Risk of amniocentesis in women screened positive for Down syndrome with second trimester maternal serum markers

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In routine obstetrical practice, prior to offering invasive prenatal diagnosis, it is crucial to weigh the risks attendant on amniocentesis against the individual's risk of aneuploidy. We took advantage of a policy of follow-up of patients undergoing Down syndrome maternal serum screening to compare the rates of fetal loss before 24 weeks and of early premature delivery at 24–28 weeks between women who underwent amniocentesis and women who did not. A total of 54 902 patients entered the study, of whom 4039 (7.35%) were lost to follow-up and 387 were excluded because of a severe fetal abnormality. Of the 50 476 remaining patients, 3472 had an amniocentesis whereas 47 004 had not and served as controls. In the amniocentesis group, the fetal loss rate before 24 weeks was 1.12% (95% CI = 1.08–1.15) and the 24–28 weeks premature delivery rate was 0.40% (95% CI = 0.39–0.41) which was significantly higher than in controls (0.42% with 95% CI 0.41–0.43 and 0.24% with 95% CI 0.23–0.25, respectively). The 0.86% difference in adverse outcome rates between the amniocentesis and control groups may be attributable to amniocentesis and compares favourably with the positive predictive value of maternal serum markers (1.70%) observed in the present study. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS: fetal loss; prenatal diagnosis; trisomy 21; amniocentesis; premature birth

INTRODUCTION

Evaluating the risk attendant on amniocentesis is crucial in routine obstetrical practice to weigh an individual's risk of aneuploidy against the risk of unintentional fetal loss. However, it is difficult to evaluate the risk attendant on amniocentesis. A randomised trial with a non-amniocentesis arm in at-risk patients would be impossible to design nowadays for ethical reasons. Observational studies of fetal loss rates following amniocentesis fail to resolve the question of the number of losses that are truly attributable to the procedure. This can be obviated by comparing loss rates in women having amniocentesis and in controls. We took advantage of a policy of follow-up of patients undergoing Down syndrome maternal serum screening to assess the rates of fetal loss before 24 weeks and of early delivery at 24–28 weeks among women who underwent amniocentesis. Mothers screened in the same institutions during the same period but who had no amniocentesis served as controls.

PATIENTS AND METHODS

The cohort population consisted of 54 902 women with singleton pregnancies included in a trisomy 21 maternal serum marker screening program over a 3-year period (1997–1999). Data were provided by six accredited laboratories: Laboratoire Séry, Le Havre (17 067); Hotel Dieu Hospital, Lyon (16 626 women); Ambroise Paré Hospital, Paris (13 694 women); Pitié Salpétrière Hospital, Paris (4570 women); Poitiers Hospital (1714 women); and Laboratory Réal-Carrié, Béziers (1231 women). Information collected for each pregnancy included: maternal age, gestational age at maternal serum sampling, calculated risk for Down syndrome based on maternal serum markers combined with maternal age, maternal choice to have an amniocentesis or not, results of fetal karyotyping when appropriate and pregnancy outcome.

Gestational age was estimated by first-trimester ultrasonography in all cases. Maternal serum screening was based on two markers, α-fetoprotein (AFP) and free β-human chorionic gonadotrophin (ß-hCG) (Perkin Elmer, dual kit), or AFP and total hCG (Abbott, Axym; Perkin Elmer; Bayer, ACS180). Trisomy 21 risk calculation was based on the combination of risk due to maternal age and of risk due to maternal serum markers as described by Wald *et al.* (1988). The same 1/250 cut-off was used in all centres to define the at-risk group. Amniocentesis was offered in the at-risk group, or when structural abnormalities were detected at second trimester ultrasound screening. However, the mother's choice to ask for an amniocentesis or to refuse it was respected.

Amniocenteses were performed under ultrasound guidance using a 20 gauge needle.

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Information on pregnancy outcome was obtained by mailing to the obstetrical units a data sheet for each patient. 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.

Cases with a severe fetal malformation or with fetal aneuploidy were analysed separately in order to avoid confusion due to losses related to termination of pregnancy or to fetal abnormalities.

Fetal loss was defined as spontaneous abortion or stillbirth occurring between the time of second trimester maternal serum marker screening and 24 weeks of gestation. Early premature delivery was defined as birth occurring between 24 and 28 weeks of gestation (livebirth or intrauterine fetal death). Pregnancy losses and premature deliveries occurring after 28 weeks were not analysed.

Fetal loss and early premature delivery rates were compared in women having amniocentesis and in controls using the Pearson chi-square test.

RESULTS

A total of 54 902 patients entered the study. Median maternal age was 29 years (range 13–44 years), and 97.8% of patients were aged under 35 years. Median gestational age at maternal serum sampling was 16 weeks (range 14–18 weeks). A total of 4039 women (7.35%) were lost to follow-up and 387 had severe fetal abnormalities (Table 1) including abnormal karyotype ($n = 121$, of which 84 were trisomy 21), cardiac abnormality ($n = 60$), neural tube defect ($n = 33$), cerebral malformation ($n = 30$) and other anomalies ($n = 143$).

The remaining 50 476 women form the study database (Table 2), 3472 of whom had an amniocentesis and form the study group while the other 47 004 served as controls.

In the study group ($n = 3472$), gestational age at amniocentesis ranged from 15 to 24 weeks (median 18 weeks). The indications for amniocentesis were maternal serum marker-derived risk $>1/250$ ($n = 3151$) and minor sonographic markers or maternal anxiety ($n = 321$).

In the control group ($n = 47 004$), 44 586 patients had a maternal serum marker-derived risk $<1/250$ and 2418 patients elected not to have an amniocentesis despite the fact they belonged to the at-risk group.

Table 1—Study population

	No amniocentesis	Amniocentesis
Number of patients ($n = 54 902$)	50 970	3932
Lost to follow-up ($n = 4039$)	3794	245
Severe fetal malformations ($n = 387$)	182	205

Table 2—Unexplained fetal loss before 24 weeks and premature birth at 24–28 weeks in the two groups, with amniocentesis and without

	No amniocentesis	Amniocentesis
Patients included ($n = 50 476$)	47 004	3472
Fetal loss before 24 weeks ($n = 235$)	197 (0.42%)	$31 + 8^a$ (1.12%)
Premature delivery between 24 and 28 weeks ($n = 129$)	115 (0.24%)	14 (0.40%)

[a] Spontaneous fetal death before amniocentesis.

The two groups differed significantly with respect to distribution of maternal age (33 years vs 29 years).

The fetal loss rate before 24 weeks was 39/3472 (1.12%; 5% CI = 1.08–1.15) in the amniocentesis group. Of these 39 fetal deaths, eight were diagnosed by ultrasonography before amniocentesis. In controls, fetal loss rate was 197/47 004 (0.42%; 5% CI = 0.41–0.43). The difference between the two groups was significant ($p < 0.001$).

The rate of severe premature delivery was 14/3472 (0.40% 5%CI = 0.39–0.41) in the amniocentesis group and 115/47 004 (0.24%; 5% CI = 0.23–0.25) in controls. The difference between the two groups was significant ($p < 0.001$).

The total rate of adverse outcome was 1.52% in the amniocentesis group and 0.66% in controls, suggesting that amniocentesis carries an additional risk of 0.86%. This compares favourably with the overall positive predictive value of maternal serum markers of 1.70%. In the population we studied, 61 trisomy 21 cases were detected by maternal serum screening at the cost of 30 cases of adverse obstetrical outcome attributable to amniocentesis.

In the at-risk group, the median calculated risk for Down syndrome, the median AFP value in multiples of the median (MoM), and the median free ß-hCG value in MoM were similar in the cases with (1/160, 0.88, 2.62, respectively) and without amniocentesis (1/162, 0.83, 2.67, respectively).

Similarly, in the at-risk group, the median calculated risk for Down syndrome, the median AFP value in MoM, and the median free ß-hCG value in MoM were similar in the cases with adverse outcome (1/165, 1.10, 2.45, respectively) and good outcome (1/160, 0.83, 2.67, respectively).

DISCUSSION

The present results suggest that the number of trisomy 21 cases detected by maternal serum screening is not outweighed by the rate of adverse obstetrical outcome induced by amniocentesis.

In assessing the amniocentesis-related rate of adverse outcome it is essential to take into account the background rate of adverse outcome. This cannot be achieved by studies lacking a control group without an invasive

procedure (Johnson *et al.*, 1996; CEMAT, 1998; Horger *et al.*, 2001). The main problem is to find adequate controls. The ideal study would randomly allocate women at risk for aneuploidy to undergo amniocentesis or not. However, this would not be acceptable nowadays for obvious ethical reasons. Nonetheless, in their pioneering randomized trial in low-risk patients, Tabor *et al.* (1986) found a significantly higher fetal loss rate (1.7%) in women with amniocentesis than in controls (0.7%). The Medical Research Council (MRC) (1977) found a higher amniocentesis-related fetal loss rate, which might be accounted for by an underestimation of the fetal loss rate among controls. Since then a number of major studies have attempted to evaluate the rate of adverse outcome attributable to amniocentesis. For instance, Tongsong *et al.* (1998), in a case-control study of 2045 matched pairs, found no significant difference in fetal loss rate, premature deliveries, or placenta abruptio between the amniocentesis and control groups. However, this study did not have enough statistical power to identify a difference smaller than 1%. Several other studies (Simpson *et al.*, 1976; Lowe *et al.*, 1978; Golbus *et al.*, 1979; Bartsch *et al.*, 1980; Crandall *et al.*, 1980; Sant-Cassia *et al.*, 1984; Antsaklis *et al.*, 2000) reported similar results but carried similar limitations.

Because of its large number of patients (47 004 controls and 3472 cases), the present study had the power to identify a significant difference in adverse outcomes of less than 1%. The drawback of the relatively large size of the study is the lost to follow-up rate. However, this is unlikely to have skewed the results substantially. The order of magnitude of the rate of adverse outcome was similar in the present study than in others. Follow-up rates were similar in amniocentesis cases and in controls. There is no reason to speculate that adverse outcomes would be more likely to be overlooked in either group, suggesting that the difference between amniocentesis and controls would remain valid.

In order to estimate the difference in lost rate between the amniocentesis and the non-amniocentesis groups we took into account all losses occurring between maternal serum sampling and 24 weeks. Therefore the difference between those global loss rates represents the losses that can be attributed to amniocentesis (0.86%). However, the overall 1.12% lost rate in the amniocentesis group does not represent only losses occurring after amniocentesis, because it includes eight fetal losses occurring prior to amniocentesis. Therefore, the value of 0.86% (0.70% fetal loss and 0.16% premature birth) is a pessimistic estimation of adverse outcomes attributable to amniocentesis.

The rate of adverse outcome attributable to amniocentesis may have been overestimated for other reasons. The indication for amniocentesis may have selected patients at increased risk of adverse outcome, because increased maternal serum markers-derived risk for trisomy 21 is also associated with non-chromosomal adverse outcomes such as preterm delivery, preeclampsia, and perinatal death (Muller *et al.*, 1993, 1996; Van Rijn *et al.*, 1999; Ogle *et al.*, 2000). Similarly, mean maternal age was significantly greater (33 years) in the amniocentesis group than in controls (29 years), and higher rates of

adverse outcome are observed in older patients (Collins *et al.*, 1998; Hollier *et al.*, 2000; Nybo-Andersen *et al.*, 2000). These biases may have been at least partly corrected by excluding patients with major fetal malformations and by excluding losses above 28 weeks. However, this latter choice prevents use of the data in counselling patients about the potential complications of amniocentesis that might arise during the third trimester.

Overall, the 0.86% rate of adverse outcomes attributable to amniocentesis in the present study compares favourably with the positive predictive value of maternal serum markers (1.70%), but largely exceeds the positive predictive value of maternal age alone at 35–38 years (0.3–0.5%). This underscores the interest of using non-invasive screening procedures to evaluate as precisely as possible the risks of aneuploidy, even in women aged over 35 years.

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