—Mini Review—

Prenatal Diagnosis of Chromosomal Abnormalities through Amniocentesis

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Abstract: *The incidence of major chromosome abnormalities in newborns is about 0.7 percent and increases with maternal age. Amniocentesis is the most common invasive prenatal procedure for the detection of fetal chromosomal abnormalities. Amniocentesis is a relatively safe procedure and fetal loss related to amniocentesis is about 0.5%. An advanced maternal age is the most common reason for using amniocentesis. The use of amniocentesis because of abnormal fetal ultrasound findings has increased recently. Fluorescence in situ hybridization (FISH) is currently a powerful tool in the area of prenatal cytogenetics. The number of amniocentesis procedures in Japan is about ten thousand per year and it is generally recognized to be a great benefit for pregnant women who have a risk of fetal chromosomal abnormalities.*

Key words: *Amniocentesis, Prenatal diagnosis, Chromosomal abnormalities*

In amniocentesis, amniotic fluid is withdrawn from the amniotic sac around the fetus. This is currently the most commonly performed invasive prenatal procedure used for diagnosing fetal genetic disorders. The first diagnosis of Down syndrome by means of amniocentesis was reported in 1968 by Valenti *et al.* [1]. Amniocentesis has subsequently become the "gold standard" for invasive prenatal diagnostic tests. The amniotic fluid obtained in the procedure is used for a variety of analyses, the most common of which is a karyotype analysis from cultured amniotic fluid cells. This article reviews the area of prenatal cytogenetic diagnosis through amniocentesis.

Received: December 11, 2003
Accepted: February 25, 2004
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Safety of Amniocentesis

The procedure is most commonly performed between 16 and 20 weeks of gestation and is referred to as midtrimester amniocentesis. Under real-time ultrasound guidance, a 22-gauge needle is inserted into the amniotic sac and approximately 20 ml of amniotic fluid is removed by aspiration.

Some of the complications associated with amniocentesis include the leakage of fluid, cramping, bleeding, infection, and miscarriage. The risk of miscarriage after amniocentesis is related to the experience of the operator, the size of the needle used, the number of times the needle is inserted, and other factors [2]. Several studies have evaluated the safety of midtrimester amniocentesis. A prospective, nonrandomized study sponsored by the National Institute of Health found an overall loss rate of 3.5% between the time of the procedure and delivery compared to a loss rate of 3.2% in matched controls [3]. The first prospective randomized study known as "the Danish study" reported that the loss rate for the amniocentesis group was 1.7% compared to 0.7% in controls [4]. In this study, amniocenteses were performed with a 20-gauge needle (compared to a 22-gauge needle in other studies), which may be a factor in the increased loss rate. Most practitioners quote a procedure-related loss rate of 0.5% [5].

Incidence of Chromosome Abnormalities

Combined surveys during the period 1969 to 1982 involving 68,159 livebirths found that 0.65 percent of newborns had major chromosomal abnormalities (Table 1) [6]. The most common of these were trisomy 21, Down syndrome, with an incidence of 0.12 percent of liveborns. The next most common were sex chromosome aneuploidies, with one XYY or XXY per

Table 1. Chromosomal abnormalities in liveborn babies [ref 6]

Type of Abnormality	Rate (%)
Sex chromosomes, males	
47, XYY	0.103
47, XXY	0.103
Other	0.073
Sex chromosomes, females	
45, X	0.024
47, XXX	0.109
Other	0.036
Autosomal trisomies	
47, +21	0.120
47, +18	0.013
47, +13	0.004
Other	0.002
Structural balanced	0.204
Structural unbalanced	0.063
Total abnormalities	0.648

1,000 male livebirths and one XXX per 1,000 female livebirths. Structural balanced rearrangements were found with an incidence of 0.2 percent of liveborns.

It is well known that the incidence of trisomy 21, as well as the incidence of other chromosomal abnormalities, increases with maternal age. The magnitude of this maternal age effect is shown in the Table 2 [7]. At a maternal age of 35 years, a 0.26 percent risk of having a livebirth with trisomy 21 and a 0.49 percent risk of having a livebirth with any chromosomal abnormality exists. At 40 years of age, both risks rise, to 0.94 percent for trisomy 21 and to 1.59 percent for any chromosomal abnormality. A maternal age of 35 has traditionally been used as a cutoff for the definition of advanced maternal age, because the risk of a fetal chromosome abnormality at this age is roughly equivalent to the risk of procedure-related loss rates after amniocentesis.

The incidence of Down syndrome, as well as that for all chromosomal abnormalities in amniocentesis, is approximately 50 percent higher than those in liveborns. These differences can be attributed to an increased spontaneous fetal loss in chromosomal abnormalities, subsequent to the time of amniocentesis. Thirty percent of fetuses with trisomy 21, 43 percent of fetuses with trisomy 13, 68 percent of fetuses with trisomy 18, and 75 percent of fetuses with 45, X were found to be spontaneously aborted during the second trimester [6].

Indications for Amniocentesis

The Japanese Societies related to genetics list the indications for amniocentesis as follows [8]: 1. Chromosomal rearrangement in either member of a couple. 2. A previous child with a chromosome abnormality. 3. Advance maternal age. 4. Carriers of an X-linked disorder. 5. Carriers of a congenital metabolic disorder. 6. Carriers of a genetic disorder for which a DNA test is available. 7. Pregnancy at increased risk for serious fetal abnormalities.

Pregnancy at an increased risk for serious fetal abnormalities includes positive maternal serum marker screening and abnormal ultrasound findings. A prenatal ultrasound examination during the first and second trimesters is now routine in obstetrical care. A variety of

Table 2. Maternal age-specific rates of chromosomal abnormalities [ref 7]

Maternal Age (y)	Liveborn		Amniocentesis	
	47, +21	All Chromosomal Abnormalities	47, +21	All Chromosomal Abnormalities
33	0.16	0.29	0.24	0.48
34	0.20	0.36	0.30	0.66
35	0.26	0.49	0.40	0.76
36	0.33	0.60	0.52	0.95
37	0.44	0.77	0.67	1.20
38	0.57	0.97	0.87	1.54
39	0.73	1.23	1.12	1.89
40	0.94	1.59	1.45	2.50
41	1.23	2.00	1.89	3.23
42	1.56	2.56	2.44	4.00
43	2.00	3.33	3.23	5.26
44	2.63	4.17	4.00	6.67
45	3.33	5.26	5.26	8.33

Table 3. Ultrasound abnormalities and frequencies of fetal aneuploidy [ref 6]

Defect	Frequency (%)
Abdominal wall defects	24.1
Agenesis of corpus callosum	38.1
Congenital heart disease	49.0
Cystic hygroma	63.0
Diaphragmatic hernia	16.8
Duodenal atresia	33.3
Facial cleft	42.7
Holoprosencephaly	33.7
Hydrocephaly	15.7
Hydrops(nonimmune)	22.3
IUGR	19.6
Limb anomalies	37.3
Microcephaly	16.0
Nuchalthickness	30.4
Oligohydramnios	13.5
Polyhydramnios	12.3
Renal anomalies	10.8
Esophageal atresia	62.5

abnormal fetal ultrasound findings are associated with an increased risk of chromosome abnormalities. Fetal aneuploidies are more commonly detected in a fetus with multiple abnormal ultrasound findings than in a fetus with isolated abnormal findings. A combined survey of four large studies concludes that ultrasound abnormalities are highly associated with fetal aneuploidy (Table 3) [6].

Advanced maternal age is the most common reason for performing an amniocentesis. Amniocentesis due to abnormal fetal ultrasound findings has recently increased.

Molecular Cytogenetics

Fluorescence in situ hybridization (FISH), a combination of cytogenetics and molecular biology, has become a powerful tool in prenatal cytogenetics. FISH is a technique that allows DNA sequences to be detected on a metaphase chromosome or in interphase nuclei with DNA probes [9]. Applications of FISH include aneuploidy analysis, translocation and structural breakpoint analysis, marker chromosome analysis, and microdeletion analysis. The most common type is the interphase FISH with probes specific for chromosomes 13, 18, 21, X and Y. A standard cytogenetic analysis of amniotic fluid requires about 2 weeks because of the need for cell culturing. An interphase FISH analysis permits the rapid detection of the aneuploidy status of chromosomes 13, 18, 21, X and Y because, in this case, there is no need to culture cells, but this is still considered to be an adjunct to a standard chromosome analysis [10].

Table 4. The Status of prenatal diagnosis in Japan from 1998 to 2000 [ref 12]

Year	1998	1999	2000
Invasive procedure (total)	10,607	10,701	10,816
Amniocentesis	10,419	10,516	10,627
FISH (total)	929	1,082	1,200
FISH (rapid)	888	1,034	1,149
Chorionic Villus Sampling	76	58	96
Fetal Blood Sampling	112	127	93

FISH (rapid): The interphase FISH analysis for the rapid detection of aneuploidy for chromosomes 13, 18, 21, X, Y.

Comparative genome hybridization (CGH) is a new technique for measuring differences in the copy number or dosage of a particular chromosome segment between two different DNA samples. Recently, array-based CGH has been developed to screen the genome for submicroscopic single copy changes [11]. A CGH array is capable of detecting not only large aneuploidies but microdeletion or microduplication as well, by the identification of a decreased or increased copy number of a whole chromosome or a chromosomal segment. In addition, array-CGH can be performed with a relatively small amount of DNA. In the near future, it is likely that array-CGH will be applied for clinical prenatal cytogenetics, and it may replace the standard G-banding method.

Amniocentesis in Japan

Our group that is sponsored by the government, surveyed the status of prenatal diagnosis in Japan during the period 1998 to 2000 [12]. The number of amniocentesis procedures was about ten thousand per year, increasing slightly with time (Table 4). Roughly less than 1 percent of pregnant women had an amniocentesis procedure. The number of CVS was less than one hundred per year. Amniocentesis was used in 98 percent of the invasive prenatal diagnostic procedures. Amniocentesis is the most common invasive prenatal diagnosis procedure in Japan, but the number performed in Japan is far less than that in the USA or in Europe.

FISH studies were carried out in about ten percent of the amniocentesis procedures. The number of FISH procedures increased with time. Ninety-six percent of the FISH analyses involved an interphase FISH

analysis for the rapid detection of the aneuploidy status of chromosomes 13, 18, 21, X and Y.

Conclusions

The incidence of major chromosomal abnormalities in newborns is about 0.7 percent and increases with maternal age. Midtrimester amniocentesis is the most common invasive prenatal procedure for detecting chromosome abnormalities. Advanced molecular cytogenetics technologies have been applied to prenatal cytogenetics. Prenatal diagnosis through amniocentesis is a great aid in cases of pregnant women who have a risk of fetal chromosomal abnormalities.

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