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Impact of a regional screening programme using maternal serum α fetoprotein (AFP) and human chorionic gonadotrophin (hCG) on the birth incidence of Down's syndrome in the west of Scotland

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Abstract

Objectives **– To evaluate the impact of a large scale population screening programme on the birth incidence of Down's syndrome in the west of Scotland over a 12 month period.**

Methods **– Biochemical screening for Down's syndrome using maternal serum α fetoprotein, chorionic gonadotrophin, and maternal age was offered to a pregnant population of 37 226 women in the west of Scotland between 1991 and 1992. The combined risk of Down's syndrome pregnancy was reported for each of the 30 084 women who opted for screening.**

Results **– When a threshold risk of 1:220 was used 1523 women (5·1% of the screened population) were assigned to the high risk group, of whom 1070 (70%) proceeded to diagnostic ammiocentesis or mid-trimester chorionic villus sampling. When multiple sources of ascertainment were used 37 Down's syndrome pregnancies were identified within the screened population, 26 (70%) of which were within the high risk group and 21 (57%) of which were prenatally diagnosed. In addition, three Down's syndrome pregnancies were diagnosed by first trimester chorionic villus sampling before biochemical screening. A further 10 Down's syndrome pregnancies were identified at birth, eight to women who had not had a screening test and two to women who had moved into the area, making a total of 50 Down's syndrome pregnancies in the whole pregnant population of 37 226. Thus the potential prenatal detection rate in the screened population was 70% (26/37), the actual prenatal detection rate in the screened population was 57% (21/37), and the overall prenatal detection rate in the total (screened and unscreened) population was 48% (24/50).**

Conclusion **– Biochemical screening for Down's syndrome is practical and effective in routine clinical practice, enabling women to make an informed choice about prenatal diagnosis and providing better use of scarce resources when a suitable protocol is applied to the whole pregnant population. Its maximum potential for the reduction of the birth incidence of Down's syndrome is limited by incomplete uptake of screening and compliance with diagnostic testing in the high risk group.**

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Prenatal screening for neural tube defects (since 1976) and for chromosome abnormalities (since 1987) is an established part of routine antenatal care in the eight health board areas in the west of Scotland. In this region there are around 37 500 births each year, and biochemical screening is taken up by 80% of women. All maternal serum assays, reporting, and follow up are carried out by a single centre, which also provides the regional cytogenetics service.

Before the introduction of biochemical screening, prenatal diagnosis of Down's syndrome depended on selection of women for diagnostic testing on the basis of their increased risk associated with advanced maternal age. Around 7% of all pregnancies and 30% of Down's syndrome pregnancies are in women aged 35 years and over, but in the west of Scotland region uptake of amniocentesis by this group has been poor (typically less than 40%), resulting in detection rates for Down's syndrome of only 12–15%.[1]

Biochemical screening uses additional risk information derived from the analysis of various pregnancy markers in maternal serum to define a high risk group. Retrospective studies, using various combinations of α fetoprotein (AFP), unconjugated oestriol (uE_3), human chorionic gonadotrophin (hCG), and the free β subunit of hCG together with maternal age risks, have indicated that around 60% of affected pregnancies might be detected by following up the 5% of women at highest risk.[2–5] Almost all studies have used AFP and either hCG or the free β subunit of hCG, but opinion is divided on the additional contribution of uE_3 to detection.[6–8]

At least five prospective studies have reported on the performance of a triple marker screening protocol using AFP, uE_3, and hCG.[9–13] Three other studies have used double marker protocols – one uE_3 and hCG[14] and two AFP and the free β subunit of hCG.[15,16] We report here the impact of another double marker screening protocol (AFP and hCG) on the birth incidence of Down's syndrome in a large screened population in the west of Scotland.

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Materials and methods

Maternal serum samples were received from 30 084 women at 15–20 weeks' gestation. α Fetoprotein was assayed by a two site immunoradiometric assay as previously described.[17] Human chorionic gonadotrophin was assayed by immunoradiometric assay (Serono MAIAclone, which measures predominately intact hCG) after dilution of serum samples 1:500. The assay protocols were semi-automated with sample, buffer, and dilution steps carried out on a Kemble sample processor followed by manual label addition and separation. Analyte levels were reported both as concentrations and as multiples of the appropriate gestational median (MOM). Interassay precision (coefficient of variation) for AFP was 5·4% at 15 kU/l and 5·0% at 78 kU/l. Coefficients of variation for hCG were 6·9% at 20 IU/l and 5·5% at 40 IU/l.

The combined risk of Down's syndrome pregnancy was calculated from the hCG (MOM)/AFP(MOM) ratio and the woman's population age risk at mid-trimester.[4] A cut off risk of 1:220 was used. Gestation was estimated in completed weeks from the date of the last menstrual period or from ultrasound scan.

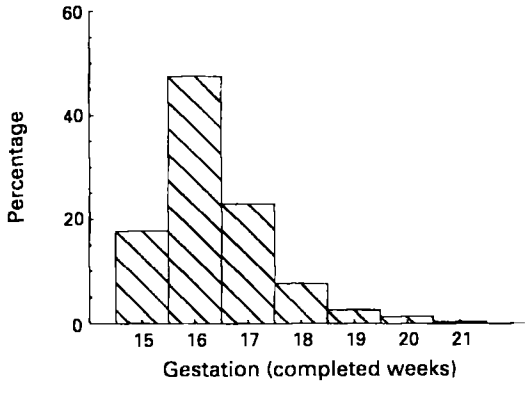

Figure 1 Distribution of gestations at which screening samples were taken.

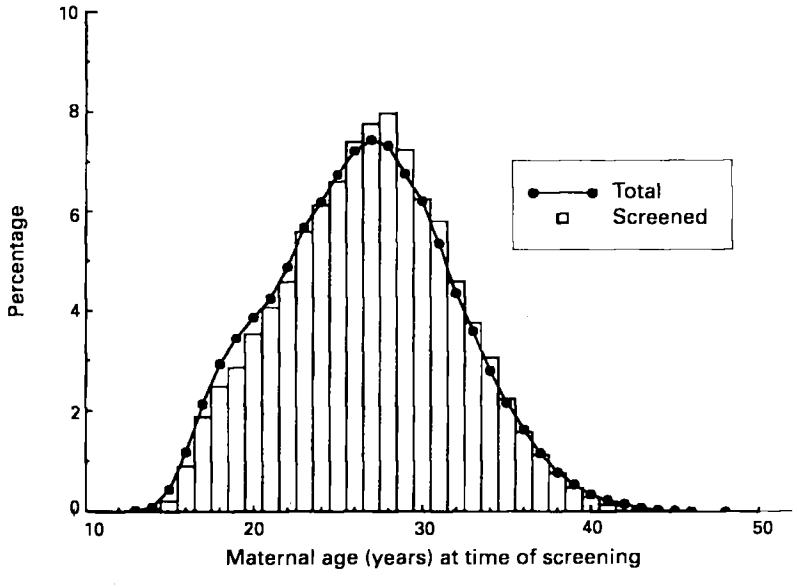

Figure 2 Distribution of maternal ages in the screened population, compared with that of the whole pregnant population adjusted to age at the time of screening.

Overall, 11% of women had information about their last menstrual period only, 9% had ultrasound information only, and 80% had gestational estimates from both the date of their last menstrual period and ultrasound (48% concordant and 32% discordant). If two different gestational estimates were obtained in the same pregnancy "certain" dates were used as the primary determinant, unless the ultrasound estimate was one or more completed weeks greater, or two or more completed weeks less, than the gestation by last menstrual period, in which case the ultrasound estimate was used. Gestations derived from "uncertain" last menstrual period(s) were only used where no ultrasound was available. When this system was used, of the 32% of women with discordant last menstrual period and ultrasound estimates of gestation, last menstrual period information was used in 12% and ultrasound information in 20% for risk calculation.

Ascertainment of Down's syndrome cases (and other abnormalities) in the region was by (*a*) the use of congenital malformation notification cards returned by the maternity unit whenever such an abnormality was suspected at birth or abortion; (*b*) inspection of prenatal and abortus cytogenetic results and postnatal cytogenetic results up to a period of six months beyond the date of delivery of the final patient screened; and (*c*) inspection of pathology reports on all fetal and neonatal deaths relating to the same period. Only those cases where there was definite cytogenetic confirmation of Down's syndrome were included. A system of cross checking then allowed the cases which had been screened and detected or missed and those not screened to be identified.

Results

Figure 1 shows the distribution of gestations at which samples were taken; 88% of all samples were received at 15–17 weeks. Figure 2 shows the distributions of maternal ages at the time of screening in single years in the screened and total pregnant populations. Median ages for each population were 26·4 and 26·1 years respectively, and 6·8% of women in the screened population and 7·1% in the whole population were aged 35 years or over.

Table 1 summarises the performance of AFP/hCG/age screening for Down's syndrome. The false positive rate was 5·1% after reassessment of gestation by ultrasound and recalculation of risks had removed 381 women from the initial high risk group. Of those remaining, 70% opted to have diagnostic amniocentesis or mid-trimester chorionic villus sampling, and 21 cases of Down's syndrome were identified. A further five cases were identified at birth among these women in the high risk group who had declined diagnostic testing. Additionally, there were 11 Down's syndrome cases born to women who had had screening and were given a risk less than the high risk cut off of 1:220, making a total of 37 cases in the screened population and a sensitivity for the screening test of 70% (26/37, 95% confidence interval 53 to 84%). Table 2 shows the individual risks and maternal

Table 1 Performance of hCG/AFP/age screening for Down's syndrome in the west of Scotland (September 1991 to September 1992)

Total No of pregnancies screened	30 084
*No (%) in high risk group	
Initial	1 904 (6·3)
After gestation reassessed	1 523 (5·1)
Uptake of diagnostic testing	1 070 (70)
No of Down's syndrome in screened population	37
No (%) in high risk group	26 (70)
95% CI	(53 to 84%)
No (%) prenatally diagnosed	21 (56)
95% CI	(39 to 72%)
Overall odds of being affected if in the high risk group	1:59
Overall odds of being affected if in the low risk group	1:2600

*Cut off risk 1:220.

Table 2 Risks from hCG/AFP/age for Down's syndrome pregnancies screened between September 1991 and September 1992

High risk group				*Low risk group*	
Prenatally diagnosed		*Born*		*Born*	
Age	*Risk*	*Age*	*Risk*	*Age*	*Risk*
20	1:110	23	1:190	21	1:270
21	1:210	33	1:120	21	1:950
23	1:110	34	1:43	23	1:3100
26	1:160	38	1:17	25	1:970
30	>1:43	38	1:160	25	1:1100
30	>1:43			27	1:250
30	1:68*			29	1:500
31	1:100†			29	1:500
31	1:170			30	1:3800
32	1:50			31	1:660‡
34	>1:22			37	1:650
34	>1:22				
34	1:24				
36	>1:15				
36	1:47				
37	1:100				
37	1:140				
38	>1:10				
38	1:60				
39	1:52				
40	1:44				

*Translocation Down's 46XY, −14, +t(14;21).
†Mosaic 46XY/47XY + 21.
‡Mosaic 46XY/47XY + 21.

Table 3 Efficacy of screening for Down's syndrome using hCG/AFP/age in different maternal age groups

	Maternal age (years)		
	<25	*25–34*	*≥35*
Total No of Down's syndrome	7	19	11
No (%) in high risk group	4 (57)	12 (63)	10 (91)

Table 4 Summary of ascertainment of Down's syndrome pregnancies in the west of Scotland for the period screened September 1991 to September 1992

	Maternal age			
	<25	*25–34*	*≥35*	*All*
Screened-HR-PND	3	10	8	21
Screened-HR-born	1	2	2	5
Screened-LR-born	3	7	1	11
First trimester-CVS-MA-PND	—	—	3	3
Not screened-born	1	5	2	8
Moved into area-born	1	1	—	2
Actual No (%) in age group	9 (18)	25 (50)	16 (32)	50
Expected %	(19)	(52)	(29)	

HR = high risk group (risk ≥1:220); LR = low risk group (risk <1:220); PND = prenatally diagnosed; CVS = chorionic villus sampling; MA = maternal age ≥35 years.

ages for each of the 37 cases. The detection rate and false positive rate vary according to maternal age group with 57% detection in women aged less than 25 years and 91% in women aged 35 years and over (table 3). Among the screened population there was only one case of Down's syndrome in a woman aged over 35 years who had been assigned to the low risk group.

Before the screening period of 15–20 weeks' gestation, 131 women aged 35 years and over had chorionic villus sampling in the first trimester on the basis of their advanced maternal age, and three cases of Down's syndrome were diagnosed. Of the remainder with viable, karyotypically normal pregnancies, 114 subsequently had routine serum screening at 15–20 weeks. In addition, a further 125 other women aged 35 years and over proceeded directly to second trimester amniocentesis and did not have a serum screening test. No Down's syndrome pregnancies were found in this group.

Table 4 summarises the ascertainment of Down's syndrome pregnancies identified cytogenetically in the whole pregnant population relating to the screening period. There were 50 cases of Down's syndrome: 24 were prenatally diagnosed, five were identified by screening and proceeded to term, 11 were missed by screening, eight were not screened, and two others moved into the area.

Discussion

The introduction of AFP/hCG/age screening for women of all ages in this region has made a significant impact on the birth incidence of Down's syndrome during the first year of the programme. Almost half of all cases of Down's syndrome (24/50) were prenatally diagnosed – most as a result of the screening programme – compared with 12–15% using the former single criterion of maternal age. Improved uptake of screening by the pregnant population beyond the current 80% and improved compliance with diagnostic testing beyond the current 70% for those found to be at increased risk would have resulted in an even greater prenatal detection rate.

If the criterion of age (≥35 years) alone had been used and with full uptake of diagnostic testing, 2643 women (7·1% of the pregnant population) would have required diagnostic testing to detect 16 cases of Down's syndrome, a sensitivity of 32%. Reporting combined risks has reduced this high risk group to 5·1% (equivalent to 1899 women in the whole population) and at the same time increased the sensitivity of detection to 70% (equivalent to 35 cases of Down's syndrome). The overall odds of finding an affected fetus if the age criterion alone were used would be 1:165, but when AFP/hCG/age is used this ratio improves to 1:59.

When the 1:220 risk threshold was used 70% of women aged 35 years and over who would previously have been candidates for diagnostic testing on the grounds of age alone were classified as at low risk. Fifteen per cent of these low risk pregnancies were investigated by amniocentesis or chorionic villus sampling carried out at the patient's request, and no cases of

fetal chromosome abnormality were found. One patient aged 37 years, who had a risk of 1:650 and did not have diagnostic testing, gave birth to a Down's syndrome infant (table 2). Overall, 91% of all cases of Down's syndrome in women $\geqslant 35$ years who had screening were detected (table 3).

Before the introduction of biochemical screening for Down's syndrome in this region an increasing proportion of older mothers ($\geqslant 35$ years) were offered diagnostic testing in the form of first trimester chorionic villus sampling, usually around 10 weeks' gestation. Cases of fetal Down's syndrome detected by this method were therefore diagnosed and terminated at a very early stage of pregnancy. The displacement of maternal age screening by multimarker biochemical screening now results in most older mothers awaiting biochemical screening at 15 weeks' gestation before making a decision about diagnostic testing. This has meant that although 70% of this group aged $\geqslant 35$ years will avoid the hazards of diagnostic testing, those women found to be carrying an affected fetus will have to endure the trauma of abortion in the late second trimester. This emphasises the need to develop screening programmes which can be used in the first trimester.[18]

The performance of our double marker AFP/intact hCG screening protocol compares favourably with those previously reported which have used uE_3 in addition to AFP and intact hCG[9 13] and with those which have used the free β subunit of hCG[15 16] instead of intact hCG. On the other hand, the results from one study suggest that uE_3 may be an acceptable alternative screening marker to AFP in combination with hCG for the detection of Down's syndrome pregnancies,[14] though this combination cannot detect open neural tube defect pregnancies, which would be revealed by raised maternal serum AFP. Comparisons of performance are difficult, however, as detection rates are quoted for different false positive rates in each study and not all studies have been in truly representative pregnant populations encompassing women of all ages.

Ascertainment bias can also affect conclusions about performance. As a check on the completeness of ascertainment of trisomy 21 in the population reported here, the expected number of Down's syndrome births was calculated from the age distribution of the total pregnant population shown in fig 2, giving a total of 45 (1·2/1000, 95% confidence interval 33 to 60). Owing to the known fetal loss rate of affected pregnancies between the first and second trimester and between the second trimester and birth,[19 20] however, the actual number of Down's syndrome cases ascertained will depend on the stage of pregnancy at which they are diagnosed. Of the 50 Down's syndrome cases identified here, three were diagnosed in the first trimester, 21 in the second trimester, and 26 at birth. If a fetal loss rate of 21% between 10 and 16 weeks' gestation[19] and of 20% from mid-trimester to term is allowed for,[20] this is equivalent to 45 Down's syndrome births, identical with the expected number.

The establishment of large scale population screening programmes for Down's syndrome allows women of all ages to make an informed choice about prenatal diagnosis and makes better use of prenatal diagnosis services by limiting the number of women requiring diagnostic testing while at the same time improving the sensitivity of the test.[21] The best measure of the actual performance of a screening test, however, is its impact on the birth incidence of Down's syndrome in the entire pregnant population. This provides information not only on the performance of the test but also on the relative acceptability of the screening programme as measured by uptake of screening and compliance with diagnostic testing.

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