

A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free β -human chorionic gonadotropin and pregnancy-associated plasma protein-A

K. Spencer, V. Souter*, N. Tul*, R. Snijders* and K. H. Nicolaidis*

Endocrine Unit, Clinical Biochemistry Department, Harold Wood Hospital, Essex; *Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, London, UK

Key words: TRISOMY 21, BIOCHEMICAL SCREENING, ULTRASOUND SCREENING, PRENATAL SCREENING, NUCHAL TRANSLUCENCY, FREE β -HCG, PAPP-A, FIRST TRIMESTER

ABSTRACT

Objective To examine the potential impact of combining maternal age with fetal nuchal translucency thickness and maternal serum free β -human chorionic gonadotropin (β -hCG) and pregnancy-associated plasma protein-A (PAPP-A) in screening for trisomy 21 at 10–14 weeks of gestation.

Methods Maternal serum free β -hCG and PAPP-A were measured by Kryptor, a random access immunoassay analyzer using time-resolved amplified cryptate emission, in 210 singleton pregnancies with trisomy 21 and 946 chromosomally normal controls, matched for maternal age, gestation and sample storage time. In all cases the fetal crown–rump length and nuchal translucency thickness had been measured by ultrasonography at 10–14 weeks of gestation and maternal blood had been obtained at the time of the scan. The distributions (in multiples of the median; MoM) of free β -hCG and PAPP-A (corrected for maternal weight) and fetal nuchal translucency (NT) were determined in the trisomy 21 group and the controls. Likelihood ratios for the various marker combinations were calculated and these were used together with the age-related risk for trisomy 21 in the first trimester 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.

Results In a population with the maternal age distribution of pregnancies in England and Wales, it was estimated that, using the combination of maternal age, fetal nuchal translucency thickness and maternal serum free β -hCG and PAPP-A, the detection of trisomy 21 pregnancies would be 89% at a fixed false-positive rate of 5%. Alternatively, at a

fixed detection rate of 70%, the false-positive rate would be 1%. The inclusion of biochemical parameters added an additional 16% to the detection rate obtained using NT and maternal age alone.

Conclusions Rapid diagnostic technology like Kryptor, which can provide automated reproducible biochemical measurements within 30 min of obtaining a blood sample, will allow the development of interdisciplinary one-stop clinics for early fetal assessment. Such clinics will be able to deliver improved screening sensitivity, rapidly and more efficiently, leading to reduced patient anxiety and stress.

INTRODUCTION

Prenatal screening for trisomy 21 based on the analysis of biochemical markers in maternal serum during the second trimester of pregnancy has become an established part of obstetric practice in many countries^{1,2}. A combination of maternal age with serum α -fetoprotein and free β -human chorionic gonadotropin (β -hCG) or maternal age with serum α -fetoprotein, total hCG and unconjugated estriol can identify approximately 65% of affected pregnancies for a 5% false-positive rate^{3–6}.

Recent interest in prenatal screening for trisomy 21 has focused on the first trimester. Of the biochemical markers that have been investigated, only free β -hCG^{7,8} and pregnancy-associated plasma protein-A (PAPP-A) have been shown to be of any value^{9,10}. Several retrospective studies have estimated that the detection rate for pregnancies with Down's syndrome, by a combination of maternal age with

serum free β -hCG and PAPP-A, would be around 60–65% for a 5% false-positive rate¹⁰. In terms of sonographic markers, in the early 1990s several reports of small series in high-risk pregnancies demonstrated a possible association between an increase in fetal nuchal translucency (NT) thickness at 10–14 weeks of gestation and the presence of a chromosomal defect¹¹. A recent multicenter study involving 100 000 pregnancies has demonstrated that, in 72% of the trisomy 21 pregnancies, fetal NT thickness was above the 95th centile of the normal range, and screening by a combination of maternal age with fetal NT identified 77% of affected pregnancies, for a false-positive rate of 5%¹².

In this retrospective study of 210 cases of trisomy 21 and 946 controls, we examine the potential impact of combining fetal NT with maternal free β -hCG and PAPP-A in screening at 10–14 weeks. With the availability of new immunodiagnostic technology based on time-resolved cryptate emission¹³, it has now become possible to obtain reproducible measurements of free β -hCG and PAPP-A within 30 min of taking a blood sample. It is therefore possible to combine the information from the ultrasound scan and maternal blood in the same clinical visit, so that parents can receive counselling concerning their combined estimated risk for an affected pregnancy.

METHODS

The study population was derived from two groups of women. The first comprised women with singleton pregnancies who were referred to the Harris Birthright Research Centre for Fetal Medicine for fetal karyotyping, because screening by a combination of maternal age and fetal NT at 10–14 weeks in their hospital identified these patients as being at high risk for Down's syndrome¹². The second group comprised self-referred women for assessment of risk. Blood samples were collected from women at the time of the scan and the serum was aliquoted and stored at -20°C prior to blinded retrospective analysis. Gestational age was determined by measurement of fetal crown–rump length (CRL). Pregnancy outcome was ascertained in all women.

Serum samples from 210 pregnancies affected by trisomy 21 were available for biochemical analysis. A control group of 946 cases was selected from pregnancies resulting in the birth of an unaffected baby; these samples were collected within the same time scale as the affected group, and they were matched for the gestational age and maternal age distribution of the trisomy 21 group.

Maternal serum free β -hCG and PAPP-A were measured over a period of 5 days on the Kryptor analyzer – a random access immunoassay analyzer using time-resolved amplified cryptate emission (TRACE) technology – and the CIS automated immunofluorescent assays (CIS UK Ltd., High Wycombe, Bucks, UK). The between-day precision of these assays (coefficient of variation; CV) was 2.8% for PAPP-A at 1.68 mIU/ml and 3.2% for free β -hCG at 21.2 ng/ml.

Statistical analysis

Regression analysis was carried out to derive the relation between free β -hCG and PAPP-A in MoM with gestational age. Correction of each MoM for maternal weight was also performed using the reciprocal-linear regression weight correction procedure of Neveux and co-workers¹⁴. Assessment of the performance of various marker combinations as potential screening procedures was examined using standard statistical modelling techniques¹⁵. We used the measured parameters (corrected for maternal weight) for PAPP-A and free β -hCG and the reported parameters for nuchal translucency from 95 476 normal and 326 trisomy 21 pregnancies^{12,16}. 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 unaffected pregnancies. These values were used to calculate likelihood ratios¹⁷ for the various marker combinations. The likelihood ratios were then used together with the age-related risk for trisomy 21 in the first trimester¹⁸ 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 Wales¹⁹.

RESULTS

The characteristics of the trisomy 21 and control pregnancies are shown in Table 1 and the observed and regressed median values for free β -hCG and PAPP-A are shown in Table 2. Free β -hCG MoM and PAPP-A MoM both in the trisomy 21 and the control groups fitted a Gaussian distribution after \log_{10} transformation, with Kolmogorov–Smirnov and Anderson Darling tests showing linearity at the 0.01 probability level. Similarly, NT MoM in both trisomy 21 and unaffected cases were previously shown to fit a Gaussian distribution after \log_{10} transformation¹⁶.

In order to be combined directly with maternal age in a multivariate risk algorithm, each marker must be independent of maternal age. There was no significant correlation between maternal age in either the control or the trisomy 21 pregnancies for PAPP-A ($r = 0.036$ and $r = 0.103$, respectively), for free β -hCG ($r = 0.036$ and $r = -0.092$, respectively) or for NT thickness ($r = -0.010$ and $r = -0.125$, respectively). Table 3 summarizes the statistical parameters associated with the distributions of the biochemical markers, along with those established for NT¹⁶. Table 4 shows the distribution of analyte levels in the control group with various maternal weight bands. Each

Table 1 Median and range for maternal age, gestational age, fetal crown–rump length and sample storage time in the group with trisomy 21 and the controls

	Trisomy 21 (<i>n</i> = 210)	Controls (<i>n</i> = 947)
Maternal age (years)	38 (19–46)	36 (15–47)
Gestational age (days)	87 (73–97)	86 (76–85)
Crown–rump length (mm)	61 (42–85)	60 (38–85)
Sample storage time (days)	889 (5–1659)	546 (102–1811)

Table 2 Observed and regressed median levels of pregnancy-associated plasma protein-A (PAPP-A) and free β -human chorionic gonadotropin (β -hCG) at 10–14 weeks of gestation

Gestation (week)	Mean gestation (week)	n	Median PAPP-A		Median free β -hCG	
			Observed	Regressed*	Observed	Regressed†
10	10.57	37	1.482	1.528	38.05	38.30
11	11.57	304	2.199	2.239	36.88	35.50
12	12.43	427	3.130	3.106	29.36	31.42
13	13.43	187	4.708	4.551	28.05	27.19

*, Calculated from the formula: median PAPP-A = $0.0269 \times \exp(0.3821 \times \text{gestation})$; †, calculated from the formula: median free β -hCG = $0.4675 \times \text{gestation}^3 - 17.213 \times \text{gestation}^2 + 206.31 \times \text{gestation} - 771.36$. Gestation is measured in decimal weeks

Table 3 Statistical parameters for the various marker distributions in trisomy 21 and control pregnancies. Values for nuchal translucency (NT) from reference 16

	Free β -hCG	PAPP-A	NT
Log ₁₀ mean controls	0.004	-0.004	0.000
Log ₁₀ SD controls	0.2558	0.2431	0.120
Log ₁₀ mean affected	0.322	-0.306	0.305
Log ₁₀ SD affected	0.2783	0.2803	0.235
10th centile controls	0.47	0.48	0.69
50th centile controls	1.00	1.00	1.00
90th centile controls	2.16	1.98	1.40
10th centile affected	0.93	0.21	1.10
50th centile affected	2.15	0.51	2.27
90th centile affected	4.61	1.05	4.32

β -hCG, β -human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A; SD, standard deviation

Table 4 Variation of biochemical marker levels with maternal weight

Weight (kg)	n	Median weight (kg)	Median free β -hCG (MoM)	Median PAPP-A (MoM)
35–44	5	40.54	1.88	1.75
45–54	112	51.68	1.21	1.21
55–64	375	57.71	1.01	1.09
65–74	227	68.72	0.96	0.90
75–84	75	78.39	0.86	0.85
85–94	29	88.06	0.74	0.63
> 95	18	106.22	0.77	0.78

β -hCG, β -human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A; MoM, multiple of the median

marker showed a small but significant negative correlation with maternal weight (PAPP-A, $r = -0.278$; free β -hCG, $r = -0.146$). Using the procedure of Neveux and colleagues¹⁴ to derive a correction factor for varying maternal weight and applying this to the study populations resulted in a slight tightening of the distributions; the SD for free β -hCG in the controls was 0.249 and in the trisomy 21 group was 0.271 and the respective values for PAPP-A were 0.237 and 0.275. When individual marker levels (as MoM) were compared against each other, there was no significant correlation between NT (as MoM) either in the control or in the trisomy 21 pregnancies for PAPP-A ($r = 0.000$ and $r = -0.089$, respectively) and free β -hCG ($r = -0.057$ and $r = 0.000$, respectively). There was a small but significant correlation between free β -hCG in MoM and PAPP-A in MoM (controls, $r = 0.160$; trisomy 21, $r = 0.216$).

Table 5 First-trimester detection rates with various marker combinations at a 5% fixed false-positive rate

Marker combination	Detection rates (%)			
	11 weeks	12 weeks	13 weeks	All weeks
Free β -hCG	28	34	40	33
PAPP-A	46	38	24	38
NT thickness	74	67	44	64
Maternal age and free β -hCG				46
Maternal age and PAPP-A				48
Maternal age and NT thickness				73
Maternal age, free β -hCG and PAPP-A				67
Maternal age, NT thickness and free β -hCG				81
Maternal age, NT thickness and PAPP-A				82
Maternal age, NT thickness, free β -hCG and PAPP-A				89

β -hCG, β -human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A; NT, nuchal translucency

Table 6 First-trimester false-positive rates (%) with various marker combinations at various detection rates

Detection rate (%)	False-positive rate (%)			
	Free β -hCG and PAPP-A	NT and free β -hCG	NT and PAPP-A	NT, free β -hCG and PAPP-A
90	23.0	12.0	12.0	6.0
85	16.0	7.0	7.0	3.5
80	11.0	5.0	4.0	2.1
75	8.0	3.0	2.7	1.5
70	6.0	2.2	1.5	1.0

β -hCG, β -human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A; NT, nuchal translucency

In the 210 trisomy 21 pregnancies, free β -hCG was above the 95th centile of the controls in 70 (33%) cases and PAPP-A was below the 5th centile in 79 (38%) cases. 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 5% varied from 46% with maternal age and free β -hCG to 89% with maternal age and all three markers (Table 5). Alternatively, at a fixed detection rate of 70%, the false-positive rate varied from 6% with maternal age, free

β -hCG and PAPP-A to 1% with maternal age, fetal NT, free β -hCG and PAPP-A (Table 6).

DISCUSSION

This study has demonstrated the potential value of screening for trisomy 21 at 10–14 weeks of gestation by a combination of maternal age, fetal NT and maternal serum free β -hCG and PAPP-A; for a 5% invasive testing rate, about 90% of trisomy 21 pregnancies can be identified. Alternatively, at a fixed detection rate of 70%, the false-positive rate would be 1%.

Maternal serum free β -hCG in trisomy 21 pregnancies was increased. The median value was 2.15 MoM (95% CI, 1.94–2.33), which is compatible with the results of previous smaller series that also measured free β -hCG in the first trimester (Table 7). However, the value is lower than in affected cases in the second trimester; in a large series of 480 trisomy 21 cases, the median MoM was 2.64⁵. The increase with gestation in the difference between trisomy 21 and normal pregnancies has also been shown in studies of paired samples from trisomy 21 pregnancies collected in the first and second trimesters³³. In our study, there was an increasing trend in values with gestation (Figure 1), even within the narrow range of 11–13 weeks, although this was not statistically significant. This may offer an explanation for the difference between our overall median of 2.15 and the 1.75 reported in a previous study¹⁰, in which more than 60% of the cases were prior to 11 weeks of gestation. The standard deviation in both the control and the trisomy 21

populations was tighter than that of previous studies^{10,33,36} and this may reflect better assay precision and also a tighter distribution of gestational ages in our study population. At a 5% false-positive rate, the detection rate using free β -hCG alone was 33% and, in combination with maternal age, detection increased to 46%; these findings are similar to those of previous reports^{27–30,32,33,35,37}.

Maternal serum PAPP-A in trisomy 21 pregnancies was decreased. The median value was 0.51 MoM (95% CI, 0.44–0.56), which is compatible with the results of

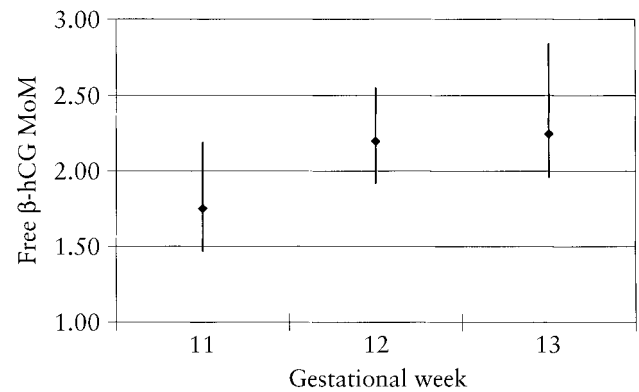


Figure 1 Variation of median multiples of the median (MoM) for free β -human chorionic gonadotropin (β -hCG) in cases of trisomy 21 across the first-trimester window of 11–13 weeks' gestation. Median MoM (symbols) and 95% confidence interval (bars) are shown. The 11-week median was 1.75 (95% CI, 1.47–2.19; $n = 65$); the 12-week median was 2.20 (95% CI, 1.92–2.55; $n = 97$); the 13-week median was 2.25 (95% CI, 1.96–2.84; $n = 45$)

Table 7 Median multiples of the median (MoM) in published studies of free β -human chorionic gonadotropin (β -hCG) in trisomy 21 cases

	Number of cases	Median MoM	Weeks' gestation (median and range)
<i>Studies with median gestation < 11 weeks</i>			
Ozturk, 1990 ²⁰	9	1.62	10 (9–12)
Spencer, 1992 ⁷	13	1.85	10 (7–13)
Macintosh, 1994 ²⁴	21	2.10	10 (8–14)
Brambati, 1994 ²⁵	13	1.13	10 (8–12)
Biagiotti, 1995 ²⁸	41	2.00	10 (8–12)
Wald, 1996 ¹⁰	77	1.79	10 (8–14)
Total	174	1.82	
<i>Studies with median gestation \geq 11 weeks</i>			
Macri, 1993 ²¹	25	2.34	11 (9–13)
Brizot, 1995 ²⁷	41	2.00	11 (10–13)
Noble, 1995 ²⁹	61	2.13	11 (10–13)
Krantz, 1996 ³⁰	22	2.09	11 (10–13)
Scott, 1996 ³¹	8	2.00	11 (10–13)
Spencer, 1997 ³²	22	1.72	11 (10–13)
Berry, 1997 ³³	54	1.99	11 (7–13)
Haddow, 1998 ³⁶	48	2.08	11 (9–13)
Total	281	2.06	
Iles, 1993 ²²	25	1.52	(8–14)
Pescia, 1993 ²³	5	2.03	(8–12)
Kellner, 1994 ²⁶	5	2.20	(8–14)
Forest, 1997 ³⁴	18	1.92	(9–13)
Wheeler, 1998 ³⁵	17	2.06	(9–12)
All studies	525	1.95	

Table 8 Median multiples of the median (MoM) in published studies of pregnancy-associated plasma protein-A (PAPP-A) in trisomy 21 cases

	Number of cases	Median MoM	Weeks' gestation (median and range)
<i>Studies with median gestation < 11 weeks</i>			
Brambati, 1993 ³⁸	14	0.27	8 (6–11)
Hurley, 1993 ³⁹	7	0.33	10 (8–12)
Macintosh, 1994 ²⁴	14	0.34	10 (8–14)
Brambati, 1994 ²⁵	13	0.31	10 (8–12)
Bersinger, 1994 ⁴⁰	20	0.47	11 (10–11)
Wald, 1996 ¹⁰	77	0.43	10 (8–14)
Total	145	0.396	
<i>Studies with median gestation \geq 11 weeks</i>			
Muller, 1993 ⁴¹	17	0.42	14 (10–14)
Spencer, 1994 ³⁷	21	0.62	12 (7–14)
Brizot, 1994 ⁴²	45	0.50	11 (10–13)
Bersinger, 1994 ⁴⁰	9	0.85	13 (12–13)
Casals, 1996 ⁴³	19	0.42	12 (10–13)
Krantz, 1996 ³⁰	22	0.41	11 (10–13)
Berry, 1997 ³³	52	0.50	11 (7–13)
Haddow, 1998 ³⁶	48	0.41	11 (9–13)
Total	233	0.485	
Wald, 1992 ⁴⁴	19	0.23	(9–12)
Iles, 1993 ²²	25	0.38	(8–14)
Forrest, 1997 ³⁴	18	0.46	(9–13)
Wheeler, 1998 ³⁵	17	0.43	(9–12)
All studies	457	0.437	

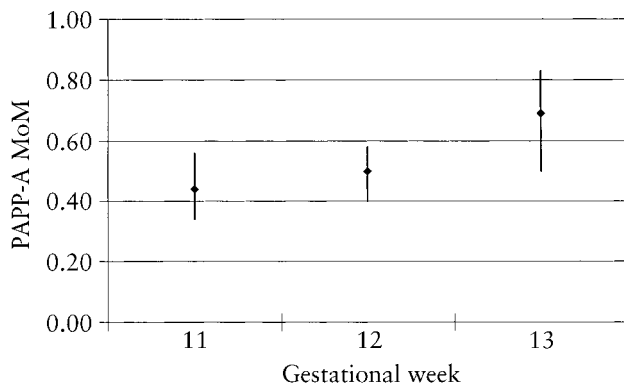


Figure 2 Variation of median multiples of the median (MoM) for pregnancy-associated plasma protein-A (PAPP-A) in cases of trisomy 21 across the first-trimester window of 11–13 weeks' gestation. Median MoM (symbols) and 95% confidence interval (bars) are shown. The 11-week median was 0.44 (95% CI, 0.34–0.56; $n = 65$); the 12-week median was 0.50 (95% CI, 0.40–0.58; $n = 97$); the 13-week median was 0.69 (95% CI, 0.50–0.83; $n = 45$)

previous smaller series that also measured PAPP-A in the first trimester (Table 8). The difference in PAPP-A between trisomy 21 and normal pregnancies decreases with advancing gestation and this may account for the variation in the reported median MoM between the various studies, because there was a considerable variation in the gestational age range of the populations that were examined. In our study, there was a significant trend in values even across the narrow gestational age range of 11–13 weeks (Figure 2). Consequently, population parameters derived from studies using a wide gestational age range will not be appropriate if screening is to be focused at the optimal time for nuchal translucency measurement (10–14 weeks)⁴⁵. The standard deviation in both the control and the trisomy 21 populations was tighter than that of previous studies and this may reflect the narrower gestational range of our population, compared to previous reports. At a 5% false-positive rate, the detection rate using PAPP-A alone was 38% and, in combination with maternal age, detection increased to 48%; these findings are remarkably similar to those of previous reports, despite differences in median MoM and standard deviation in the various studies^{30,33,36,42}. The observed variability of detection rate with the biochemical markers across the narrow gestational window makes it clear that risk algorithms will need to be designed with different statistical models for possibly each week of gestation across the first trimester, as we have suggested previously⁴⁵.

When consideration is given to combining biochemical markers together, it is necessary to take into account the degree of correlation between the markers. In the major published series^{10,30,33,36}, the correlations varied between -0.223 and 0.065 for the trisomy 21 pregnancies, and between -0.086 and 0.154 in the normal group; in our study, the correlations were 0.216 and 0.160 , respectively. Combining free β -hCG and PAPP-A with maternal age in our study produced a modelled detection rate of 67% at a 5% false-positive rate; the estimated detection in previous

studies was 55–63%^{10,30,33,34,36,46}. When biochemistry is combined with nuchal translucency, a further level of correlation needs to be taken into account. Our findings confirm the previous studies of Brizot and colleagues^{27,42}, indicating insignificant correlation of both PAPP-A and free β -hCG with nuchal translucency. Our estimated sensitivity of 89% for a 5% false-positive rate in screening by a combination of maternal age, maternal serum free β -hCG and PAPP-A and fetal nuchal translucency was similar to the estimate of 87% by Cuckle⁴⁷ and the 87% observed by Orlandi and co-workers⁴⁶, and considerably better than the 80% estimated by Wald and Hackshaw⁴⁸. Combining biochemistry with NT increases the modelled detection rate by a further 16% over and above that observed with NT and age alone.

Dunstan and Nix⁴⁹ have provided a methodology to compare detection rates between the first and second trimesters, taking into account fetal loss rates. Essentially, for the first-trimester test to be considered superior to that in the second trimester, the detection rate should be at least 8.3% higher. Clearly, if this is the case, first-trimester biochemistry alone (detection of 67%) would not be better than second-trimester biochemistry (detection of 65%), but the combination of NT and biochemistry in the first trimester (89%) is considerably more sensitive than biochemical screening in the second trimester.

The new technology for biochemical analysis, which provides automated, precise and reproducible measurements within 30 min of obtaining a blood sample, makes it possible to combine biochemical and ultrasonographic testing as well as counselling in one-stop clinics for early fetal assessment. Such interdisciplinary clinics would have the advantage of providing a more efficient service, bringing the benefit of improved sensitivity over previous methods of screening as well as the ability to deliver this quickly, so that patient anxiety and stress may be reduced.

ACKNOWLEDGEMENTS

We acknowledge the support of CIS in providing the reagents for this study and CIS (UK) for providing funding for the OSCAR (One Stop Counseling, Assessment and Risk) clinic at Harold Wood Hospital. The work at the Harris Birthright Centre is supported by a grant from the Fetal Medicine Foundation (Charity no. 1037116).

REFERENCES

1. Cuckle HS, Ellis AR, Seth J. Provision of screening for Down's syndrome. *Br Med J* 1995;311:512
2. Palomaki GE, Knight GJ, McCarthy JE, Haddow JE, Donhove JM. Maternal serum screening for Down syndrome in the United States: a 1995 survey. *Am J Obstet Gynecol* 1997;176:1046–51
3. Spencer K, Coombes EJ, Mallard AS, Milford Ward A. Free beta human chorionic gonadotropin in Down's syndrome screening: a multicentre study of its role compared with other biochemical markers. *Ann Clin Biochem* 1992;29:506–18
4. Spencer K. Dépistage de la trisomie 21 à l'aide de la beta hCG libre: notre expérience sur trois ans. *Med Foetale Echographie Gynecol* 1994;20:67–9

5. Macri JN, Spencer K, Garver K, Buchanan PD, Say B, Carpenter NJ, Muller F, Boue A. Maternal serum free beta hCG screening: results of studies including 480 cases of Down syndrome. *Prenat Diagn* 1994;14:97-108
6. Macri JN, Spencer K. Towards the optimal protocol for Down's syndrome screening. *Am J Obstet Gynecol* 1996;174:1668-9
7. Spencer K, Macri JN, Aitken DA, Connor JM. Free beta hCG as a first trimester marker for fetal trisomy. *Lancet* 1992;339:1480
8. Spencer K. hCG and its subunits in first trimester Down syndrome screening. In Grudzinskas JG, Ward RHT, eds. *Screening for Down Syndrome in the First Trimester*. London: RCOG Press, 1997:117-31
9. Brambati B, Lanzani A, Tului L. Ultrasound and biochemical assessment of first trimester pregnancy. In Chapman M, Grudzinskas JG, Chard T, eds. *The Embryo: Normal and Abnormal Development and Growth*. New York: Springer-Verlag, 1991:181-94
10. Wald NJ, George L, Smith D, Densum 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;104:407-12
11. Snijders RJM, Nicolaides KH. *Ultrasound Markers for Fetal Chromosomal Defects*. Carnforth, UK: Parthenon Publishing, 1996
12. Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH, for the Fetal Medicine Foundation First Trimester Screening Group. 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;351:343-6
13. Mathis G. Probing molecular interactions with homogenous techniques based on rare earth cryptates and fluorescence energy transfer. *Clin Chem* 1995;41:1391-7
14. Neveux LM, Palomaki GE, Larivee DA, Knight GJ, Haddow JE. Refinements in managing maternal weight adjustment for interpreting prenatal screening results. *Prenat Diagn* 1996;16:1115-19
15. Royston P, Thompson SG. Model based screening for risk with application to Down's syndrome. *Stats Med* 1992;11:257-68
16. Nicolaides KH, Snijders RJM, Cuckle HS. Correct estimation of parameters for ultrasound nuchal translucency screening. *Prenat Diagn* 1998;18:519-21
17. Reynolds TM, Penney MD. The mathematical basis of multivariate risk screening; with special reference to screening for Down's syndrome associated pregnancy. *Ann Clin Biochem* 1990;27:452-8
18. Snijders RJM, Holzgreve W, Cuckle H, Nicolaides KH. Maternal age-specific risk for trisomies at 9-14 weeks' gestation. *Prenat Diagn* 1994;14:543-52
19. Office of Population Censuses and Surveys. *Birth Statistics, Series FM1, Nos 13-21*. London: HMSO, 1986-1994
20. Ozturk M, Milunsky A, Brambati B, Sachs ES, Miller SL, Wands JR. Abnormal maternal levels of hCG subunits in trisomy 18. *Am J Med Genet* 1990;36:480-3
21. Macri JN, Spencer K, Aitken DA, Garver K, Buchanan PD, Muller F, Boue A. First trimester free beta-hCG screening for Down syndrome. *Prenat Diagn* 1993;13:557-62
22. Isles RK, Sharma K, Wathen NC, Campbell J, Ward H, Muller F, Grudzinskas JG, Chard T. hCG, free subunit and PAPP-A composition in normal and Down's syndrome pregnancies. In *Fourth Conference: Endocrinology and Metabolism in Human Reproduction*. London: RCOG, 1993:32
23. Pescia G, Marguerat PH, Weihs D, The HN, Maillard C, Loertscher A, Senn A. First trimester free beta-hCG and SP1 as markers for fetal chromosomal disorders: a prospective study of 250 women undergoing CVS. In *Fourth Conference: Endocrinology and Metabolism in Human Reproduction*. London: RCOG, 1993:45
24. Macintosh MCM, Iles R, Teisner B, Sharma K, Chard T, Grudzinskas JG, Ward RHT, Muller F. Maternal serum human chorionic gonadotrophin and pregnancy associated plasma protein A, markers for fetal Down syndrome at 8-14 weeks. *Prenat Diagn* 1994;14:203-8
25. Brambati B, Tului L, Bonacchi I, Shrimanker K, Suzuki Y, Grudzinskas JG. Serum PAPP-A and free beta hCG are first-trimester screening markers for Down syndrome. *Prenat Diagn* 1994;14:1043-7
26. Kellner LH, Weiss RR, Weiner Z, Neur M, Martin G. Early first trimester maternal serum AFP, UE3, hCG and free beta-hCG measurements in unaffected and affected pregnancies with fetal Down syndrome. *Am J Hum Genet* 1994;55:A281
27. Brizot ML, Snijders RJM, Butler J, Bersinger NA, Nicolaides KH. Maternal serum hCG and fetal nuchal translucency thickness for the prediction of fetal trisomies in the first trimester of pregnancy. *Br J Obstet Gynaecol* 1995;102:127-32
28. Biagiotti R, Cariati E, Brizzi L, D'Agata A. Maternal serum screening for Down syndrome in the first trimester of pregnancy. *Br J Obstet Gynaecol* 1995;102:660-2
29. Noble PL, Abrahama HD, Snijders RJM, Sherwood R, Nicolaides KH. Screening for fetal trisomy 21 in the first trimester of pregnancy: maternal serum free β -hCG and fetal nuchal translucency thickness. *Ultrasound Obstet Gynecol* 1995;6:390-5
30. Krantz DA, Larsen JW, Buchanan PD, Macri JN. First trimester Down syndrome screening: free β human chorionic gonadotropin and pregnancy associated plasma protein A. *Am J Obstet Gynecol* 1996;174:612-16
31. Scott F, Wheeler D, Sinosich M, Boogert A, Anderson J, Edelman D. First trimester aneuploidy screening using nuchal translucency, free beta human chorionic gonadotrophin and maternal age. *Aust NZ Obstet Gynaecol* 1996;36:381-4
32. Spencer K, Noble P, Snijders RJM, Nicolaides KH. First trimester urine free beta hCG, beta core and total oestriol in pregnancies affected by Down's syndrome: implications for first trimester screening with nuchal translucency and serum free beta hCG. *Prenat Diagn* 1997;17:525-38
33. 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;104:811-17
34. Forest J-C, Masse J, Moutquin J-M. Screening for Down syndrome during the first trimester: a prospective study using free β -human chorionic gonadotropin and pregnancy associated plasma protein-A. *Clin Biochem* 1997;30:333-8
35. Wheeler DM, Sinosich MJ. Prenatal screening in the first trimester of pregnancy. *Prenat Diagn* 1998;18:537-43
36. Haddow JE, Palomaki GE, Knight GJ, Williams J, Miller WA, Johnson A. Screening of maternal serum for fetal Down's syndrome in the first trimester. *N Engl J Med* 1998;338:955-61
37. Spencer K, Aitken DA, Crossley JA, McGaw G, Berry E, Anderson R, Connor JM, Macri JN. First trimester biochemical screening for trisomy 21: the role of free beta hCG, alpha fetoprotein and pregnancy associated plasma protein A. *Ann Clin Biochem* 1994;31:447-54
38. Brambati B, Macintosh MCM, Teisner B, Maguiness S, Shrimanker K, Lanzani A, Bonacchi I, Tului L, Chard T, Grudzinskas JG. Low maternal serum levels of pregnancy associated plasma protein A (PAPP-A) in the first trimester in association with abnormal fetal karyotype. *Br J Obstet Gynaecol* 1993;100:324-6
39. Hurley PA, Ward RHT, Teisner B, Isles RK, Lucas M, Grudzinskas JG. Serum PAPP-A measurements in first trimester screening for Down's syndrome. *Prenat Diagn* 1993;13:903-8

40. Bersinger NA, Brizot ML, Johnson A, Snijders RJM, Abbot J, Schnieder H, Nicolaides KH. First trimester maternal serum pregnancy associated plasma protein A and pregnancy specific beta-1 glycoprotein in fetal trisomies. *Br J Obstet Gynaecol* 1994;101:970-4
41. Muller F, Cuckle HS, Teisner B, Grudzinskas JG. Serum PAPP-A levels are depressed in women with fetal Down's syndrome in early pregnancy. *Prenat Diagn* 1993;13:633-6
42. Brizot ML, Snijders RJM, Bersinger NA, Khun P, Nicolaides KH. Maternal serum pregnancy associated plasma protein A and fetal nuchal translucency thickness for the prediction of fetal trisomies in early pregnancy. *Obstet Gynecol* 1994;84:918-22
43. Casals E, Fortuny A, Grudzinskas JG, Suzuki Y, Teisner B, Comas C, Sanllehy C, Ojuel J, Borrell A, Soler A, Ballesta AM. First trimester biochemical screening for Down syndrome with the use of PAPP-A, AFP and β -hCG. *Prenat Diagn* 1996;16:405-10
44. Wald N, Stone R, Cuckle HS, Grudzinskas JG, Barkai G, Brambati B, Teisner B, Fuhrmann W. First trimester concentrations of pregnancy associated plasma protein A and placental protein 14 in Down's syndrome. *Br Med J* 1992;305:28
45. Reynolds TM, Dunstan F, Nix B, Williams K, Crossley J, Holding S, Krantz D, Wright D, Bray I, Spencer K. Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome. Response to: Wald NJ, Hackshaw AK (1997). *Prenat Diagn* 1998;18:511-15
46. Orlandi F, Damiani G, Hallahan TW, Krantz DA, Macri JN. First trimester screening for aneuploidy: biochemistry and nuchal translucency. *Ultrasound Obstet Gynecol* 1997;10:381-6
47. Cuckle H. Fetal nuchal translucency test for Down's syndrome. *Lancet* 1997;350:1629-30
48. Wald NJ, Hackshaw AK. Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome. *Prenat Diagn* 1997;17:821-9
49. Dunstan FDJ, Nix ABJ. Screening for Down's syndrome: the effect of test date on the detection rate. *Ann Clin Biochem* 1998;35:57-61