefficient		
PAPP-A & free β-hCG	0.229* (5551)	0.221
PAPP-A & AFP	0.070* (5156)	0.144
Free β-hCG & AFP	−0.02** (5180)	0.198

* $p < 0.0001$.
** $p = 0.10$.

follows: NT alone, 61 and 62%; PAPP-A and free β-hCG with or without AFP, 68 and 69%; NT, PAPP-A and free β-hCG, 82 and 77%; all four markers, 82 and 73%.

Of the 24 pregnancies with other types of aneuploidy, 16 (67%) had a Down syndrome risk exceeding 1 in 250 based on NT alone and 17 had high risk (71%) in combination with serum markers. But only 3 (12%) had high risk based on serum markers alone.

DISCUSSION

Our study shows that in France, first-trimester screening, with NT and two or more maternal serum markers, is likely to achieve a high screening efficiency. This has important implications for the national screening policy.

There have been five large prospective intervention studies that used both NT and first-trimester serum markers—PAPP-A and free β-hCG. Spencer *et al*. (2000) observed detection and FPRs of 86% (7 cases) and 6.7% (3762 pregnancies); Krantz *et al*. (2000) found rates of 91% (33) and 7.9% (5223); Bindra *et al*. (2002) reported rates of 92% (82) and 6.8% (14 200); Schuchter *et al*. (2002) reported rates of 86% (14) and 5.2% (4939) and Crossley *et al*. (2002) reported rates of 80% (34) and 5% (17 229). A smaller study (Tsai *et al*., 2001) found similar results. In addition, there was a non-intervention study in a routinely presenting obstetrics population with a similar design to our own. Niemimaa *et al*. (2001) observed detection and false-positive rates of 80% (5 cases) and 5.4% (1602 pregnancies). The false-positive rates in some of these studies are higher than ours, using the same markers, and the detection rates are also somewhat higher. This is probably due in part to our choice of 1 in 250 at term as the cut-off, whereas they have generally used 1 in 270 or 300 during the first trimester. The relatively small number of affected pregnancies will also have contributed to the between-study differences in detection rate.

The French National Screening Programme was implemented in January 1997 using second-trimester serum markers. A review of the first 854 000 women screened in the first 2 years showed that 65% of women were screened, with an observed 73% detection rate and 6.9% false-positive rate (Muller *et al*., 2002). In the current non-intervention study, the combined use of NT and serum markers in the first trimester achieved a much lower false-positive rate (4.7%) and although the observed DR was not increased (73%), the total number of Down syndrome cases was small and the model-derived rate was much higher (81%). First-trimester screening also has the benefits of earlier reassurance and diagnosis, with a safer termination of pregnancy if required. There is now a need to consider moving the National Screening Programme to the first trimester. A prospective intervention study of first-trimester screening is currently underway in France and will be completed within a year.

Although NT measurement is not currently part of the National Screening Programme, a large number of women throughout France are now being scanned, mostly in small centres. A prerequisite for accurate NT measurement and the correct interpretation of results is standardisation and quality control. While comparable standards for laboratory measurements have been in place in France since 1970, they are not widely available for ultrasonography. Even within our own study there is

Table 2—Detection and false-positive rates with a 1 in 250 term risk cut-off: estimated using a model and directly observed[a]

Combination	Modelled		Directly observed	
	DR (%)	FPR (%)	DR [95% CI]	FPR [95% CI]
NT alone	64	6.0	62% (16/26) [43–80%]	5.0% (281/5605) [4.4–5.6%]
PAPP-A & free-β	72	6.9	69% (18/26) [51–87%]	8.0% (451/5633) [7.3–8.7%]
PAPP-A, free-β & AFP	73	6.9	69% (18/26) [51–87%]	8.0% (425/5277) [7.3–8.8%]
NT, PAPP-A & free-β	81	4.5	73% (19/26) [56–90%]	4.7% (263/5598) [4.1–5.3%]
NT, PAPP-A, free-β & AFP	81	4.5	73% (19/26) [56–90%]	4.7% (246/5243) [4.1–5.3%]

DR, detection rate; FPR, false-positive rate.
[a] Based on retrospectively calculated risks.

evidence that the NT measurements were not as precise as they could be, since the NT standard deviation (0.16) was much wider than in the large multi-centre FMF study (0.12). The FMF provides training and ongoing audit of NT measurement and there is a need to have a similar scheme in France.

In addition to first-trimester NT measurement and second-trimester serum screening, French women are also identified as having a high enough risk to warrant invasive prenatal diagnosis on the basis of age alone (over 38) and the presence of ultrasound signs at 22-weeks gestation. These approaches are complementary and when considered independently they generate a large number of unnecessary invasive procedures. If more than one approach is taken, it is important to incorporate the results from all the tests in a final risk calculation, which is then used to decide on the next step.

To date the most effective first-trimester screening method is to combine NT and serum biochemistry. In the near future, this may be improved upon by the addition of new markers such as ultrasound nasal bone determination (Cicero *et al*., 2001). Another, perhaps more long-term possibility would be to incorporate the use of fetal DNA in the maternal circulation. However, studies to date suggest that this may take up to 10 years before it is generally applicable.

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APPENDIX

French collaborative group

Amiens (C Lemay, N Roussel); Caen (M Herroux) (Chambéry (B Dingeon, C Doche); Dreux (JC Cartron, MH Ramaroasy); Le Havre (E Berreville, JY Col, D Bouige); Lille (G Renom, JM Perini, E Paux); Lyon Croix-Rousse (S Guibaud, C Boisson); Lyon Hôtel Dieu (F Poloce, MC Gelineau); Paris A. Béclère (C Benattar, F Audibert); Paris A. Paré (F Muller, S Dreux); Paris Institut de Puériculture (F Forestier, V Olin); Paris Pitié (M Bernard, D Vauthier-Brouzes); Paris R. Debré (J Guibourdanche, D Luton); Tours (D Galliano).