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Down syndrome maternal serum marker screening after 18 weeks' gestation

Françoise Muller[1]*, Sophie Dreux[1], Jean-François Oury[2], Dominique Luton[2], Serge Uzan[3], Michèle Uzan[4], Michel Levardon[5] and Marc Dommergues[6]

[1]*Service de Biochimie, Hôpital Ambroise Paré, Boulogne, France*
[2]*Maternité, Hôpital Robert Debré, Paris, France*
[3]*Maternité, Hôpital Tenon, Paris, France*
[4]*Maternité, Hôpital Jean Verdier, Paris, France*
[5]*Maternité, Hôpital Beaujon, Paris, France*
[6]*Maternité, Hôpital Necker, Paris, France*

Women having access to prenatal care late in pregnancy may still wish to benefit from maternal serum screening for Down syndrome. Therefore, we established reference values for α-feto protein (AFP) and free β-human chorionic gonadotrophin (β-hCG), and assessed the diagnostic value of maternal serum marker screening at 18–35 weeks' gestation based upon a series of 4072 sera from unaffected pregnancies and 118 sera from pregnant women with fetuses affected by Down syndrome. Using a 1/250 risk cut-off, a detection rate of 72.9% (95% CI = 71.5–74.3%) was achieved with a false-positive rate of 7.51% (95% CI = 6.71–8.3%). This was not significantly different from the percentages observed in our 14–17 weeks routine screening (50 596 patients): 71.9% (95% CI = 71.5–72.3%) and 6.48% (95% CI = 6.28–6.68%), respectively. Detection and screen-positive rates were, respectively, 51.3% (95% CI = 35.6–67.0%) and 5.95% (95% CI = 5.12–6.68%) in women aunder 35 years of age, and 84.8% (95% CI = 76.9–92.7%) and 24% (95% CI = 20.7–27.3%) in women aged 35 years and over. In conclusion, maternal serum marker screening is feasible at 18 weeks' gestation and later, which may be of interest in selected cases. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS: prenatal diagnosis; trisomy 21; amniocentesis; maternal serum markers; Down syndrome screening

INTRODUCTION

The efficacy of Down syndrome screening based on maternal serum markers has now largely been demonstrated (Haddow *et al*., 1992; Aitken *et al*., 1993; Muller *et al*., 1993a; Palomaki *et al*., 1997; Walton *et al*., 1999). Second trimester screening is usually done at 15–17 weeks' gestation. However, pregnant women who have access to prenatal care only later in pregnancy may still wish to benefit from Down syndrome screening. Therefore, after establishing reference values adjusted for gestational age, α-feto protein (AFP) and free β-human chorionic gonadotrophin (β-hCG), we studied the efficacy of maternal serum markers at 18–35 weeks' gestation using a second data set for sera from women with a trisomy 21-affected fetus.

PATIENTS AND METHODS

Maternal serum screening was based on two markers, AFP and free β-hCG, assayed in a single laboratory using the Perkin Elmer dual kit (Turku, Finland). Maternal serum samples were collected at 18–35 weeks' gestation from two sets of patients. The first set consisted of 4072 sera from women with singleton pregnancies unaffected by trisomy 21. We studied AFP and free β-hCG between 18 and 35 weeks' gestation in a logistic regression model. Median values thus defined allowed us to express AFP and free β-hCG as multiples of the median (MoM).

The second data set consisted of 118 sera from women with singleton pregnancies affected by trisomy 21 without mosaicism or translocation. Gestational ages (in weeks) were distributed as follow: 18 ($n = 20$), 19 ($n = 18$), 20 ($n = 20$), 21 ($n = 10$), 22 ($n = 8$), 23–24 ($n = 6$), 26–30 ($n = 15$) and 31–35 weeks ($n = 11$). In 15 cases, maternal serum had been sampled for screening purposes but gestational age turned out to be above 18 weeks. In 103 cases, blood was sampled from women immediately before amniocentesis indicated for advanced maternal age (57 cases), abnormal ultrasound findings (44 cases), or for a previously affected pregnancy (anencephaly in one and Turner syndrome in the second). AFP and free β-hCG were expressed as MoMs.

In both data sets, a risk calculation was performed using Perkin Elmer software as described by Wald *et al*. (1988). AFP and free β-hCG were entered as MoM based on our reference data set. Maternal weight was the only confounding factor taken into account. Using a 1/250 risk cut-off, the sensitivity and specificity of maternal serum markers at 18–35 weeks were compared with those observed in our routine 14–17 weeks trisomy 21 maternal serum marker screening programme (50 596 patients) over a 3-year period (1997–1999) in the same laboratory.

Screen-positive and detection rates were compared taking into account maternal age, which has an impact on these parameters (Muller *et al*., 1999, 2000).

*Correspondence to: Françoise Muller, Biochimie, Hôpital Ambroise Paré, 92104 Boulogne, France.
E-mail: francoise.muller@apr.ap-hop-paris.fr

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Gestational age was estimated by early first trimester ultrasonography in 95% of cases and was based on dates alone in the remaining 5%.

For each case included in the study, information on pregnancy outcome was obtained by mailing a data sheet to the obstetrical units. Up to five reminders were sent over a 6-month period. When no information could be obtained, a letter was sent to the patient herself.

Statistical analysis was performed using Statview software (SAS Institute Inc., Berkeley, CA, USA).

RESULTS

Reference values (4072 cases) of AFP and free β-hCG are displayed as a function of gestational age in Figures 1 and 2. Raw and regressed values are presented in Table 1.

In the subset of 118 sera from women with trisomy 21-affected pregnancies (Table 2) the median value of AFP was 0.87 MoM ($\log_{10} = -0.060$; SD = 0.259). AFP expressed as a MoM did not vary significantly with gestational age ($p > 0.05$). The median value of free β-hCG was 2.79 MoM (log = 0.446; SD = 0.309). Free β-hCG expressed as MoM did not vary significantly with gestational age ($p > 0.05$).

Using a 1/250 cut-off, risk calculation would have led to a 72.9% (95% CI = 71.5–74.3%) detection rate with a 7.51% (95% CI = 6.71–8.31%) false-positive rate (Table 2). This was not significantly different from the percentages observed in our 14–17 weeks routine screening (50 596 patients) 71.9% (95% CI = 71.5–72.3%) and 6.48% (95% CI = 6.28–6.68%), respectively.

In women under 35 years of age, detection and false-positive rates were, respectively, 51.3% (95% CI = 35.6–67.0%) and 5.95% (95% CI = 5.12–6.68%) when screening was performed at 18 weeks or later and 64.2% (95% CI = 60.7–67.7%) and 5.22% (95% CI = 5.02–5.42%) in the 14–17 weeks screening programme.

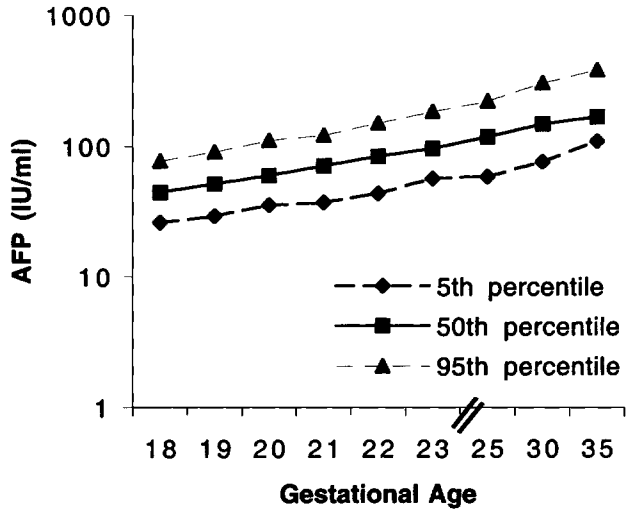

Figure 1—Normal values of AFP (IU/ml) as a function of gestational age

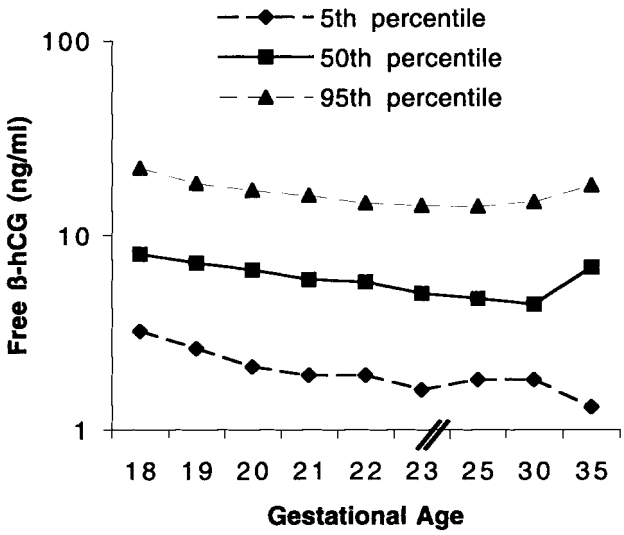

Figure 2—Normal values of free β-hCG (ng/ml) as a function of gestational age

In women aged 35 years and above, detection and screen-positive rates were, respectively, 84.8% (95% CI = 76.9–92.7%) and 24% (95% CI = 20.7–27.3%) when screening was performed at 18 weeks or later and 84.8% (95% CI = 81.6–88.0%) and 27.84% (26.84–28.84%) in the 14–17 weeks screening programme.

DISCUSSION

Down syndrome maternal serum marker screening was initially described at 15–17 weeks of gestation (Wald *et al.*, 1988), and its efficacy has been validated in large prospective series (Haddow *et al.*, 1992; Muller *et al.*, 1993a; Cuckle, 1996, 2000; Palomaki *et al.*, 1997; Wald *et al.*, 1997; Walton *et al.*, 1999). Although recent research has focused mainly on early screening methods allowing for first trimester prenatal diagnosis (Muller *et al.*, 1993b, Krantz *et al.*, 2000; Spencer *et al.*, 2000), in rare situations it may be desirable to evaluate the risk of aneuploidy later in the second trimester or even during the third trimester. For instance, women presenting late in gestation may wish to benefit from maternal serum screening. Reappraisal of an individual's risk of carrying an aneuploid child may also be of interest if minor sonographic abnormalities are found at second or third trimester routine ultrasound. Few studies have analysed the value of maternal serum screening after 16 weeks. In a study of 67 015 singleton pregnancies, Spencer observed a lower detection rate for the 22 cases observed at 17–19 weeks (59.1%) than for the 85 cases observed at 14–16 weeks (78.8%), and no results were provided after 19 weeks (Spencer, 1999).

The present study provides reference values for AFP and free β-hCG throughout gestation, and evaluates the potential efficacy of late maternal marker screening. At 18–35 weeks, overall sensitivity and specificity of markers were similar to those observed in the 14–17 weeks screening programme (respectively, 72.9% vs 71.9%)

Table 1—Maternal serum raw and regressed values of AFP and free β-hCG at each gestational age

Gestational age (weeks)	AFP (median IU/ml)	AFP (regressed)	Free β-hCG (median ng/ml)	Free β-hCG (regressed)
18 ($n = 2042$)	44.3	43	8	8
19 ($n = 901$)	51.1	51.1	7.2	7.2
20 ($n = 417$)	59.3	62.5	6.6	6.6
21 ($n = 254$)	69.8	72	5.9	6.1
22 ($n = 177$)	83.1	83.1	5.7	5.6
23 (n 103)	95.6	95.6	5	5.2
24–25 ($n = 112$)	117	112	4.7	4.6
26–30 ($n = 54$)	146	147	4.4	4.25
31–35 ($n = 12$)	166	166	6.8	4

Table 2—Maternal serum markers in controls and trisomy 21-affected pregnancies sampled after 18 weeks[a]

	All maternal ages		Women <35 years of age		Women ≥35 years of age	
	Unaffected pregnancies	T21-Affected pregnancies	Unaffected pregnancies	T21-Affected pregnancies	Unaffected pregnancies	T21-Affected pregnancies
n	4072	118	3440	39	632	79
Maternal age (median)	29	38	28	29	36	40
Gestational age (median)	19	21	18	25	19	20
MoM AFP (median)	1.00	0.87	1.00	0.84	1.01	0.88
MoM free β-hCG (median)	1.00	2.79	1.00	2.79	1.00	2.75
Patients at risk ≥1/250	7.51%	72.9%	5.95%	51.3%	24%	84.8%

[a] For 95% CI, see text.

and (7.51% vs 6.48%). Of the 118 Down syndrome cases, one-third of the samples were collected at 18–19 weeks, one-third at 20–22 weeks and one-third at 23–35 weeks. Nonetheless, more data based on large numbers of Down syndrome pregnancies at each gestational age are required to confirm that the performance at say 19 weeks is the same as that at say 33 weeks.

From a pathophysiological point of view, the present results suggest that the abnormalities in placental metabolism causing low AFP and high free β-hCG in trisomy 21 cases (Frendo *et al*., 2000) are not restricted to the early second trimester.

Because sensitivity of maternal serum markers increases and specificity decreases with maternal age (Muller *et al*., 1999, 2002), the relatively large number of women aged 35 years and above in the present series may have increased both the screen-positive and the detection rates. However, when the results were adjusted for maternal age, the efficacy of maternal serum markers was not significantly different between 18–35 and 14–17 weeks.

In conclusion, trisomy 21 screening by means of maternal serum markers is feasible throughout gestation, which may be of clinical value in selected patients.

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