Prenatal diagnosis of genetic disease in Canada: report of a collaborative study*

NANCY E. SIMPSON, PH D; L. DALLAIRE,† MD, PH D; J.R. MILLER,† PH D; L. SIMINOVICH,† PH D; J.L. HAMERTON,† D SC; JANET MILLER, MA; CAROL MCKEEN, B SC, B ED

A study of 1223 amniocenteses carried out during 1020 pregnancies in 990 women showed that 2nd-trimester amniocentesis at about 16 weeks' gestation is a safe, accurate and reliable procedure for the diagnosis of certain classes of genetic disease when it is monitored by ultrasound, performed by a trained obstetrician and carried out in a major health sciences centre. The percentage of fetal losses (4.7%) and neonatal deaths (0.5%) during the study was not greater than in control samples for women 35 years of age and older. The best results were obtained when needles of gauge 20 or 21 were used. The use of needles of gauge 19 or larger and more than two insertions during a single amniocentesis were associated with a significantly greater frequency of fetal loss than a second or even a third amniocentesis during the same pregnancy. For 39 fetuses (3.8%) a diagnosis of a genetic abnormality was made and 23 male fetuses were found to be potentially hemizygous for an X-linked gene. There were 51 therapeutic abortions as a result of the diagnosis. Sixty-six tests (5.4%) gave an inconclusive result and seven (0.6%) gave an erroneous diagnosis; five of the latter (two false-positives and three false-negatives) resulted from the α_1-fetoprotein test for neural-tube defects and in two cases the sex was incorrectly determined. The frequency of all chromosome abnormalities was 1:20 when the mother's age was 40 years or more and 1:60 when the mother's age was between 35 and 39 years. When a mother had previously had a child with a chromosome abnormality the risk of recurrence of such an abnormality was 1:100 when the age of the mother was 35 years or more.

Une étude de 1223 amniocentèses pratiquées au cours de 1020 grossesses survenant chez 990 femmes a révélé que l'amniocentèse au 2ème trimestre, après environ 16 semaines de gestation, est une intervention sûre, précise et fiable pour diagnostiquer certaines classes de maladies génétiques quand elle est effectuée dans un centre hospitalier important par un obstétricien qualifié, sous contrôle échographique. Les pourcentages de mortinatalité (4.7%) et de décès chez les nouveaux-nés (0.5%) durant l'étude n'ont pas excédé ceux de groupes témoins composés de femmes âgées de 35 ans et plus. Les meilleurs résultats ont été obtenus avec des aiguilles de calibres 20 ou 21. L'emploi d'aiguilles d'un calibre égal ou supérieur à 19 et le recours à plus de deux insertions pendant une seule amniocentèse ont été reliés à un pourcentage significativement plus élevé de mortinatalité qu'une deuxième ou même une troisième amniocentèse pendant la même grossesse. Un diagnostic d'anomalie génétique a été rendu pour 39 foetus (3.8%), et 23 foetus de sexe masculin ont été trouvés possiblement hémizygote pour un gène situé sur le chromosome X. Cinquante et un avortements thérapeutiques ont été pratiqués suite aux diagnostics posés. Soixante-six tests (5.4%) ont donné des résultats non concluants et sept (0.6%) ont donné un diagnostic erroné; cinq de ces derniers (deux faux-positifs et trois faux-négatifs) sont provenus du test des α_1-foetoprotéines pour les anomalies du canal dorsal de l'embryon et dans deux cas le sexe a été incorrectement diagnostiqué. La fréquence des aberrations chromosomiques a été de 1:20 quand l'âge de la mère était de 40 ans ou plus et de 1:60 quand l'âge de la mère était de 35 à 39 ans. Quand la mère a eu précédemment un enfant porteur d'une anomalie chromosomique le risque de récidive a été de 1:100 quand l'âge de la mère était de 35 ans ou plus.

In November 1971 the Medical Research Council of Canada established a working group on prenatal diagnosis of genetic disease, with wide terms of reference. This group invited Canadian centres to collaborate in a study of genetic amniocentesis to determine the appropriateness of the indications, the risk to mother and fetus, and the efficiency and reliability of the procedures. The study was also designed to provide prospective data on the risk of chromosome abnormalities among offspring of high-risk mothers and to monitor the introduction of new tests on the fetus. During the course of the study it became clear that pregnancies at high risk for neural-tube defects could be monitored by determining the

From the division of medical genetics, Queen's University, Kingston (N.E.S., J.M. and C.M.); the section of medical genetics, Hôpital Sainte-Justine, Montreal (L.D.); the division of genetics, University of Manitoba, Winnipeg (J.L.H.); the department of medical genetics, University of British Columbia, Vancouver (J.R.M.); and the department of medical genetics, University of Toronto (L.S.)

*While the collaborating centres gave general approval to this report, the authors are responsible for its final content.

†Member of the working group on prenatal diagnosis of genetic disease of the Medical Research Council of Canada

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Reprint requests to: Dr. Nancy Simpson, Department of pediatrics, Queen's University, Kingston, ON K7L 3N6

concentration of α_1-fetoprotein (AFP) in amniotic fluid; advantage was taken of the introduction of this test to clinical practice to study its efficiency and efficacy.

This report summarizes the preliminary findings of the collaborative study.

Collection of data

Amniocentesis cases

Each of the 13 collaborating centres was associated with a major health sciences complex and was requested to register all amniocenteses performed with a central data bank at Queen's University, Kingston. The Canadian guidelines for genetic amniocentesis[1] were adhered to, and each centre followed up all cases to the completion of pregnancy (that is, to the delivery of a live or stillborn infant or to elective or spontaneous abortion). Apr. 1 to Dec. 31, 1972 was the pilot study period; thereafter, the original questionnaire was modified slightly and the main study commenced in January 1973, follow-up of all cases being completed by February 1976. Data on 1020 pregnancies, representing the majority of pregnancies in which genetic amniocentesis was performed in Canada during the period January 1972 to mid-1975, were obtained.

The study was monitored for quality and objectivity throughout by means of regular review of the data by the working group and consultation with participating centres, and by annual meetings between the working group and the principal investigator and nurse-coordinator from each centre.

Each centre coordinated its own data on a five-part questionnaire: part 1 contained personal data, identifying information, obstetric history and indication for amniocentesis; part 2 provided information on the amniocentesis itself and was completed by the obstetrician performing the procedure; part 3 provided information about the outcome of the pregnancy; part 4 gave the laboratory results; and part 5 gave the results of a physical examination of the newborn infant.

Control data

It was decided at the outset not to attempt to use matched pregnancies as controls because of the difficulties encountered in studies in the United States and the United Kingdom in obtaining suitable age-matched controls and because of the cost of such an undertaking. The working group believed that many of the variables could be controlled adequately by comparison within the study and by using control data from vital statistics records available from various agencies in Canada.[2-5]

The numbers of stillbirths* and neonatal deaths* among offspring of women 35 years of age and older who underwent amniocentesis were compared with control data for women of the same age obtained from the vital statistics records of the provinces of British Columbia (G. Renwick and J. Rowe: personal communication, 1976) for the years 1973 and 1974 and Manitoba[6] for the years 1970 through 1974. These two provinces were chosen because the appropriate statistics were available by the age of the mother.

The incidence of spontaneous abortions* at 16 through 19 weeks' gestation in the study population was compared with that in several hospital populations in Toronto and Hamilton. The records of all women aged 35 and older who were admitted to the Toronto General Hospital and three hospitals in Hamilton with a pregnancy-related diagnosis in 1973 and 1974 were selected. In addition, a prenatal clinic in Hamilton was screened for patients aged 35 years or more who had registered for prenatal care before 20 weeks' gestation. (In all instances records of women who had had a therapeutic abortion or genetic amniocentesis were excluded.) For each record the pregnancy outcome, maternal age, parity, gravidity, number of previous stillbirths and spontaneous abortions, sex and gestational age at term were recorded.

Amniocentesis procedure and follow-up

Most amniocenteses† were carried out at a university-affiliated teaching hospital. Genetic counselling and advice were provided by the appropriate genetic centre. Continuing prenatal care usually reverted to the family physician or obstetrician after the amniocentesis had been performed.

Results

Characteristics of the women

During this study 1020 pregnancies requiring 1223 amniocenteses were monitored. Of the 990 women 30 had two amniocenteses in different pregnancies. Twenty-seven percent of the women who underwent amniocentesis had completed a university education, while 19% had completed only grade 8; the comparable figures for the general Canadian female population were 7.5% and 44%. The head of the household had a professional, technical or managerial position almost twice as often in the amniocentesis group as in the general Canadian population.[2] Frequencies of other occupational categories were similar in the amniocentesis and control groups.

In the amniocentesis group over 50% of the women were Protestant and 30% Catholic, compared with 37% and 46%, respectively, in the general Canadian population. Racial distribution of the amniocentesis group was similar to that of the general Canadian population, over 93% of the women being Caucasian.[3,4]

Indications for amniocentesis

Maternal age was the indication for over half the procedures; the second most common indication was the previous birth of a child with a chromosome abnormality, usually Down syndrome. The number of women undergoing amniocentesis for either reason increased steadily during the study (Fig. 1). The number undergoing amniocentesis because of the previous birth of a child with a neural-tube defect increased gradually from the end of 1973, when measurement of AFP in amniotic fluid became routine. In contrast, the numbers of amniocenteses performed because of the birth of a child with a biochemical or X-linked disease showed little change during the study.

Geographically, delivery of this service was not uniform and the numbers of women undergoing amniocentesis were only a small fraction of those at risk in the age groups 35 to 39 years

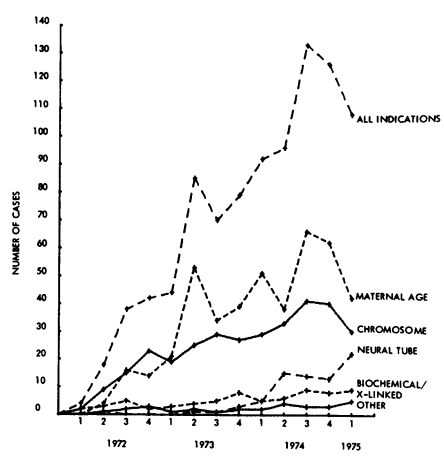

FIG. 1—Distribution of amniocentesis cases by indication for the procedure in 3-month periods from January 1972 to March 1975.

*In this report stillbirth is defined as the delivery of a dead fetus of 20 weeks' or more gestational age. Neonatal death is defined as death within 7 days after delivery. Spontaneous abortion is defined as the delivery of a fetus between the amniocentesis and 19 weeks' gestational age.

†Throughout this report "amniocentesis" refers to an attempt to obtain amniotic fluid on a given day. "Insertion" and "needle insertion" refer to actual insertions of the needle into the abdomen at a single amniocentesis. More than one needle insertion per amniocentesis and more than one amniocentesis per pregnancy may be required to obtain fluid. A successful amniocentesis is one in which some amniotic fluid was obtained.

Table I—Number of women per 1000 giving birth* who underwent amniocentesis in 1973 and 1974

Region	1973 Mother's age (yr)		1974 Mother's age (yr)	
	35-39	≥ 40	35-39	≥ 40
Maritimes	6.0	2.6	8.6	18.9
Quebec	1.5	8.3	5.6	17.2
Ontario	6.1	12.7	9.5	38.1
Manitoba	9.0	28.6	1.4	24.9
Saskatchewan	0.0	10.9	1.5	8.4
Alberta	13.0	26.6	20.6	59.9
British Columbia	17.7	56.7	10.6	106.2
All provinces†	5.9	15.2	8.4	34.1

*Statistics Canada data for 1973 and 1974; includes live births and stillbirths.
†Excluding Newfoundland.

and 40 years or over (Table I).

Amniocentesis technique

The technique of amniocentesis varied. Number of needle insertions, needle size, use of placental localization, and gestational age by uterine size were important variables.

Needle insertions: Among the successful amniocenteses 76% required one, 14% two and 10% three or more needle insertions. Insertions were fewer with placental localization by ultrasonography and when a 20- or 21-gauge needle was used. The percentage of successful amniocenteses requiring one insertion to obtain fluid varied directly with gestational age as measured by uterine size, from 56% at 14 weeks or less to 81% at 18 weeks or more. At 16 weeks' gestation 70% of amniocenteses required a single insertion to obtain fluid. When more than two insertions were made during one amniocentesis there were significantly more spontaneous abortions and stillbirths ($P < 0.001$).

Needle size: Amniocentesis was significantly ($P < 0.01$) more likely to be successful when needles of gauge 20 or 21 rather than 19 or larger were used. The use of 19-gauge or larger needles resulted in a significantly higher ($P < 0.001$) incidence of spontaneous abortion or stillbirth. Use of a needle larger than gauge 20 may have resulted in more maternal complications within 72 hours of the procedure ($0.05 > P > 0.02$).

In certain cases a small needle (smaller than gauge 21) led to relatively poor results in obtaining fluid. Obstetricians were arbitrarily divided into two groups: those who obtained fluid in at least 85% (group A) and those who obtained fluid in less than 85% (group B) of their amniocenteses. The group B obstetricians used a small needle in 66%, whereas the group A obstetricians used a small needle in only 24% of their amniocenteses. Both groups A and B used placental localization and only those who registered more than five cases were considered in these comparisons. There were no differences in performance of obstetricians working in the centres, compared with those working outside, except that those working outside did fewer amniocenteses. Furthermore, there was no apparent correlation between the rate of success in obtaining fluid and the number of amniocenteses done by a given obstetrician.

Placental localization: Only one amniocentesis was required in 86% of the pregnancies in which the placenta was localized, compared with 76% of those in which the placenta was not localized ($P < 0.001$). There were significantly more ($P < 0.01$) successful amniocenteses when ultrasonography was used.

Gestational age by uterine size: Success in obtaining amniotic fluid was directly proportional to gestational age. At 14 weeks 76% of amniocenteses were successful; at 15 weeks, 86%; at 16 weeks, 91%; at 17 weeks, 93%; and at 18 weeks, 95% ($P < 0.001$). At 15 weeks or less a diagnosis was made in 92%; at 16 weeks, in 94%; and at 17 weeks or more, in 96%. Most results of chromosome analysis were obtained within 21 days of amniocentesis, whereas most enzyme assays took more than 22 days to complete (Table II).

Reasons for repeated amniocenteses

One amniocentesis was needed for diagnosis in 84%, two in 14% and three in 2.5% of the pregnancies for which a diagnosis was obtained. Of the 277 instances in which amniocentesis failed to provide a diagnosis the procedure was repeated in 203. The reasons for repetition (and number of instances) were as follows: culture failure (89), failure to obtain fluid (60), blood obtained instead of fluid (34), and inconclusive result or for confirmation of an abnormal result (15); in 5 instances no reason was stated.

There was significantly more ($P < 0.001$) success in karyotyping when mixed epithelial- and fibroblast-like cells or fibroblast-like cells alone were cultured than when epithelial-like cells only were cultured.

Maternal complications within 72 hours of amniocentesis

Of the 1223 amniocenteses amniotic fluid leakage per vaginam occurred after 16 and abdominal swelling or tenderness after 11; uterine contractions, vaginal bleeding, spontaneous abortion or other complications occurred in 17 instances (1.4%), and four women had two of the above complications each. There were significantly more ($P < 0.001$) complications (7.3%) when the amniocentesis was carried out at a gestational age of 15 weeks or less than when the age was 16 weeks or more (2.8%).

One mother in this series died post partum. Her infant, born at the 30th week of gestation, 14 weeks after the amniocentesis, had severe Rh disease and died subsequently. The mother was well at first post partum but died suddenly on the 7th day of pulmonary embolism.

Diagnostic results of amniocentesis

Diagnostic results are listed in Table III.

From the tests done to determine the sex of a fetus at risk for an X-linked disease, 23 fetuses were found to be male and potentially hemizygous for the mutant gene.

Of the 11 inconclusive tests chromosome analysis showed a 46,XX/46,XY karyotype in 3; the cause was thought to be contamination of the amniotic fluid with maternal cells. In each of

Table II—Interval between final amniocentesis and diagnosis

Interval (days)	Diagnostic test (no. of amniocenteses [and % of total])	
	Enzyme assay	Chromosome analysis
1 - 7	1 (2.9)*	14 (1.4)
8 - 14	1 (2.9)	401 (39.3)
15 - 21	6 (17.1)	288 (28.2)
22 - 28	11 (31.4)	94 (9.2)
29 - 35	6 (17.1)	14 (1.4)
≥ 36	6 (17.1)	10 (1.0)
No information†	4 (11.4)	199 (19.5)
Total	35 (100.0)	1020 (100.0)

*In testing for G_{M1} gangliosidosis β-galactosidase was measured directly in amniotic fluid; cells were not cultured.
†Includes no date and no result obtained.

Table III—Diagnostic results from final amniocentesis

Diagnosis	No. (and %) of amniocenteses
Normal	
Normal, including minor variants	907* (88.9)
Balanced translocations	6 (0.6)
Biochemical heterozygotes	2 (0.2)
Subtotal	915 (89.7)
Affected	
Chromosome abnormality	23 (2.3)
Biochemical abnormality	9 (0.9)
High α_1-fetoprotein value	7 (0.7)
Subtotal	39 (3.8)
Inconclusive	
Culture failure	38 (3.7)
Amniocentesis failure	17 (1.7)
Inconclusive test†	11 (1.1)
Subtotal	66 (6.5)
Total	1020 (100.0)

*Includes 23 males with 50% risk for X-linked disease.
†See text.

the three instances a normal boy was delivered. Three cell cultures showed possible chromosome artefacts: the first showed a normal female karyotype except for one colony with a C;D translocation; the second showed a 47,XX,+9 karyotype in cells from the first amniocentesis, while cells from two successive repeat amniocenteses showed a 46,XX pattern (spontaneous abortion occurred and confirmation was not possible); and the third showed a possible mosaic pattern (46,XX/47,XXX) but a 46,XX girl was born. AFP values were high in two instances. In one instance this was thought to have resulted from contamination of amniotic fluid with fetal blood. In the second the AFP value was in the high borderline range; the parents elected not to have the test repeated and to continue the pregnancy. In both instances normal girls were delivered. Three cultures failed to give a satisfactory diagnosis; a normal boy and two normal girls were delivered at term.

The fetal abnormalities observed when the indication for amniocentesis was advanced maternal age are given in Table IV. Sixteen of the 18 affected fetuses had a chromosome abnormality: trisomy 21 in 9, trisomy 18 in 3, trisomy 13 in 1, 7q+ in 1, de novo t(5;16) in 1 and XY/XXY in 1. One fetus had hypophosphatasia diagnosed by ultrasound. One had a high AFP value, which was interpreted as due to fetal death; the pregnancy terminated at 29 weeks in a stillbirth; the fetus had an open myelocele.

When the indication for amniocentesis was a chromosome abnormality in the family (Table V) four of the six abnormal fetuses had chromosome abnormalities and two had neural-tube defects. In 14 families in which one parent was carrying a chromosome translocation, apparently balanced translocations were found in six fetuses and possibly unbalanced translocations in one; in the last instance abortion was performed because a sibling with the same karyotype had major congenital malformations.

When the mother's age was 35 to 39 years the frequency of all chromosome abnormalities was 1:60 (Table VI). When the mother was 40 or older the frequency was more than 1:20 and two thirds of the fetuses had trisomy 21. When the indication was the previous birth of a child with trisomy 21 and the mother's age was less than 35 years about 1 in 100 fetuses had a chromosome abnormality. With increasing maternal age and the previous birth of an affected child the rate of chromosome abnormality appeared to be greater than for the same maternal ages but a negative family history; the numbers involved, however, were extremely small.

The results in the 191 pregnancies monitored for nonchromosomal indica-

Table IV—Abnormalities diagnosed when indication for amniocentesis was advanced maternal age

Mother's age (yr)	No. of pregnancies tested	No. (and %) of fetuses affected	Diagnosis*
35 - 39	249	5 (2.0)	Trisomy 21 Trisomy 18 46, XY, 7q+ ? unbalanced 46, XX,t (5;16) (p13;q24) Hypophosphatasia†
≥ 40	217	13 (6.0)	Trisomy 21 (8 fetuses) Trisomy 18 (2 fetuses) Trisomy 13 XY/XXY High α_1-fetoprotein value (fetal death)
Total	466	18 (3.9)	

*Abortion was performed in all instances except in one case of trisomy 21 and in the XY/XXY case (both diagnoses were confirmed at birth) and in the case of high α_1-fetoprotein value thought due to fetal death (stillbirth at 29 weeks; myelocele). All diagnoses were confirmed at abortion or birth except in one case of trisomy 21 (macerated fetus; tissue culture failed).
†First identified by ultrasound.

Table V—Abnormalities diagnosed when indication for amniocentesis was a chromosome abnormality in the family

Indication	No. of pregnancies tested	No. (and %) of fetuses affected or carrier	Diagnosis*
Down syndrome†			
Mother's age (yr)			
< 35	238	3 (1.3)	Trisomy 21‡ XXY Neural-tube defect
35-39	33	1 (3.0)	XXY
≥ 40	12	1 (8.3)	Trisomy 21
Other chromosome abnormality	66	1 (1.5)	Neural-tube defect
Translocation in a parent	14	1 (7.1)	? unbalanced 46,XX,t(1;4) (p32;q31)§
		6 (42.9)	Balanced t (D; G) (3 fetuses) t (D; D) t (5;9) t (13;14)
Total	363	13	

*Abortion was performed in all instances except in one case of trisomy 21 (Down syndrome confirmed at birth). All diagnoses were confirmed at abortion or birth except two of neural-tube defect (no apparent neural-tube defect at abortion) and in one XXY case (macerated fetus at abortion).
†Down syndrome in a previous child or another family member or both (excludes familial translocation).
‡Mother subsequently shown to have mosaic pattern for trisomy 21.
§? unbalanced because of previous birth of abnormal child with 46, XY,t (1;4) (p34:q31) karyotype.

tions are shown in Table VII. Included in the 90 pregnancies monitored for neural-tube defects is one that was monitored routinely by ultrasound, which showed an anencephalic fetus; this finding was subsequently confirmed by a high AFP value in the amniotic fluid. Of the 89 pregnancies in which the indication for amniocentesis was the previous birth of a child with a neural-tube defect, three fetuses had a neural-tube defect, one had trisomy 21 and one had a chromosome rearrangement. Thus, for neural-tube defects, 3.4% of fetuses were affected when the indication was an affected child in the family, compared with 0.2% (2:931) when there was no affected child. These frequencies do not depart significantly from expectation.[7] Enzymes were assayed in 35 pregnancies monitored because of high risk of a metabolic disease: eight fetuses (22.9%) were considered to be affected and two, heterozygous. The sex of the fetus was determined in 33 pregnancies because of the risk of a male being affected with an X-linked disease; 23 males were detected.

Abortion was performed in 51 of the 62 instances (Table III) in which the fetus was considered to be abnormal (Table VIII). In four instances in which the fetus was considered to be normal an abortion was performed at the request of the mother. Six of the other 11 "abnormal" fetuses were potentially hemizygous for an X-linked gene; all were phenotypically normal at birth but in 1 the Wiskott-Aldrich syndrome developed later. In three instances (two of trisomy 21 and one of an XY/XXY mosaic pattern) the decision not to abort, although the fetus had a chromosome abnormality, was taken by the mother. In two instances of fetuses with high AFP values spontaneous abortion occurred (at less than 20 weeks and at 29 weeks [see Table IV]); both fetuses had open neural-tube defects. The diagnosis could not be confirmed in nine fetuses because of the condition of the abortus.

Outcome of pregnancy

The outcomes of the 1020 pregnancies are given in Table IX. Thirty-three pregnancies (3.2%) resulted in stillbirth or neonatal death and an additional 10 pregnancies (1.0%) terminated in spontaneous abortion.

Table VI—Occurrence of fetal chromosome abnormalities by indication for amniocentesis

Indication	No. of pregnancies tested	Number (and %) of fetuses affected: Trisomy 21	Number (and %) of fetuses affected: All chromosome abnormalities
Mother's age (yr)			
35 - 39	249	1 (0.4)	4 (1.6)
≥ 40	217	8 (3.7)	12 (5.5)
Trisomy 21 in sibling			
Mother's age (yr)			
< 35	211	1* (0.5)	2 (0.9)
35-39	24	0 (0.0)	1 (4.2)
≥ 40	7	1 (14.3)	1 (14.3)
Nonchromosomal abnormality in family	191	1 (0.5)	2 (1.0)
Total	913†	12 (1.3)	22 (2.4)

*Mother subsequently found to have mosaic pattern for trisomy 21.
†"Chromosome" indications other than maternal age or Down syndrome in sibling are excluded.

Table VII—Abnormalities diagnosed when indication for amniocentesis was nonchromosomal

Indication	No. of pregnancies tested	No. (and %) of fetuses affected	Diagnosis*
Neural-tube defect	90	6 (6.7)	Neural-tube defect (4 fetuses) Trisomy 21 ? unbalanced 46,XX,t (2;4) (p14;q35)
Metabolic disease	35	8 (22.9)	Tay-Sachs disease (4 fetuses) G_{M1} gangliosidosis Metachromatic leukodystrophy Sandhoff's disease Lesch-Nyhan disease
X-linked disease	33	23 (69.7)	Male
Other†	33	0 (0.0)	
Total	191	37 (19.4)	

*Abortion was performed in all instances except six of possible X-linked disease (six males, all apparently normal at birth). One spontaneous abortion (diagnosed neural-tube defect) occurred. All diagnoses were confirmed at abortion or birth except two: in one case of X-linked disease (no information on abortus) and in the Lesch-Nyhan fetus (no information on abortus other than a balanced t[13;14] from amniotic fluid cells).
†Includes a variety of indications, such as radiation exposure and repeated abortion. These will be discussed in detail in the final report of the working group.

Table VIII—Indications for elective abortion

Indication (diagnostic result)	No. with indication	No. confirmed	Reason for lack of confirmation
Sex for X-linked disease	17	16	No info. on abortus
Chromosome abnormality	20	17	Macerated fetus
Biochemical abnormality	9	8	No info. on abortus
High α_1-fetoprotein value	5	3	No apparent neural-tube defect
Subtotal	51	44	
Inconclusive result	2*	1	No info. on abortus
None; diagnosis, normal	2*	1	Apparently normal fetus; sex indeterminate
Total	55	46	

*Elective abortion requested by mother.

Table IX—Outcome of pregnancy

Outcome	No. (and %)
Normal male	458 (44.9)
Normal female	398 (39.0)
Twins	13 (1.3)
Undetectable abnormality*	49 (4.6)
Detectable abnormality†	4 (0.6)
Neonatal death	10 (1.0)
Stillbirth	23 (2.3)
Spontaneous abortion	10 (1.0)
Elective abortion	55 (5.4)
Total	1020 (100.0)

*Includes spina bifida occulta, closed meningocele, club foot, cleft palate, osteogenesis imperfecta, cystic fibrosis, etc.
†Trisomy 21, 2 cases; 46, XY/47,XXY, 1 case: all detected; mother elected to continue pregnancy. Anencephalic stillbirth: missed — no fluid obtained in two attempts.

Table X—Outcome of pregnancy when indication for amniocentesis was maternal age 35 years or more, compared with outcome in two provincial control populations of women aged 35 years or more

Outcome of pregnancy	% of all births		
	Amniocentesis group	Manitoba controls*	BC controls†
Live birth‡	96.2	97.4	98.3
Neonatal death (≤ 7 days)	0.5	1.2	1.3
Stillbirth (≥ 20 weeks)	3.8	2.6	1.7
No. of births	443	6066	3098

*1970-74 vital statistics data for Manitoba.
†1973-74 vital statistics data for British Columbia.
‡Includes neonatal deaths.

Table XI—Outcome of pregnancy when indication for amniocentesis was maternal age 35 years or more, compared with outcome in two hospital control populations of women 35 years or more

Outcome of pregnancy	% of all births		
	Amniocentesis group	Hamilton controls*	Toronto controls†
Live birth	96.2	96.3	97.8
Stillbirth	3.8	3.7	2.2
Spontaneous abortion‡	0.9	1.9	1.5
No. of births	443	431	275

*1973-74, one prenatal clinic, three hospitals.
†1973-74, Toronto General Hospital.
‡16 through 19 weeks' gestation only.

Table XII—Erroneous diagnoses

Indication for amniocentesis	Diagnosis	Outcome	Type of error	Probable reason for erroneous diagnosis
Down syndrome	Normal female	Spontaneous abortion; male	Sexing	Maternal contamination or erroneous pathology report
Mother's age, 40 years	Normal female	Normal male	Sexing	Maternal contamination
Down syndrome	Neural-tube defect	No neural-tube defect	False +	Test limitation
Trisomy 18	Neural-tube defect	No neural-tube defect	False +	Test limitation
Neural-tube defect	Normal female	Closed neural-tube defect	False −	Test limitation
Neural-tube defect	Normal female	Closed neural-tube defect	False −	Test limitation
Maternal age, 45 years	Death	Stillbirth; open neural-tube defect	? false −	Incorrect interpretation

Fetal losses and neonatal deaths: The percentage of stillbirths (for women aged 35 years or more) in the amniocentesis group was significantly greater than that in the BC control population ($P < 0.01$) but was not significantly greater than that in the Manitoba control population (Table X). The percentage in the Manitoba group, however, was significantly greater than that in the BC group ($P < 0.01$). The percentage of neonatal deaths in the amniocentesis group was not significantly different from those in the two control groups. Comparison of the incidence of stillbirths in the amniocentesis group and in the Hamilton and Toronto control groups showed no differences (Table XI). The incidence of spontaneous abortions in the amniocentesis group was low but did not differ significantly from those in the two hospital control groups.

Because of the apparent increase in the stillbirth rate in the amniocentesis group compared with the BC group the data for the amniocentesis group and the Hamilton and Toronto groups were divided into 5-week periods. The period of 20 to 24 weeks' gestational age included 6 of the 17 stillbirths in the amniocentesis group but only 3 of the 13 stillbirths in the Hamilton control group and 1 of the 6 stillbirths in the Toronto control group. When, however, the fetal losses in the amniocentesis group between 16 and 24 weeks were added (10) and compared with those in the hospital control groups the rate in the amniocentesis group (2.3%) was not increased significantly over that in the control groups (1.9% for the Hamilton controls and 1.8% for the Toronto controls).

The data on stillbirths and spontaneous abortions suggest that when there is a fetal loss at about 20 weeks there may be some differences in reporting. There may have been more of a commitment to report accurately the gestational age of a fetal loss in the amniocentesis group than in the controls. There were more stillbirths in the amniocentesis group reported from 20 to 24 weeks than in the two hospital control groups and this appeared to be somewhat balanced by the fewer fetal losses reported from 16 through 19 weeks among the women who had had amniocentesis. However, comparison of the fetal losses between 16 and 24 weeks in the amniocentesis group with those in the two control groups revealed no differences. Thus, the differences seen in Table X are probably due to variability in the reporting of fetal loss when it occurred around 20 weeks' gestational age.

Among the fetal losses were two cases of amnionitis that may have been the result of the procedure. One pregnancy terminated with the spontaneous abortion of a pair of female twins 60 hours after the amniocentesis. This was the only amniocentesis and only one needle insertion was required to obtain fluid. In the other case a male infant was born at 28 weeks and died at 4½ hours. This woman had had four amniocenteses, one each at 15, 16, 19 and 20 weeks. The first two were carried out by an obstetrician outside the genetic centre, who used three needle insertions in each instance. The third and fourth amniocenteses were performed by an obstetrician in the genetic centre. The placenta was not localized at any time. It was only on the fourth amniocentesis that fluid was obtained and it was bloody. The multiple procedures and needle insertions may have caused the amnionitis and death of the infant. This case occurred near the beginning of the study and the difficulties encountered were undoubtedly due to lack of experience.

Erroneous diagnoses

Seven erroneous or missed diagnoses occurred (Table XII). Two may have been due to contamination of the amniotic fluid with maternal cells or, in one case, an error in the pathology report. Five instances involved prenatal detection of neural-tube defects. No error in chromosome analysis per se was reported.

Discussion

In one other collaborative amniocentesis study, the NICHHD (National Institute of Child Health and Human Development) National Registry for Amniocentesis,[8] 1040 pregnancies in which amniocentesis was undertaken and a similar number of matched control pregnancies were monitored. The results can be compared with those of our study.

Delivery of amniocentesis service

Both our study and the NICHHD study indicated that, at present, and for a variety of sociologic reasons, amniocentesis is used by only a small proportion of the female population at risk and that the mothers taking advantage of its availability are likely to be well educated and in a professional family. In this respect the delivery of this service does not differ from that of other innovative health services. Our study indicated that Protestants are more likely than Catholics to undergo amniocentesis.

Amniocentesis procedure

Both our study and the NICHHD study indicated that the procedure is safe for both the fetus and the mother. In the NICHHD study no difference in percentage of fetal loss was observed between the amniocentesis and control series. In our study the percentage of fetal loss in the amniocentesis group was not greater than that in the control groups for women 35 years of age and older. The fetal losses in the amniocentesis groups in the American study and in our study for women of all ages were 36 out of 1040 and 33 out of 1020, respectively; furthermore, in the US matched control sample there were 32 fetal losses in 992 pregnancies.

Our study indicated that multiple needle insertions during a single amniocentesis were more often followed by a spontaneous abortion or stillbirth than were two insertions or fewer. It is not clear whether the difficulty in obtaining amniotic fluid was related to potential fetal loss or whether the fetal loss was related to the multiple insertions. No such clear relation was observed in the NICHHD study; however, in that study an increased number of insertions was associated with an increased incidence of maternal complications within 72 hours of the procedure. The number of separate amniocenteses in a single pregnancy did not, however, appear to be important in either series. As might be expected, greater success in obtaining fluid was directly correlated to increase in uterine size; success decreased significantly when the gestational age was less than 16 weeks. Furthermore, tests carried out at 15 weeks or less had a greater chance of resulting in complications. Sixteen weeks seems to be the optimal time to carry out genetic amniocentesis. This, however, has to be balanced against the desirability of obtaining a fetal diagnosis as early as possible, particularly when this depends on enzyme assays.

A comparison of amniocenteses that were monitored by ultrasonography with those that were not, indicated that ultrasonography is important for a number of reasons, including a higher frequency of single amniocenteses being required and greater success in obtaining fluid. Ultrasonography did not, however, affect fetal loss or frequency of maternal complications.

Both the Canadian and United States studies showed that the use of large needles was contraindicated. In the Canadian series use of a needle size greater than gauge 19 led to fewer successful amniocenteses, more spontaneous abortions and stillbirths, and perhaps an increased frequency of maternal complications. In the American series the use of a needle of gauge 17 or greater resulted in a significant increase in number of fetal losses. It appears that needles of gauge 20 or 21 are best. Use of a small needle (gauge 21 or less) was not associated with unsuccessful amniocenteses in the total group. On the other hand, a small needle had been used more often when fluid was not obtained by the group B obstetricians than by the group A obstetricians. The lack of success among the group B obstetricians was not due to fewer insertions or fewer amniocenteses per patient; in fact, they did significantly more than the group A obstetricians ($P < 0.001$). Group B obstetricians did not perform their amniocenteses earlier than group A obstetricians. Thus, needle size appears to be the most important factor contributing to their higher failure rate.

Because of the high level of maternal anxiety and the desirability of performing abortion as early as possible when the fetus is affected, it is important that the interval between the test and the delivery of the results be as short as possible. The results reported here and those obtained in the United States indicated that, particularly for biochemical disease, further work is needed to try to reduce this interval.

The results of our study indicated that high accuracy and reliability can now be obtained in diagnosing both chromosome abnormalities and biochemical disease. There are, however, clear limitations to the use of AFP values in amniotic fluid for detection of neural-tube defects; five out of seven of the erroneous diagnoses were related to the test for AFP. Further work is therefore needed to improve the accuracy of this test.

When data from our study and the NICHHD study were pooled, an increase in frequency of chromosome abnormalities at advanced maternal ages, compared with the accepted frequency in the general population (based on live births) became evident. This increase applied primarily to trisomy 21, but other chromosome abnormalities, especially trisomy 18, were also increased in frequency. In the 2nd trimester when the mother's age was 40 years or more a frequency of 1:20 was found for all types of chromosome abnormalities, while at ages 35 to 39 the frequency was about 1:60. If these results can be taken as representative for the general population they may indicate a greater increase in risk than was previously supposed. The numbers were, however, relatively small and further data are needed.

Conclusions

Amniocentesis for the diagnosis of certain classes of genetic disease can now be considered safe, accurate and reliable when carried out at about 16 weeks' gestation, monitored by ultrasound and performed by an obstetrician trained to carry out the procedure during the 2nd trimester of pregnancy. Needles of gauge 20 or 21 should be used. More than two insertions during a single amniocentesis in an attempt to obtain fluid should be avoided because this seems to be associated with a greater frequency of fetal loss than a second or even a third amniocentesis during the same pregnancy. Further research is clearly needed to shorten the time required to obtain a laboratory result, particularly in respect to biochemical disease, and to improve the accuracy of the AFP test for neural-tube defects. However, both the NICHHD study and our study were carried out in major university centres under careful control, so that whether these conclusions would apply to universal delivery of amniocentesis is doubtful, and continuing efforts to maintain standards will be needed.

Both the lay public and physicians must be better informed of the availability of this procedure and the indications for which it should be offered. With the decrease in maternal age across Canada, the reduction in the number of women over 40 giving birth, and the increased risk of chromosome abnormalities at maternal ages between 35 and 39, consideration must be given to the widespread offering of amniocentesis to pregnant women over the age of 35. However, even now, when most laboratories in Canada are providing this service primarily to a low proportion of pregnant women over 40,

the laboratory facilities and personnel are working at a maximum level consistent with reliability, safety and speed. Thus, if the availability of the test is to be extended, an extension of laboratory facilities will also be needed. Clearly the demonstration that this test is safe and accurate will increase the pressure for its widespread provision to women at risk. If this goal is to be achieved on an equitable and efficient basis, then increasing provision must be made for these services in health care programs across the country.

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