PhD Theses

Perinatal autopsy as the quality control of praenatal diagnosis. Experiences with major fetal trisomies.

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Introduction

To rule out fetal aneuploidy is the commonest indication for invasive prenatal diagnosis either by amniocentesis, chorionic villus sampling or percutan umbilical blood sampling. Since invasive fetal testing is associated with a risk of miscarriage of about 1-2%, these procedures are recommended only for pregnancies considered to be at high risk for chromosomal defects. One of the screening methods to identify the high-risk group is second trimester ultrasound examination. Most fetuses with major chromosomal defects develop either external or internal abnormalities by the second trimester of pregnancy and these anomalies can possibly be recognized with detailed and targeted ultrasound examination. Hence sonography offers the opportunity to select fetuses who possibly need to be karyotyped by using different major (“structural”) or minor (“soft marker”) abnormalities that are present more frequently in aneuploid than in euploid fetuses. However, prenatal sonographic findings have been demonstrated to have some rates of false-positive and false-negative diagnosis.

One possible way of the feedback of prenatal diagnosis is a thorough pathologic examination of the fetus. In pregnancies ending up with termination, prenatal ultrasound diagnosis may be confirmed or revised by postmortem examination of aborted fetuses. If the termination of the pregnancy (TOP) occurs due to fetal aneuploidy, postmortem autopsy may reveal additional major or minor structural
anomalies that have potential for aneuploidy-screening at the mid-trimester scan. Thus, perinatal autopsy plays an important role in the quality control of the sonographic examination. It can also help to describe a characteristic spectrum of major and minor anomalies of fetal aneuploidies that can be possible seen on prenatal sonography.

In the present study I evaluate the correlation of prenatal ultrasound findings with the results of subsequent pathologic examinations in fetuses with trisomy 21, trisomy 18 and trisomy 13. In addition, we try to position perinatal autopsy in a multidisciplinary management of fetal malformations.

**Objectives**

1. How have changed the indications of fetal karyotyping during the last 15 years?

2. How big was the degree of correlation between prenatal ultrasound findings and the results of subsequent pathologic examinations in fetuses with major aneuploidies? Has the correlation changed during the time period of 1999-2004?

3. What is the importance of those anomalies that were diagnosed with autopsy, but were not seen with sonography („false negative sonographic signs”)?

4. What is the importance of those ultrasound findings that were not confirmed at autopsy? („false positive” sonographic
signs)? I also sought to evaluate whether fetal autopsy is always “superior” to sonography?

5. According to the results of perinatal autopsy, what kind of anomalies of the fetus can be used to improve the effectiveness of sonographic screening for fetal trisomies?

**Patients and methods**

The database of the Fetal Diagnosis Unit of our department was reviewed during this study after obtaining the approval of the Institutional Ethics Committee. Cytogenetic records of fetuses were collected between the time period of 1990-2004 and the data of karyotypically documented aneuploid fetuses were analyzed. Of the 22,150 fetal chromosome-analyses performed, 514 (2.3%, 514/22,150) chromosome-abnormalities were found. Among them 207 fetuses with trisomy 21 (40.3%, 207/514), 70 fetuses with trisomy 18 (13.6%, 70/514) and 28 fetuses with trisomy 13 (5.4%, 28/514) were identified prenatally. Singleton fetuses, who underwent genetic testing following prenatal sonography during the second trimester in our institution, and had trisomy 21, trisomy 18 or trisomy 13 constituted the study-population (n = 305). Mothers were referred to our genetic counseling department for different reasons: advanced maternal age, family history of genetic problems in previous pregnancies, „suspect” ultrasound findings found in other institutions
and abnormal triple test results. In this respect our study population represents a high-risk group of patients.

Two ultrasound equipments were used for the examinations: an ATL Ultramark 9 HDI 3000 (Phillips Medical Systems, Bothell, WA) and a GE Voluson 730 (GE Medical Systems, Milwaukee, WI). The course of the examinations followed the guidelines of the Hungarian Society of Ultrasound in Obstetrics and Gynecology, and routinely targeted basically all structures of the fetus. All the abnormalities of each organ were recorded. We offer fetal echocardiography as part of our prenatal diagnostic service from 1994. It is performed by a perinatal cardiologist with special experience in neonatology. If a patient underwent more than one sonographic examination, only the results of the first examination were included in the analysis. To rule out any bias, the sonographic examinations were done before the results of the cytogenetic evaluations of the fetuses were revealed.

Each sonographic finding was classified as „major” structural anomaly and „minor” („soft”) sonographic marker. Major structural anomalies were defined as central nervous system (CNS) anomalies, cardial defects, cystic hygroma, abdominal defects, renal anomalies, limb defects and facial anomalies. Among minor anomalies, we paid special attention to: nuchal fold thickness > 6 mm, short femur or humerus (< 10th percentile), pyelectasis (> 4 mm), hyperechoic bowel, echogenic intracardiac focus (EIF) and choroid plexus cyst
(CPC). We also detected intrauterine growth restriction (IUGR, if estimated fetal weight <10th percentile), amniotic fluid abnormalities (oligo- or polyhydramnios) and umbilical cord abnormalities. (EIF and hyperechoic bowel were described as soft markers in the mid-1990s, so we did not examine them consistently in the first years of the study.)

Cytogenetic evaluation of the fetuses was performed either by amniocentesis (AC), or chorionic villus sampling (CVS) followed by amniotic cell culture (in case of AC) or direct analysis method (in case of CVS) using standard techniques. G or R banding was used and karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN 1995). A minimum of 20 metaphases from at least five cultures were analyzed.

Therapeutic TOP was performed following proper genetic counseling. Parental consent was gained before each autopsy.

Standard protocol for the autopsies was followed for each examination. The results of the sonographic examinations were available to the pathologists prior to the pathologic examinations. Until autopsy was performed, the corpses were stored at low temperature (4 °C). The time that elapsed between abortion and autopsy was 1-3 days. All cases had histologic examination from the organs and the placenta.

The ultrasound and pathology reports were analyzed by organ system, and the findings were compared. The correlation was
assigned to 1 of 3 categories: class A, full agreement between the findings on sonography and autopsy; class B, autopsy findings not detected by ultrasound (“false negative sonographic findings”); class C, ultrasound findings not confirmed at autopsy (“false positive sonographic findings”).

**Results**

Between 1990 and 2004 22,150 fetal karyotypings were performed and 514 (2.3%) chromosome abnormalities were diagnosed. Among them, 207 fetuses with trisomy 21, 70 fetuses with trisomy 18 and 28 fetuses with trisomy 13 were identified prenatally. All of the fetal aneuploidies were diagnosed by AC (83.3%, 254/305), or CVS (16.7%, 51/305) after detailed sonographic examinations.

The mean maternal age of the patients was 32.4 years (range: 17-44 yrs) at the time of the ultrasound examination. The median gestational age at the time of the sonogram was 19.5 weeks (13-25 wks). The main indications for referral to our Prenatal Diagnosis Unit included advanced maternal age (66.3%), abnormal maternal serum screening (8.4%) and abnormal sonographic findings in other institutions (15.3%).

The couples opted for therapeutic TOP in all cases but five after proper genetic counseling. (Three cases with trisomy 18 and two with trisomy 21. All three cases with trisomy 18 resulted in preterm labor, the newborns died within a week and perinatal autopsies were
performed. The two children with trisomy 21 are mentally retarded and show minor anomalies of Down syndrome.) The parental consent rate for perinatal autopsy was 100% following TOP. The mean gestational age at the time of the abortion was 22 weeks (range 14-25 wks). TOP were done with the method of dilatation and evacuation (D+E) in 27 cases (three fetuses with trisomy 13, three fetuses with trisomy 18 and 21 fetuses with trisomy 21). Proper pathologic evaluation of these fetuses could not be performed because of the fragmented specimens. We excluded these cases from the study-population. The autopsy data of the remaining 276 fetuses were analyzed in the present study.

Of the 25 fetuses with trisomy 13, all had 1 or more major structural findings at post-abortion pathologic examination. There were 79 separate major structural abnormalities detected on autopsy. Of the 79 abnormalities, sonography detected 48 (60.8%). All fetuses with trisomy 18 had one or more abnormal structural findings at post-abortion pathologic examination. There were 185 separate major structural abnormalities found on autopsy. Of them, sonography detected 93 (50.3%). Of the 184 fetuses with trisomy 21, 153 (83.1%) had one or more abnormal structural findings at post-abortion pathologic examination, whereas in 16.9% of the cases fetal pathology did not reveal any defects in fetal anatomy. There were 347 separate structural abnormalities found at autopsy. Of them, sonography detected 91 (26.2%). Altogether 611 separate structural
malformations were diagnosed during pathology examination in fetuses with trisomies. Of them, sonography detected 232 (37.9%).

The rate of additional findings at autopsy was 64.2% (392/611) and involved mainly two organ systems: facial abnormalities (including ears and eyes) and extremities (including hands and feet). If we remove the data of these two organs, the rate of additional findings would be 24.7% (72/291). Of the additional findings on autopsy, 30 were cardiac defects.

A small portion of ultrasound findings was not confirmed at autopsy (8%, 49/611).

I evaluated the sensitivity of second trimester sonography in the identification of major or minor abnormalities found at autopsy by organ system.

Conclusions

1. Besides maternal age, fetal anomalies detected by ultrasound have gained more and more importance among the indications of fetal karyotyping during the last 15 years. Examining the 3 most common autosomal trisomies during two time-periods (I. 1990-1998. and II. 1999.2004.), I have found the following data: The ratio of karyotypings done to mothers younger than 35 years increased from 15.4% to 40% (p=0.22) in case of Trisomy 13; from 41% to 50% (p=0.65) in
case of Trisomy 18; and from 23.8% to 31.1% (p=0.30) in case of Trisomy 21.

2. Altogether 611 separate major structural malformations were diagnosed during autopsy in fetuses with major trisomies. Full agreement was achieved between sonography and autopsy in 36% of the malformations, whereas additional findings at autopsy (64%) involved mainly two organ systems: face (including ears and eyes) and extremities (including hands and feet). High agreement rate was observed between sonography and pathology in cases of the following organ-systems: central nervous system anomalies (72.5%), cardiac anomalies (65%), fetal hydrops (90%) and cystic hygroma (96%). The detection rates of these fetal anomalies have increased in the second time-period. The difference was significant in cases of cardiac defects and renal anomalies. Concordance rates between sonography and autopsy findings regarding soft markers were considerably high in some markers (increased nuchal fold thickness – 71%, short femur/humerus – 68%, fetal pyelectasis - 52%, and choroid plexus cysts – 61%). On the other hand, fetal autopsy had limited value regarding hyperechoic bowel and echogenic intracardiac foci.

3. The concordance rate was low between the sonographic signs and the pathologic features of face abnormalities and
anomalies of the extremities, 11.2% and 6.9%, respectively. Interestingly, unlike in trisomy 21 cases, a relatively high agreement rate (76.5%) was revealed between sonography and autopsy findings of facial abnormalities in fetuses with trisomy 13. The possible explanation of this can be the different spectrum of facial defects in the two types of aneuploidies. Midline defects (certain defects of the closure of the embryo) are typical features of trisomy 13, whereas the incidence of these anomalies is low in Down-syndrome individuals. Instead, fetuses with trisomy 21 have more subtle anomalies of the face.

4. We observed that fetal autopsy following termination of pregnancy has limited value regarding hyperechoic bowel and hyperechogenic intracardiac foci. These sonographic signs were not confirmed at autopsy. I believe that regarding these markers, fetal autopsy is not “superior” to sonography. In other words, autopsy findings are not always the gold standard to gauge ultrasound accuracy especially regarding soft sonographic markers. The same applies to some cases of major structural defects, such as anomalies of the brain (since pathologic evaluation of the brain is often hampered by autolytic processes) or “functional” anomalies of the heart (like “dilated ventricles” or “insufficiency of the tricuspid valves”). These examples well illustrate that sonography and
autopsy can be complementary methods in a prenatal diagnostic center.

5. It can be drawn two conclusions from the low concordance rate between sonographic findings and pathology features regarding face anomalies and abnormalities of the extremities: 1. Anomalies of the extremities and the face were not efficient sonographic markers for fetal aneuploidies during the past. 2. The effectiveness of sonographic screening for trisomies can be improved with the detailed examination of the fetal face (including ears and eyes) and fetal extremities (including hands and feet). In addition, my data support, that fetal echocardiography plays important role in the prenatal diagnosis of fetal chromosome aberrations.

This series confirms that perinatal autopsy provides additional information in many fetuses with aneuploidy and indicates possible directions of sonographic screening for major chromosome aberrations. The contribution of perinatal pathologist is important in the process of multidisciplinary management of prenatally diagnosed fetal abnormalities. However, prenatal sonography and perinatal autopsy should be considered as complementary ways of increasing our knowledge about the possible symptoms of fetal aneuploidies developed by the second trimester of pregnancy.
Publications

Publications pertinent to the topic of the dissertation


**IF: 0,892**


**IF: 0,761**


**Other publications**


**IF: 0,889**


**IF: 0,867**


Total IF: 19,221
IF as first author: 6,053