Clin Genet 2004: 65: 11–16
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CLINICAL GENETICS

Short Report

Screening for Down's syndrome in early and late first and second trimester using six maternal serum markers

Christiansen M, Larsen SO, Oxvig C, Qin Q-P, Wagner JM, Overgaard MT, Gleich GJ, Sottrup-Jensen L, Nørgaard-Pedersen B. Screening for Down's syndrome in early and late first and second trimester using six maternal serum markers.
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The efficiency of six maternal serum markers for Down's syndrome (DS), alpha fetoprotein (AFP), human chorionic gonadotropin (hCG), free β-hCG, pregnancy-associated plasma protein-A (PAPP-A), the proform of eosinophil major basic protein (ProMBP), pregnancy-specific-β-1-glycoprotein (SP_1), and combinations thereof, was examined. Discriminant analysis in 156 DS pregnancies and 546 controls defined three effective combinations of serum marker logMoMs (multiples of the median in control samples) in three gestational age windows, i.e. Index I (weeks 7–9) = 0.52 logMoM ProMBP + 0.28 logMoM PAPP-A − logMoM SP_1; Index II (weeks 10–12) = 1.94 logMoM free β-hCG − logMoM SP_1, and Index III (weeks 15–19) = 0.78 logMoM free β-hCG + 1.12 logMoM ProMBP − logMoM AFP. The estimated detection rates of indices and age for a false-positive rate (FPR) of 5% were 73% for Index I, 69% for Index II, and 60% for Index III. Including the ultrasound marker nuchal translucency, using a DS at term risk of 1 : 400 as cut-off, the detection rates of the indices increased to 86, 83, and 82% for FPRs of 4.3, 4.1, and 5.8%, respectively. The indices are promising markers for screening for DS.

M Christiansen[a], SO Larsen[b], C Oxvig[c], Q-P Qin[a], JM Wagner[d], MT Overgaard[c], GJ Gleich[d], L Sottrup-Jensen[c] and B Nørgaard-Pedersen[a]

[a]Department of Clinical Biochemistry, Statens Serum Institut, [b]Department of Biostatistics, Statens Serum Institut, Copenhagen, [c]Department of Molecular Biology, Aarhus University, Aarhus, Denmark, and [d]Department of Immunology and Medicine, Mayo Clinic and Foundation, Rochester, NY, USA

Key words: AFP – chromosome disease – first trimester – hCG – nuchal translucency – PAPP-A – prenatal diagnosis – ProMBP – screening – SP_1

Corresponding author: Dr Michael Christiansen, MD, Department of Clinical Biochemistry, 5 Artillerivej, DK-2300 Copenhagen S, Denmark.
Tel.: +45 32 68 36 51;
Fax: +45 32 68 38 78; e-mail: mic@ssi.dk

Received 6 May 2003, revised and accepted for publication 22 August 2003

Maternal serum screening for fetal malformations and Down's syndrome (DS) in 14–18 weeks of pregnancy is well established in many countries in the form of 'Triple screening' (1–3), with a detection rate of 66% for a false-positive rate (FPR) of 5% (4). The use of new markers, such as free β-hCG, free α-hCG, inhibin, and others may increase the detection rate for DS to about 80% with the same FPR (5–9). Screening for DS in first trimester (7–14 weeks of pregnancy) has become possible using pregnancy-associated plasma protein A (PAPP-A) and free β-hCG (10–14). The ultrasound marker nuchal translucency (NT) measured in week 10–14 has been shown (together with age) to detect 82.2% of DS for a FPR of 8.3% (15) and in combination with PAPP-A and β-hCG to achieve detection rates (DRs) in excess of 90% for a FPR of 5% (16).

The purpose of this study was to evaluate six maternal serum markers: AFP, hCG, free β-hCG, PAPP-A, ProMBP (the proform of eosinophil major basic protein), and SP_1 (pregnancy-specific β-1-glycoprotein) as markers of fetal DS pregnancies through pregnancy.

Materials and methods

Serum samples

Serum samples were obtained from 348 pregnant women with a normal pregnancy outcome (controls), i.e. without fetal chromosomal disease, and from 156 pregnant women carrying a DS fetus

(DS pregnancies), as verified by karyotyping following amniocentesis or chorion villus sampling (n = 120) or birth (n = 36). The samples were obtained through serum screening programs for syphilis and DS and fetal malformations performed at Statens Serum Institut, Copenhagen. All serum samples were obtained prior to any invasive diagnostic procedures, i.e. CVS or AC, as these may cause an elevation of marker values *per se*. The total number of serum samples from pregnancies with normal outcome was 546 as 198 women had samples taken both in the first and in the second trimester. The Danish Cytogenetic Central Registry (DCCR, Aarhus, Denmark) that registers all chromosomal diseases was routinely used to ascertain that none of the controls were pregnancies with a chromosomally diseased fetus. Table 1 summarizes the gestational age distribution of samples. ProMBP was only measured in 311 normal sera and 131 DS sera; however, these sera had largely the same distribution with respect to gestational age as the total material. Gestational age was determined from last menstrual period (LMP) and in most cases verified by ultrasound examination. DS and control samples were matched for length of storage and number of freeze–thaw cycles.

Biochemical measurements

Pregnancy-associated plasma protein-A (PAPP-A) and Schwangerschaftsprotein 1 (SP_1) were determined by in-house sandwich immunoassays, as previously described (14, 17). The ProMBP was determined by a two-site immunoradiometric assay (IRMA) after the reduction of the samples, as described elsewhere (18). Human chorionic gonadotropin (hCG) (nicked and non-nicked) and alpha fetoprotein (AFP) were determined by an Autodelfia analytical system (Wallac OY, Turku, Finland) using the appropriate kits. For some determinations of AFP and all of free β-hCG (nicked and non-nicked) determinations, the dual label kit was used (19) (Wallac OY).

Table 1. Distribution of serum samples from normal and Down's syndrome pregnancies in different gestational weeks

		Down's syndrome		
Weeks	Normals	Born	Aborted	Total
4	8	0	0	0
5	31	1	3	4
6	44	1	5	6
7	71	0	4	4
8	56	2	3	5
9	50	0	6	6
10	26	1	6	7
11	11	1	1	2
12	9	1	4	5
13	4	0	0	0
14	5	2	5	7
15	53	6	26	32
16	93	2	25	27
17	55	7	14	21
18	18	9	7	16
19	8	2	9	11
20	4	1	2	3
Total	546	36	120	156
Total weeks (4–12)	306	7	32	39

Statistical methods

All concentrations were $\log_{10}$ transformed. Compatibility with the normal distribution was assessed by probit diagrams. Correlations were calculated using Spearman's correlation coefficient. LogMOM (multiple of the median of control samples) values were calculated separately for first and second trimester. For weeks 13–20, a log-linear regression of normals was used to determine log median values for each gestational week. These median values were then subtracted from the observed value ($\log_{10}$ values) to give logMoM values for both normals and DS pregnancies. For the first trimester, the same procedure was used for ProMBP, PAPP-A, and AFP, but for the other three markers (SP_1, free β-hCG, and hCG), with no log-linear relationship in first trimester, the empiric $\log_{10}$ mean values for each week were used. Discriminatory ability was assessed by calculating the Mahalanobis distance as

$$D = \frac{m_{\mathrm{d}} - m_{\mathrm{n}}}{\sqrt{\frac{\left(s_{\mathrm{d}}^2 + s_{\mathrm{n}}^2\right)}{2}}},$$

where m denotes the mean logMoM value, s^2 the variance and d and n refer to DS and normal pregnancies, respectively. The Mahalanobis distance is a measure of the difference between distributions normalized for the difference in variation. A Monte Carlo simulation was used to estimate the efficiency of the optimized combinations of markers in screening (20). The distributional parameters of NT (21), the a priori risks for a DS pregnancy as a function of the mother's age (22), and the normalized distribution of maternal ages (23) were obtained from the literature. NT was considered independent of serum markers.

Results

Concentration and variation of markers in DS and normal pregnancies

Figure 1 shows the mean values of the $\log_{10}$ serum concentrations of each marker for normal

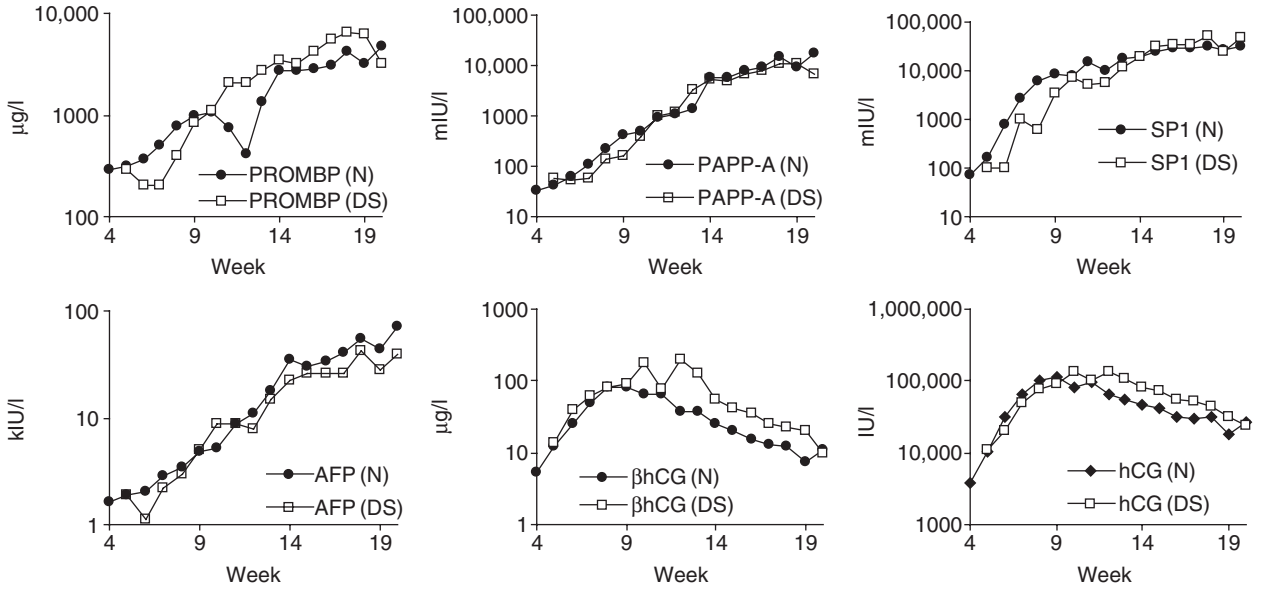

Fig. 1. The mean $\log_{10}$ concentration of maternal serum markers through gestation in normal (●) and Down's syndrome pregnancies (□).

pregnancies and for DS pregnancies for each gestational week. Apart from hCG and β-hCG where a dome-shaped development over time was seen, the markers increased through pregnancy. The within-week standard deviations of the logarithmic concentrations were calculated for the period 4–12 weeks and 13–20 weeks and were 0.2035 and 0.1571 for ProMBP, 0.2988 and 0.2595 for PAPP-A, 0.4324 and 0.1738 for SP_1, 0.2195 and 0.1785 for AFP, 0.3359 and 0.2597 for β-hCG, and 0.3215 and 0.2520 for hCG in normal pregnancies. In DS pregnancies, the within-week standard deviations in weeks 4–12 and 13–20 were 0.2313 and 0.2553 for ProMBP, 0.2697 and 0.2568 for PAPP-A, 0.5215 and 0.2363 for SP_1, 0.3414 and 0.2025 for AFP, 0.3965 and 0.3571 for β-hCG, and 0.2741 and 0.2902 for hCG. The standard deviations were similar for the two periods, although for SP_1, free β-hCG, and intact hCG, the variances were definitely higher in early pregnancy as compared with late pregnancy. The exponential rise in maternal serum concentrations of these markers in early pregnancy may account for this. For DS (Fig. 1), some of the log mean values are based upon few observations (Table 1) and the standard deviations were higher than in normal pregnancies.

In DS pregnancies (Fig. 1), serum concentrations of ProMBP, PAPP-A, and SP_1 were low in weeks 7–9. In weeks 10–12, free β-hCG and SP_1 were low and in weeks 15–20, free β-hCG, hCG, and ProMBP were high in DS pregnancies, whereas AFP was low. Generally, the $\log_{10}$MoM values tended for both trimesters to follow Gaussian (normal) distributions. However, as described elsewhere (17), the distributions for SP_1 were clearly skewed to the left so that some caution should be exercised in risk calculations based on this marker.

Definition of discriminatory indices

Based on the six marker profiles for normal and DS pregnancies throughout gestation, three combinations of markers were selected, e.g. Index I (weeks 7–9), Index II (weeks 10–12), and Index III (weeks 15–19), and linear combinations of logMoM values were optimized by discriminant analysis. The variances and covariances used were taken as the average value for normals and DS.

The final optimized indices were as follows:

$$\text{Index I} = 0.52\ \text{logMoM ProMBP} + 0.28\ \text{logMoM PAPP-A} - \text{logMoM SP}_1$$

$$\text{Index II} = 1.94\ \text{logMoM free}\beta\text{-hCG} - \text{logMoM SP}_1$$

$$\text{Index III} = 0.78\ \text{ogMoM free}\beta\text{-hCG} + 1.12\ \text{logMoM ProMBP} - \text{logMoM AFP}$$

Table 2 summarizes the mean values (*m*) and standard deviations (SD) for these indices for DS and for normals together with the Mahalanobis

Table 2. Three different gestational age windows and corresponding indices with Mahalanobis distances and detection rates for the indices (without maternal age) for two false-positive rates

Index	I (7–9 weeks)	II (10–12 weeks)	III (15–19 weeks)
Markers	ProMBP, PAPP-A, and SP_1	Free β-hCG and SP_1	
Free β-hCG, ProMPB, and AFP			
m_{downs}	0.6546	1.1956	0.5446
SD_{DOWNS}	0.4352	1.1304	0.4223
n	10	13	93
m_{normal}	−0.0004	0.0185	0.0123
$SD_{NORMALS}$	0.2699	0.4817	0.3064
n	89	46	146
D	1.81	1.35	1.44
DR (5% FPR)	69	63	53
DR (1% FPR)	52	52	33

D, Mahalanobis distance; DR, detection rate; FPR, false-positive rate; *m*, mean logMoM index; n, number of samples; SD, standard deviation logMoM index.

distance (*D*). The *D*-values in weeks 10–12 and weeks 15–19 were nearly identical (1.35 and 1.41, respectively), but in weeks 5–9, the *D*-value was higher (1.81). No difference in index values was found between DS cases born or aborted. In normal plots, all indices were normally distributed, both among controls and DS pregnancies. The detection rate of each index for two FPRs is also given in Table 2.

Table 3 summarizes the estimates of the performance of each index when used in combination with age in population screening, using a standardized age distribution of pregnant women (23) and a priori risks of DS (22). The performance seems to decrease from Index I to Index III.

Figure 2 shows receiver–operator characteristics curves for the indices in combination with age and nuchal translucency. The FPR is reduced from approximately 5 to 1% for a constant DR when adding nuchal translucency as marker.

Discussion

Biochemical screening for DS in the first trimester seems to be superior to second trimester screening. As all the DS cases were identified either at birth (36/156, 23.1%) or as a result of second trimester routine screening (120/156, 76.9%), the performance demonstrated relates directly to the reduction in the prevalence of DS in second trimester or at term. This is important, as a large proportion of DS fetuses are spontaneously aborted between first trimester and term (24). However, the number of DS pregnancies in the first trimester gestational age windows is small as reflected in the broad confidence intervals in Table 3.

The performance of screening in the first trimester may be improved by using serum markers such as SP_1 and ProMBP and the ultrasound marker nuchal translucency (25–27). SP_1 has previously been shown to be an excellent marker for DS in early first trimester (<10 weeks) (17); however, SP_1 as a single marker is not effective in discriminating between DS and normal pregnancies in weeks 10–12 (28). It is thus surprising that we have included SP_1 in Index II (10–12 gestational week). However, the performance of a marker combination is not predicted directly from the performance of the individual markers in one-dimensional screening (20).

The PAPP-A concentrations determined in the present study were obtained using a double polyclonal assay (14), and the discrimination between DS and normal pregnancies would no doubt have been better if a double monoclonal assay had

Table 3. Estimated performance of serum screening for Down's syndrome using indices in combination with maternal age

	1 : 400		1 : 250		5% FPR
Index (markers)	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (90% CI)
I (ProMBP, SP_1, and PAPP-A)	76.2	6.4	71.3	4.0	73 (50–96)
II (β-hCG and SP_1)	70.2	5.5	65.3	3.1	69 (48–90)
III (β-hCG, AFP, and ProMBP)	69.2	8.9	61.8	5.4	60 (52–68)

Detection rates and false-positives rates are given for the risk cut-offs 1 : 400 and 1 : 250 (for giving birth to a Down's syndrome child), respectively, and the detection rate for a false-positive rate of 5% is also given together with 90% confidence interval of the DR.

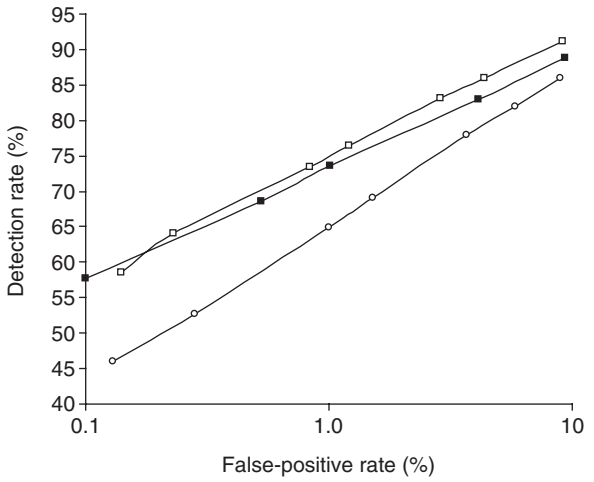

Fig. 2. Receiver–operator characteristics curves of Index I + age + NT (□), Index II + age + NT (■), and Index III + age + NT (○) as screening markers for Down's syndrome.

been used (29, 30). It is surprising that PAPP-A does not contribute significantly to the marker combination in week 10–12, but it may reflect that PAPP-A is a better marker in early first trimester than in late first trimester (21). However, the efficacy of β-hCG and SP_1 should be tested more thoroughly before a change of marker from PAPP-A to SP_1 can be recommended.

The good screening results obtained with different parameters indicate that it may be possible to design screening programs adapted to different communities, where the time of entry to prenatal care varies and where the ultrasound resources and expertise vary.

A tempting scenario for a prenatal screening program for chromosomal disease and fetal malformations would be an early (7–9 weeks) first trimester serum screening using either SP_1 alone (17) or Index I (ProMBP, PAPP-A, and SP_1) followed by a later first trimester (10–12 weeks) serum screening using PAPP-A and β-hCG or Index II (free β-hCG and SP_1) and nuchal translucency screening. After the latter, a decision could be made whether to perform CVS. If no invasive diagnostics is performed, serum 'triple screening' or Index III could be offered in second trimester. Furthermore, the combination of tests could be performed as 'integrated screening' (31) or 'contingent screening' with improved cost-effectiveness (32). These approaches – and the new indices – should be tested in prospective studies.

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