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Second trimester trisomy 21 maternal serum marker screening. Results of a countrywide study of 854 902 patients

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Objectives In France, maternal serum marker screening is governed by specific legislation. We conducted a study of the countrywide trisomy 21 screening based on second trimester maternal serum markers.

Methods We reviewed the medical records of 854 902 patients prospectively screened for second trimester maternal serum markers in the 60 authorised laboratories over the two-year period 1997–1998. All patients screened in France were included. The risk of trisomy 21 was calculated from the combination of maternal age and maternal serum markers. The same cut-off (1/250) was used in all laboratories.

Results In 1998, 65% of pregnant women underwent maternal serum screening. In the 837 765 patients under 38 years of age who were screened, 54 321 (6.48%; 5% CI 6.42–6.53%) had a calculated risk >1/250. Of the 884 Down syndrome cases observed, 626 were detected by maternal serum markers (70.8%; 5% CI 67.8–73.8%). These good results can be explained by a strict quality control of all steps. For the 13 891 patients over 38 years of age, the Down syndrome detection rate was 98.9% for a 34% false-positive rate.

Conclusions Strict rules covering prenatal trisomy 21 screening are of benefit to patients, practitioners and laboratories alike, and ensure good quality control, a high trisomy 21 detection rate and a low amniocentesis rate. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS: Down syndrome; aneuploidy; biochemical markers

INTRODUCTION

Prenatal diagnosis of trisomy 21 is based on fetal karyotyping. Because of cost and the risks associated with fetal tissue sampling, karyotyping is limited to patients at increased risk. Maternal age, abnormal ultrasound findings and maternal serum markers are used as criteria, either alone or preferably in combination (Merkatz *et al*., 1984; Bogart *et al*., 1987; Cuckle *et al*., 1987; Wald *et al*., 1988; Haddow *et al*., 1992; Muller *et al*., 1993; Goncalves *et al*., 1994; Halliday *et al*., 1995). Numerous articles have underlined the problems of ethics and patient understanding intrinsic to maternal serum marker screening (Platt and Carlson, 1992; Gekas *et al*., 1999; Al-Jader *et al*., 2000). In France, this debate was conducted openly over a nine-year period (1988 to 1997) and resulted in the issue of specific legislation. The legal obligations are described below. Following implementation of this legislation, we reviewed countrywide data on trisomy 21 screening based on maternal serum markers. Data are presented for 854 902 cases recorded during 1997 and 1998.

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MATERIALS AND METHODS

Study population

In France every year there are 730 000 pregnancies in a population of 60 million. Each pregnant woman is entitled to a monthly medical examination. All patients are offered prenatal screening for Down syndrome, toxoplasmosis and rubella, as well as blood group determination. Three ultrasound examinations are performed in normal pregnancies at the first, second and third trimesters.

Prenatal diagnosis is regulated, and each at-risk pregnancy has to be managed in a multidisciplinary healthcare centre accredited by the Ministry of Health (39 centres). Each centre must include a maternity unit employing an expert in fetal ultrasonography, a medical geneticist, and a paediatrician. The centre must work in collaboration with a psychiatrist or psychologist, a fetal pathologist and laboratories accredited in one or more specialities: cytogenetics (80 laboratories), maternal serum markers for Down syndrome screening (60), infectious diseases (44), molecular biology (43), biochemistry (38), haematology (3) and immunology (3).

Prenatal karyotyping is available free of charge to all pregnant women aged 38 years and over. For patients under 38, different indications are taken into account: patients with a previous trisomy-affected pregnancy, couples with an abnormal karyotype, abnormal fetal

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ultrasound findings and abnormal maternal serum markers. The latter are subject to strict regulations.

For the present study all 60 maternal serum screening laboratories were sent a standard questionnaire covering: method of screening (markers and software), number of patients for each maternal age range, number of patients in the at-risk group, number of trisomy 21 cases detected by maternal serum screening, by second- and third-trimester ultrasonography, and detected at birth. All questionnaire data were processed in a central laboratory. In order to be sure that Down syndrome cases were not missed among live births, two methods were undertaken: (1) a questionnaire was sent to the maternity unit concerning Down syndrome status, because all newborns undergo a paediatric examination at birth, and at one, three and six months after birth and; (2) a questionnaire was sent to the 80 French cytogenetics laboratories which perform karyotyping pre- and postnatally. However, stillbirths without karyotyping may have been missed. Due to the absence of a national registry of Down syndrome cases, these two methods are the only ways of checking for trisomy 21. As some centres performed first trimester screening based on nuchal translucency measurement followed by second trimester maternal serum screening, the number of trisomy 21 cases expected from the maternal age distribution will not be relevant.

Organisation in France of maternal serum marker screening for trisomy 21

Maternal serum markers have been used in France since 1988, but national coordination was initiated by a decree dated 27 January 1997, governing laboratory practices, the obligations of the practitioner and patient, and reimbursement of the cost of screening and karyotyping.

RESULTS

Population screened

During 1997, 378 941 patients underwent maternal serum trisomy 21 screening, and in 1998, 475 961. This means that 52% of pregnant women were screened in 1997 and 65% in 1998.

There were 851 656 single pregnancies in which 83 176 patients were aged 35 years and over (and of these 13 891 were over 38 years). Only 3246 (0.86%) twin pregnancies were included because this screening was routinely performed for twin pregnancies in only five of the laboratories. In 98% of cases, gestational age was determined by early ultrasound scanning. Gestational age was between 14 weeks and 17 weeks + 6 days (weeks of amenorrhoea) in 98% of cases, the legally stipulated period.

Laboratories

Of the 60 laboratories, 23 were in university teaching hospitals, seven in regional hospitals and 30 were in the private sector. Each laboratory processed an average of 3700 cases per year (range 900 to 100 000). Nearly twice as many women were screened annually by the private laboratories (6212) as by the public laboratories (3500). All 60 laboratories used recommended tests (software and assay kit from the same supplier): Perkin Elmer (Turku, Finland) (28% of cases); Bayer (Tarrytown, NY, USA) (20% of cases); Ortho Clinical Diagnosis (Rochester, USA) (20%); Abbott (Chicago, USA) (15%); Cis Bio (France) (13%); Roche (Mannheim, Germany) (4%). Nine (15%) of these laboratories used the triple test (AFP, oestriol, hCG) representing 216 242 patients (25%) and 51 (85%) the double test (AFP and hCG in 27 or AFP and free ß in 23, hCG and oestriol in one).

Down syndrome risk was calculated using the software proposed by the manufacturers. In all cases this software combines Down syndrome risk due to maternal age alone and risks due to biochemical markers. The same cut-off 1/250 was used in all laboratories. Risk was calculated by taking into account patient body weight in 38 laboratories (62%) in 1997 and by all laboratories in 1998, and ethnic background in addition in 20 (33%).

Data sheets sent to the practitioners indicated a calculated risk for trisomy 21, using a cut-off of 1 in 250 calculated at sampling, and gave a written interpretation of the data. In addition, AFP values over 2.5 MoM indicated an increased risk of neural tube defect and detailed ultrasonography was recommended for these patients. The practitioner is asked for the karyotyping results one month after maternal serum screening, thereby allowing confirmation that the practitioner has offered karyotyping to all at-risk patients. One month after the expected date of birth, the practitioner is asked for the outcome of pregnancy. This follow-up is an intrinsic part of quality assurance as recommended by the Ministry of Health.

Results for patients under 38 years of age (Tables 1 and 2)

Of the 837 765 patients, 418 835 were under 30 years of age, 349 645 between 30 and 34 and 69 285 were 35 to 37. Of the 837 765 patients, 54 321 (6.48%; 5% CI 6.42–6.53%) were in the at-risk group. Inter-laboratory variations were observed: the false-positive rate was between 4 and 8% in 48 laboratories, below 4% in one, and over 8% in 11. The screened-positive rate depends on maternal age. It was 3.9% for patients under 30 years of age, 8.2% for patients aged 30 to 34 (mean for patients under 35 = 5.5%) and 26.8% for patients aged 35 to 37.

Amniocentesis was performed in 95% of patients included in the at-risk group. When amniocentesis was refused, refusal was generally by the patient for personal reasons, and very occasionally by the practitioner for medical reasons (hepatitis C or HIV-positive). Among the 837 765 patients under 38 years of age, 884 cases of trisomy 21 were observed (prevalence 1/950). Of the 54 321 patients included in the at-risk group, 626 had a trisomy 21-affected pregnancy, giving a 70.8% detection rate (67.8 to 73.8% for a 95% confidence interval). Parents did not opt for termination of pregnancy in

Table 1—Results of maternal serum trisomy 21 screening in France (1997 and 1998) (total patients 854 902)

Patients <38 years of age (single pregnancies)	
Total patients	837 765
Patients at risk (⩾1/250)	54 321 (6.48%)
Total number of trisomy 21	884
Trisomy 21 at risk ⩾1/250	626
Detection rate	70.8%
Positive predictive value	1/87
Patients ⩾38 years of age (single pregnancies)	
Total patients	13 891
Patients at risk (⩾1/250)	4763 (34%)
Total number of trisomy 21	93
Trisomy 21 at risk ⩾1/250	92
Detection rate	98.9%
Positive predictive value	1/52
Patients with twin pregnancy (all maternal ages)	3246

three of the 626 cases of trisomy 21 detected. The positive predictive value (PPV) was 1/87, meaning that 87 karyotypings are necessary to detect 1 case of trisomy 21.

Screened positive rates and detection rates as a function of maternal age are presented in Table 2. Detection rate and amniocentesis rate in the double versus triple test were respectively 70.8% and 6.3% versus 71% and 6.61%—values not significantly different.

Results for patients aged 38 and over

Of 13 891 patients aged 38 and over, 4763 (34%) were included in the at-risk group, thus circumventing 66% of amniocenteses. Ninety-three trisomy 21 cases were observed in this population of which 92 were included in the at-risk group (98.9% detection rate). Parents did not opt for termination of pregnancy in two (2.1%) of the 92 cases of trisomy 21 detected.

Twin pregnancies

Of the 60 laboratories, five screen for twin pregnancies using their own normal values. Maternal serum screening was performed in 3246 twin pregnancies, of which 333 (10.2%) were in the at-risk group. In the five trisomy 21-affected twin pregnancies, seven fetuses were trisomy 21-affected. Both twins were affected in two of the five pregnancies, and maternal serum marker screening detected one of these two pregnancies. One of each pair of twins was trisomy-affected in the three other pregnancies, and all three were detected by maternal serum marker screening. Too few cases of trisomy 21 were observed to allow a firm conclusion to be drawn.

Table 2—False-positive and detection rates

Maternal age	False-positive rate	Detection rate
<30 years	4.0%	62.4%
30–34 years	8.2%	71.0%
35–37 years	17.8%	73.1%
⩾38 years	34.3%	97.1%

Neural tube defect screening

This screening was performed by 26 laboratories in 1997 and by 35 in 1998, covering 72% of the population screened for trisomy 21. All of these laboratories use the same AFP cut-off value (2.5 MoM). One per cent (9283) of patients were included in this high-risk group, in which 178 cases of neural tube defects were identified, indicating a 1 in 52 positive predictive value of AFP. These neural tube defects comprised 118 spina bifida, 32 anencephaly, two encephalocele and one exencephaly; in 12 cases data were unavailable.

DISCUSSION

As techniques analysing fetal DNA or fetal blood cells in maternal serum are still investigational (Al-Mufti *et al*., 1999; Lo Dym *et al*., 1999), trisomy 21 prenatal diagnosis is currently based on karyotyping. Invasive sampling is therefore unavoidable and patients at increased risk of aneuploidy must be identified. The three criteria applied—maternal age, abnormal ultrasound findings and maternal serum markers—can be used alone or in combination. Large discrepancies are observed on a countrywide scale. In most countries, prenatal karyotyping is proposed to older patients with a cut-off at 35 years or (in France) 38 years. Offering amniocentesis to older women is the most expensive method and has the highest rate of miscarriage per case detected. In contrast, the present study demonstrates that maternal serum marker screening in these older patients allows the detection of 98.9% of trisomy 21 cases, thereby preventing amniocentesis in 66% of cases. In other words, when maternal age is the only criterion used for trisomy 21 screening, 200 amniocenteses are necessary to detect one case of trisomy 21, but when age is combined with maternal serum markers, only 87 (under 38 years of age) or 52 (over 38) amniocenteses are required. Therefore, the use of maternal serum markers reduces invasive sampling three- to five-fold. Hence, for these older patients, maternal serum screening can be considered as

an alternative, but does not constitute a diagnostic test and clear information must be given to the patient.

Since the first use of Wald's model (Wald *et al*., 1988), trisomy 21 maternal serum screening has become very widely used, giving a 60% trisomy 21 detection rate for a 5% false-positive rate (Cuckle, 1996). Few large-scale prospective study reports give both false-positive and detection rates. The largest (Palomaki *et al*., 1997) included the experience of 316 laboratories in the USA for about 2 million patients but only reported laboratory practice and false-positive rates (6.7% for double test and 6.5% for triple). The 5.5% false-positive rate and 74% detection rate we observed in a population of 754 869 patients under 35 years of age confirm the values predicted by modelling. However, the detection rate depends on the accuracy of the total number of trisomy 21 cases. Prospective studies necessarily overestimate the detection rate since cases with negative results that miscarry are not ascertained. The strengths of our study are that the results of karyotyping of at-risk patients are known in all cases, and that the outcome (trisomy 21 or not) in the population not at-risk was checked by two different approaches (maternity unit and cytogenetic laboratories). This was possible because of the relatively small number (80) of prenatal cytogenetics laboratories. Our high detection rate (71%) is probably due to very frequent use of ultrasonography to determine gestational age (98% of cases compared with 35% in most studies). In addition, we paid particular attention to individual and countrywide quality control. Furthermore, each assay kit was validated by the Medicines Control Agency, as was the accompanying software.

An indirect method to validate the detection rate consists of calculating the theoretical number of cases of trisomy 21 expected on the basis of maternal age. In the 837 762 patients under 38 years of age, 1027 cases of trisomy 21 are expected. The 626 trisomy 21 cases detected by maternal serum marker screening gave a detection rate of 61%. However, because first trimester nuchal translucency is used in France, the population with second trimester maternal serum markers is biased, since it comprises two different groups of patients: those with a normal nuchal translucency measurement and those with no nuchal translucency measurement, patients with abnormal nuchal translucency (including trisomy 21 cases) being excluded. Therefore, the anticipated 1027 cases constitute an upper limit and the 61% detection rate a lower limit. The discrepancy between the 884 trisomy 21 cases observed and the 1027 expected can be explained by nuchal translucency screening performed in an estimated 20% of patients.

Trisomy 21 maternal serum screening is an integral part of public health policy and has led to the creation of multidisciplinary fetal medicine centres in which patients at risk are monitored. The utility of second trimester maternal serum markers in predicting adverse pregnancy outcome (Muller *et al*., 1996; Walton *et al*., 1999) was not analysed in this study, apart from detection of neural tube defects. In our series, the proportion of spina bifida was elevated (66%), probably due to the detection of major neural tube defects (anencephaly, exencephaly, encephalocele) at the first trimester ultrasound examination when performed at 11–13 weeks.

Ours is one of the largest second trimester Down syndrome screening studies and is the only one covering a whole country. However, the good results observed should not distract us from the need to make three essential improvements: earlier screening, higher sensitivity and greater specificity.

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APPENDIX

ABA Study Group (association of the 60 French laboratories authorized by the Ministry of Health to screen prenatally for trisomy 21): Albi (C Gassier, MB Bleuven); Amiens (C Lemay, N Roussel); Angers (H Puissant, A Larget-Pied); Arras (A Gruson, M Baillet); Aubergenville (MF Pétavy, M Mintz); Avignon (V Gras, T Roudon); Bézier (JY Réal, P Dumas); Bordeaux (J Souby, C Mathieu); Bordeaux (A Ruffié); Brest (MP Moineau, JF Morin); Caen (P Leymarie, M Herrou); Chambéry (B Dingeon, C Doche); Clermont-Ferrand (P Chatron, P Lochu); Dijon (J Desgres, X Frigère); Dreux (JC Chartron); Epinal (G Lefaure, JP Gonand); Grenoble (AS Gauchez-Quenin, M Comet); La Réunion (H Caillens); Le Havre (D Bouige); Le Havre (D Thibaud); Le Mans (F Duprey, P Sigogneau); Lille (JL Dhondt, JM Perini); Lille (G Couplet, A Mainardi-Leduc); Lille (P Jaumain, JM Deswartes); Longjumeau (F Gras); Lorient (F Cornu); Lyon Croix-Rousse (S Guibaud, C Boisson); Lyon Hôtel Dieu (F Poloce, J Pichot); Lyon Mérieux (C Abel, C Sault); Marseille Saint-Joseph (MP Brechard, P Yerokine); Marseille (F Roux, MF Pelissier); Metz (ME Larcher, M Wasel); Nancy (F Feldem, C Baillet); Nantes (A Baret); Nantes (MF Dubin); Nice (P Soubiran); Nîmes (G Renier-Vigouroux); Orléans (B Luthier); Paris A. Béclère (C Benattar, J Taieb); Paris A. Paré (F Muller, S Ngo); Paris Hôpital Américain (JM Costa, T Connois); Paris Cerba-Pasteur (I Lacroix, F Hamida); Paris Cochin (J Ingrand, Y Fulla); Paris D'Eylau (A Lemeur, JC Aidembaum); Paris Diaconesse (D Francoual, S Prince); Paris Institut de Puériculture (F Forestier, MP Beaupard); Paris LCL (M Roger, L Druard); Paris Pitié (M Bernard); Paris Poissy (L Malagrida); Paris R. Debré (J Guibourdanche, D Porquet); Poitiers (C Millet); Reims (E Nowak); Rennes (C Massart, P Fergelot); Saint Etienne (H Dupoizat, P Guiardiola); Saint-Etienne (J Frey, N Rabi); Strasbourg (G Coumaros, C Koehl); Toulouse (E Carles, M Prola); Toulouse (A Blanchet, F Fortenfant); Tours (D Dudragne, B Cara).