

# Screening for Trisomy 18 by Fetal Nuchal Translucency and Maternal Serum Free $\beta$ -hCG and PAPP-A at 10–14 Weeks of Gestation

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In a study of 50 cases of trisomy 18 compared with 947 controls we have found the median multiple of the median (MoM) of maternal serum free  $\beta$  human chorionic gonadotrophin to be significantly decreased (0.281 MoM) in samples collected between the 10th and 14th week of gestation. Similarly, maternal serum pregnancy associated plasma protein A (PAPP-A) levels are also decreased (0.177 MoM), whilst the median nuchal translucency is significantly higher (3.272 MoM). Free  $\beta$ -hCG MoM was less than the 5th centile of normal in 64 per cent of cases of trisomy 18 and for PAPP-A was less than the 5th centile in 78 per cent of cases. Also, in 78 per cent of cases the nuchal translucency was above the 95th centile. When combined together in a multivariate algorithm with maternal age, we predict that 89 per cent of cases of trisomy 18 could be detected at a 1 per cent false-positive rate. We conclude that specific trisomy 18 risks should be part of developing risk algorithms combining maternal serum biochemistry and nuchal translucency for use in first trimester screening alongside those for trisomy 21. Copyright © 1999 John Wiley & Sons, Ltd.

KEY WORDS: trisomy 18; biochemical screening; ultrasound screening; prenatal screening; nuchal translucency; free  $\beta$ -hCG; PAPP-A; first trimester

## INTRODUCTION

Trisomy 21 pregnancies are associated with increased maternal age, increased fetal nuchal translucency (NT) thickness, increased maternal serum free  $\beta$  human chorionic gonadotrophin ( $\beta$ -hCG) and decreased maternal serum pregnancy associated plasma protein A (PAPP-A). Screening for trisomy 21 between the 10th and 14th week of pregnancy, by a combination of maternal age, fetal NT, and maternal serum  $\beta$ -hCG and PAPP-A, identifies about 90 per cent of affected pregnancies for a screen-positive rate of 5 per cent (Spencer *et al.*, 1999b).

In the second trimester, screening programmes for trisomy 18 have been proposed based on the finding of low maternal serum free  $\beta$ -hCG and alpha-fetoprotein (AFP) (Spencer *et al.*, 1993) or based on the findings of low total hCG, AFP and unconjugated oestriol (Canick *et al.*, 1990) in those cases of trisomy 18 uncomplicated by neural tube defects. These programmes typically can detect 50–60 per cent of cases for a 0.5–1.0 per cent false-positive rate.

Trisomy 18 is the second most common autosomal trisomy and at 10–14 weeks of gestation the relative proportion of trisomy 21 to trisomy 18 is about three to one (Snijders *et al.*, 1995b). Ultrasonographic features of trisomy 18 in the first trimester include increased nuchal translucency, reported in about 75 per cent of cases (Sherod *et al.*, 1997), early onset

intra-uterine growth retardation and exomphalos, found in about 30 per cent of fetuses (Snijders *et al.*, 1995a). Early reports of biochemical markers have suggested that trisomy 18 is associated with a decrease in maternal serum free  $\beta$ -hCG and PAPP-A (Spencer *et al.*, 1992, 1994). Since then several reports on small numbers of affected pregnancies have confirmed that trisomy 18 is associated with a decrease in maternal serum free  $\beta$ -hCG and PAPP-A (Bersinger *et al.*, 1994; Brizot *et al.*, 1994, 1995; Scott *et al.*, 1996; Jauniaux *et al.*, 1996; Zimmerman *et al.*, 1996; Spencer *et al.*, 1997; Biagiotti *et al.*, 1998).

The aim of this study was to examine the effectiveness of screening for trisomy 18 by a combination of maternal age, fetal NT, and maternal serum free  $\beta$ -hCG and PAPP-A at 10–14 weeks of gestation.

## METHODS

From 1994 onwards maternal serum samples were collected at the Harris Birthright Centre from women prior to chorionic villus sampling (CVS) because of advanced maternal age or increased risk for chromosomal abnormality after NT measurement at 10–14 weeks. Serums were stored at  $-20^{\circ}\text{C}$ . At the time of ultrasound examination CRL and NT were measured as previously described (Snijders *et al.*, 1998). In total some 50 cases of trisomy 18 maternal serum samples were available. In 44 cases the remarks on fetal anatomy were also recorded. Maternal age, weight, duration of the pregnancy based on last menstrual period and all ultrasound findings were collected in a

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Table 1—Maternal age, gestational age and CRL for the trisomy 18 and control groups

	Trisomy 18, mean (min–max)	Controls, mean (min–max)
Maternal age (years)	34.9 (18–46)	35.1 (15–47)
Gestational age (days)	85.6 (79–94)	85.1 (72–99)
CRL (mm)	52.6 (36–71.1)	60.4 (38–85)
Median length of storage (days)	815 (32–1395)	546 (102–1811)
Fetal heart rate	161.56	161.87
Number of cases	50	947

database. Outcome of pregnancy and fetal karyotypes were added as soon as available.

During the same period, serum from women attending the clinic for the assessment of risk for chromosomal abnormality or for CVS were also taken, and the same data were entered in the database. From the stored sera 947 controls were selected with matching for maternal and gestational age. The inclusion criteria were normal karyotype at CVS or birth of a baby without abnormalities.

Maternal serum free  $\beta$ -hCG and PAPP-A were measured using the Kryptor analyser—a rapid random access immunoassay analyser using time resolved amplified cryptate emission (TRACE) technology and the CIS automated immunofluorescent assays (CIS UK Ltd, High Wycombe, Bucks, U.K.). The samples were measured over a period of five days. The between day precision of these assays has been previously reported (Spencer *et al.*, 1996b).

### Statistical analysis

Regression analysis was carried out to derive the relationship between free  $\beta$ -hCG and PAPP-A with gestational age. All analyte measurements were converted to MoMs using the derived medians from normal pregnancies at the same gestation. Correction of each MoM for maternal weight was also performed using the reciprocal-linear regression weight correction procedure of Neveux *et al.* (1996). Assessment of the performance of various marker combinations as potential screening procedures was examined using standard statistical modelling techniques (Royston and Thompson, 1992). We used the measured parameters for PAPP-A and free  $\beta$ -hCG and the reported parameters for nuchal translucency from 95 476 normal control pregnancies (Snijders *et al.*, 1998; Nicolaidis *et al.*, 1998) and the measured parameters for the trisomy 18 cases. Using these population parameters, a series of 15 000 random MoM values were selected for each marker from within the distributions of the affected and the unaffected pregnancies. These values were then used to calculate likelihood ratios for the various marker combinations. The likelihood ratios were then used together with the age-related risk for trisomy 18 in the first trimester (Snijders *et al.*, 1994) to calculate the expected detection rate of affected

pregnancies, at a fixed false-positive rate, in a population with the maternal age distribution of pregnancies in England and Wales (OPCS, 1986–1994).

### RESULTS

Table 1 summarizes the data of the trisomy 18 and the control groups. The mean maternal age was 34.9 years for the trisomy 18 group and 35.1 years for the controls; the gestational age based on crown–rump length (CRL) was 85.6 days for the trisomy 18 group and 85.1 for the controls. These differences were not statistically significant. The mean CRL was statistically ( $p < 0.0001$ ) significantly shorter in the trisomy 18 group.

Free  $\beta$ -hCG, PAPP-A and nuchal translucency all fitted a Gaussian distribution after  $\log_{10}$  transformation in both the control group (Spencer *et al.*, 1996b; Nicolaidis *et al.*, 1998) and the trisomy 18 group, with Kolmogorov–Smirnov and Anderson–Darling tests showing linearity at the 0.01 probability levels. Figs 1, 2 and 3 show the normal probability plots for the markers in the trisomy 18 group.

In cases of trisomy 18 the median MoM was significantly lower than the controls for the markers free  $\beta$ -hCG and PAPP-A with medians of 0.281 and 0.177, respectively. For nuchal translucency the median MoM was significantly higher (3.272 MoM) than in the controls. The standard deviations of  $\log_{10}$  free  $\beta$ -hCG and PAPP-A MoM in the trisomy 18 group were 0.322 and 0.306, respectively, whilst that for  $\log_{10}$  nuchal translucency MoM was 0.254. These values are slightly wider than those observed in a large series of trisomy 21 cases (Spencer *et al.*, 1999b). As reported in our previous study (Spencer *et al.*, 1999b), in the control group no significant correlation was found between maternal age and  $\log_{10}$  MoM of the markers NT, free  $\beta$ -hCG and PAPP-A (correlation coefficients  $-0.010$ ,  $0.036$ ,  $0.036$ , respectively) or between NT and free  $\beta$ -hCG and PAPP-A (correlation coefficients  $-0.057$  and  $0.000$ ). There was a small significant correlation between free  $\beta$ -hCG and PAPP-A ( $r = 0.160$ ).

In the trisomy 18 group, no significant correlation was found between  $\log_{10}$  MoM of the markers free  $\beta$ -hCG and PAPP-A ( $0.0735$ ), and NT and PAPP-A

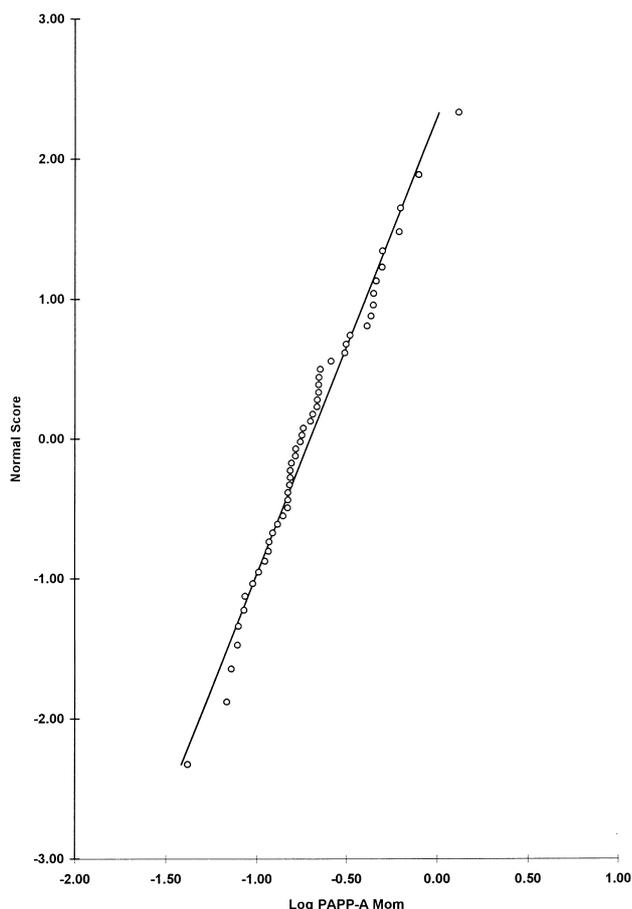


Fig. 1—Probability plot of log PAPP-A MoM in cases of trisomy 18

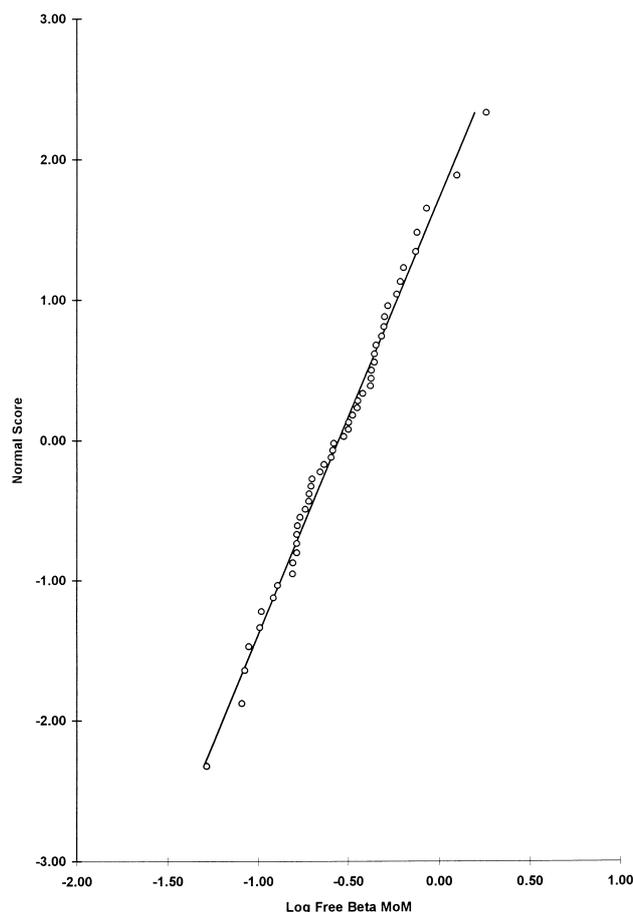


Fig. 2—Probability plot of log free  $\beta$ -hCG MoM in cases of trisomy 18

(0.0927). A significant positive correlation was found between NT and free  $\beta$ -hCG (0.4164).

Table 2 summarizes the distribution parameters for the control population from our previous study (Spencer *et al.*, 1999b) and for the trisomy 18 cases from this study.

Figs 4, 5 and 6 show the individual cases of trisomy 18 plotted against gestational age for each of the markers. From these figures it was evident that there was a discernible decrease in MoM with gestational age for each of the markers. For free  $\beta$ -hCG, if the data were split into two groups based on those with a gestation of 11 weeks or earlier and those with a gestation of 12 weeks or later, the median MoM was higher in the earlier group (0.319) compared with the later group (0.254), although this was not significant ( $p=0.212$ ). For PAPP-A, the earlier median was again higher (0.222) than the later median (0.150) and this was significantly different ( $p=0.0037$ ). Also, for NT, the earlier median was 4.333 compared with 2.444, and this was highly significant ( $p<0.0001$ ). The correlation of NT MoM with gestational age (weeks) was 0.444, and for PAPP-A MoM and gestational age was 0.267, and for free  $\beta$ -hCG and gestational age was 0.104.

In 32 out of 50 cases (64 per cent) of trisomy 18, free  $\beta$ -hCG MoM was under the 5th centile ( $<0.397$  MoM)

and in 38 cases (76 per cent) under 10th centile ( $<0.471$  MoM). PAPP-A was under 5th centile ( $<0.385$  MoM) in 39 cases (78 per cent) and under the 10th centile (0.48 MoM) in 44 (88 per cent) cases of trisomy 18. In 39 out of 50 cases (78 per cent) of trisomy 18 the nuchal translucency was over the 95th centile (1.57 MoM) and over the 90th centile (1.40 MoM) in 45 cases (90 per cent).

When the observed statistical parameters were used in the mathematical model of a population with the maternal age distribution of pregnancies in England and Wales, the estimated detection rates using various marker combinations with maternal age at a fixed false-positive rate of 1 per cent varied from 58 per cent with maternal age and free  $\beta$ -hCG to 89 per cent with maternal age, NT, PAPP-A and free  $\beta$ -hCG (see Table 3).

We have recorded ultrasound findings of fetal anatomy in 44 cases. In 7 cases no fetal anatomy was recorded. Exomphalos was present in 15 (34.1 per cent) cases and in these cases none of the median marker values were different between those with exomphalos and those without (see Table 4).

In 7 cases (15.9 per cent) we observed fetal oedema. In these cases PAPP-A was lower than in cases without oedema and NT was significantly higher in those cases (see Table 5).

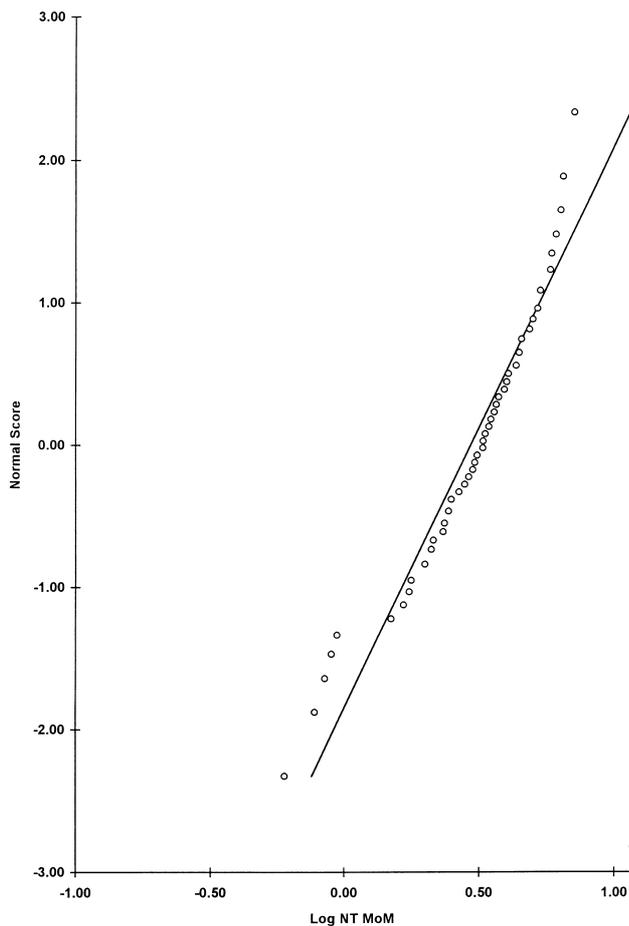


Fig. 3—Probability plot of log NT MoM in cases of trisomy 18

## DISCUSSION

Table 6 summarizes the published data thus far with respect to PAPP-A and free  $\beta$ -hCG in cases of trisomy 18. The median free  $\beta$ -hCG observed in our large series of 0.281 is very close to the median value by meta analysis of the published data (0.292). Similarly, for PAPP-A, our study median of 0.177 is very close to that in the meta analysis (0.204). However, care is needed when combining data from various studies, especially when the median gestational ages of the

studies vary. We have shown in a previous study with trisomy 21 (Spencer *et al.*, 1999b) that there is a gradual change in the median MoM of both free  $\beta$ -hCG and PAPP-A in affected cases across even narrow gestational windows in the first trimester. In this present study of cases of trisomy 18, whilst a trend towards lower MoMs appears to be apparent (but not statistically significant) for free  $\beta$ -hCG, for PAPP-A the trend towards lower values as the pregnancy gets older is a significant change, as is the change for NT. This move to lower values of PAPP-A also continues through the second trimester (Spencer *et al.*, 1999a). This pattern sets trisomy 18 apart from trisomy 21 when values of PAPP-A in the second trimester approach normal.

Studies that have tried to model detection rates in the first trimester using biochemical markers have shown similar levels of performance for trisomy 18. Biagiotti *et al.* (1998) showed detection rates of 45.8 per cent for PAPP-A and 32.5 per cent for free  $\beta$ -hCG at a 0.5 per cent false-positive rate compared with 68 per cent and 44 per cent for our larger series. When combined together, PAPP-A and free  $\beta$ -hCG gave a detection rate of 74 per cent in the Biagiotti *et al.* (1998) study at a 0.5 per cent false-positive rate, which was identical to that in our study. Compared with Biagiotti *et al.* (1998) we found a tighter distribution of PAPP-A and free  $\beta$ -hCG in the control population, and for the trisomy 18 group our distribution for PAPP-A was significantly tighter (0.306 versus 0.384) and was similar for free  $\beta$ -hCG (0.322 versus 0.309). The observed median values in our study were also lower than in the Biagiotti *et al.* (1998) study. Compared with second-trimester screening for trisomy 18 using either AFP and free  $\beta$ -hCG (Spencer *et al.*, 1993), or AFP, total hCG and unconjugated oestriol (Canick *et al.*, 1990), first-trimester biochemical screening would appear to be able to detect a much higher proportion of cases than the 50–60 per cent achievable in the second trimester.

Nuchal translucency has already been shown (Sherod *et al.*, 1997) to be increased in cases of trisomy 18 and in conjunction with maternal age could identify 80 per cent of cases in a trisomy 21 screening programme set to provide a 5 per cent false-positive rate.

Table 2—Distribution parameters for the trisomy 18 and control populations

	Free $\beta$ -hCG	PAPP-A	NT
Log <sub>10</sub> mean controls	0.004	-0.004	0.000
Log <sub>10</sub> SD controls	0.2558	0.2431	0.120
Log <sub>10</sub> mean affected	-0.554	-0.704	0.469
Log <sub>10</sub> SD affected	0.3219	0.306	0.254
10th centile controls (MoM)	0.47	0.48	0.69
50th centile controls (MoM)	1.00	1.00	1.00
90th centile controls (MoM)	2.16	1.98	1.40
10th centile affected (MoM)	0.103	0.083	1.222
50th centile affected (MoM)	0.281	0.177	3.272
90th centile affected (MoM)	0.693	0.498	5.833

NT data for control population from Nicolaides *et al.* (1998).

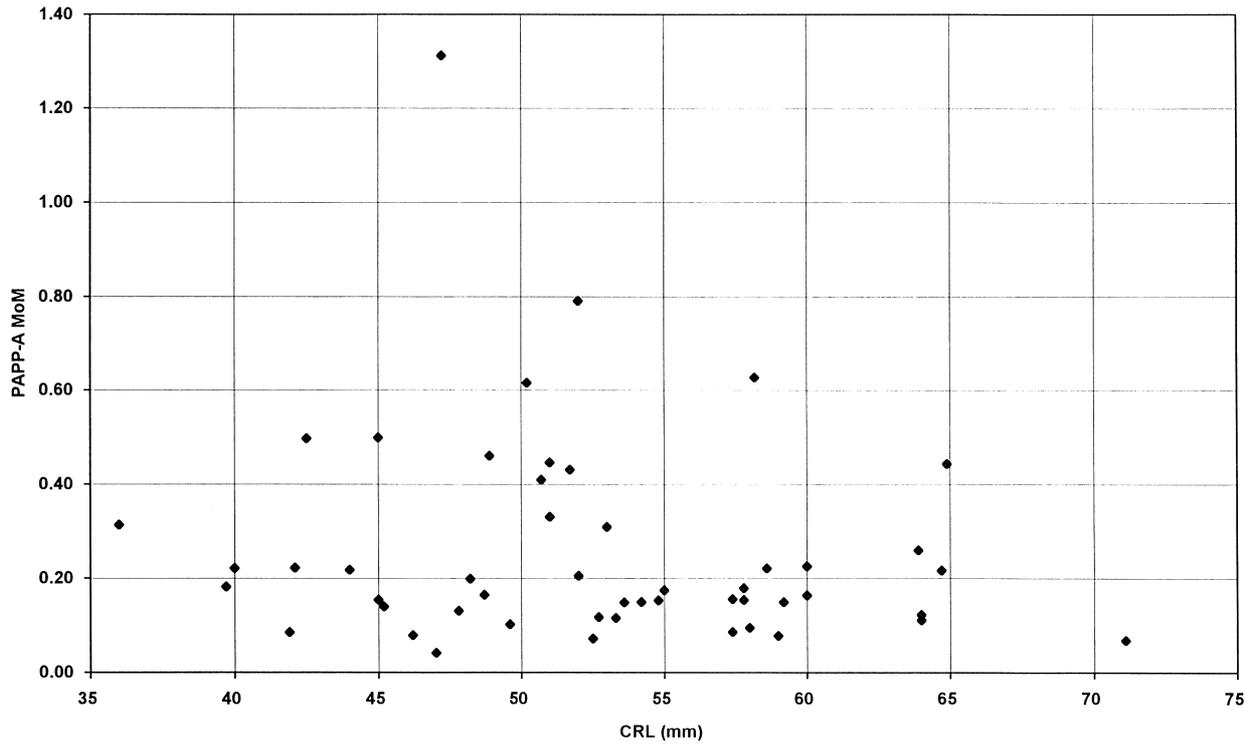


Fig. 4—PAPP-A MoM in 50 cases of trisomy 18 in the first trimester

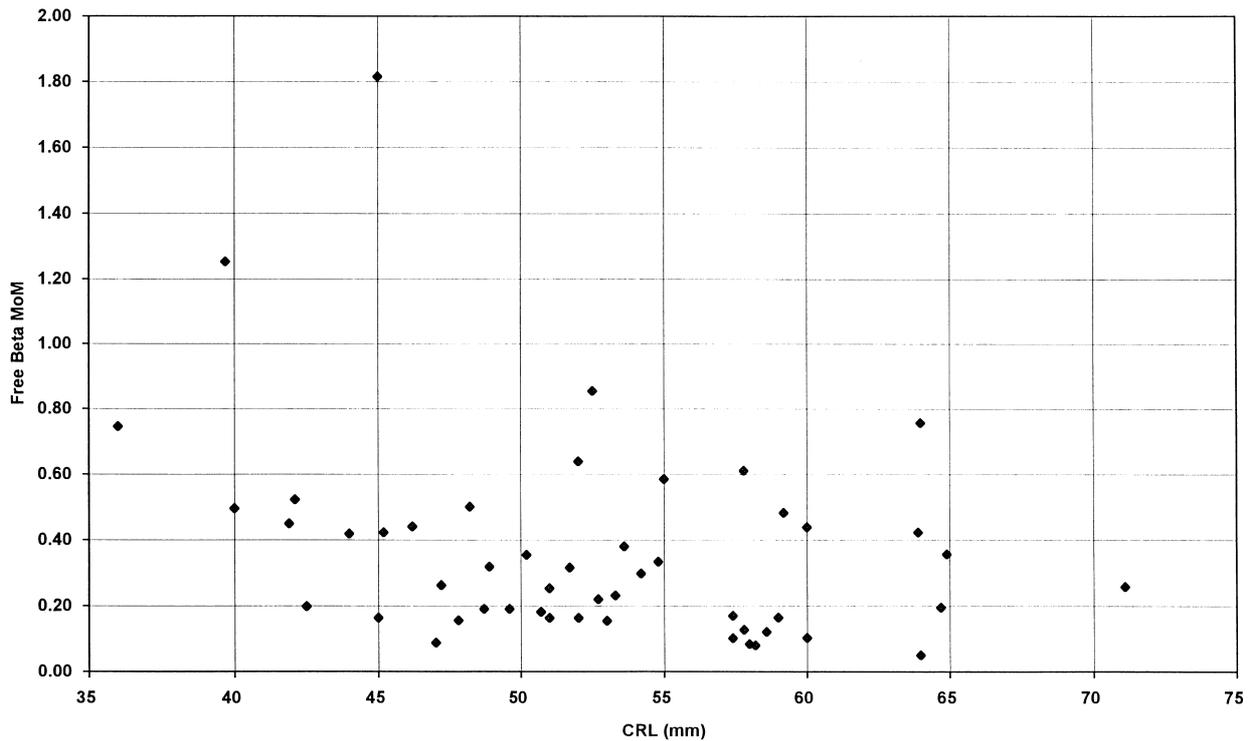


Fig. 5—Free  $\beta$ -hCG MoM in 50 cases of trisomy 18 in the first trimester

It might be argued that our study design in pre-selecting women with increased NT will have biased the distribution parameters for NT, however, in routine screening women of all ages in the obstetric population at Harold Wood over the last three years,

we have observed 20 cases of trisomy 18 in which the NT was measured and the population parameters for these cases are in agreement with this study findings (K. Spencer *et al.* unpublished data). We therefore are confident that the current estimation of detection rate

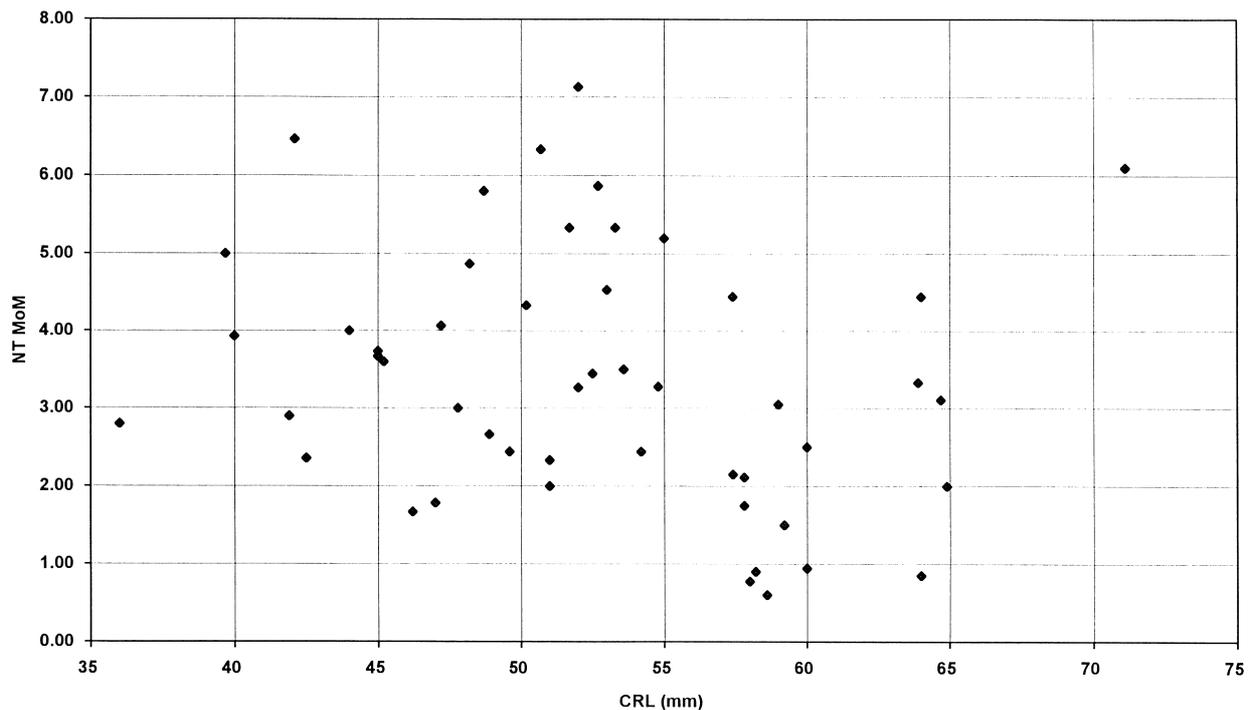


Fig. 6—NT MoM in 50 cases of trisomy 18 in the first trimester

Table 3—Trisomy 18 detection rates at various false-positive rates for different marker combinations modelled against the age distribution of pregnancies in England and Wales

Marker combination	Detection at an FPR of 1%	Detection at an FPR of 0.5%
MA+free $\beta$ -hCG	58%	55%
MA+PAPP-A	70%	68%
MA+NT	70%	67%
MA+NT+free $\beta$ -hCG	73%	68%
MA+NT+PAPP-A	78%	75%
MA+free $\beta$ -hCG+PAPP-A	77%	74%
MA+NT+free $\beta$ -hCG+PAPP-A	89%	86%

Table 4—Median values of free  $\beta$ -hCG MoM, PAPP-A MoM and NT MoM in cases with and without exomphalos

Trisomy 18 exomphalos	<i>N</i>	Free $\beta$ -hCG median MoM	PAPP-A median MoM	NT median MoM
Yes	15	0.299	0.1657	3.12
No	29	0.261	0.189	3.27
		$p=0.8662$	$p=0.2286$	$p=0.6216$

will apply to a general unscreened population. The fact that the biochemical pattern allows a more specific separation of trisomy 21 and trisomy 18 makes it logical therefore to combine the ultrasound information and the biochemical information to provide specific risks for trisomy 18. When this is done, detection rates very similar to those achieved for trisomy 21 (Spencer *et al.*, 1999b) are possible at a much lower

false-positive rate. These detection rates are far superior to those obtained in the second trimester and are a further argument for moving screening into this earlier time window. However, the actual reduction in birth prevalence of trisomy 18 is likely to be less than the first-trimester detection rate because of the high fetal loss of trisomy 18 cases occurring between the first trimester and term.

Table 5—Median values of free  $\beta$ -hCG MoM, PAPP-A MoM and NT MoM in cases with and without oedema

Trisomy 18 oedema	<i>N</i>	Free $\beta$ -hCG median MoM	PAPP-A median MoM	NT median MoM
Yes	7	0.2595	0.1232	4.444
No	37	0.2992	0.1823	3.000
		<i>p</i> =0.8348	<i>p</i> =0.065	<i>p</i> =0.0168

Table 6—Summary of published data on maternal serum free  $\beta$ -hCG and PAPP-A in cases of trisomy 18

Study	Gestation (weeks)	Number of cases	Free $\beta$ -hCG median MoM	PAPP-A median MoM
Spencer <i>et al.</i> (1992)	7–13	5	0.17	
Spencer <i>et al.</i> (1994)	6–14	5		0.49
Bersinger <i>et al.</i> (1994)	10–13	9		0.07
Brizot <i>et al.</i> (1994)	10–13	19		0.17
Brizot <i>et al.</i> (1995)	10–13	19	0.30	
Scott <i>et al.</i> (1996)	10–13	4	0.30	
Jauniaux <i>et al.</i> (1996)	10–11	5	0.19	
Zimmermann <i>et al.</i> (1996)	10–13	5	0.33	0.08
Brambati <i>et al.</i> (1997)	8–13	8	0.12	0.32
Biagiotti <i>et al.</i> (1998)	8–13	23	0.34	0.25
Spencer <i>et al.</i> (1997)	11–14	7	0.24	
Median ( <i>n</i> )			0.287 (76)	0.211 (69)

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