Papers

Second trimester screening for Down's syndrome using maternal serum dimeric inhibin A

James E Haddow, Glenn E Palomaki, George J Knight, Deirdre L Foster, Louis M Neveux

Abstract

***Objectives*—To determine the second trimester Down's syndrome screening performance of maternal serum dimeric inhibin A, both alone and in combination with existing serum markers.**

***Setting*—A case-control set of serum samples from patients with Down's syndrome (52) and subjects with matched unaffected pregnancies obtained in a previous cohort study before second trimester amniocentesis and karyotyping. The amniocenteses were performed for reasons other than a positive serum screening test result.**

***Methods*—For each serum from a Down's syndrome pregnancy, five serum samples from pregnancies with a normal karyotype were matched for recruitment centre, gestational age, maternal age, and date of amniocentesis. A specific form of inhibin (dimeric inhibin A) was measured using monoclonal antibodies. Measurements of α fetoprotein, unconjugated oestriol, and human chorionic gonadotrophin and its free β subunit were already available. Screening performance was modelled using distribution variables of the analytes coupled with the 1993 age distribution of pregnant women in the United States.**

***Results*—The median dimeric inhibin A level was 2.10 times higher in Down's syndrome pregnancies. When dimeric inhibin A was combined with maternal age and three other serum markers (α fetoprotein, unconjugated oestriol, and human chorionic gonadotrophin) the Down's syndrome detection rate increased to 75% (from 66%) at a 5% false positive rate. If dimeric inhibin A could be added for less than $31 (ranging from $16 to $39 depending on the detection rate, markers chosen, and method of dating), the cost of detecting each Down's syndrome pregnancy and the number of procedure related fetal losses would both be reduced.**

***Conclusions*—The addition of dimeric inhibin A to prenatal screening programmes for Down's syndrome should be considered, or possibly it could be substituted for an existing serum marker. One barrier to implementation in the United States, however, is the unavailability of kits with Food and Drug Administration approval.**

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Keywords: Down's syndrome; prenatal screening; dimeric inhibin A

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Second trimester maternal serum screening for Down's syndrome is now routinely offered in various areas in the world.[1 2] In the United States most programmes base the risk of Down's syndrome on a combination of maternal age and serum measurements of α fetoprotein, unconjugated oestriol, and human chorionic gonadotrophin.[1] Current screening programmes identify about 60% of Down's syndrome pregnancies by offering amniocentesis to the 5% of screened women at highest risk.[3] In order to improve on this performance substantially, a fourth serum marker would need to be strongly associated with Down's syndrome and also be relatively independent of the existing markers. Dimeric inhibin A has been reported to meet these criteria.[4–11]

Inhibins are circulating dimeric glycoprotein hormones synthesised by the gonads and the placenta. The α subunit can combine with one of two β subunits (βA and βB) to form inhibin A or inhibin B. Inhibin precursors are also present in maternal circulation. Non-specific assays that cannot distinguish between these forms of inhibin were often used in the early reports of the association between inhibin and Down's syndrome in the second trimester.[12–14] Monoclonal assays can now selectively measure the various inhibin precursors and the two dimeric inhibins. The assay that selectively measures dimeric inhibin A is more discriminatory for Down's syndrome in the second trimester than the non-specific assays.[6 7] Dimeric inhibin A is comparable in univariate screening performance to human chorionic gonadotrophin or its free β subunit. The current study was undertaken to investigate further the Down's syndrome screening performance of maternal serum dimeric inhibin A alone and in combination with other serum markers in a well characterised study population with complete ascertainment of Down's syndrome.

Methods

SELECTION OF CASES AND CONTROLS

This case-control study is based on a collaborative multicentre cohort study which has been described in detail elsewhere.[15] In that study 5385 women provided informed consent before a scheduled second trimester amniocentesis. Serum samples were obtained before the procedure and sent to a central laboratory (Scarborough, Maine, USA) for immediate measurement of α fetoprotein, unconjugated oestriol, and human chorionic gonadotrophin. Excess serum was immediately divided into aliquots and stored at −20°C. Free β subunit was subsequently measured on all of the stored samples.[16] Karyotypes and gestational age estimates based on biparietal diameter measurements were available for all pregnancies; gestational age estimates based on last menstrual period dating were available for nearly all pregnancies. Each of the maternal serum samples from the 52 Down's syndrome pregnancies with gestational age between 14 and 21 completed weeks (cases) was matched with five serum samples from the pool of pregnancies with a normal karyotype (controls). The following variables were used for matching: recruitment centre, completed week of gestation (last menstrual period), month of enrolment, and maternal age (usually to within one year). Overall, 256 control samples were available for analysis (in four instances, the selected control sample was not available).

BIOCHEMICAL MEASUREMENTS

Dimeric inhibin A was measured in duplicate using a solid phase sandwich enzyme linked immunosorbent assay (ELISA) (inhibin A dimer, medium sensitivity; Serotec, Oxford, UK) according to the manufacturer's instructions, with a two hour colour development. Assays were performed over a three week period without knowledge of whether the sample was from a case or control pregnancy. The between assay coefficient of variation for a pool of second trimester pregnancy serum samples with an average concentration of 152.6 pg/ml was 8.6%, similar to the variability found over a five month period in another laboratory (J Canick, personal communication). Methods for measuring maternal serum α fetoprotein, unconjugated oestriol, and human chorionic gonadotrophin and its free β subunit have been described elsewhere.[15 16]

CONVERSION OF DIMERIC INHIBIN VALUES TO MULTIPLES OF THE MEDIAN (MOM)

Gestational age specific median values for dimeric inhibin A were computed using measurements from the controls. The observed medians were slightly higher at earlier and later gestational ages. This effect has been reported in a large series of unaffected pregnancies in which the data were fitted to a quadratic model.[17] The same model was used to calculate dimeric inhibin A median values in the present study. Individual assay measurements were then converted to MoM and adjusted for maternal weight using a published method.[18] Median values were established separately according to whether gestational age was based on last menstrual period dates or ultrasound measurements.

Table 1 Number and percentage of unaffected and Down's syndrome pregnancies at or above selected dimeric inhibin A multiples of the median (MoM)

Dimeric inhibin A MoM cut off level	Unaffected pregnancies		Down's syndrome pregnancies	
	Number	%	Number	%
≥1.0	128	50.0	48	92.3
≥1.2	85	33.2	43	82.7
≥1.4	57	22.3	40	76.9
≥1.6	32	12.5	37	71.2
≥1.8	25	9.8	33	63.5
≥2.0	21	8.2	27	51.9
≥2.2	15	5.9	22	42.3
≥2.4	12	4.7	17	32.7
≥2.6	10	3.9	14	26.9
≥2.8	10	3.9	12	23.1
≥3.0	8	3.1	12	23.1

MODELLING SCREENING PERFORMANCE

In order to reliably estimate Down's syndrome screening performance for dimeric inhibin A, both alone and in combination with other serum markers, we employed a published model[19] that uses each analyte's distribution variables (and the correlations between them) along with the maternal age distribution in the United States in 1993.[20] The modelling has been described in more detail elsewhere.[15] The only change from the existing variables[16] is that the lower truncation limit for unconjugated oestriol was raised from 0.4 to 0.5 in order to avoid excessively high likelihood ratios. The method of DerSimonian and Laird was used to estimate the consensus medians.[21] Consensus standard deviations were estimated using the pooled variances, weighted by the number of samples. The consensus correlation coefficients were estimated after a z transformation.[22]

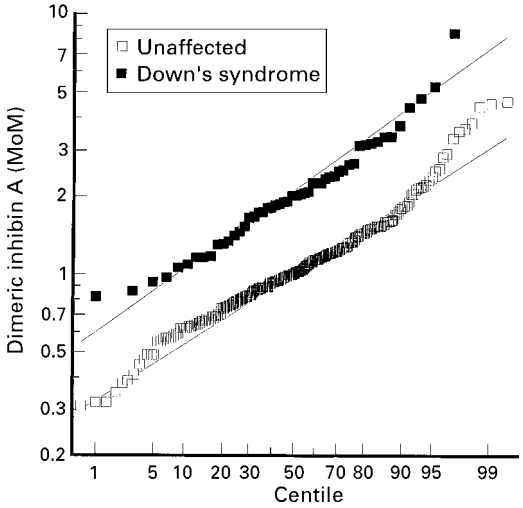

Figure 1 Probability plot for dimeric inhibin A in unaffected and Down's syndrome pregnancies. The dimeric inhibin A levels (expressed as multiples of the median (MoM)) for individual pregnancies are plotted vertically on a logarithmic scale and horizontally on the expected Gaussian centile scale, the latter based on the rank of the observation within its category. When the points plotted for each category follow an approximate straight line, the distribution is, by definition, log Gaussian. The MoM levels are based on last menstrual period dating and have been adjusted for maternal weight.

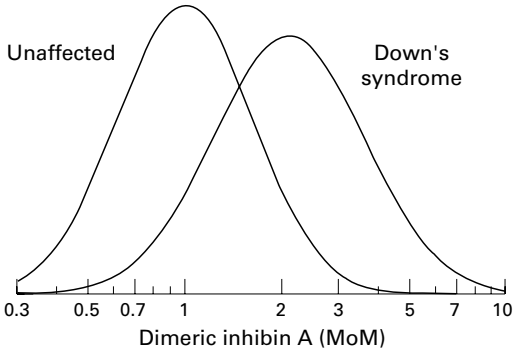

Figure 2 Distribution of dimeric inhibin A levels for unaffected pregnancies and pregnancies affected by Down's syndrome. The overlapping curves show the relative frequency of Down's syndrome and unaffected pregnancies at various dimeric inhibin A levels. The multiples of the median (MoM) levels are based on last menstrual period dating and have been adjusted for maternal weight.

Results

OBSERVED DIMERIC INHIBIN A SCREENING PERFORMANCE

Table 1 shows the observed univariate screening performance of dimeric inhibin A measurements. Overall, 51.9% of Down's syndrome and 8.2% of unaffected pregnancies had dimeric inhibin levels of 2.0 MoM or higher. Figure 1 shows a probability plot of the dimeric inhibin A levels in both Down's syndrome (logarithmic mean and standard deviation of 0.3222 and 0.2340) and unaffected pregnancies (0.0000, 0.2095). Both groups fitted straight lines reasonably well. At higher dimeric inhibin A levels, the unaffected pregnancies were above that predicted by the line. For this reason, truncation limits were placed at 0.7 and 2.5 MoM. Figure 2 shows the distributions of dimeric inhibin A in both unaffected and Down's syndrome pregnancies.

Table 2 Modelled Down's syndrome detection rates at three false positive rates for combinations of maternal age and α fetoprotein (AFP), unconjugated oestriol (uE_3), human chorionic gonadotrophin (hCG), and dimeric inhibin A (DIA)

False positive rate (%)	*Maternal age and serum markers*	*Detection rate (%)**	
		LMP	*BPD*
3	DIA	42	44
	AFP and DIA	60	61
	AFP and hCG	55	58
	AFP, uE_3, and DIA	65	67
	AFP, hCG, and DIA	65	66
	AFP, uE_3, and hCG	59	64
	AFP, uE_3, hCG, and DIA	69	71
5	DIA	52	55
	AFP and DIA	68	69
	AFP and hCG	62	65
	AFP, uE_3, and DIA	73	75
	AFP, hCG, and DIA	72	74
	AFP, uE_3, and hCG	66	71
	AFP, uE_3, hCG, and DIA	75	78
7	DIA	61	63
	AFP and DIA	73	74
	AFP and hCG	68	70
	AFP, uE_3, and DIA	77	79
	AFP, hCG, and DIA	77	78
	AFP, uE_3, and hCG	71	76
	AFP, uE_3, hCG, and DIA	80	82

*When gestational age is estimated by last menstrual period (LMP) or biparietal diameter (BPD). Statistical parameters necessary for modelling the detection and false positive rates (when dating is by last menstrual period) are found in table 5 and reference 16.

Table 3 Modelled false positive rates at three Down's syndrome detection rates for combinations of maternal age and α fetoprotein (AFP), unconjugated oestriol (uE_3), human chorionic gonadotrophin (hCG), and dimeric inhibin A (DIA)

Detection rate (%)	*Maternal age and serum markers*	*False positive rate (%)**	
		LMP	*BPD*
60	DIA	6.5	6.1
	AFP and DIA	2.9	2.9
	AFP and hCG	4.3	3.5
	AFP, uE_3, and DIA	2.1	1.8
	AFP, hCG, and DIA	2.0	1.9
	AFP, uE_3, and hCG	3.3	2.3
	AFP, uE_3, hCG, and DIA	1.6	1.3
70	DIA	11.3	10.4
	AFP and DIA	5.7	5.5
	AFP and hCG	8.1	6.9
	AFP, uE_3, and DIA	4.2	3.7
	AFP, hCG, and DIA	4.2	3.9
	AFP, uE_3, and hCG	6.5	4.7
	AFP, uE_3, hCG, and DIA	3.4	2.8
80	DIA	19.8	18.9
	AFP and DIA	11.5	11.0
	AFP and hCG	15.3	13.2
	AFP, uE_3, and DIA	8.6	7.4
	AFP, hCG, and DIA	9.0	8.1
	AFP, uE_3, and hCG	12.9	9.7
	AFP, uE_3, hCG, and DIA	7.2	6.0

*When gestational age is estimated by last menstrual period (LMP) or biparietal diameter (BPD).

MODELLED SCREENING PERFORMANCE OF DIMERIC INHIBIN A

There was no association between dimeric inhibin A and maternal age in either the unaffected ($r = 0.01$) or Down's syndrome ($r = -0.09$) pregnancies. Table 2 shows the estimated Down's syndrome detection rates at three false positive rates using various combinations of maternal age, dimeric inhibin A, and other maternal serum markers. Selected combinations that do not include dimeric inhibin A are included for comparison and are slightly different from a previous publication[16] because of modifications to the modelling software and the use of a higher truncation limit for unconjugated oestriol (0.5 rather than 0.4 MoM) as described above. At a 5% false positive rate, the combination of maternal age and dimeric inhibin A identified 52% of the Down's syndrome pregnancies. Adding α fetoprotein measurements increased detection to 68%. A triple test consisting of α fetoprotein, unconjugated oestriol, and dimeric inhibin A further increased detection to 73%. This detection rate is 7% higher than that for the combination of α fetoprotein, unconjugated oestriol, and human chorionic gonadotrophin. A four marker test was associated with the highest detection rate of 75%. When gestational ages were estimated by ultrasound, the detection rates were the same or slightly higher (parameters for these calculations are available on request).

Table 3 shows false positive rates at three Down's syndrome detection rates for the same combinations of markers. At a 70% detection rate, the combination of maternal age, α fetoprotein, unconjugated oestriol, and dimeric inhibin was associated with a false positive rate

Table 4 Modelled Down's syndrome detection and false positive rates at four risk cut off levels for combinations of maternal age and α fetoprotein (AFP), unconjugated oestriol (uE_3), human chorionic gonadotrophin (hCG), and dimeric inhibin A (DIA)

Down's syndrome term (second trimester) risk cut off level	*Maternal age and serum markers*	*Detection rate (%)**		*False positive rate (%)**		*OAPR (1:n)**	
		LMP	*BPD*	*LMP*	*BPD*	*LMP*	*BPD*
200 (150)	DIA	43	45	3.1	3.1	52	50
	AFP and DIA	61	62	3.1	3.2	37	38
	AFP and hCG	59	62	4.0	4.0	49	47
	AFP, uE_3, and DIA	65	68	2.8	3.1	31	33
	AFP, hCG, and DIA	66	67	3.0	3.1	33	34
	AFP, uE_3, and hCG	61	67	3.6	3.7	43	40
	AFP, uE_3, hCG, and DIA	68	71	2.8	2.9	30	30
250 (190)	DIA	50	53	4.7	4.7	68	64
	AFP and DIA	65	66	4.1	4.1	46	45
	AFP and hCG	63	65	5.1	5.1	59	57
	AFP, uE_3, and DIA	68	71	3.7	4.0	40	41
	AFP, hCG, and DIA	69	70	3.9	3.9	41	41
	AFP, uE_3, and hCG	65	70	4.6	4.7	51	49
	AFP, uE_3, hCG, and DIA	71	74	3.5	3.7	36	36
300 (230)	DIA	59	63	6.4	6.8	79	78
	AFP and DIA	68	69	5.0	5.0	53	53
	AFP and hCG	66	68	6.2	6.1	68	65
	AFP, uE_3, and DIA	71	74	4.5	4.8	46	47
	AFP, hCG, and DIA	71	73	4.7	4.7	48	47
	AFP, uE_3, and hCG	68	72	5.6	5.6	60	57
	AFP, uE_3, hCG, and DIA	73	76	4.3	4.4	43	42
350 (270)	DIA	65	65	8.2	7.6	92	85
	AFP and DIA	70	71	5.9	6.0	61	61
	AFP and hCG	68	71	7.3	7.2	78	74
	AFP, uE_3, and DIA	73	76	5.3	5.5	53	53
	AFP, hCG, and DIA	74	75	5.5	5.5	54	53
	AFP, uE_3, and hCG	70	74	6.6	6.5	69	64
	AFP, uE_3, hCG, and DIA	75	78	5.0	5.1	48	48

OAPR, Odds of being affected given a positive result.
*When gestational age is estimated by last menstrual period (LMP) or biparietal diameter (BPD).

of 4.2%. This is lower than the 6.5% false positive rate found when human chorionic gonadotrophin was used in place of dimeric inhibin A. The combination of all four markers was associated with a false positive rate of 3.4%, the lowest of all combinations tested. False positive rates were lower when gestational age was based on ultrasound measurements.

Table 4 shows the screening performance of the same combinations of markers at four selected Down's syndrome term (and second trimester) risk cut off levels. The detection and false positive rates and the odds of being affected given a positive result are provided. In table 4 (as well as in tables 2 and 3) screening performance estimates were equivalent or slightly less efficient when free β subunit measurements were substituted for human chorionic gonadotrophin. For example, at a 5% false positive rate, the detection rate for α fetoprotein, unconjugated oestriol, and free β subunit was 62% (4% lower) and when dimeric inhibin A was added, the detection rate was 73% (2% lower).

COMPARISON WITH OTHER PUBLISHED STUDIES

Table 5 provides a summary of the results of published trials of dimeric inhibin A and allows a comparison between these results[4–8 10 11] and those of this study. The estimate in this study of the separation between affected and unaffected pregnancies was near the consensus of the other seven studies and the standard deviations were slightly smaller. The correlation coefficients were also consistent with the other seven studies. In most of the studies the highest correlations occur between dimeric inhibin A and human chorionic gonadotrophin (or its free β subunit). When the false positive rate was held constant at 5% the estimated univariate Down's syndrome detection rates ranged from 30 to 50% (computed using the medians and standard deviations reported for each study). The consensus estimate of 41% (based on the weighted medians and standard deviations) is similar to that of this study (46%).

COST ANALYSIS

The present study can be used to estimate allowable costs of adding dimeric inhibin A to a screening panel by setting an upper limit to the added screening costs equal to the savings realised by reducing the number of diagnostic tests (amniocenteses and karyotypes). This can be achieved by holding the detection rate constant at, for example, 70% (table 3). Based on last menstrual period dating, the combination of maternal age and α fetoprotein, unconjugated oestriol, and human chorionic gonadotrophin was associated with a 6.5% false positive rate. When dimeric inhibin A was added the false positive rate was reduced to 3.4%. Thus for 10 000 pregnancies, 310 fewer amniocenteses (650−340) would be needed to detect the same number of Down's syndrome pregnancies. If diagnostic testing costs $1000 per screen positive woman, then $310 000 would be available for adding dimeric inhibin A measurements for the 10 000 women. This translates into an allowable additional cost, for all laboratory expenses, of $31 per woman tested. If dimeric inhibin A could be added for less than that cost, the screening and diagnostic costs would be lower. If the uptake of amniocentesis were less, the allowable additional cost would be lower—for example, if the

Table 5 Population distribution parameters and univariate Down's syndrome screening performance for dimeric inhibin A (DIA)

	Unaffected pregnancies							*Down's syndrome pregnancies*							
				Correlation with DIA							*Correlation with DIA*				*Detection rate (%)**
Reference	*No*	*Median*	*SD*	*AFP*	*uE_3*	*hCG*	*Free β*	*No*	*Median*	*SD*	*AFP*	*uE_3*	*hCG*	*Free β*	
Wald *et al*[10]	1355	1.00	0.2154	0.12	0.02	0.24	0.28	77	1.79	0.1986	0.09	−0.11	0.28	0.37	30
Aitken *et al*[5]	202	1.00	0.2967	0.24	NR	0.27	0.15	44	2.06	0.3521	0.24	NR	0.39	0.23	31
Cuckle *et al*[6]	280	1.00	0.2000	0.11	−0.02	0.41	0.38	56	1.62	0.2500	0.08	−0.08	0.29	0.18	32
Lambert-Messerlian *et al*[7]	100	1.00	0.2330	0.15	0.03	0.33	NR	20	1.95	0.2610	0.29	−0.20	0.67	NR	36
Spencer *et al*[8]	367	1.00	0.1945	0.15	NR	NR	0.24	157	1.77	0.2284	0.06	NR	NR	0.39	37
Wallace *et al*[4]	150	1.00	0.2700	NR	NR	NR	NR	21	2.60	0.2000	NR	NR	NR	NR	44
This study	256	1.00	0.2095	0.23	−0.07	0.42	0.46	52	2.10	0.2340	−0.03	−0.04	0.51	0.44	46
Wenstrom *et al*[11]	313	1.02	0.2431	0.07	0.02	0.36	0.33	33	2.57	0.2579	NR	NR	NR	NR	50
Consensus	3023	1.00	0.2245	0.14	0.01	0.30	0.31	460	2.06	0.2463	0.09	−0.09	0.39	0.35	41

*Estimated Down's syndrome detection rate at a 5% false positive rate using the parameters specified in that row.
AFP, α fetoprotein; uE_3, unconjugated oestriol; hCG, human chorionic gonadotrophin; Free β, free β subunit of hCG; NR, not reported.

uptake were 80%, the allowable cost in the previous example would be $25 rather than $31. Since fewer amniocenteses would be necessary to maintain the same detection rate, fewer procedure related fetal losses would occur. Among the 10 000 women tested, about six fetal losses would occur (650 amniocenteses × 1/111 procedure related losses[23]) if three markers were used compared with about three fetal losses (340/111) if dimeric inhibin A were included as the fourth marker. Allowable costs vary according to detection rate, method of dating, and serum markers chosen. The costs of the most likely combinations would range between $16 and $39.

Discussion

Three major advantages of the present dataset are that all pregnancies were karyotyped in the second trimester (eliminating the possibility of biased sample selection), the indications for diagnostic testing did not include abnormal screening test results (leading to unbiased estimates of analyte levels in cases and control), and three of the other serum marker measurements were made on the fresh samples over a two year period (simulating routine practice). In addition, gestational dating data based on both last menstrual period and biparietal diameter measurements were available, allowing a direct comparison of screening performance. The performance based on last menstrual period dating may be slightly overestimated because study participants would not have been eligible for a second trimester amniocentesis if their last menstrual period dates were found to be grossly inaccurate. Another study which looked at the relative performance of screening by dating method found a slightly greater improvement in performance when ultrasound dating was used.[10]

This study confirms previous results indicating that dimeric inhibin A is among the best second trimester maternal serum markers yet described. In contrast with the other serum markers, its levels do not vary greatly with gestational age during the second trimester. This is an advantage in that interpretation of the results will not be greatly influenced by incorrect estimates of gestational age, resulting in more reliable estimates of Down's syndrome risk. For those considering incorporating this marker into their existing routine, sufficient data exist in the literature to allow reliable interpretation in the case of diabetic pregnancies,[24 25] twin pregnancies,[26] and various racial groups.[27] It is also possible to adjust for maternal weight.[9] Adding dimeric inhibin A measurements to existing multiple marker screening programmes improves performance and will be cost effective if the added expense is less than $31 per pregnancy tested. This cost estimate is based on the reduction in false positives that can be achieved by adding dimeric inhibin A measurements while holding the detection rate constant (achieved by varying the risk cut off level). Some laboratories may wish to consider this strategy instead of focusing on increasing detection (achieved by holding the risk cut off level constant). In the United States it is necessary at present for laboratories to establish their own assays for dimeric inhibin A, as no manufacturer is licensed to sell kits for clinical use.

1 Palomaki GE, Knight GJ, McCarthy JE, *et al*. Maternal serum screening for Down syndrome in the United States: a 1995 survey. *Am J Obstet Gynecol* 1997;**176**:1046–51.
2 Macintosh M, Ellis A, Cuckle H, *et al*. Variation in biochemical screening for Down's syndrome in the United Kingdom. *Br J Obstet Gynaecol* 1998;**105**:465–7.
3 Palomaki GE, Neveux LM, Haddow, JE. Can reliable Down's syndrome detection rates be determined from prenatal screening intervention trials? *J Med Screen* 1996;**3**: 12–17.
4 Wallace EM, Grant VE, Swanston IA, *et al*. Evaluation of maternal serum dimeric inhibin A as a first trimester marker of Down's syndrome. *Prenat Diagn* 1995;**15**:359–62.
5 Wallace EM, Swanston IA, McNeilly AS, *et al*. Second trimester screening for Down's syndrome using maternal serum dimeric inhibin A. *Clin Endocrinol* 1998;**44**:17–21.
6 Cuckle HS, Holding S, Jones R, *et al*. Combining inhibin A with existing second-trimester markers in maternal serum screening for Down's syndrome. *Prenat Diagn* 1996;**16**: 1095–100.
7 Lambert-Messerlian GM, Canick JA, Palomaki GE, *et al*. Second trimester levels of maternal serum inhibin-A, total inhibin, α inhibin precursor and activin in Down's syndrome pregnancies. *J Med Screen* 1996;**3**:58–62.
8 Spencer K, Wallace EM, Ritoe S. Second trimester dimeric inhibin-A in Down's syndrome screening. *Prenat Diagn* 1996;**16**:1101–10.
9 Wald NJ, Densem JW, George L, *et al*. Prenatal screening for Down's syndrome using inhibin-A as a serum marker. *Prenat Diagn* 1996;**16**:143–53.
10 Wald NJ, Densem JW, George L, *et al*. Inhibin-A in Down's syndrome pregnancies: revised estimate of standard deviation. *Prenat Diagn* 1997;**17**:285–90.
11 Wendstrom KD, Owen J, Chu DC, *et al*. α-Fetoprotein, free β-human chorionic gonadotropin, and dimeric inhibin A produce the best results in a three-analyte, multiple-marker screening test for fetal Down syndrome. *Am J Obstet Gynecol* 1997;**177**:987–91.
12 Van Lith JM, Pratt JJ, Beekhuis JR, *et al*. Second trimester maternal serum immunoreactive inhibin as a marker for fetal Down's syndrome. *Prenat Diagn* 1992;**12**:801–6.
13 Spencer K, Wood PJ, Antony FW. Elevated levels of maternal serum inhibin immunoreactivity in second trimester pregnancies affected by Down's syndrome. *Ann Clin Biochem* 1993;**30**:219–20.
14 Cuckle HS, Holding S, Jones R. Maternal serum inhibin levels in second-trimester Down's syndrome pregnancies. *Prenat Diagn* 1994;**14**:387–90.
15 Haddow JE, Palomaki GE, Knight GJ, *et al*. Reducing the need for amniocentesis in women 35 years of age or older with serum markers for screening. *N Engl J Med* 1994;**330**: 1114–18.
16 Knight GJ, Palomaki GE, Neveux LM, *et al*. HCG and the free β-subunit as screening tests for Down syndrome. *Prenat Diagn* 1998;**18**:235–45.
17 Watt HC, Wald NJ, Huttly WJ. The pattern of maternal serum inhibin-A concentrations in the second trimester of pregnancy. *Prenat Diagn* (in press).
18 Neveux LM, Palomaki GE, Larrivee DA, *et al*. Refinements in managing maternal weight adjustment for interpreting prenatal screening results. *Prenat Diagn* 1996;**16**:1115–19.
19 Royston P, Thompson, SG. Model-based screening by risk with application to Down's syndrome. *Stat Med* 1992;**11**: 257–68.
20 National Center for Health Statistics. *Vital statistics of the United States, 1993*. Vol 1. *Natality*. Washington, DC: Government Printing Office, 1997:103. (DHHS publication No 1 (PHS) 97.)
21 Berlinn JA, Laird NM, Sacks HS, *et al*. A comparison of statistical methods for combining event rates from clinical trials. *Stat Med* 1989;**8**:141–51.
22 Sokal RR, Rohlf FJ. *Biometry: the principles and practice of statistics in biological research*. 3rd ed. New York: WH Freeman and Co., 1995:575–81.
23 Wald NJ, Kennard A, Hackshaw A, *et al*. Antenatal screening for Down's syndrome. *J Med Screen* 1997;**4**:181–246.
24 Wald NJ, Watt HC, George L. Maternal serum inhibin-A in pregnancies with insulin-dependent diabetes mellitus: implications for screening for Down's syndrome. *Prenat Diagn* 1996;**16**:923–6.
25 Wallace EM, Crossley JA, Ritoe SC, *et al*. Maternal serum inhibin-A in pregnancies complicated by insulin dependent diabetes mellitus. *Br J Obstet Gynaecol* 1997;**104**:946–8.
26 Watt HC, Wald NJ, George L. Maternal serum inhibin-A levels in twin pregnancies: implications for screening for Down's syndrome. *Prenat Diagn* 1996;**16**:927–9.
27 Watt HC, Wald NJ, Smith D, *et al*. Effect of allowing for ethnic group in prenatal screening for Down's syndrome. *Prenat Diagn* 1996;**16**:691–8.