

Genetic amniocentesis in twin pregnancies: results of a multicenter study of 529 cases

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ABSTRACT

To evaluate the risk of abortion after genetic amniocentesis in twin pregnancies, a retrospective study of 15 centers was performed. The spontaneous abortion rate up to 20 completed weeks of gestation was 2.3%; the abortion rate up to 28 completed weeks, as defined by WHO, was 3.7%. The abortion rate could not be correlated either with the number of needle insertions or with the type of marker dye used. There was also no correlation between the abortion rate and the gestational age at which amniocentesis was performed. A significant association was shown between congenital intestinal obstructions and the application of methylene blue intra-amniotically as a marker dye. Considering the increased natural loss rate in multiple gestations, amniocentesis in twin pregnancies seems to be a safe and reliable technique.

INTRODUCTION

Amniocentesis is now an established method for the exclusion of chromosomal and metabolic disorders in the unborn. Within the indication spectrum, the age of the mother represents the major reason for carrying out amniocentesis in 75–85% of all examinations. Over the last few years, moreover, the proportion of women with multiple pregnancies who have requested amniocentesis has markedly increased. While the risk of abortion following amniocentesis in a singleton pregnancy is known from large-scale studies, the results of studies concerning the rate of fetal loss following amniocentesis

in multiple pregnancies show great variations, perhaps because of the small sample size.

A twin pregnancy involves a higher genetic risk in comparison with a singleton pregnancy. This risk has been calculated to be $5/3 \times X$ by Hunter and Cox¹. Their calculation is based on the empirical relationship between monozygotic and dizygotic twins of 1/3 to 2/3; X represents the empirical age risk. Thus, a 33-year-old woman pregnant with twins has the same statistical risk of having a child with a clinically relevant chromosomal aberration as a 35-year-old woman with a singleton pregnancy (Figure 1).

The value of the calculated risk of an abortion following amniocentesis must, therefore, be accurate in order to be able to balance it against the undoubtedly higher genetic risk, and thereby provide a family with adequate counselling. This paper provides information on the average abortion rate after amniocentesis and on the safety of the procedure in twin pregnancies.

MATERIALS, PATIENTS AND METHODS

In a retrospective multicenter study involving 15 centers with a high yearly amniocentesis frequency, 529 twin pregnancies in which amniocentesis had been carried out were recorded between January 1985 and December 1991. In order to be able to compare the collected data with that in the literature (Table 1), the loss of both twins up to the 20th and the 28th weeks of pregnancy, as

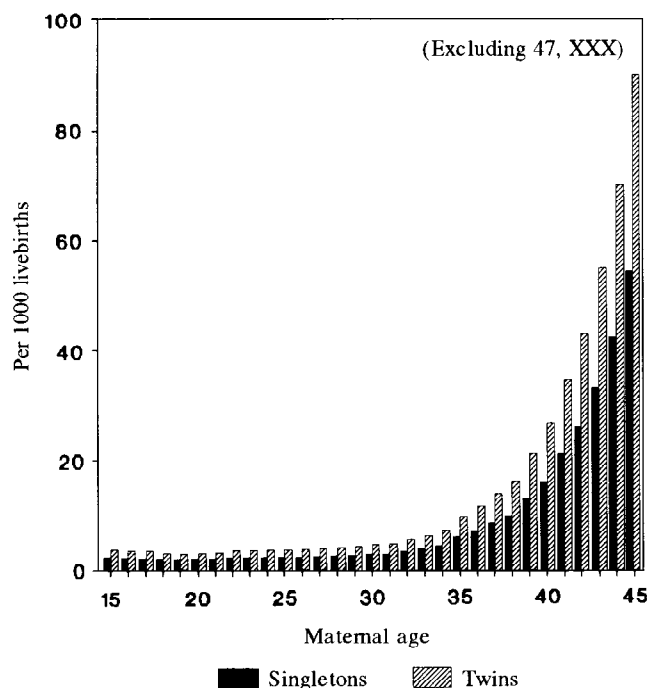


Figure 1 Risk of clinically relevant chromosomal abnormality occurring in singleton and twin pregnancies. Based on the empirical data by Hook¹⁹ for singleton pregnancies and the formula by Hunter and Cox¹ for the probability of at least one of the twins having a chromosomal abnormality at birth

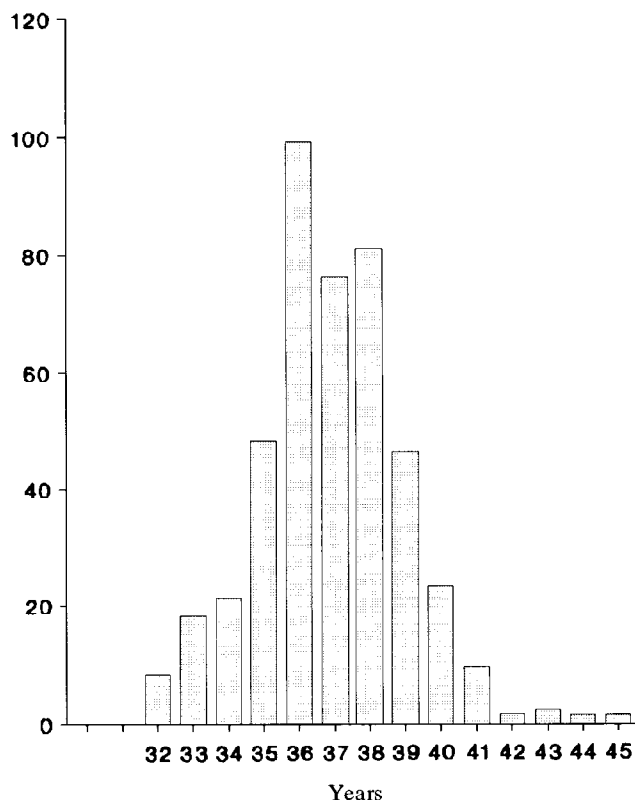


Figure 2 Distribution of maternal age (years)

Table 1 Amniocentesis results in twin pregnancies

Reference	Number of cases	Period of data accumulation	Rate of abortion %	Cut-off (weeks)
3	19	since 1979	0.0	20
4	13	since 1978	5.3	28
5	29	1973-79	17.0	28
6	70	1972-83	4.3	20
			10.0	28
7	28	1976-82	3.6	28
8	48	1977-83	2.0	28
9	48	1980-83	12.5	28
10	82	1980-85	4.9	28
11	330	1969-90	3.6	28
Present study	529	1985-90	2.3	20
			3.7	28

defined by the World Health Organization, was recorded. Only those twin pregnancies, in which two viable and sonographically unremarkable fetuses were present at the time of amniocentesis, were incorporated into the study. Pregnancies involving chromosomal and sonographically abnormal fetuses were excluded. Readily available documentation regarding the outcome of each pregnancy was a condition for accepting data into the study. Postnatally discovered defects of the children were, as far as possible, noted, as were the age of the mother, the time during the pregnancy at which amniocentesis was carried out, the number of insertions of the sampling needle and the application of a marker dye.

The sampling procedure itself has now been standardized in most hospitals, including those taking part in this study. Amniocentesis was carried out transabdomi-

nally under sonographic guidance using a 20–22-gauge needle with a trocar. A sample of 15–20 ml amniotic fluid was removed from each amniotic cavity. In cases where it was necessary to mark the first cavity in order to identify both sacs, 1–2 ml indigo carmine or methylene blue were injected into the first sac after aspiration of amniotic fluid. Subsequently, the second amniotic sac was punctured in the same manner. If dye-stained fluid was obtained, ultrasound examination was repeated and another attempt was made to obtain fluid from the second sac.

RESULTS

In total, 529 twin pregnancies in which amniocentesis had been carried out were evaluated. The maternal age varied between 31 and 45 years (average 36.7 years, see Figure 2). The time during the pregnancy at which amniocentesis was performed varied between the 14th and 19th gestational weeks (average 16.8 weeks, see Figure 3). A recent development at some centers (from the beginning of 1991) has been the tendency to undertake amniocentesis at an earlier gestational stage. The number of needle insertions per procedure is shown in Figure 4 (average 1.99). The type of dye used was known in 524 of the 529 cases. Indigo carmine was employed to mark the first amniotic sac in 351 cases, whereas methylene blue was used in 123 cases; no marker was injected in 50 cases (Figure 5). In this respect, there has been a recent trend to avoid using a dye and to rely solely on sonographic differentiation of the amniotic sacs.

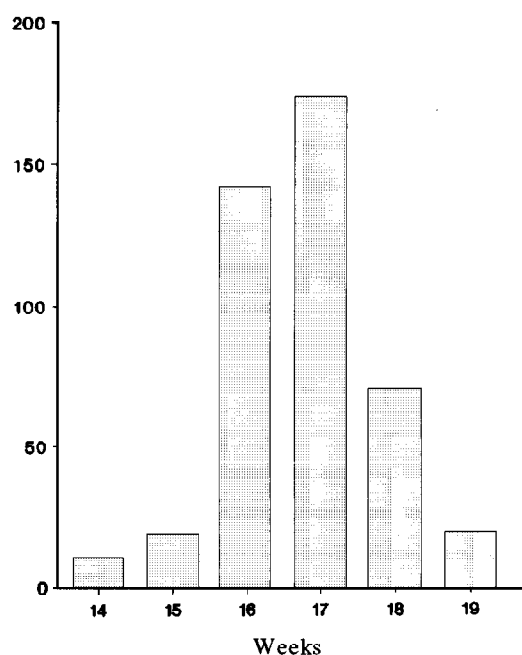


Figure 3 Distribution of gestational ages

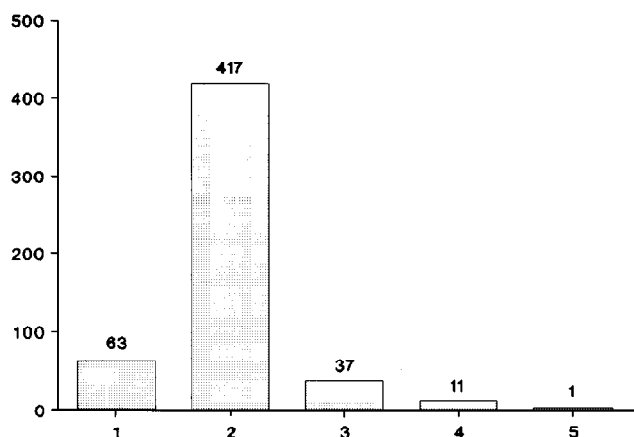


Figure 4 Number of needle insertions

In the 529 studied twin pregnancies, there were 12 cases (2.3%) in which the pregnancy terminated in a spontaneous abortion of both twins before 20 completed weeks of gestation. Eight of the studied mothers aborted between 20 and 28 completed weeks of gestation (1.5%). In total, this gave an abortion rate of 3.5% (20/529), as defined by WHO. Eleven pregnancies were lost after the 28th week. The loss of one twin with the subsequent birth of a living healthy child occurred in 31 pregnancies (Table 2).

The recorded intervals between amniocentesis and spontaneous abortion are given together with the number of needle insertions, the dye used and the suggested reason for abortion (as far as could be determined) in Table 3. This shows that abortion cannot be correlated either with the number of needle insertions or with the type of dye used.

The list of postnatally observed malformations cannot, of course, be considered as complete; slight deform-

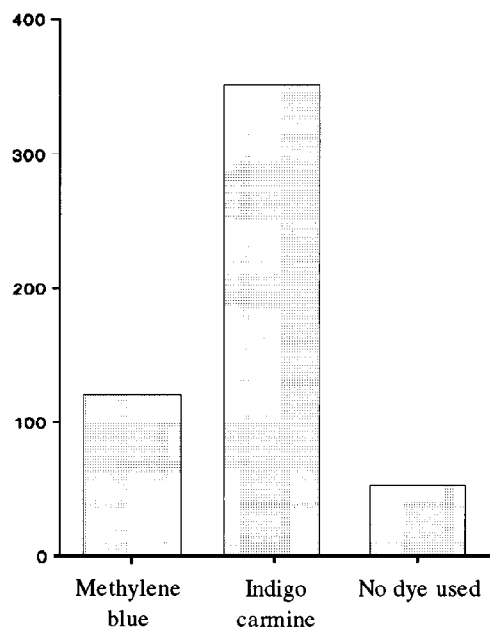


Figure 5 Frequency of dye used

Table 2 Fetal outcome

	n	%
Twin pregnancies	529	100.0
Pregnancy loss (both babies)		
up to 20 completed weeks	12	2.3
up to 28 completed weeks	8	1.5
Total	20	3.7
Stillborn after 28 completed weeks (both babies)	11	2.1
Demise of one fetus	31	5.8
Perinatal deaths	4	0.4
Livebirths, surviving children	961	90.8

ities and illnesses manifesting themselves later in the neonatal period were certainly not comprehensively registered. However, the significant occurrence of jejunal atresia is noteworthy. One case of omphalocele (but with no karyotypic abnormality) was not detected at the time of amniocentesis (Table 4).

Even at the beginning of the study, a correlation between the dye used and the occurrence of jejunal atresia had been noted (by J. G. Van der Pol). However, a significant association was only manifest between this malformation and the application of methylene blue at the beginning of 1991 (Figure 6). Of the 123 children whose amniotic sac was marked with 1–2 ml 1% methylene blue, 21 children required a postnatal operation to correct jejunal atresia (17.0%). In those children whose amniotic sac was marked with indigo carmine, there was one child (who was chromosomally normal) with duodenal stenosis (Figure 6). This difference is significant (Fisher's exact test: $p < 0.0001$). A possible cause of the correlation and the suggested etiology of the methylene blue-induced obstructions of the small intestine will be reported elsewhere (J. G. Van der Pol).

Table 3 Intervals between amniocentesis and abortion

Maternal age (years)	Number of insertions per case	Dye	Gestational age (weeks)		Suspected cause of abortion
			Of amniocentesis	At abortion	
<i>Up to 20 completed weeks</i>					
38	2	MB	16	17 + 5	AC ?
37	2	—	16/18	17 + 5	TS
38	2	IC	17/18	18 + 2	loss of amniotic fluid
37	2	IC	17/18	19	TS
37	2	IC	17/18	20	AC ?
44	2	IC	17/18	18	AC ?
38	2	IC	17/18	19	AC ?
36	2	IC	16	18	AC ?
34	2	IC	17	17 + 5	loss of amniotic fluid
36	2	MB	16	19	AC ?
37	2	IC	16	19	TS
33	1	IC	19	20	AC ?
<i>Up to 28 completed weeks</i>					
38	4	MB	16 + 18	25	sepsis
38	2	MB	17	28	TS
34	2	MB	19	23	AC ?
39	2	IC	18	24	AC ?
38	2	IC	17	21	TS
33	2	IC	19	22	AC ?
33	2	IC	17	23	AC ?
36	2	IC	16	21	AC ?

MB = methylene blue; IC = indigo carmine; TS = transfusion syndrome; AC = amniocentesis

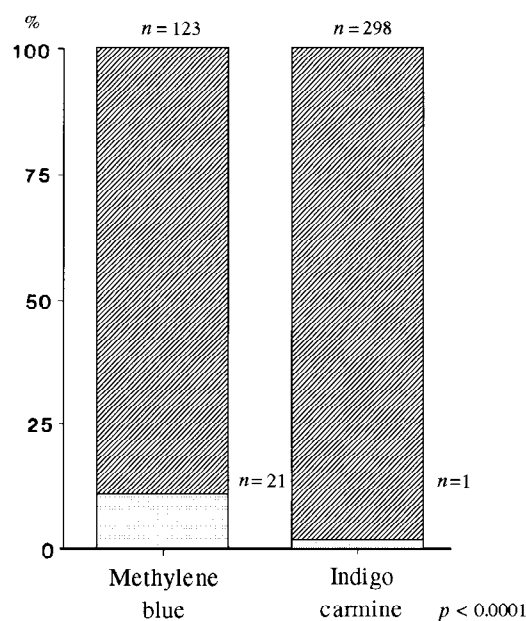
Table 4 Malformations detected postnatally

Jejunal atresia	21
Duodenal atresia	1
Jeune's syndrome	1
Pylorospasm	2
Clubfoot	2
Hypospadias	1
Omphalocele	1
Inguinal hernia	1
Total	30

DISCUSSION

Although the frequency of monozygotic twins is almost constant, the rate of occurrence of dizygotic twin pregnancies increases with maternal age². Because the most frequent indication for amniocentesis is advanced maternal age, twins will be encountered more frequently in this group.

The results of numerous small studies indicate that there is an increase in the risk of pregnancy loss for a couple undergoing amniocentesis for a twin gestation (Table 1). Recently, Anderson and colleagues¹¹ have reported a large single-center review of 336 multiple gestations. The authors show that the abortion rate following amniocentesis is 3.6% up to 28 completed weeks of gestation. This result agrees well with the data from our multicenter study (3.7%).

**Figure 6** Relation between the dye used and intestinal obstructions. Lighter shading indicates intestinal obstruction

We have not been able to confirm the suggested association between the abortion rate and the number of needle insertions¹²; neither could a correlation be established between maternal age and the abortion rate. This disagrees with studies on the spontaneous abortion rate in singleton pregnancies^{13,14}. Gilmore and McNay¹³ found an increase of the loss rates in singleton pregnancies with advancing maternal age. Nevertheless, the authors could find no differences in the spontaneous rate of abortion between age classes 30–34 and 35–39 years. A marked increase in spontaneous abortion in singleton pregnancies only occurred in the age class over 40 years.

The lack of an increase in the abortion rate in mothers over 40 years of age in our study can partly be explained because we eliminated data of all chromosomally and sonographically abnormal pregnancies.

In order to establish the identity of the procedural risk, the abortion rate during twin pregnancies without amniocentesis should be considered. Unfortunately, little reliable information exists concerning the loss rate between the 14th and 28th week of twin pregnancy. Coleman and colleagues¹⁵ reported a spontaneous abortion rate of 5% in pregnancies where the diagnosis of twins was first made at 20.5 mean gestational age. Kohl and Casey¹⁶ have combined the results of 6503 sets of twins and noted a 3.4% loss of pregnancies weighing under 500 g. Prömpeler and colleagues¹⁷ have estimated the spontaneous abortion rate in twin pregnancies with two viable fetuses at 16 and 17 weeks of gestation to be 4.5% until 28 completed weeks. Their sample, with 90 cases, was however small.

The results of our large multicenter study show that the abortion rate after amniocentesis in twin pregnancies is markedly higher than in singleton pregnancies, the latter rate being 1.7% according to Tabor¹⁸. Nevertheless,

it is unlikely that this higher abortion rate greatly exceeds that of the normal biological loss rate in twin pregnancies. During genetic counselling, the couple requesting advice should be informed, on the one hand, of the higher genetic risk of one of the twins having a relevant chromosomal abnormality but, on the other hand, of the higher abortion rate following amniocentesis.

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