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Second-trimester Down syndrome maternal serum screening in twin pregnancies: impact of chorionicity

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Objective To evaluate the diagnostic value of second-trimester maternal serum screening for Down syndrome in twin pregnancies.

Method On the basis of a prospective study of second-trimester maternal serum screening, we studied the distribution of alpha-fetoprotein (AFP) and free ß hCG in 3043 twin pregnancies with known outcome. There were 1561 dichorionic and 244 monochorionic pregnancies. The placental type was not available in 1238 cases. We compared 5 screening policies with the same risk, 1/250, cut-off: maternal age, maternal age corrected for the risk of having at least one affected twin in dichorionic pregnancies, maternal serum marker screening using observed AFP and free ß-hCG values divided by a factor of 2, by using the median values actually observed in the global twin population, or by the median values specific to mono- or dichorionic twins.

Results When expressed in singleton-derived MoMs, the median was 2.10 for AFP and 2.11 for free ß-hCG. The median AFP did not differ between monochorionic and dichorionic pregnancies. The distribution of free ß-hCG was significantly shifted towards greater values in monochorionic (2.16 MoM) compared to dichorionic (2.07) pregnancies ($p < 0.0001$). Screened-positive and detection rates were, respectively, 6.6% and 27.3% using maternal age alone, 24.6% and 54.5% using maternal age corrected for the risk of having at least one affected twin in dichorionic pregnancies, 7.75% and 54.5% using observed AFP and free beta-hCG values divided by a factor of 2, 8.05% and 54.5% using the median values actually observed in the global twin population, and 7.75% and 54.5% using the median values specific to mono- or dichorionic twins.

Conclusion Trisomy 21 second-trimester maternal serum screening is feasible in twins, and is better than a policy based on maternal age alone. Copyright © 2003 John Wiley & Sons, Ltd.

KEY WORDS: Down syndrome; prenatal screening; twin pregnancies; trisomy 21; AFP; hCG; multiple gestation

INTRODUCTION

Down syndrome screening in pregnant women expecting twins can be based on maternal age alone, on first-trimester nuchal translucency measurement, or on maternal serum markers combining the risk related to maternal age and to maternal serum markers. Early reports suggested that maternal serum screening for Down syndrome could be achieved in twins on the basis of the concept that maternal serum concentration of markers in twins would be twice that in singletons (Spencer *et al*., 1994; Wald and Densem, 1994; Barnabei *et al*., 1995; Neveux *et al*., 1996; Râty *et al*., 2000). Thus, an estimate of the risk of Down syndrome in twins could be made by entering half of the observed concentration of markers in a software designed for singletons. However, this approach is problematical for a number of reasons. In twin pregnancies, maternal serum marker levels are a reflection of both twins and may be confounded by the presence of an unaffected co-twin, resulting in a lower detection rate than in singleton pregnancies. In addition, data on the distribution of maternal serum markers are scarce in normal and in trisomy 21–affected twin pregnancies. Furthermore, while the maternal age-specific risk of having affected monozygotic twins is the same as for singletons, in dizygotic twins the age-related risk of having at least one affected child is higher (Meyers *et al*., 1997). Unfortunately, it is not possible to assess zygosity prenatally except following the sonographic diagnosis of a monochorionic placenta or of discordant fetal gender.

Taking advantage of a policy of offering maternal serum screening in twin pregnancies, we established reference ranges for alpha-fetoprotein (AFP) and free ß-hCG and assessed the screened-positive rate and the detection rate of maternal serum markers in twins, taking into account the impact of chorionicity.

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MATERIAL AND METHODS

Between January 1997 and June 2000, 3292 women with twin pregnancies underwent maternal serum marker screening at 14 to 22 weeks of gestation (median 15 weeks). There was no family history of trisomy 21. The outcome of pregnancy was known in the 3043 cases (92.4%) that form our database. All samples were assayed in the same laboratory. Maternal serum AFP and free ß-hCG were measured using the two-site fluoroimmunometric PerkinElmer kit (PerkinElmer, Turku, Finland). The intraassay and interassay precision was <3.5% for AFP and <4% for free ß-hCG. Trisomy 21 risk was calculated using the PerkinElmer software. Maternal age, maternal weight, gestational age, chorionicity, race-ethnicity, and smoking status were recorded. Gestational age was determined by first-trimester ultrasonography in 2891 cases (95%) and by dates in 152 cases. The diagnosis of chorionicity could be made in 1562 cases on the basis of either on first-trimester ultrasound evaluation of the T or lambda sign (Fisk and Bennett, 1995) or on post-natal placental examination.

From a practical point of view, the factor used to normalise serum marker concentrations was progressively corrected as our database increased, allowing us to use the median concentration observed in twins expressed in multiples of median (MoM) derived from singletons. When the risk of trisomy 21 was estimated as greater than 1/250, amniocentesis was offered.

Retrospective analysis of our database enabled us to reassess the distribution of free ß-hCG and AFP in twins.

We then estimated the screened-positive rate and the detection rate of maternal serum markers in twins using either a simple correction factor of 2 or using the values we observed in normal twins. In the subgroup in which chorionicity was known, we simulated a risk calculation on the basis of the specific maternal serum marker distribution that we observed in dichorionic and in monochorionic pregnancies.

We also simulated a screening policy on the basis of maternal age alone, using a cut-off of 37 years, which corresponds to a 1/250 risk in singletons.

In the subgroup in which chorionicity was known, we applied the correction proposed by Meyers *et al.*, 1997 to the subgroup of dichorionic twins. This consists in calculating the risk of having at least one affected fetus assuming that the dichorionic twins were dizygotic. Using a risk threshold of 1/250 of having at least one affected twin, this would lead to the offer of amniocentesis to women aged ≥34 years with dichorionic twins.

Statistical analysis of data was performed using the StatView 5 software (SAS Institute, Berkeley, USA). MoM distributions from monochorionic and dichorionic twin pregnancies were compared by the F-test of unequal variance. The chi-square test was used for comparison of percentages.

RESULTS

Twin pregnancies included in the study consisted of 3032 trisomy 21–unaffected pregnancies, 4 pregnancies with both twins affected by trisomy 21, and 7 with one trisomy 21–affected fetus. Demographic data are presented in Table 1.

The log-transformed distribution of AFP and free ß-hCG expressed in singleton-derived MoMs was analysed in pregnancies unaffected by trisomy 21 (Figures 1 and 2). The median was 2.10 for AFP (SD = 0.096) and 2.11 (SD = 0.183) for free ß-hCG (Table 2). When

Table 1—Demographic data

	Total	Monochorionic	Dichorionic
n	3043	245	1317
Maternal age (median, extremes)	30 (16–44)	29 (19–38)	31 (16–44)
Maternal weight (kg, median, extremes)	61 (39–138)	61 (43–137)	62 (43–137)
No. of patients <35 years (%)	82.3%	84%	81.8%
Smokers (%)	6.5%	4.5%	6.5%
Patients of Asian origin (%)	0.66%	1.29%	0.77%

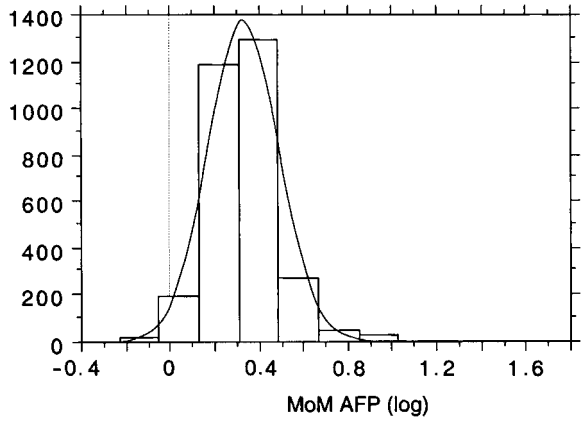

Figure 1—Distribution of maternal serum AFP (log MoM) in twin pregnancies. Log median MoM = 2.10 and standard deviation = 0.096

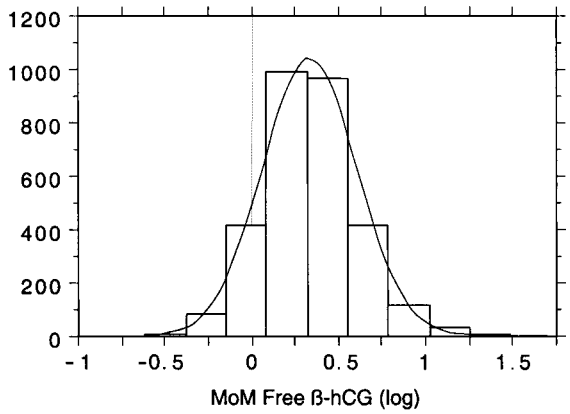

Figure 2—Distribution of maternal serum free ß-hCG (log MoM) in twin pregnancies. Log median MoM = 2.11 and standard deviation = 0.183

Table 2—Median values of AFP and free ß-hCG in twin pregnancies. Results are expressed in singleton-derived MoMs

	Total	Monochorionic	Dichorionic
n	3043	245	1317
AFP (median of MoM and 95% CI)	2.10 (2.05–2.15)	2.10 (1.92–2.28)	2.10 (2.02–2.18)
Free ß-hCG (median of MoM)	2.11 (2.03–2.19)	2.16 (1.81–2.51)	2.07 (1.97–2.17)

CI, Confidence interval.

Table 3—Maternal serum markers and risks calculated using 5 different protocols in 11 trisomy 21–affected pregnancies

Case no.	Mat age	Chorionicity	Nb T21-affected	AFP MoM*	Free ß-hCG MoM*	Risk at	Risk at age Meyers[a]	Risk MoM divided by 2	Risk MoM divided by 2.11	Risk adjusted for chorionicity
1	28	DC	1	2.04	30.1	897	448	242	219	223
2	32	DC	1	2.06	1.57	569	284	3837	3655	3677
3	33	DC	1	2.18	3	483	241	978	979	974
4	34	DC	1	1.78	2.36	403	201	876	852	868
5	36	DC	1	0.99	3.04	265	132	90	92	90
6	38	DC	1	1.51	15.25	165	82	24	22	23
7	44	DC	1	1.99	5.86	38	20	<10	<10	<10
8	37	DC	2	2.31	4.20	210	210	202	208	205
9	32	MC	2	1.97	1.14	569	569	5619	5292	5720
10	34	MC	2	1.85	16.22	403	403	90	81	83
11	35	MC	2	2.10	3.69	329	329	352	367	409

MC, Monochorionic; DC, dichorionic.
* Values observed for singleton pregnancies. Risk per pregnancy at amniocentesis expressed as 1/X.
[a] Meyers *et al*., 1997.

Table 4—Efficacy of fetal trisomy 21 screening depending on five different policies

	Maternal age alone	Maternal age corrected[a]	MSM MoM medians divided by 2	MSM MoM medians adjusted for twins	MSM MoM medians adjusted for chorionicity
All twins ($n = 3043$)					
Screened-positive rate (5% CI)	6.6%	24.6%	7.75% (6.80–8.70)	8.05% (7.08–9.02)	7.75% (6.42–9.08)
Detection rate ($n = 11$)	27.3%	54.5%	54.5%	54.5%	54.5%
Sub-group of MC ($n = 244$)					
Screened-positive rate (5% CI)	6.97%	6.97%	6.14% (3.13–9.15)	6.96% (3.77–10.15)	5.73% (2.81–8.65)
Detection rate ($n = 3$)	0/3	0/3	1/3	1/3	1/3
Sub-group of DC ($n = 1561$)					
Screened-positive rate (5% CI)	6.68%	34.3%	7.11% (5.84–8.38)	7.88% (6.54–9.22)	7.75% (6.42–9.08)
Detection rate ($n = 8$)	3/8	6/8	5/8	5/8	5/8

MSM, Maternal serum marker screening.
[a] Maternal age corrected according to Meyers (see reference) among dichorionic twins.

chorionicity was taken into account, the median MoM of AFP did not differ between monochorionic and dichorionic pregnancies. The distribution of free ß-hCG was significantly shifted ($p < 0.0001$) towards greater values in monochorionic (2.16 MoM) compared to dichorionic (2.07) pregnancies (Table 2).

Table 3 provides a detailed description of the 11 pregnancies with at least one trisomy 21–affected fetus, including singleton-derived MoMs of AFP and free ß-hCG and the risk of having an affected pregnancy according to 5 different policies. The screened-positive rates and detection rates of maternal serum markers in twins according to these different policies are given in Table 4.

DISCUSSION

Down syndrome screening using maternal serum markers in twins is controversial. So far, results from reasonably large-scale observational studies in the clinical setting have not been reported. Although previous papers focused on the distribution of markers or on the potential strategies of interpretation, our study is the first to

provide data on the sensitivity of second-trimester maternal serum screening in twins.

Of the 3043 pregnancies included prospectively in routine maternal serum screening, 11 were affected by at least one case of trisomy 21 (15 fetuses), a number (15–16 cases) similar to that expected for the distribution of maternal age in the study population. This observation is in accordance with the analysis of Cuckle based on 106 Down syndrome cases (Cuckle, 1998).

Nuchal translucency was measured in 7 of the 11 affected pregnancies, a proportion similar to that observed in the overall population. Nuchal translucency was considered normal ($\leq$2.1 mm) in both twins in each of the 7 cases.

Assessment of the risk of fetal aneuploidy in twins is made difficult by several factors. The mechanism of twinning has a substantial impact on the risk, but the diagnosis of zygosity cannot always be made prenatally. Overall, 20 to 30% of twin pregnancies are monozygotic, but the rate of dizygotic twinning increases with maternal age, and is affected by the ethnic background. In monozygotic pregnancies, the risk of having both twins affected by a chromosomal abnormality is related to maternal age, and it follows the same curve as in singleton pregnancies. In dizygotic twins in contrast, since each twin is the result of one egg being fertilized by one sperm, the risk of each twin being affected is an independent probability. Thus, the risk of having at least one affected baby can be estimated as grossly twice the risk of a singleton pregnancy (Meyers *et al*., 1997). Zygosity can be only partly inferred from the sonographic diagnosis of chorionicity, which is based on first-trimester ultrasound (Fisk and Bennett, 1995). Monochorionic pregnancies are necessarily monozygous. Dichorionic pregnancies with discordant fetal genders are dizygotic. Dichorionic pregnancies with concordant fetal genders may be either monozygotic or dizygotic.

When considering maternal serum markers in twins, additional difficulties are encountered. The primary question is to establish reference values for AFP and free beta-hCG in twins. Initially, Wald *et al*. (1991) proposed the simple method of the 'pseudo-risk' to calculate the risk of Down syndrome in twin pregnancies. This method was originally introduced as a stopgap until more information became available on the distribution of markers in twins. It has been outdated by an approach based on the actual distribution of markers in twin pregnancies as described by Cuckle (1998). A meta-analysis of 8 published studies (1314 twins) indicated medians of 2.26 MoM for AFP, 2.06 for hCG, and 2.07 for free ß-hCG, the results being expressed as multiples of the medians that had been observed in singleton pregnancies (Cuckle, 1998). We have no explanation as to why these values differ from ours. In a first-trimester biochemical study, Spencer raised the question of whether chorionicity had an impact on marker values and found no difference in free ß-hCG, and a small but not significant difference in PAPP-A distribution between 45 monochorionic and 135 dichorionic cases (Spencer, 2001). Such differences between monochorionic and dichorionic twins might also exist in the second trimester. In our study, normal values of free ß-hCG differed significantly between monochorionic and dichorionic pregnancies (2.16 MoM vs 2.07 MoM). The spread of the distribution was similar to that previously reported by Spencer in the first trimester (Spencer, 2000; Spencer, 2001). No difference was observed for AFP. The discordance between the distribution of free ß-hCG values in monochorionic twins in our study (median = 2.16 MoM) and the study of Spencer (median = 2.0 MoM) is probably explained by the fact that these studies were carried out at different stages of development.

Several hypotheses can be proposed to explain the differences in free ß-hCG between monochorionic and dichorionic pregnancies. Experimental evidence suggests that three factors can influence the placental production of hCG: the number of trophoblastic cells, their degree of oxygenation (Alsat *et al*., 1996), and inflammatory cytokines. *In vitro*, placental production of hCG is up-regulated by a low oxygen pressure. Vascular disturbances, which are more frequent in monochorionic twins, could induce placental hypoxia thus increasing hCG production. They could also facilitate the transfer of hCG to the maternal circulation. Chorionicity-related vascular disturbances have been considered as a potential explanation for the observation by Sebire *et al*. that the 95th percentile of nuchal translucency thickness was 1.5 times higher in monochorionic than in dichorionic pregnancies (Sebire *et al*., 1996). In a smaller study, Monni *et al*. (2000) also reported a greater relative incidence (1.66) of raised nuchal translucency in monochorionic twins.

The major limitation of maternal serum markers in twins results from the fact that they are a reflection of both twins. Raised hCG or decreased AFP production by the affected twin may be confounded by the presence of an unaffected co-twin, resulting in a lower detection rate than in singleton pregnancies.

All this may explain why the efficacy of serum markers is not as good in twins as in singletons, using for instance as a reference an 800 000-case collaborative study published recently showing a 72% sensitivity and a 6.5% detection rate in singletons (Muller *et al*., 2002). However, maternal serum screening in twins compares favourably with the sensitivity and specificity observed using a policy based on maternal age alone, in which the sensitivity is exactly the same but the false-positive rate is three times higher. Although highly significant, the differences in free ß-hCG distribution between monochorionic and dichorionic twins had only a small impact on the sensitivity and specificity of screening, perhaps due to small study populations.

When it is available, screening based on first-trimester nuchal translucency measurement might be the technique of choice in twins. In 1996, Sebire *et al*. showed that, using a 95th-percentile cut-off established in singletons, nuchal translucency screening in twins would lead to a 5.4% screened-positive rate in dichorionic twins and to a 8.4% screened-positive rate in monochorionic twins (Sebire *et al*., 1996). Nuchal translucency measurement detected seven out of eight cases of Down syndrome, an encouraging result although

the numbers are too small to draw definitive conclusions on the sensitivity of nuchal translucency in twins. More recently, it has been calculated that screening for trisomy 21 in twin pregnancies in the first trimester using free ß-hCG and PAPP-A, combined with nuchal translucency measurement, would give detection rates approaching 80% for a 5% false-positive rate (Spencer, 2000). This now has to be confirmed by large prospective studies, taking into account the potential complications of chorionic villous sampling in twin pregnancies.

In conclusion, although much hope can be placed on first-trimester screening combining ultrasound and serum markers, this approach is not yet widely available nor has it been evaluated in large prospective studies. Our results suggest that trisomy 21 maternal serum marker screening during the second trimester is feasible in twins, and provides data that could be used to inform mothers about the risks and benefits of this screening strategy.

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