PRENATAL DIAGNOSIS
Prenat Diagn 2008; **28**: 1105–1109.
Published online 11 November 2008 in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/pd.2145

Second-trimester Down syndrome maternal serum marker screening: a prospective study of 11 040 twin pregnancies

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Objective To analyze the value of Down syndrome (DS) second-trimester maternal serum screening in large series of twin pregnancies.

Methods Prospective study of second-trimester maternal serum markers [alpha fetoprotein (AFP) and free β-human chorionic gonadotrophin (β-hCG)] in 11 040 twin pregnancies, 27 of which were trisomy 21-affected. Comparison with 64 815 singleton pregnancies, of which 86 were trisomy 21-affected. Markers were expressed in multiple of median (MoM) corrected by a previously defined coefficient (2.1 for AFP and 2.07 or 2.16 for free β-hCG, dichorionic or monochorionic, respectively).

Results Trisomy 21 frequency was 1/649 for twins and 1/754 in singletons (NS). Mean detection rate was 63% (71% when both twins were affected and 60% when one was affected), versus 74.4% in singletons. False-positive rates were 10.8% in twins versus 10.3% in singletons (NS). No significant differences in MoM AFP and free β-hCG values were noted between twins and singletons (0.92 and 0.78 for AFP and 1.54 and 2.68 for free β-hCG, respectively).

Conclusion Our study demonstrates that second-trimester DS maternal serum marker screening can be performed in twin pregnancies. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: multiple pregnancy; trisomy 21; prenatal screening

INTRODUCTION

The general approach to prenatal screening for Down syndrome (DS) is to estimate a woman's risk of having a trisomy 21-affected pregnancy on the basis of factors such as maternal age, maternal serum markers (MSMs), and first-trimester nuchal translucency (NT) measurement. In the past decade, DS screening based on risk calculation combining maternal age, second- or first-trimester MSMs, and NT measurement has been widely used. Depending on the combinations, 60 to 90% of fetuses with DS are detected with a 5% false-positive rate (Cuckle, 2000; Muller *et al*., 2002, 2003a; Spencer *et al*., 2003; Wald *et al*., 2003; Malone *et al*., 2005; Nicolaides *et al*., 2005; Chasen *et al*., 2007).

Maternal serum screening for DS in twin pregnancies is fraught with difficulties (Cuckle, 1998; Wald and Rish, 2005). Firstly, serum marker levels in unaffected twin pregnancies are considered to be double those observed in singleton pregnancies. In addition, the distributions of the serum markers in DS-affected twin pregnancies are not known with any degree of reliability. Secondly, MSM levels in twins 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 a singleton pregnancy, while NT measurement is specific to each fetus. Thirdly, the patient-specific maternal age-related risk in twin pregnancies depends on zygosity and chorionicity. In monochorionic twin pregnancies, DS risk due to maternal age is the same as in singleton pregnancies (but both fetuses are DS-affected), whereas in dichorionic twin pregnancies, the risk of having at least one aneuploid fetus is doubled (each with an a priori risk of aneuploidy). For example, at 31 years of age the risk for DS is 1/193 in twins, which is comparable to the risk of 1/192 observed at 35 years of age in singleton pregnancies (Meyers *et al*., *1997*). However, the observed prevalence of DS in twin pregnancies is much less than predicted by theoretical calculations (Doyle *et al*., 1991).

The aim of our study was to analyze second-trimester MSMs for DS screening in a prospective series of twin pregnancies.

PATIENTS AND METHODS

During the period 1998–2006, a routine prospective countrywide study of 11 040 twin pregnancies for DS maternal serum screening was performed. Chorionicity was known in 4961 cases (45%). When unknown, the pregnancy was assumed to be dichorionic because in

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Received: 26 February 2008
Revised: 9 September 2008
Accepted: 23 September 2008
Published online: 11 November 2008

cases of unknown chorionicity, the necessary adjustment factor is similar to the one in dichorionic pregnancies, as previously described (Muller *et al*., 2003b). Four trisomy 21-affected cases were excluded because they were referred to our institution. In addition, patients with NT measurement >3 mm [in fetuses with a crown-rump length (CRL) between 45 and 85 mm] were considered to be at increased risk for chromosomal anomalies and were not included in the maternal serum screening program. The 64 815 singleton pregnancies included in the screening program during the same period were considered as a control population. Whereas, amniocentesis is routinely offered to patients 38 years of age and over, some patients prefer to undergo maternal serum screening and were included in our study.

In accordance with French law, maternal consent was obtained in all cases. Maternal age, maternal weight, maternal smoking status, gestational age, NT, CRL and chorionicity were recorded. Gestational age was determined by first-trimester ultrasonography (CRL) in the vast majority of cases. When unknown, the date of last menstrual period or gestational age known by the patient was taken into account.

According to French regulations concerning maternal serum DS screening (Muller *et al*., 2002), first-trimester maternal serum screening is allowed only in research programs. Second-trimester MSM screening was performed in all cases in our laboratory based on alpha fetoprotein (AFP) and free β-human chorionic gonadotrophin (β-hCG) (Dual kit PerkinElmer, Turku, Finland) using the AutoDELFIA automatic immunoassay system (PerkinElmer, Turku, Finland). AFP and free β-hCG were expressed in multiples of median (MoM). MultiCalc software (PerkinElmer, Turku, Finland) was used in all cases for DS risk calculation. The raw value of each marker was expressed in MoM by dividing the raw value by the median raw value observed in non-DS-affected singletons for the same gestational age. This MoM was first corrected by factors corresponding to maternal weight and smoking status. This corrected MoM was divided by factors we previously defined for twin pregnancies, 2.1 for AFP (independently of chorionicity) and 2.07 or 2.16 for free β-hCG (dichorionic and monochorionic, respectively) (Muller *et al*., 2003b). Because of the lack of precision concerning the age-specific prevalence of DS in twin pregnancies, we assume that the prior term risk for twins does not differ from that of singletons. A 1/250 cutoff at sampling was used to define the DS high-risk group. Amniocentesis was offered free of charge to all high-risk patients. Risk assessment combining maternal age, MSMs, and NT measurement was not routinely performed.

DS status was recorded by our laboratory. For high-risk patients (patients ≥38 years old, and patients with MSM screening >1/250), a questionnaire was sent to cytogenetics laboratories (76 in France). When amniocentesis was not performed (patient refusal or medical reason), and for patients not at high risk, DS cases were collected in maternity units and in multidisciplinary centers for prenatal diagnosis (45 centers in France). In addition, blood karyotyping was performed in all DS cases observed after birth. These abnormal results were collected from cytogenetics laboratories and cross-referenced with our MSM files. According to French law, in the case of severe fetal anomaly, after multidisciplinary consultation, termination of pregnancy (TOP) is allowed at the patient's request whatever be the gestational age. Detection rate, false-positive rate, sensitivity, and specificity, were calculated. The χ^2 test was used for comparison between percentages and Student's t test was used for comparison of means ($p < 0.05$ considered as significant).

RESULTS

Trisomy 21 frequencies

Study groups are presented in Table 1. Maternal serum screening was performed in 11 040 twin pregnancies, of which 27 were trisomy 21-affected, 20 with one trisomy 21-affected fetus (dichorionic), and 7 with both fetuses trisomy 21-affected (5 monochorionic and 2 dichorionic). Of the 27 trisomy 21-affected twin pregnancies, 11 were obtained after *in vitro* fertilization. The control group consisted of 64 815 singleton pregnancies, of which 86 were trisomy 21-affected.

Maternal age distribution was not significantly different ($p = 0.58$) between trisomy 21-affected twin pregnancies and trisomy 21-affected singleton pregnancies (median 35.5 years, range 18–44 years, 7 aged ≥38 years versus median 35 years, range 17–43 years, 26 aged ≥38 years respectively). Maternal age distribution was not significantly different ($p = 0.28$) between DS-unaffected twin pregnancies and DS-unaffected singleton pregnancies (respectively median 30 years, range 15–46 years, versus median 29 years, range 13–49 years). Trisomy 21 frequency was 1/649 for twins and 1/754 for singletons, which is a nonsignificant difference ($\chi^2 = 0.54$; $p = 0.45$).

Maternal serum markers

Using a 1/250 cutoff, overall mean trisomy 21 detection rate in twin pregnancies was 63% (17/27) (95% CI: 44.8–81.2) (Tables 2, 3 and 4). When both twins were affected, detection rate was 71%, and when only one was affected, detection rate was 60%. In singleton pregnancies, detection rate was 74.4% (64/86) (95% CI: 65.2–83.6). The difference in detection rates between twin and singleton pregnancies was not significant ($\chi^2 = 1.32$; $p = 0.25$). In the subgroup of patients aged

Table 1—Twin pregnancies and control groups

		Trisomy 21 (pregnancies)	Trisomy 21 (fetuses)
Twin pregnancies (total)	11 040	27	34
Monochorionic	1271	5	10
Dichorionic	3690	22	24
Unknown	6079	0	0
Singleton pregnancies	64 815	86	86

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DOI: 10.1002/pd

Table 2—Down syndrome detection rate and second-trimester maternal serum markers in 27 cases of trisomy 21-affected twin pregnancies and 86 trisomy 21-affected singleton pregnancies

	Detection rate	AFP MoM median (range)	Free β-hCG MoM median (range)
Down syndrome-affected twin pregnancies ($n = 27$)			
Monochorionic ($n = 5$)	3/5	0.92 (0.42–1.03)	1.88 (0.59–8.69)
Dichorionic ($n = 22$)			
Two trisomy 21-affected fetuses ($n = 2$)	2/2	ND (0.77–1.13)	ND (0.89–2)
Subtotal for both twins trisomy 21-affected ($n = 7$)	5/7(71%)	0.92 (0.42–1.13)	1.88 (0.59–8.69)
One trisomy 21-affected fetus (n = 20)	12/20 (60%)	0.94 (0.44–1.63)	1.40 (0.38–14.55)
Total ($n = 27$)	17/27 (63%)	0.92 (0.42–1.63)	1.54 (0.38–14.55)
Down syndrome-affected singleton pregnancies ($n = 86$)	64/86 (74.4%)	0.78 (0.20–1.83)	2.68 (0.54–29.60)

MoM, multiple of median.

Table 3—Details of both fetuses with Down syndrome (all monochorionic except 6 and 7, which were dichorionic)

	Maternal age	AFP adjustedMoM[a]	Free β-hCG adjustedMoM[a]	NT and CRL fetus 1 (mm)	NT and CRL fetus 2 (mm)	DS risk (1/x) Maternal age + MSM
1.	35	1.03	1.88	NM	NM	260
2.	34	0.92	8.69	NM	NM	70
3.	36	0.60	4.40	2.0/77	2.3/72	16
4.	36	0.42	1.80	NM	NM	30
5.	32	0.97	0.59	NM	NM	4800
6.	33	0.77	0.89	NM	NM	243
7.	37	1.13	2.00	NM	NM	200

[a] Observed MoM divided by median MoM in unaffected twin pregnancies (2.10 for AFP and 2.16 for free β-hCG in monochorionic; 2.10 for AFP and 2.07 for free β-hCG in dichorionic).
MoM, multiple of median; NT, nuchal translucency; CRL, crown-rump length; NM, not measured; DS, Down syndrome; MSM, maternal serum markers.

Table 4—Details of DS cases with one affected fetus (all dichorionic)

	Maternal age	AFP adjustedMoM[a]	Free β-hCG adjustedMoM[a]	NT and CRL fetus 1	NT and CRL fetus 2	DS risk (1/x) Maternal age + MSM
8	39	1.42	1.71	NM	NM	310
9	28	0.99	14.55	1.5/53	1.9/53	190
10	37	1.20	0.69	NM	NM	2500
11	38	1.06	1.54	2.0/59	2.9/62	245
12	39	0.60	1.21	NM	NM	89
13	36	0.44	0.87	1.2/58	1.4/58	149
14	18	0.84	5.08	NM	NM	210
15	36	0.49	0.80	1.6/50	1.9/52	230
16	32	1.00	4.06	1.8/68	2.3/71	114
17	32	0.77	1.10	1.5/57	1.7/56	982
18	31	0.80	4.16	0.8/44	0.7/45	83
19	44	0.98	2.58	NM	NM	10
20	32	1.01	0.75	2.1/66	2.1/66	3800
21	38	0.74	6.67	1.2/53	1.7/53	20
22	33	1.07	1.33	1.6/67	1.5/65	1100
23	38	1.63	0.38	NM	NM	743
24	39	0.90	2.11	1.0/52	1.1/55	49
25	37	1.06	1.22	1.3/47	1.9/45	570
26	36	0.49	1.46	NM	NM	80
27	33	0.64	0.98	1.2/68	0.5/58	566

[a] Observed MoM divided by median MoM in unaffected twin pregnancies (2.10 for AFP and 2.07 for free b-hCG in dichorionic).
MoM, multiple of median; NT, nuchal translucency; CRL, crown-rump length; NM, not measured; DS, Down syndrome; MSM, maternal serum markers.

<38 years, detection rate was 60% (12/20) in twin pregnancies and 66.6% (40/60) in singleton pregnancies, a nonsignificant difference ($\chi^2 = 0.29$; $p = 0.6$).

False-positive rate was 10.8% (1199/11 040) in twins and 10.3% (6682/64 815) in singletons, a nonsignificant difference ($\chi^2 = 3.08$; $p = 0.10$). However, in the

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DOI: 10.1002/pd

subgroup of patients aged <38, false-positive rate was 9.2% (967/10 494) in twins and 7.9% (4887/61 429) in singletons, a significant difference ($\chi^2 = 19.04$; $p < 0.001$). Maternal age distribution explained these high false-positive rates, patients 35 years old and over representing 30.3% of cases in twin pregnancies, and 16% in singletons. For the same 5% false-positive rate, detection rates were 37% for twins versus 45.4% for singletons, a nonsignificant difference ($\chi^2 = 0.42$; $p = 0.50$).

No significant difference in median AFP MoM values was noted between twins and singletons (0.78 and 0.92, respectively) ($p = 0.30$) or between monochorionic and dichorionic pregnancies (0.92 and 0.94, respectively) ($p = 0.85$). The differences observed in MoM values for free β-hCG, 1.54 in twins and 2.68 in singletons, did not reach significance ($p = 0.35$) nor did the differences observed between monochorionic and dichorionic pregnancies (respectively, 1.88 and 1.40) ($p = 0.89$).

DISCUSSION

Second-trimester MSM screening for DS detected 17 of the 27 trisomy 21 twin pregnancies out of a total of 11 040 twin pregnancies. The observed prevalence (1/649) was similar to that in singletons (1/754), as previously observed by Cuckle (1998) and Morris *et al.* (2002). The mean DS detection rate was 63% for a 10.8% false-positive rate, percentages not significantly different from the 74.4% and 10.3% observed in the singleton control group of 64 815 patients. When a fixed 5% false-positive rate is used, detection rates were 37and 45.4%, respectively, which is a nonsignificant difference. There are as yet too few prospective maternal serum screening studies including enough trisomy 21-affected twin pregnancies to allow detection rate comparisons. Detection rates were evaluated using multivariate Gaussian modeling techniques, which estimate a 51% detection rate for a 5% false-positive rate (Cuckle, 1998).

In France, 80% of patients <38 years of age and 40% of older patients undergo routine second-trimester DS maternal serum screening in singleton pregnancies. However, the use of such screening in twin pregnancies remains controversial due to the potential limits stated by different studies (Wald and Rish, 2005 review). The mechanism of twinning has a substantial impact on the risk. The age-related risk is the same as in singleton pregnancies in monozygotic twins, but in dizygotic twins, the risk of having at least one affected baby can be theoretically estimated as twice the risk of a singleton pregnancy (Meyers *et al.*, 1997). However, the observed prevalence does not confirm this hypothesis (Doyle *et al.*, 1991). In addition, risk calculation based on MSMs is problematic because in twins, the values reflect both twins. Raised hCG or decreased AFP related to the trisomy 21-affected twin may be confounded by the presence of the unaffected co-twin, resulting in a lower detection rate than in singleton pregnancies. Among the difficulties encountered in twin pregnancies, the risk of fetal loss related to amniocentesis appears to be higher (2.7%) than that of singletons (0.6%) (Yukobowich *et al.*, 2001).

Trisomy 21 risk related to NT measurement is also controversial in twins. Whereas the sensitivity is similar to that in singleton pregnancies, the false-positive rate is higher, mainly due to a higher prevalence of increased NT in chromosomally normal fetuses from monochorionic pregnancies (8.4% vs 5.4% in dichorionic twins and 5.2% in singleton pregnancies) (Pandya *et al.*, 1995; Sebire *et al.*, 1996). Different studies have assessed the value of the combination of NT measurement with first-trimester MSMs in twin pregnancies. On the basis of a modeling study, Cuckle (2000) showed that, in the first trimester, a combination of pregnancy-associated plasma protein-A (PAPP-A) and free β-hCG will yield a 64.9% detection rate, a rate that increases to 86.5% if ultrasound NT is used as an additional marker, with respective FPR of 4.7 and 2.3%. Spencer (2000) analyzed the distribution of free β-hCG and PAPP-A in 159 twin pregnancies in comparison with 3466 singleton pregnancies. The predicted detection rate for a 5% false-positive rate with MSMs would be 52% for dichorionic pregnancies, 55% for monochorionic pregnancies, 75% with NT measurement alone, and 80%, with the combination of both, some 10% less than in singleton pregnancies.

In conclusion, the rate of twin pregnancies is increasing due to higher mean maternal age and greater use of assisted reproduction techniques (Tandberg *et al.*, 2007), and the potential benefit of DS maternal serum screening in twins is still questioned. Our study based on the largest published series demonstrates that second-trimester DS MSM screening can be performed in twin pregnancies. Because of the better results observed, the method of choice for DS screening is probably NT measurement, provided quality control is performed. MSM screening would therefore be designated for patients who do not undergo this screening. Whatever the method, because of specific problems related to twin pregnancies (double amniocentesis, selective TOP), DS screening in twins must be performed in tandem with multidisciplinary centers and clear information must be given to patients by a geneticist and an obstetrician.

ACKNOWLEDGEMENTS

We thank the clinical staff who gave us all the details concerning follow-up of patients and children. We also thank all the French cytogenetics laboratories that provided us with the karyotyping results.

APPENDIX

1. ABA Study Group

Association of the French laboratories authorized by the Ministry of Health to carry out DS screening. The following laboratories provide us with data on DS screening in twin pregnancies: Amiens (C Lemay); Amiens (JM Bourdrel); Avignon (V Gras, T Roudon); Béziers (JY Réal, P Dumas); Bordeaux (E Ruedas, J Souby); Calais (P Andlauer, E Gaeremynck); Chalon sur Saône (B Duchêne, F Barba, C Pomel); Chambéry (B Dingeon, C Doche); Dax (I Peraud, H Chahine); Dreux

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DOI: 10.1002/pd

(C Finot, MH Ramaorasy); La Rochelle (H Lallaoui); Le Blanc-Mesnil (P Clément, L Lohman, M Mintz); Le Havre (E Berreville); Le Mans (P Sigogneau, F Duprey); Lille (G Couplet, A Mainardi); Limoges (T Chianéa); Lons le Saunier (B Veyrat, A Piedimonte); Lyon Mérieux (C Sault); Marseille (F Roux, A Boulbina); Marseille (Giorgetti, Caparros); Martinique (M Sainte-Rose); Metz (ME Larcher, M Wasel); Mulhouse (O Michotey); Nantes (A Baret); Nantes (S Mirallié); Nice (Delpech); Nîmes (M Cabrol); Orléans (L Got); Nouméa (E Choblet, Y Barguil); Paris-APHP Robert Debré (I Czerkiewicz, S Dreux, F Muller); Paris Argenteuil (D Khalfon); Paris Drouot (G Cassuto, B Brethome); Paris Hôpital Américain (MT Pannecière, S Palleau); Paris D'Eylau (JC Aidenbaum); Paris LCL (C Hamberger, L Druart); Paris APHP Pitié-Salpétrière (M Bernard, C Brochet); Poitiers (C Millet, MP Bounaud); Saint-Etienne (P Guiardiola, P Antoine, G Belot); Saint-Etienne (A Chamson); Tahiti (H Mulot, C Roy); Toulouse CHU (F Fortenfant, A Blancher); Tours (D Dudragne, B Cara); Wattignies (P Duchateau, H Odaert).

2. Clinical Study Group

Paris-Boulogne (Angotti-Jalladeau); Paris-Créteil (CHIC, C Touboul); Saint-Brieuc (CH, B Le Fiblec); Toulon (CH Fontpré, A-M Frances); Paris AP-HP (D Luton, CHU Beaujon; D Mahieu-Caputo, CHU Bichat; M Uzan, CHU Jean Verdier; Y Dumez, CHU Necker Enfants Malades; B Carbone, CHU Saint-Antoine; F Lewin, CHU Saint-Vincent de-Paul; S Uzan, CHU Tenon, JM Jouannic, CHU Trousseau).

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