Ultrasound Obstet. Gynecol. 4 (1994) 199–204

Life without amniocentesis: elevated maternal serum α-fetoprotein in the Manitoba program 1986–91

P. M. Kyle, C. R. Harman, J. A. Evans, K. MacDonald*, S. M. Menticoglou, F. A. Manning and B. N. Chodirker**

Departments of Obstetrics, Gynecology and Reproductive Sciences and *Human Genetics, University of Manitoba, Winnipeg, Canada

Key words: AMNIOCENTESIS, MATERNAL α-FETOPROTEIN, FETAL ULTRASOUND, MANITOBA PROGRAM

ABSTRACT

Pregnant women demonstrating an elevated maternal serum α-fetoprotein level are at increased risk for fetal neural tube defect or other anomaly. Diagnostic procedures to evaluate these pregnancies include high-resolution ultrasound and amniocentesis to measure amniotic fluid levels of α-fetoprotein and N-acetylcholinesterase. We wished to examine the efficacy of detailed ultrasound examination alone, in evaluation of women with 'unexplained' elevation of maternal serum α-fetoprotein. The results showed that no neural tube defects were missed in the assessment of 1325 pregnancies with a raised level of maternal serum α-fetoprotein over 6 years, despite complete reliance on ultrasound in 98%. Detailed fetal ultrasound evaluation by experienced personnel is adequate to identify all cases of neural tube defects in a selected high-risk population.

INTRODUCTION

Raised levels of α-fetoprotein in amniotic fluid and maternal serum are associated with fetal neural tube defects, and ultrasound can detect these anomalies. The sensitivity of the procedure to identify anencephaly is much greater than 90%, but is lower (60–80%) for the detection of spina bifida[1]. This disparity exists because the exclusion of spina bifida with certainty requires detailed visualization of the entire spine, an examination which takes time and skill. Therefore, cases of spina bifida can be missed by 'routine' ultrasound examination. Nevertheless, recognition that cases of spina bifida are almost invariably associated with ultrasound cranial signs (scalloping of the frontal bones, absence of the cisterna magnum) has increased the detection efficiency of the procedure[2].

Following the introduction of widespread maternal serum α-fetoprotein screening programs, many centers have established protocols of step-wise assessment to establish a diagnosis[1,3,4]. Most published guidelines recommend the use of ultrasound to exclude several known causes of raised maternal serum α-fetoprotein, such as incorrect assessment of gestational age at sampling, multiple pregnancy and fetal demise, but these protocols do not rely exclusively on ultrasound for the assessment of fetal structure. Amniocentesis is required to measure amniotic fluid α-fetoprotein and/or acetylcholinesterase levels, to rule out a diagnosis of neural tube defect with certainty.

When performed at 16–20 weeks' gestation, amniocentesis carries a procedure-related risk of pregnancy loss of 0.5–1.5%, and this may be increased in pregnancies with a raised level of maternal serum α-fetoprotein[5]. In addition, there is a small but definite incidence of false-positive raised levels of amniotic fluid α-fetoprotein and acetylcholinesterase, which can be a problem when considering pregnancy termination as an action for the abnormality[6,7].

With the availability of high-resolution ultrasound and increasing operator experience, it may be that routine amniocentesis is now redundant in women with an unexplained raised level of serum α-fetoprotein[8–10]. In order to answer this question with more certainty, we reviewed the results of the Manitoba maternal serum α-fetoprotein screening program, which commenced high-resolution fetal ultrasound examination in 1986. The program's policy was to rely on ultrasound examination almost exclusively for the assessment of the fetus, with only occasional and selected use of amniocentesis.

METHODS

The publicly funded Manitoba maternal serum α-fetoprotein screening program was introduced provincially in 1985, following a pilot study in 1983, and it conforms

Correspondence: Dr C. R. Harman, Room WR-120, Women's Hospital, 735 Notre Dame Avenue, Winnipeg, Manitoba, Canada R3E OL8

Received 22–11–93
Revised 21–2–94
Accepted 1–3–94

meticulously to international screening program standards. The program is applied across the entire health-care jurisdiction, leading to inclusion of women from a wide range of socioeconomic and racial backgrounds.

This evaluation of the program is a retrospective study of the years 1986–91, inclusive. Maternal serum samples were drawn between 15 and 24 weeks' gestation. From 1986 to November 1989, the cut-off value for definition of elevated maternal serum α-fetoprotein was 2.5 multiples of the median (MoM) (serum α-fetoprotein analysis using a commercially available radioimmunoassay kit (Amersham, Illinois, USA) and from December 1989 onwards, the cut-off level was lowered to 2.3 MoM, following conversion to an enzyme immunoassay technique (IMX, Abbott Laboratories, Illinois, USA). A detailed comparison between the two methods showed that these cut-off levels selected a similar proportion of women with 'elevated' α-fetoprotein. Women demonstrating a raised level were referred at 17–24 weeks' gestation for a 'first-visit' detailed ultrasound examination. All examinations were performed by one of three experienced perinatologists in a single fetal assessment unit, using either an Acuson Model 128 or ATL Ultramark 9 machine with a 3.5-MHz linear array transducer and/or a transvaginal probe. Fetal viability, order of pregnancy, and measurements to determine gestational age were ascertained before the detailed assessment of fetal anatomy. For this, particular attention was paid to the shape of the cranium (Figure 1a), the cerebral ventricles, the cerebellum and the spine in transverse and longitudinal views (Figure 1b). If the immediate fetal position precluded optimal views of the cranium or spine, the length of examination was extended until adequate views were obtained. In a small number of cases, this meant that the woman returned for subsequent examination within the next few days. Furthermore, on occasion the use of a transvaginal probe made possible an adequate examination of the lower spine deep within the maternal pelvis. Once incorrect dates were excluded (maternal serum α-fetoprotein repeated), abnormal pregnancies associated with a raised level of maternal serum α-fetoprotein were identified and subsequent management was arranged. These pregnancies included multiple gestation, fetal demise, structural defects, placental abnormalities and oligohydramnios. For the remaining singleton pregnancies with a technically adequate ultrasound scan, the policy was not to determine amniotic fluid α-fetoprotein and acetylcholinesterase levels for reassurance. Such measurements were routinely made when amniocentesis was performed for some other reason – such as karyotyping (advanced maternal age) or rhesus disease, but amniocentesis specifically for amniotic fluid α-fetoprotein and acetylcholinesterase was only done when the technical quality of the scan did not satisfy strict ultrasound criteria, or on occasions where unusually high levels of maternal serum α-fetoprotein and specific perinatal concerns coincided.

Follow-up data from all pregnancies with a raised maternal serum α-fetoprotein level were obtained from the hospital records, supplemented by information from the Manitoba Congenital Anomalies Registry. For each year, the data were examined to determine pregnancies screened, number with elevated maternal serum α-fetoprotein level, and the frequencies of fetal ultrasound evaluation and amniocentesis. The diagnosis made at fetal assessment was identified, in addition to the reason for amniocentesis. Finally, the birth and adjusted (i.e. births plus abortions) prevalence of neural tube defects for each year were determined.

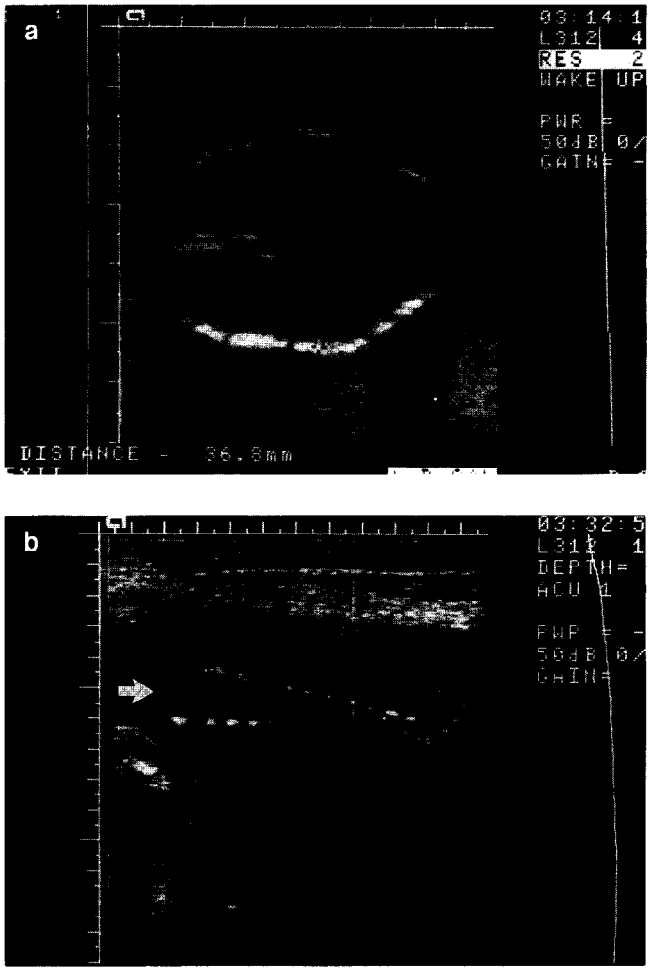

Figure 1 (a) Marked 'lemon sign' with skull contour scalloped by collapse of frontal bones, due to posterior brain herniation; (b) large open spina bifida in lumbosacral region, demonstrating broad splaying of dysraphic vertebra and disorganized floating nervous tissue (arrow)

RESULTS

During the period 1986–91, 49 982 women were screened, 48% of total births for the period (Table 1). The proportion of screened pregnancies has increased from 28% in 1986 to 60% in 1991. Of those women screened, 1671 (3.3%) were shown to have a raised serum α-fetoprotein level; this proportion was consistent throughout the testing period (range 2.4–3.9%). Of women with raised levels of maternal serum α-fetoprotein, 1325 (79%) went on to have a detailed fetal assessment. The reasons why the remaining 21% had no fetal assessment performed are listed in Table 2. Of those who had a fetal assessment, 64 (4.8%) were shown to have incorrect dates, which

Table 1 Annual numbers of deliveries for the Province of Manitoba, number of women who underwent screening for maternal serum α-fetoprotein, number of raised levels, and finally those with a high level who underwent detailed ultrasound evaluation

Year	*Deliveries*	*Screened*	*Raised maternal serum α-fetoprotein*	*Fetal assessment*
1986	17 112	4 798	165	131
1987	17 064	6 716	228	187
1988	17 129	8 450	259	222
1989	17 445	9 231	222	180
1990	18 011	10 362	389	313
1991	17 520	10 425	408	292
Total	104 281	49 982	1671	1325

Table 2 The reasons for no diagnostic fetal ultrasound evaluation performed in women with a raised level of maternal serum α-fetoprotein

Reason	*n*	*%*
Known multiple pregnancy	65	18.8
Incorrect dates	23	6.6
Fetal demise	35	10.1
Post-procedural rise in maternal serum α-fetoprotein	21	6.1
Follow-up by another center	36	10.4
Repeat maternal serum α-fetoprotein normal†	55	15.9
Lost to follow-up	111	32.0

*, Following chorionic villus sampling or amniocentesis. Levels normalized on repeat sampling; †, the policy of repeating an initially raised but borderline maternal serum α-fetoprotein level was discontinued after 1988

'normalized' the maternal serum α-fetoprotein levels, and 349 (26.3%) had a pregnancy abnormality (Table 3). Multiple pregnancy accounted for at least 50% of these abnormalities. Among the 70 fetal structural anomalies, 42 were neural tube defects (20 anencephaly, 22 spina bifida).

During this period, amniocentesis was performed 73 times (5.5% of fetal assessments, 4.4% of raised maternal serum α-fetoprotein). There were two primary reasons for amniocentesis. First, it was indicated for risk factors unrelated to the raised level of maternal serum α-fetoprotein, which included karyotype and other amniotic fluid studies in the assessment of ultrasound-diagnosed anomalies (35.6%), karyotype for advanced maternal age alone (37%) or amniotic fluid ΔOD_{450} in rhesus disease (2.7%), and accounted for 55 (75.3%) of all amniocenteses (Table 4). Second, amniocentesis procedures were carried out when technical quality was not satisfactory due to inadequate visualization (15.1%), or when an extremely raised level of maternal serum α-fetoprotein (> 5.0 MoM) was associated with another risk factor, such as a strong family history of neural tube defects (9.6%) (Table 5).

In the 912 women with an unexplained raised level of maternal serum α-fetoprotein in a singleton pregnancy, only 18 amniocenteses (2.0%) were performed specifically because of poor visualization of the fetus or accentuated risk. In most of these cases the maternal serum α-fetoprotein level was markedly increased to a mean 6.2 MoM (range 2.5–15.4). Maternal obesity limited the technical aspects of the scan; 60% of the women with an inadequate scan had required weight adjustment (instituted at 73 kg) for interpretation of their maternal serum α-fetoprotein level, compared to 29% of the total screened group. In

Table 3 The number of women with a high level of maternal serum α-fetoprotein who were shown to have a cause for the raised level on ultrasound scan (total = 1325)

	Number	*% Women having fetal assessment*
Incorrect dates	64	4.8
Multiple pregnancy	183	13.8
Fetal death	35	2.6
Fetal anomaly*	70	5.3
Placental anomaly	31	2.3
Oligohydramnios	30	2.3
Total	413	31.2

*, Includes 42 neural tube defects accounting for 3.2% of fetal assessments (20 anencephaly and 22 spina bifida)

Table 4 Amniocentesis performed for a known reason in combination with a raised level of maternal serum α-fetoprotein for the period 1986–91. These accounted for 75.3% of all amniocenteses performed in women with a raised level of maternal serum α-fetoprotein

Assessment of anomalies	*Number*
Spina bifida*	7
Ventral wall defects	7
Multiple congenital anomalies, trisomy 18	1
Abnormal placenta/chorioangioma triploidy	5
Hydrops	1
Bilateral choroid plexus cysts	2
Cystic hygroma	1
Other (increased V/H ratio, IUGR)	2
Advanced maternal age or rhesus disease	29
Total	55

*, Included one triploid fetus; V/H, ventricular/hemisphere ratio; IUGR, intrauterine growth retardation

Table 5 The number of amniocenteses performed to evaluate the fetus, in women with a singleton pregnancy considered to have an unexplained raised level of maternal serum α-fetoprotein (MSAFP)

		Amniocentesis	
Year	*Unexplained raised MSAFP, singleton*	*Poor visualization of fetus on ultrasound scan*	*Specific concern and raised MSAFP*
1986	96	1	2
1987	126	3	3
1988	145	3	0
1989	119	0	1
1990	216	4	1
1991	210	0	0
Total	912	11 (1.2%)	7 (0.8%)

Table 6 The annual prevalence (total of livebirths, stillbirths and pregnancy terminations) of neural tube defects (NTD) for the Province of Manitoba through the period 1986–91. The numbers detected by and missed by the maternal serum α-fetoprotein (MSAFP) screening program are listed, in addition to the effectiveness of detailed ultrasound scanning as a diagnostic procedure

Year	*Total number NTD*	*Detected by raised MSAFP*	*Missed by MSAFP screening*	*Missed by diagnostic ultrasound scan*
1986	22	2	1	0
1987	23	5	2	0
1988	21	9	1	0
1989	16	8	2	0
1990	24	11	3	0
1991	26	10	1	0
Total	122	44	10	0

none of these 18 cases was there amniotic fluid evidence of open neural tube defect; all had normal chromosomes. Karyotyping was performed on all 73 amniocentesis specimens regardless of indication for the tap. Karyotyping was performed on all anomalous newborn infants. Four chromosome abnormalities were discovered, three in anomalous fetuses (two by amniocentesis – trisomy 18 and triploidy; one at pregnancy termination – triploidy). The fourth aneuploidy (trisomy 21) was discovered at birth – the patient was counselled for amniocentesis regarding advanced maternal age but declined amniocentesis at 19 weeks. Detailed review of all follow-up records, including all hospital admissions of these infants within the first year of life, disclosed no other chromosome abnormalities.

The prevalence of neural tube defects (birth and adjusted) within Manitoba over this period was 122 (1.2 per 1000 births) which is the expected rate for this population with a high proportion of British and European descent (Table 6). No neural tube defect was missed by ultrasound examination. Over the 6 years, ten were not detected by α-fetoprotein screening due to the following reasons: an open lesion but maternal serum α-fetoprotein level below the cut-off level (six cases, two with associated chromosomal abnormality); closed lesions (three cases); and screened too early, at 7 weeks, with no repeat sample (one case).

DISCUSSION

With the development of high-resolution ultrasound, detailed evaluation of the fetal anatomy can be performed, enabling many congenital malformations to be diagnosed with certainty. With specific reference to neural tube defects, recognition that specific cranial signs are associated with spina bifida has increased the detection efficiency of the procedure[2,11]. The availability of the transvaginal probe now enables examination of the fetal anatomy even when it is deep within the maternal pelvis. The purpose of this review were two-fold:

(1) To analyze the results of the *prospective* application of those ultrasound principles, to the exclusion of amniocentesis when conditions were met; and

(2) To detail the experience of a large, centralized maternal serum α-fetoprotein screening program that features a single, dedicated Fetal Assessment Service.

Our results indicate that performance of high-resolution ultrasound by skilled and experienced personnel allows fetal anatomy to be examined thoroughly to make or exclude a diagnosis with confidence. No fetal neural tube defect was missed on ultrasound examination in this series. The policy of restricted amniocentesis continues to be a principle of our program. Therefore, in contrast to a previous report[12], the premise that routine amniocentesis should be performed in all unexplained elevations of maternal serum α-fetoprotein is challenged. Providing the 'null hypothesis' (i.e. that ultrasound as now practised will *never* miss a neural tube defect is an infinite process. Confidence limit (CL) calculations using the binomial formula for our population (CL = 0.0–0.4%) suggest a worst-case scenario of less than one neural tube defect per 1000 amniocenteses not done. We

believe that detection of 100% of the 42 neural tube defects among 1325 detailed fetal examinations is strong evidence.

In some centers, the supplementary measurement of amniotic fluid α-fetoprotein and/or acetylcholinesterase is performed to overcome absence of a dedicated ultrasound service contingent to the maternal serum α-fetoprotein screening program. However, with the increasing numbers of experienced scanners, this issue should be less of a problem. More likely, a greater influence on the number of amniocenteses performed is a policy to avoid litigation. It may be argued that such a policy is simpler for the physician and the patient. It would be faster in terms of appointment time, and it does not rely on the availability of scanning expertise. Furthermore, additional information such as the fetal karyotype may be provided. This 'advantage' needs to be challenged: the likelihood of karyotype abnormality of important developmental significance in the absence of any fetal structural anomaly (which would be detected by this ultrasound examination) is small (1 in 1309 normal fetuses in our study). No structurally normal newborn had a karyotype abnormality identified, but this program did not include routinely obtaining a karyotype from all babies. Other studies have shown that fetal sex chromosome abnormalities may be increased among women with increased levels of maternal serum α-fetoprotein[13], but most of these are not associated with deficits for which parents elect pregnancy termination when there are no associated ultrasound abnormalities[14].

Amniocentesis is an invasive procedure with defined risks that may be increased even further in the case of raised maternal serum α-fetoprotein[5]. Even assuming a minimum pregnancy loss rate of 0.5%, an additional four pregnancies would have been lost in this study, if the remaining 851, which had an unexplained elevated maternal serum α-fetoprotein, had proceeded to amniocentesis. This group had an increased risk for perinatal loss[15,16]. The number may even have been higher with routine amniocentesis. Such losses cannot be justified, if the detection rate of neural tube defects is 100% with non-invasive ultrasound scanning alone.

False-positive measurements of amniotic fluid α-fetoprotein and acetylcholinesterase are possible, particularly if a blood-stained amniotic sample is obtained[7], providing further unnecessary complication in 4% or more[17]. The additional advantage of a detailed fetal ultrasound examination is that other causes of a raised maternal serum α-fetoprotein may be identified, which are not detected by amniotic fluid measurements. Some even advocate a follow-up scan to identify the cause for the elevated maternal serum α-fetoprotein, such as increasing oligohydramnios and/or intrauterine growth retardation[16]. The benefits of such a policy would need to be tested in a randomized controlled trial, but use of a detailed 'first-visit' scan clearly provides the baseline for such surveillance.

The prevalence at birth of neural tube defects has decreased in developed countries since the introduction of screening using either maternal serum α-fetoprotein assessment, ultrasound, or both[18,19]. However, ultrasound used as a screening tool in large numbers of low-risk women does not have the sensitivity to detect all cases of neural tube defects[20]. In fact, such 'screening' ultrasound use is no longer recommended[21]. This review emphasizes that it is only when targeted high-risk women are evaluated by high-resolution ultrasound in an examination that takes time and skill, that all neural tube defects will be detected reliably.

The policy of relying on detailed fetal ultrasound examination, by experienced personnel, to identify all cases of open neural tube defects in women with elevated maternal serum α-fetoprotein, is validated. The experience of this large, centralized program in markedly restricting use of amniocentesis, provides a model for reducing unnecessary invasive testing.

REFERENCES

1. Burton, B. K. (1988). Elevated maternal serum alpha-fetoprotein (MSAFP): interpretation and follow-up. *Clin. Obstet. Gynecol.*, **31**, 293–305
2. Nicolaides, K. H., Gabbe, S. G., Campbell, S. and Guidetti, R. (1986). Ultrasound screening for spina bifida: cranial and cerebellar signs. *Lancet*, **2**, 72–4
3. Persson, P. H., Kullander, S., Gennser, G., Grennert, L. and Laurell, C. B. (1983). Screening for fetal malformations using ultrasound and measurements of alpha-fetoprotein in maternal serum. *Br. Med. J.*, **286**, 747–9
4. Consensus meeting (1985). Maternal serum alpha-fetoprotein screening for neural tube defects. *Prenat. Diagn.*, **5**, 77–83
5. Tabor, A., Madsen, M., Obel, E. B., Philip, J., Bang, J. and Norgaard-Pedersen, B. (1986). Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. *Lancet*, **1**, 1287–92
6. Hobbins, J. C., Venus, I., Tortora, M., Mayden, K. and Mahoney, M. J. (1982). Stage II ultrasound examination for the diagnosis of fetal abnormalities with an elevated amniotic fluid alpha-fetoprotein concentration. *Am. J. Obstet. Gynecol.*, **142**, 1026–9
7. Lee, J. E. S., Ng, W., Falk, R. E., Alfi, O., Lopez, L. and Platt, L. D. (1985). False-positive amniotic fluid acetylcholinesterase results: the need for a multifacet approach to the prenatal diagnosis of neural tube defects. *Obstet. Gynecol.*, **66**, 22–4S
8. Richards, D. S., Seeds, J. W., Katz, V. L., Lingley, L. H., Albright, S. G. and Cefalo, R. C. (1988). Elevated maternal serum alpha-fetoprotein with normal ultrasound: is amniocentesis always appropriate? A review of 26,069 screened patients. *Obstet. Gynecol.*, **71**, 203–7
9. Hogge, W. A., Thiagarajah, S., Ferguson, J. E., Schnatterly, P. T. and Harbert, G. M. (1989). The role of ultrasonography and amniocentesis in the evaluation of pregnancies at risk for neural tube defects. *Am. J. Obstet. Gynecol.*, **161**, 520–4
10. Nadel, A. S., Green, J. K., Holmes, L. B., Frigoletto, F. D. Jr and Benacerraf, B. R. (1990). Absence of need for amniocentesis in patients with elevated levels of maternal serum alpha-fetoprotein and normal ultrasonographic examinations. *N. Engl. J. Med.*, **323**, 557–61
11. Campbell, J., Gilbert, W. M., Nicolaides, K. H. and Campbell, S. (1987). Ultrasound screening for spina bifida: cranial and cerebellar signs in a high-risk population. *Obstet. Gynecol.*, **70**, 247–50
12. Drugan, A., Zador, I. E., Syner, F. N., Sokol, R. J., Sacks, A. J. and Evans, M. I. (1988). A normal ultrasound does not obviate the need for amniocentesis in patients with

elevated serum alpha-fetoprotein. *Obstet. Gynecol.*, **72**, 627–30
13. Watson, W. J., Cheschier, N. C., Katz, V. L. and Seeds, J. W. (1991). The role of ultrasound in the evaluation of patients with elevated MSAFP: a review. *Obstet. Gynecol.*, **78**, 123–8
14. Evans, J. A., MacDonald, K. and Hamerton, J. L. Sex chromosome abnormalities: prenatal diagnosis and the need for continued prospective studies. *Birth Defects*, **26**, 273 81
15. Nelson, L. H., Bensen, J. and Burton, B. K. (1987). Outcome in patients with unusually high maternal alpha-fetoprotein levels. *Am. J. Obstet. Gynecol.*, **157**, 572–6
16. Crandall, B. F., Robinson, L. and Grau, P. (1991). Risks associated with an elevated maternal serum alpha-fetoprotein level. *Am. J. Obstet. Gynecol.*, **165**, 581–6
17. Milunsky, A. and Sapirstein, V. S. (1981). Acetylcholinesterase (AChE) assay for prenatal diagnosis of neural tube defects (NTDs) (abstr.) *Pediatr. Res.*, **15**, 644
18. Cuckle, H. S., Wald, N. J. and Cuckle, P. M. (1989). Prenatal screening and diagnosis of neural tube defects in England and Wales in 1985. *Prenat. Diagn.*, **9**, 393–400
19. Davis, C. F. and Young, D. G. (1991). The changing incidence of neural tube defects in Scotland. *J. Pediatr. Surg.*, **5**, 516–18
20. Neven, P., Ricketts, N. E. M., Geirsson, R. T., Smith, R. and Crawford, J. W. (1991). Screening for neural tube defect with maternal serum alpha-fetoprotein and ultrasound without the use of amniocentesis. *J. Obstet. Gynecol.*, **11**, 5–8
21. Evans, M. I., Chervenak, F. A. and Eden, R. D. (1991). Report of the Council on Scientific Affairs of the American Medical Association: ultrasound evaluation of the fetus. *Fetal Diagn. Ther.*, **6**, 132–47