PRENATAL DIAGNOSIS, VOL. **17:2:** 101–108 (1997)

SCHWANGERSCHAFTSPROTEIN 1 (SP1) AS A MATERNAL SERUM MARKER FOR DOWN SYNDROME IN THE FIRST AND SECOND TRIMESTERS

Q.-P. QIN*, M. CHRISTIANSEN*, T. H. NGUYEN†, S. SØRENSEN‡, S. O. LARSEN§ AND B. NØRGAARD-PEDERSEN*

*Department of Clinical Biochemistry, Statens Serum Institut, Copenhagen, Denmark; †Department of Obstetrics and Gynecology, Herlev Hospital, University of Copenhagen, Denmark; ‡Department of Clinical Biochemistry, Hvidovre Hospital, University of Copenhagen, Denmark; §Department of Biostatistics, Statens Seruminstitut, Copenhagen, Denmark

Received 2 January 1996
Revised 24 June 1996
Accepted 6 July 1996

SUMMARY

The potential of the maternal serum concentration of schwangerschaftsprotein 1 (MSSP1) as a marker for Down syndrome (DS) pregnancies was evaluated in the fifth to the 20th gestational week using 156 DS pregnancies and 546 unaffected control pregnancies. In DS pregnancies, the median of the multiple of the median (MOM) of MSSP1 was 0·27 [95 per cent confidence interval (CI) 0·11–0·59] in weeks 5–9 ($n=25$) and 1·28 (CI 1·11–1·49) in weeks 14–20 ($n=117$), significantly different from controls ($P<10^{-6}$). In weeks 10–12, the median MSSP1 MOM was 0·89 (CI 0·20–2·09) ($n=14$), not different from controls ($P=0·42$). Using MSSP1 alone as a marker for DS gave—in empirical receiver-operator-characteristics (ROC) analysis—a detection rate of about 44 per cent for a false-positive rate of about 5 per cent in weeks 5–9 (using MSSP1 MOM $\leq$ cut-off), whereas a sensitivity of about 20 per cent was found for a false-positive rate of 5 per cent in weeks 14–20 (using MSSP1 MOM $\geq$ cut-off). In parameterized ROC analysis, the detection rates were 38 and 18 per cent for a false-positive rate of 5 per cent in weeks 5–9 and 14–20, respectively.

KEY WORDS: pregnancy-specific β1-glycoprotein; time-resolved immunofluorescence; Down syndrome; serum screening in first trimester; prenatal diagnosis

INTRODUCTION

Schwangerschaftsprotein 1 (SP1), also known as pregnancy-specific β1-glycoprotein, is a glycoprotein produced by placenta syncytiotrophoblast and secreted into the maternal circulation (Bohn, 1979). The concentration of SP1 in maternal serum (MSSP1) increases throughout pregnancy (Schultz-Larsen and Herz, 1978; Lenton *et al.*, 1981) and it is a prognostic indicator in pregnancy complications (Brambati *et al.*, 1991; Johnson *et al.*, 1993). In Down syndrome (DS) pregnancies, elevated MSSP1 in the second trimester has been described (Bartels and Lindemann, 1988; Wald *et al.*, 1989; Knight *et al.*, 1989; Bartels *et al.*, 1990, 1994; Petrocik *et al.*, 1990; Graham *et al.*, 1992). In the first trimester, MSSP1 has been found to be reduced (Brock *et al.*, 1990; Brambati *et al.*, 1992; Macintosh *et al.*, 1993; Bersinger *et al.*, 1994) in DS pregnancies. There is, however, still some uncertainty on the clinical importance of these findings with respect to maternal serum screening for Down syndrome (Spencer, 1991; Macintosh *et al.*, 1993), due to the few studies that have been performed and the low number of Down syndrome pregnancies included in these, especially in the first trimester.

In this study we have analysed the diagnostic accuracy of MSSP1 as a marker for Down

Addressee for correspondence: Michael Christiansen, MD, Department of Clinical Biochemistry, Statens Serum Institut, 5 Artillerivej, DK 2300 S, Copenhagen, Denmark.

CCC 0197–3851/97/010101–08

syndrome in 156 Down syndrome pregnancies as compared with 546 unaffected controls and have attempted to define optimal 'diagnostic windows', i.e. parts of gestation where MSSP1 is useful as a marker. Furthermore, we describe an assay for SP1 based on time-resolved immunofluorescence.

MATERIALS AND METHODS

Patient samples ($n=156$) from pregnancies with a Down syndrome fetus and unaffected controls ($n=546$) were collected in the course of a screening programme for Down syndrome and malformations at Statens Seruminstitut, Copenhagen. DS pregnancies were diagnosed at chorionic villus sampling or amniocentesis ($n=120$) or at birth ($n=36$). All diagnoses were confirmed by karyotyping. Gestational age was determined by the last menstrual period, and in most cases confirmed by ultrasound examination. Serum samples from early pregnancy were recovered from the screening programme for syphilis in pregnancy at Statens Seruminstitut. Serum samples from blood donors ($n=139$) were obtained from Blodbanken, Rigshospitalet, Copenhagen. Twenty serum samples from pregnant women were obtained from the Department of Clinical Biochemistry, Hvidovre Hospital, Copenhagen, where SP1 concentrations had been determined by rocket immunoelectrophoresis (Sørensen, 1978).

MSSP1 was determined by a non-competitive time-resolved immunofluorometric assay using a rabbit antibody against SP1 (A131, lot 124, DAKO A/S, Glostrup, Denmark). Briefly, the assay was carried out as follows: Maxisorp microtitre plates (Nunc A/S, Roskilde, Denmark) were coated with 10 μg/ml anti-SP1 antibody in 15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9·6 overnight (100 μl/well) at room temperature. After washing in the wash buffer [5 mM Tris–HCl, 150 mM NaCl, 0·005 per cent (w/v) Tween 20, 0·1 per cent (w/v) Germall II, pH 7·75] (Wallac OY, Turku, Finland), plates were blocked for 2 h at room temperature with 200 μl/well dilution buffer [10 mM phosphate, 150 mM NaCl, 0·25 per cent (w/v) bovine γ-globulin (Sigma G 7516, Sigma Chemical Co., U.S.A.), 1 per cent (w/v) bovine serum albumin (Sigma A4503, Sigma Chemical Co., U.S.A.)]. One hundred microlitres of either standards or samples (both in duplicate) diluted in dilution buffer (dilution factor 10–1000 times according to gestational age) were added to each well and incubated for 3 h with slow shaking. After washing, 25 ng of Eu^{3+}-chelate labelled rabbit anti-SP1 (DAKO A131) in 100 μl of dilution buffer was added to each well and incubated overnight. After washing, 200 μl of enhancement solution (Wallac OY, Turku, Finland) was added to each well and time-resolved fluorescence was measured at 613 nm. Data were analysed using the Multicalc software package (Wallac OY, Turku, Finland). Rabbit anti-SP1 (A131) was labelled using a commercial Eu^{3+}-labelling kit (Wallac OY, Turku, Finland), following the manufacturer's instructions, and an approximate content of 8·5 Eu molecules per molecule of IgG was obtained. As a standard WHO IRP 78/610 (WHO International Laboratory, Statens Seruminstitut, Denmark) was used. When the contents of one ampoule were dissolved in 0·75 ml of distilled water, the concentration of SP1 was 100 IU/l (Bohn *et al.*, 1980).

Groups were compared using the Mann–Whitney *U*-test and correlation was performed by Spearman's rank method. Due to the non-log-normal distribution of MSSP1 values in controls as a function of the gestational age, it was not possible to construct 'smoothed' medians by log-linear regression. As the distribution of MSSP1 values in each gestational week was markedly skewed (see Fig. 2), we obtained an estimate of the population median as the antilog_{10} to the mean of the $\log_{10}$ MSSP1 for each week (Table I). All the estimated population medians fell within the 95 per cent confidence interval of the empirical median. Confidence intervals were non-parametric. Diagnostic accuracy was analysed by receiver-operator-characteristics (ROC) curves, either empirically based on the actual MOM values or based on the theoretical distributions of MOM values in controls and Down syndrome pregnancies. Normality of distributions was assessed by normal plots and by Shapiro–Wilk's test.

RESULTS

A standard curve and intra-assay precision profile of the time-resolved immunofluorescence assay of SP1 is shown in Fig. 1. The detection limit was 0·25 mIU/l and the assay went up to 62·5 mIU/l. The intra-assay precision profile demonstrates that the intra-assay variability was less than 10 per cent in the dynamic range. The inter-assay coefficient of variation was 6·7 per cent. Mean recovery (range)

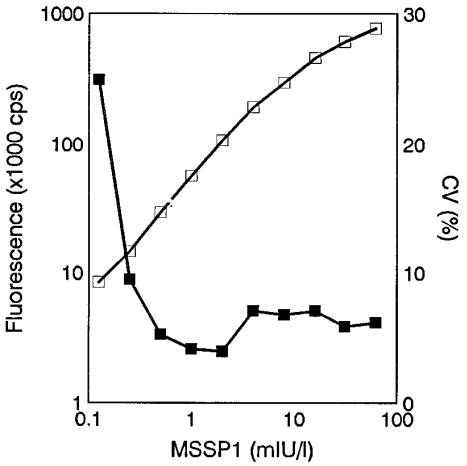

Fig. 1—Standard curve of the time-resolved immunofluorometric assay for SP1 (□——□) and the intra-assay precision profile (■——■). Each point represents the mean of eight determinations

after addition of the standard (6·20 mIU) to serum samples at concentrations 0·69, 9·32, and 41·14 mIU/l, assayed in triplicate, was 102·5 per cent (101·1–104·7 per cent). The dilution curves of three serum samples were parallel with the standard curve. Determination of MSSP1 by the time-resolved immunofluorometric assay (TrIFMA) correlated excellently with determinations by rocket immunoelectrophoresis ($r=0·99$, $P<0·01$, $n=20$). From a difference plot, it was found that the TrIFMA gave slightly higher values [mean difference (SD): 5·6 mIU/l (3·5 mIU/l)]. SP1 was only detectable in serum from 4 out of 70 male blood donors (range 0·28–1·80 mIU/l) and from 9 out of 69 female blood donors (range 0·26–0·69 mIU/l). The stability of SP1 was examined after heating serum samples to 56°C for 30 min (conditions used for inactivation of complement) and after 1, 2, 5, and 8 freeze/thaw cycles. Heat treatment caused a significant reduction (from a mean of 7·95 IU/l to 1·06 IU/l, $n=40$, $P<0·001$), whereas up to 8 freeze/thaw cycles did not have any effect on the concentration of SP1.

The distribution of MSSP1 in unaffected control pregnancies as a function of the completed gestational weeks is shown in Fig. 2 and described—with approximated population medians—in Table I. The distributions were markedly skewed and particularly so in the early first trimester.

Using the population medians, multiples of the median MSSP1 were calculated for Down syndrome pregnancies. In Table II the individual

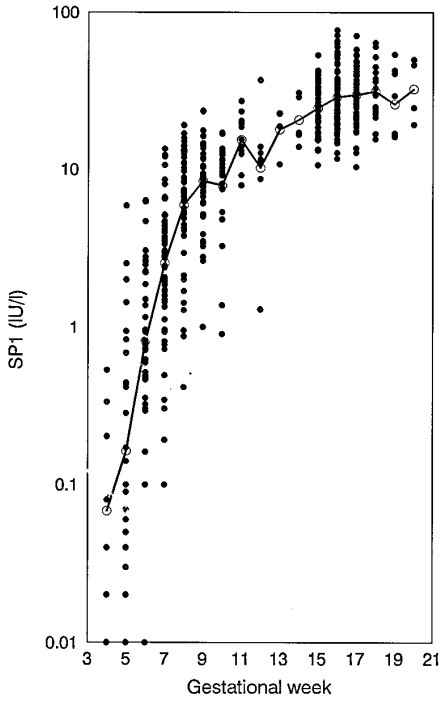

Fig. 2—Scatterplot of SP1 concentrations in the serum of normal pregnant women ($n=546$) as a function of the gestational age. The population medians are shown connected with a line (○——○)

MSSP1 MOM values for 39 Down syndrome pregnancies in the first trimester are shown, together with the medians of MOM for each gestational week of DS pregnancies in weeks 14–20 ($n=117$). Analysed week-by-week, the median MOMs were below 1 until week 10 and the difference in MOMs before (5th–9th week) and after week 10 (10th–12th week) was significant ($P=0·03$). The distribution of MSSP1 MOM values of DS pregnancies as a function of the gestational age is shown in Fig. 3. The difference in distribution of the MSSP1 MOM values between DS pregnancies and controls from the same period of gestation was significant in weeks 5–9 ($P<10^{-27}$) and in weeks 14–20 ($P<10^{-6}$), whereas the difference was not significant in weeks 10–12 ($P=0·42$) (Table II). The results indicated that DS pregnancies were characterized by reduced MSSP1 MOMs before the tenth gestational week and elevated MOMs from weeks 14–20. Log-normal distributions of MSSP1 MOMs in these two periods of gestation were estimated as detailed in Table III. The distribution of MSSP1 MOMs of controls in weeks 5–9 was not compatible with a

Table I—Medians of MSSP1 (IU/l) in control pregnancies in different gestational weeks

Week	Empirical median (CI)*	Range	*N*	Population median†
4	0·06 (0·01–0·53)	0·01–0·53	8	0·07
5	0·17 (0·05–0·43)	0·01–5·85	31	0·16
6	0·79 (0·62–0·96)	0·01–6·29	44	0·81
7	3·03 (2·40–3·68)	0·10–13·51	71	2·55
8	6·71 (5·18–8·32)	0·41–19·24	56	5·90
9	9·09 (7·29–11·59)	1·00–23·73	50	8·38
10	9·58 (7·54–11·36)	0·90–17·18	26	7·85
11	15·57 (9·10–23·50)	7·89–27·27	11	15·51
12	11·21 (8·66–14·00)	1·30–37·10	9	10·18
13	20·82 (not calc.)	10·74–22·88	4	18·02
14	17·23 (not calc.)	14·00–30·90	5	20·74
15	25·51 (21·37–30·21)	10·64–53·35	53	24·66
16	28·33 (25·59–32·28)	11·66–77·05	93	28·96
17	29·67 (27·81–40·20)	10·35–71·05	55	29·83
18	31·50 (23·48–41·58)	15·61–63·84	18	31·48
19	23·24 (16·17–53·68)	16·17–53·68	8	26·09
20	35·53 (not calc.)	19·38–49·79	4	32·43

Not calc. = not calculated (low number).
*CI = 95 per cent confidence interval of median.
†See Materials and Methods for details.

log-normal distribution according to Shapiro–Wilk's test ($P = 0{\cdot}0012$). However, Shapiro–Wilk's test is very sensitive to outliers (Altman, 1991) and as the normal plot did not show any major deviation from log-normality, we decided to estimate the population distribution of MSSP1 MOMs as a log-normal distribution. The theoretical distributions of DS and control pregnancies are depicted in Fig. 4.

There was no significant correlation between MSSP1 MOMs and maternal age in either the group of DS pregnancies from weeks 5–9 or in the 14th–20th gestational week group.

The diagnostic accuracy of MSSP1 MOM determination alone in discriminating between DS pregnancies and normal unaffected pregnancies was analysed by constructing empirical ROC curves for MSSP1 MOM values in weeks 5–9 (using MSSP1 MOM $\leq$ cut-off) and weeks 14–20 (using MSSP1 MOM $\geq$ cut-off); the resulting curves are shown in Fig. 5. From the curves it can be estimated that a detection rate of about 44–48 per cent can be obtained for a false-positive rate of about 5 per cent in weeks 5–9 and a sensitivity of about 20 per cent for a false-positive rate of 5 per cent in weeks 10–20. Using the theoretical distributions of controls and DS pregnancies (Table III and Fig. 4), parameterized ROC curves were constructed as shown in Fig. 5. From the parameterized curves the detection rate could be estimated to be 38 per cent in weeks 5–9 and 18 per cent in weeks 14–20 for a false-positive rate of 5 per cent. From Fig. 5 it can be seen that the use of estimated log-normal distributions of MSSP1 MOMs seem to underestimate rather than overestimate the detection rate, as compared with the empirical data.

DISCUSSION

The values found for MSSP1 in normal controls correspond well to values reported by others (Lenton *et al.*, 1981; Sørensen *et al.*, 1995) and the distribution as a function of the gestational age, with an early very steep rise followed by what could be considered an exponential rise with decreasing slope, has previously been suggested as a model for the development of MSSP1 over gestational age (Sørensen *et al.*, 1995). This finding, together with the good operational characteristics of the TrIFMA as described in this study, makes the TrIFMA suitable for inclusion in large volume screening programmes, particularly so, as a system for automation and simultaneous

Table II—MSSP1 MOM values of DS pregnancies

Week	Individual MOM	Median MOM*	*N*†
5	0·62	0·62	4 (1)
	0·62		
	0·00‡		
	1·73		
6	0·12	0·15	6 (1)
	0·33		
	0·18		
	0·02		
	1·10		
	0·12		
7	0·06	0·43	4 (0)
	0·27		
	0·59		
	2·81		
8	0·06	0·08	5 (2)
	0·08		
	0·03		
	0·16		
	0·47		
9	0·11	0·52	6 (0)
	0·11		
	0·46		
	1·88		
	0·67		
	0·58		
10	0·24	1·14	7 (1)
	2·09		
	0·63		
	1·14		
	2·28		
	0·14		
	5·04		
11	0·10	0·62	2 (1)
	1·14		
12	0·20	0·34	5 (1)
	0·23		
	1·51		
	1·98		
	0·34		
14		1·53 (0·05–2·70)	7
15		1·30 (0·98–1·65)	32
16		1·09 (0·97–1·74)	27
17		1·17 (1·02–1·41)	21
18		1·74 (1·16–1·91)	16
19		1·04 (0·44–2·49)	11
20		1·36 (n.c.)	3
Weeks 5–9		0·27 (0·11–0·59)[1]	25 (4)
Weeks 10–12		0·89 (0·20–2·09)[2]	14 (3)
Weeks 5–12		0·34 (0·16–0·63)	39 (7)
Weeks 14–20		1·28 (1·11–1·49)[3]	117

*95 per cent confidence interval of median. †Number diagnosed at birth. ‡Not detectable. Comparison with controls: (1) $P<10^{-27}$, (2) $P=0{\cdot}42$, and (3) $P<10^{-6}$ (Mann–Whitney).

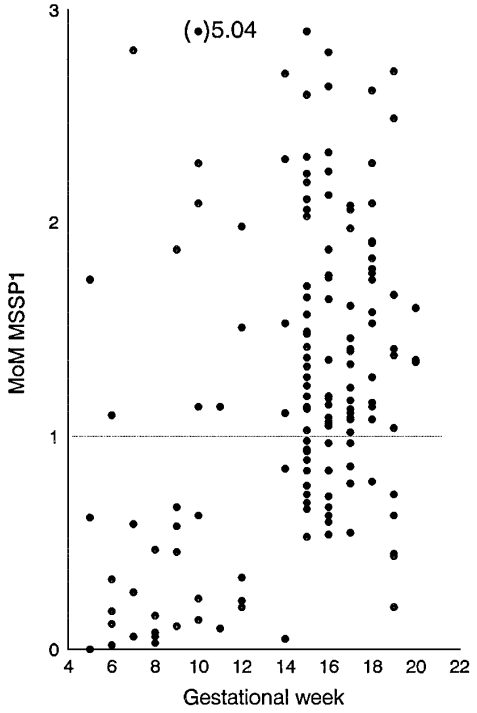

Fig. 3—Scatterplot of MOM MSSP1 values as a function of the gestational age in DS pregnancies ($n=156$)

determination of other parameters by time-resolved immunofluorescence is commercially available (Wallac OY, Turku, Finland). The close correspondence between the MSSP1 values determined by the TrIFMA and rocket immunoelectrophoresis (RIE) is important as SP1 exists in many forms—partly due to many different transcribed genes (Streydio *et al.*, 1990; McLenachan *et al.*, 1994) and partly due to aggregation and complex formation—in serum and this may result in discrepant results using different methods of quantitation (Teisner *et al.*, 1979).

The performance of MSSP1 determination in discriminating between DS and normal pregnancies is excellent, particularly in the early first trimester (Figs 4 and 5). From Table II and Figs 4 and 5 it can be seen that two diagnostic windows, namely from week 5 to week 9 and from week 14 to week 20, should be used when applying MSSP1 as a marker for Down syndrome. If samples from weeks 5–14 are analysed using the same diagnostic window, the resulting MOM will depend solely on the distribution of samples. This may—as previously suggested (Macintosh, 1994)—be the case for several of the studies published on MSSP1 in

Table III—Parameters of the estimated normal distribution of log MOM MSSP1

		Mean	SD
5th–9th week			
Controls (n=252)	Log MOM MSSP1	0·0002	0·4419
DS pregnancies (n=24)	Log MOM MSSP1	− 0·5763	0·5610
14th–20th week			
Controls (n=236)	Log MOM MSSP1	0·0001	0·1718
DS pregnancies (n=116)	Log MOM MSSP1	0·1034	0·2011

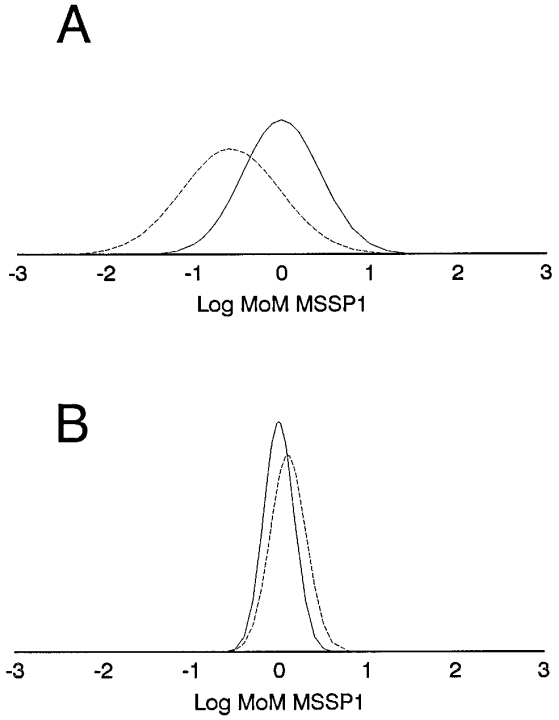

Fig. 4—The estimated log-normal distributions of MOM MSSP1 values in (A) weeks 5–9 and (B) weeks 14–20. The dashed line represents the DS pregnancies. The unbroken line represents unaffected controls. Means and standard deviations are given in Table III

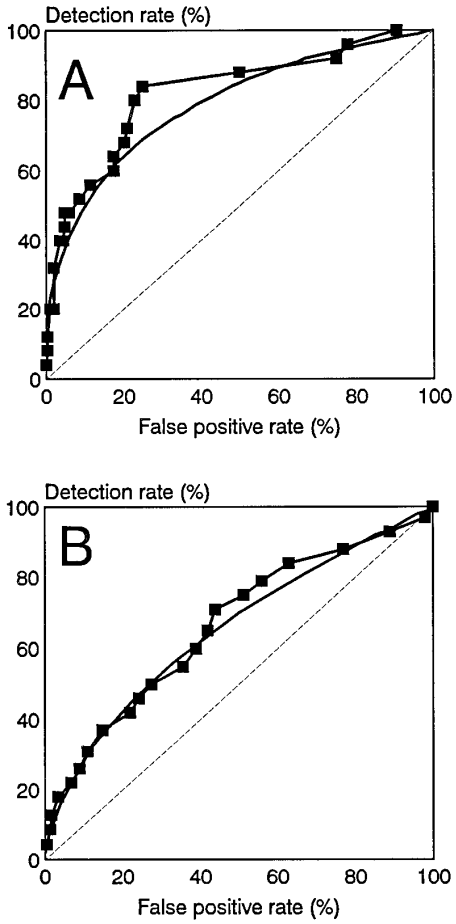

Fig. 5—Receiver-operator-characteristics (ROC) curves for the detection of DS pregnancies using (A) MOM MSSP1 ≤ cut-off in weeks 5–9 and (B) MOM MSSP1 ≥ cut-off in weeks 14–20. (■——■) Empirical ROC curves; (——) parameterized ROC curves. (See text for details)

the first trimester. Another reason for discrepant results in MSSP1 MOM values may be the use of serum samples from pregnancies where the DS diagnosis was made in the second trimester or at birth, as compared with studies only comprising samples from pregnancies terminated in the first trimester. The latter selection of DS pregnancies will contain many pregnancies that are destined for spontaneous abortion and where the MSSP1 may reflect this (Brambati *et al.*, 1991), rather than the presence of a DS fetus. In a recent meta-analysis on MSSP1 (Cuckle, 1994), it was found that in pregnancies prior to the completed 15th week of

gestation the median MSSP1 MOM in DS pregnancies was 0·60 ($n=35$), as opposed to a median MOM of 1·46 in pregnancies after the 15th week ($n=261$). In several studies in the second trimester it has been shown that MSSP1 does not add to the efficiency of a multiple-marker screening system if hCG is already included (Wald *et al.*, 1989; Bartels *et al.*, 1994). This could be caused by the high correlation between concentrations of SP1 and hCG in maternal blood found in the latter study (Bartels *et al.*, 1990) and the correlation of MOMs of SP1 and hCG, AFP and uE3 found by Wald *et al.* (1989). However, others (Petrocik *et al.*, 1990) found that MSSP1 with age alone gave a sensitivity of 71·7 per cent for a false-positive rate of 4·3 per cent and this sensitivity was increased to 78·3 per cent with a false-positive rate of 3·4 per cent when the screening also included hCG and AFP. In the present study, data on other parameters were not available, but hCG and SP1 show a different development of serum concentration with gestational age, particularly late in the first trimester (Sørensen *et al.*, 1995), and the secretion of SP1 and hCG from pre-embryos *in vitro* is discordant (Dimitriadou *et al.*, 1992). The mutually independent regulation of secretion of SP1 and hCG makes it unlikely that determination of one parameter makes it unnecessary to measure the other. This may, however, depend on the period of gestation, as the information content of MSSP1 and other markers varies greatly with gestational age.

The final solution to the clinical usefulness of including MSSP1 as a marker for DS pregnancies must depend on multiple-marker studies on large materials of fresh samples—preferably in the form of clinical trials.

In conclusion, we suggest (i) that MSSP1 is included in multimarker studies, particularly in early first trimester; and (ii) that MSSP1 is used as a marker with two diagnostic windows, i.e. from week 5 to week 9 (using a low value of MSSP1 MOM as a risk marker) and from week 14 to week 20 (using a high value of MSSP1 MOM as a risk marker). However, as quoted above, several previous studies have indicated that the gain in detection rate obtained by including MSSP1 as a serum marker in the second trimester may be marginal.

REFERENCES

Altman, D.G. (1991). *Practical Statistics for Medical Research*, 1st edn, London: Chapman & Hall, 139.

Bartels, I., Lindemann, A. (1988). Maternal levels of pregnancy-specific β_1-glycoprotein (SP-1) are elevated in pregnancies affected by Down's syndrome, *Hum. Genet.*, **80,** 46–48.

Bartels, I., Thiele, M., Bogart, M.H. (1990). Maternal serum hCG and SP1 in pregnancies with fetal aneuploidy, *Am. J. Med. Genet.*, **37,** 261–264.

Bartels, I., Bockel, B., Caesar, J., Krawczak, M., Thiele, M., Rauskolb, R. (1994). Risk of fetal Down's syndrome based on maternal age and varying combinations of maternal serum markers, *Arch. Gynecol. Obstet.*, **255,** 57–64.

Bersinger, N.A., Brizot, M.L., Johnson, A., Snijders, R.J., Abbott, J., Schneider, H., Nicolaides, K.H. (1994). First trimester maternal serum pregnancy-associated plasma protein A and pregnancy-specific beta 1-glycoprotein in fetal trisomies, *Br. J. Obstet. Gynaecol.*, **101,** 970–974.

Bohn, H. (1979). Placental and pregnancy proteins. In: Lehmann, F.G. (Ed.). *Carcino-embryonic Proteins*, Vol. 1, Amsterdam: Elsevier, 289–299.

Bohn, H., Chard, T., Grudzinskas, J.G., Klopper, A., Rosen, S., Schultz-Larsen, P., Seppäla, M., Sizaret, P., Tatarinov, Y.S., Teisner, B. (1980). Reference preparation for assay of some pregnancy and cancer associated proteins, *Lancet*, **ii,** 796.

Brambati, B., Lanzani, A., Tului, L. (1991). Ultrasound and biochemical assessment of first trimester pregnancy. In: Chapman, M., Grudzinskas, G., Chard, T. (Eds). *The Embryo*, London: Springer-Verlag, 181–194.

Brambati, B., Chard, T., Grudzinskas, J.G., Macintosh, M.C.M., Lanzani, A., Bonacchi, I. (1992). Potential first trimester biochemical screening tests for chromosome anomalies, *Prenat. Diagn.*, **12** (Suppl), S4.

Brock, D.J.H., Barron, L., Holloway, S., Liston, W.A., Hillier, S.G., Seppäla, M. (1990). First-trimester maternal serum biochemical indicators in Down syndrome, *Prenat. Diagn.*, **10,** 245–251.

Cuckle, H. (1994). Screening at 11–14 weeks of gestation: the role of established markers and PAPP-A. In: Grudzinskas, J.G., Chard, T., Chapman, M., Cuckle, H. (Eds). *Screening for Down's Syndrome*, Cambridge: Cambridge University Press, 311–324.

Dimitriadou, F., Phocas, I., Mantzavinos, T., Sarandakou, A., Rizos, D., Zourlas, P.A. (1992). Discordant secretion of pregnancy specific beta 1-glycoprotein and human chorionic gonadotrophin by human pre-embryos cultured *in vitro*, *Fertil. Steril.*, **57,** 631–636.

Graham, G.W., Crossley, J.A., Aitken, D.A., Connor, J.M. (1992). Variation in the levels of pregnancy-specific beta-1-glycoprotein in maternal serum from chromosomally abnormal pregnancies, *Prenat. Diagn.*, **12,** 505–512.

Johnson, M.R., Riddle, A.F., Grudzinskas, J.G., Sharma Collins, W.P., Nicolaides, K.H. (1993). Reduced circulating placental protein concentrations

during the first trimester are associated with preterm labour and low birth weight, *Hum. Reprod.*, **8,** 1942–1947.

Knight, G.J., Palomaki, G.E., Haddow, J.E., Johnson, A.M., Osathanondh, R., Canick, J.A. (1989). Maternal serum levels of the placental products hCG, hPL, SP1 and progesterone are all elevated in cases of fetal Down syndrome, *Am. J. Hum. Genet.*, **45,** A263.

Lenton, E.A., Grudzinskas, J.G., Gordon, Y.B., Chard, T., Cooke, I.D. (1981). Pregnancy-specific beta 1-glycoprotein and chorionic gonadotrophin in early pregnancy, *Acta Obstet. Gynecol. Scand.*, **60,** 489–492.

Macintosh, M.C.M. (1994). Schwangerschaftsprotein 1 (SP1) and biochemical screening for Down's syndrome. In: Grudzinskas, J.G., Chard, T., Chapman, M., Cuckle, H. (Eds). *Screening for Down's Syndrome*, Cambridge: Cambridge University Press, 171–180.

Macintosh, M.C.M., Brambati, B., Chard, T., Grudzinskas, J.G. (1993). First-trimester maternal serum schwangerschaftsprotein 1 (SP1) in pregnancies associated with chromosomal anomalies, *Prenat. Diagn.*, **13,** 563–568.

McLenachan, P.A., Rutherfurd, K.J., Beggs, K.T., Sims, S.E., Mansfield, B.C. (1994). Characterization of the PSG11 gene, *Genomics*, **22,** 356–363.

Petrocik, E., Wassman, E.R., Lee, J.J., Kelly, J.C. (1990). Second trimester maternal serum pregnancy specific beta-1 glycoprotein (SP-1) levels in normal and Down syndrome pregnancies, *Am. J. Med. Genet.*, **37,** 114–118.

Schultz-Larsen, P., Herz, J.B. (1978). Predictive value of pregnancy-specific beta 1 glycoprotein in threatened abortion, *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **8,** 253–257.

Sørensen, S. (1978). An electroimmunoassay of the pregnancy specific β_1-glycoprotein (SP_1) in normal and pathological pregnancies, and its clinical value compared to human chorionic somatomammotropin (hCS), *Acta Obstet. Gynecol. Scand.*, **57,** 193–201.

Sørensen, S., Momsen, G., Ruge, S., Pedersen, J.F. (1995). Differential increase in the maternal serum concentrations of the placenta proteins human chorionic gonadotrophin, pregnancy-specific beta 1-glycoprotein, human placental lactogen and pregnancy-associated plasma protein A during the first half of normal pregnancy, elucidated by means of a mathematical model, *Hum. Reprod.*, **10,** 453–458.

Spencer, K. (1991). Pregnancy specific beta 1 glycoprotein in Down's syndrome screening: does it have any value? Proceedings of the ACB Meeting, *Ann. Clin. Biochem.*, **C46,** 108.

Streydio, C., Swillens, S., Georges, M., Szpirer, C., Vassart, G. (1990). Structure, evolution and chromosomal localization of the human pregnancy-specific beta 1-glycoprotein gene family, *Genomics*, **7,** 661–662.

Teisner, B., Grudzinskas, J.G., Hindersson, P., Al-Ani, A.T.M., Westergaard, J.G., Chard, T. (1979). Molecular heterogeneity of pregnancy specific β_1 glycoprotein: the effect on measurement by radioimmunoassay and electroimmunoassay, *J. Immunol. Methods*, **31,** 141–149.

Wald, N.J., Cuckle, H.S., Densem, J.W. (1989). Maternal serum specific β1-glycoprotein in pregnancies associated with Down's syndrome, *Lancet*, **2,** 450.