PhD Thesis Abstract

PRENATAL ULTRASOUND DIAGNOSIS IN FETAL ANOMALIES

INTERDISCIPLINARY APPROACH AND THE IMPORTANCE OF PERINATAL AUTOPSY

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I. CURRENT KNOWLEDGE

In this chapter of the PhD thesis I have reviewed the latest data on my research topic, using recent published literature. I have described the use of ultrasound diagnosis (non-invasive) in obstetrics, in all pregnancy trimesters, the invasive diagnostic methods, and the conventional autopsy protocols.

Practicing obstetrics can no longer be conceived in the absence of imaging techniques. The accuracy of screening ultrasound (US) for fetal abnormalities has improved rapidly, reaching in specialized centers - high figures in sensitivity and specificity. This is due to the technological progress, but also to continuing education of doctors/nurses in the prenatal diagnosis. Nowadays the late first trimester US is widely used. It is a top performing method in screening for chromosomal abnormalities as an integral part of the combined test, but has already become also the "first ultrasound of fetal morphological abnormalities." However, the follow-up reassessment of fetal morphology in the second trimester (18-24 weeks of amenorrhea - WA) remained the "gold standard". Its value is perennial for late-onset anomalies and for conditions developing the US expression late in pregnancy. Conventional autopsy remained the most important technique for confirming / refuting / modulating the prenatal diagnosis.

II. ORIGINAL CONTRIBUTIONS

The main objective of the thesis:

The main objective was **to compare the ultrasound examination results in the diagnosis of structural anomalies with those obtained by means of the post-mortem autopsy of specimens.**

Secondary Objectives:

- **Establishing the prevalence of fetal anomalies** in the unselected population addressed to the Prenatal Diagnosis Unit (PDU) Craiova.

- **Determining the detection rates for specific congenital anomalies** in PDU Craiova.

- Finding the correlation rates of **post-mortem informative autopsies**, function of the gestational age at which they are performed.

- Determining the rates of post-mortem autopsies providing **additional information** to the prenatal diagnosis.

- Finding the amount of **additional information obtained by means of additional tests** (computed tomography scan – CT and nuclear magnetic resonance imaging examination - MRI), targeted histological examinations, conventional G-banding karyotyping using abortion products and assessing **their clinical relevance.**

- Establishing the contribution of **factors that influence the concordance between the results of the ultrasound examination and the perinatal autopsy** (gestational age, parity, amniotic fluid volume, presence of uterine scars, placental location, and body mass index)**.**

- Determining the **factors that influence the acceptability of the prenatal genetic diagnosis.**

- **Obtaining a dynamic database,** allowing the interpretation of different parameters studied, function of the timing (3 years period).

- **Description of patients' characteristics in the study group** (the enlisted mothers and the population of fetuses).

- **Standardization the protocol for medically induced abortion**, so as to provide maximum safety for the mother.

- **Standardization of fetal autopsy protocols.**

- **Obtaining data on the time interval** required for the prenatal ultrasound examination and fetal autopsy (using specimens with different gestational ages).

- **Establishing relationships** between our results and the data in published literature.

Methods

The work involved the collaboration among various specialties related to obstetrics (imaging techniques, pediatric surgery, medical genetics, biology, pathology).

The research was conducted according to **Rules of good practice in scientific research**, formulated by the Ethics Committee of University of Medicine and Pharmacy Craiova. I invested the first 6 months in increasing my **knowledge** with regards to antenatal diagnosis in early pregnancy. I subsequently prepared the papers of **informed consent** which were provided to patients enrolled in the study. They referred to all stages of diagnosis and intervention, as well as to the subsequent use of both imagistic and clinical / laboratory data for research purposes. Finally, I set up **the content of the database**.

The target population of this research project was the population of fetuses addressed to the PDU and the ones for which hospitalization in the Obstetrics and Gynecology Clinic I (OG I) was required. Both patients with US markers for congenital structural abnormalities, as well as patients in the high risk group for chromosomal abnormalities (by using the combined test) were enlisted.

The working method included the US examination of the fetal anatomy in the late first trimester (using an extended protocol) in all cases enlisted before 13 weeks + 4 days. The cases suspected for congenital anomalies were selected. Using a rhythm depending on the particularities of each case, ultrasound of the 2nd/ 3rd trimesters, clinical and lab examination of the new-born, and the postabortum / postpartum autopsy were performed. In selected cases, additional diagnostic methods: CT, MRI, histological tests, genetic testing using abortion products were also used.

Fetal autopsy was carried out within the Department of Pathology at University of Medicine and Pharmacy Craiova. It involved the identification, fixation, preservation, and processing the specimens, autopsy using dedicated surgical instruments, as well as obtaining the electronic autopsy photographic files in each case study. Autopsy was performed in multidisciplinary teams (obstetrician and fetal pathologists) under the supervision of a senior pathologist or head of the pathology department.

DATA ANALYSIS. RESULTS. DISCUSSION

Ultrasound examination and its implications

The most important consequence of improving US technique and operators' skills was the translation of all fetal morphology US targets in the second trimester of pregnancy – at the end of the first trimester. Operators must have the ability to diagnose a particular anomaly, the knowledge of associated defects and natural history of each specific condition. Moreover, they need to have deep knowledge of the counseling process, and demonstrate empathy if the US of fetal anatomy raises questions.

Abortion.

In most countries, the therapeutic termination of pregnancy for major congenital abnormalities (TOP-MCA) is allowed in the second trimester (before 22-24 WA). In this study, in every case in which the issue of the TOP-MCA was raised after 14 weeks, the parental counseling was extensive. TOP-MCA was not allowed before 18 WA except for in extreme malformations or genetic abnormalities associated with mental retardation. After 24 WA TOP-MCA was allowed in lethal abnormalities only.

Autopsy

Between all the specialties related to obstetrics, pathological anatomy is, and should remain, one of the key specialties, having the function of "quality control"; it complements and refines the information obtained by US. The final diagnosis obtained by corroborating the data (prenatal US diagnosis and anatomo-pathological examination of the specimens) should be the basis for

counseling the couples having the desire to conceive subsequently. Moreover, autopsy has a critical role in the teaching, training, research and epidemiology.

Results of the study

During the 3 years of study, 9082 births were carried out in Craiova University Hospital. 88 cases with prenatally suspected fetal abnormalities were enrolled in the prospective study. **Prevalence of congenital fetal anomalies** was 9.69 per 1,000 births in the study group. The results are consistent with those reported at a European level (EUROCAT reports show recent figures of 9.5 per 1,000 births).

Concordance between ultrasound data and autopsy data

The perinatal autopsy was performed in 62 cases. Of them, **the concordance** between the two methods was found in 59 cases (95.2%). The false negative results rate in major congenital anomalies was 0, as was the rate of major false positive abnormalities, that could lead to a therapeutic abortion. For convenience, we classified the cases as follows: it was considered "concordance" if the main diagnosis (either major or minor anomaly) was confirmed by means of autopsy, and "discordance" - if the main diagnosis was changed, thus the process becoming clinically significant. We have tried to standardize the method used for full conventional autopsy, and we discussed the case in detail with the pathology department colleagues.

The results in our study contradict those of an earlier review including 27 publications: the change in the main diagnosis upon additional findings was present in 22% to 76% of the cases. Conversely, they are consistent with those published more recently by a study group in Norway: they showed a good correlation between prenatal diagnosis and autopsy data, with a 90% accuracy of the primary US diagnosis.

The high rates of detection of congenital abnormalities, specific to PDU, are explained by the highly specialized staff, all physicians being licensed in the prenatal diagnosis, all having extensive experience in the first-trimester ultrasound and maternal-fetal medicine.

In the 1st trimester we found a 91.7% concordance between the US and autopsy data (11 cases out of 12), in the second trimester – 97.7% (43 cases of 44), and in the third – 83.3% (5 out of 6).

No statistically significant association was found between the US-autopsy concordance rate and the gestational age when the autopsy was performed (Fisher Exact test, $p = 0.200 > 0.05$). This finding, by far the most significant, reflects both the expertise of the examiners in the fetal anomaly/genetic scan in late first trimester, as well as the excellent quality of conventional microscopic autopsy on small size specimens (with a crown-rump length below 84 mm on US).

These results are unique in Romania and Europe, as most centers reported the absence/the lack of relevance of conventional autopsy in specimens below 16 – 18 weeks of amenorrhea.

The lower figures obtained in the third trimester of pregnancy are explained by the difficulties encountered in the prenatal diagnosis. These are related to the acoustic physics and third trimester particular features: the increased fetal volume and the decreased amniotic volume.

The statistical analysis shows the high percentage of cases (72.6% – 45 patients out of 62) in which the autopsy failed to provide any additional information to the US examination. In 17.7% of cases, the post-mortem autopsy provided additional information having no clinical significance. In 9.7% of cases only - the additional information had clinical significance. The reason for the high percentage of autopsies that did not provide additional information was not the difficulty of manipulating small specimens. The few cases in which the autopsy provided additional clinical information were found in all pregnancy trimesters.

Using a proper autopsy technique, strong lighting and dedicated surgical instruments, we were able to confirm the suspected diagnosis very early in pregnancy (in specimens under 14 weeks of gestation). The most sensitive area in the confirmation process is that of fetal heart abnormalities, followed by central nervous system abnormalities. In this study, classifying the two categories ("informative" vs. "non-informative" autopsy) was done by the pathologist who supervised the autopsy. The potential of pathological examination in detecting different congenital malformations may have huge variations (for instance, it is much easier to confirm extremities defects than cardiac defects, regardless the gestational age of the specimen). However, no statistically significant association was found between the percentage of informative autopsy and, respectively, the gestational age (GA) at the completion of the study (Fisher's Exact Test, p = 0.650). In rare cases, the degradation or maceration of the specimen prevented us to obtain autopsy information.

Conventional karyotyping provided additional information with clinical significance in a large number of cases: 15 out of 88 (17%). In 11 cases – the testing was performed in the second trimester. Genetic testing was accepted by more than half of the patients (48 out of 88 – 54.5%), although these tests are not subsidized in the National Health Assurance system. We hope that these results, reflecting the relevance of the cytogenetic examination in congenital structural abnormalities cases, support with concrete data the gratuitousness for this subgroup in the future. Unlike genetic testing, **computerized tomography** has been performed in a small number of cases, and none of them has provided any additional information of clinical significance (0 out of 4 cases). Similarly, **MRI** examination was rarely performed and provided clinical significant information in one case only. 5 cases studied by means of MRI belong to the central nervous system suspected anomalies group. **Postnatal clinical examination** has been shown to be particularly valuable. In 7

cases it provided additional information to the prenatal US examination, having a notable clinical significance.

The Spearman's rank correlation was calculated between the gestational age at diagnosis and the examination time interval needed for the diagnosis. The correlation value was -0.142 ($p = 0.188$), which shows a poor negative correlation, without statistical significance. As a result, in our case series, the gestational age at the diagnosis did not correlate with the US examination time interval. These results are related with the PDU policy: the fetal morphology examination is similar in first and second trimester. Furthermore, it appears that the duration of the US examination is probably more related to the scanning conditions, the fetal position, and the experience of the examiners, and less to the gestational age. All examinations were performed using the same ultrasound equipment, so the potential bias due to the use of different US systems was absent. Both the examination protocol and the ultrasound reports were standardized.

The statistical analysis failed to prove statistically significant associations between the concordance US-autopsy and the following parameters:

- the Body Mass Index (BMI) (Fisher Exact test, $p = 0.543 > 0.05$),
- the parity rank (Fisher Exact test, $p = 0.692 > 0.05$),
- the placental site (Fisher Exact test, $p = 0.625 > 0.05$),
- the amniotic fluid volume (Fisher Exact test, $p = 0.513 > 0.05$),
- the presence or absence of uterine scars (Fisher Exact test, $p = 0.774 > 0.05$).

The gestational age (GA) at study entry was statistically significantly associated with the **home** residence (urban vs. rural) (Fisher Exact Test $p = 0.021$). Rural area patients tend to address the general practitioners for pregnancy care (to be registered and, consequently, referred to the prenatal US examination) later than urban area patients. Also, the GA at the study entry was statistically significantly associated with **parity** (Fisher Exact test $p = 0.007$). In nulliparous group, 94.3% addressed the physicians early in pregnancy for prenatal care. Multiparous tended to require the first US examination later in pregnancy.

The rate of enrollment in the study was stable over the 3 years. There were no statistically significant differences between **the average time required for autopsy** during the three years (Levene test for variance homogeneity $p = 0.949$, ANOVA F = 1.300, $p = 0.280$). The time interval required for the perinatal autopsy appears to be dependent rather on the type of anomaly studied than on the operators' experience.

We did not find statistically significant differences between the **average age** of patients with fetuses with chromosomal anomalies – 29.2 years (21 to 40 years of age) and the mean age of patients with euploid fetuses – 28.9 years (17 to 42 years of age). This finding seems to be related to

the small recruitment figures, and does not invalidate the demonstrated linear dependence between the maternal age and the chromosomal anomalies incidence.

The cardiovascular system and the central nervous system were more often affected by congenital anomalies. Surprisingly, although most doctors recommend the periconception supplementation of folic acid in the urban environment, a large number of neural tube defects (10 cases – 11.3%) were present among the enlisted cases.

The number of cases with **isolated** abnormalities was comparable to the number of cases of multiple associated abnormalities (33 cases – 37.5% vs. 26 cases - 29.5%). The cases with **major** abnormalities prevailed (major versus minor 82.3% versus 12.9%).

Among the 15 cases with confirmed **chromosomal anomalies**, 6 fetuses had no structural anomaly, 8 had an isolated structural anomaly, and 7 fetuses had multiple malformations. The percentage of fetuses in which the US markers for chromosomal abnormalities have been detected is remarkable (at least one marker has been described in 42% of the autopsied cases). The association between US markers for chromosomal anomalies and the confirmed cases was statistically significant (Fisher Exact Test, $p < 0.01$).

In this study, **the average time interval of the case in observation** (the time span between enlistment and exit the study, for a specific case) was 5.9 weeks, ranging from 24 hours to 26 weeks, with a standard deviation 7.73.

The most important **limitation** of this project was the low enrollment rate (which resulted in a small number of cases entering the final analysis). This is related both to the Romanian health care system issues and to the low number of researchers involved in this project. Some of the results are biased by the small study size.

We believe that the results will help us modulate the medical practice in the prenatal diagnosis, and in particular the counseling process, both in normal pregnancies and in cases with fetal congenital anomalies. We will use in the counseling process of future parents - our own figures in US detection rates (for minor and major structural fetal anomalies), as well as the confirmation rates figures resulting after performing the conventional autopsy.

VI. CONCLUSIONS:

Ultrasound examination is a safe (risk-free) test for the intrauterine patient, which can be applied on a large scale. It remains the most efficient investigation during the prenatal period.

The physicians involved in the prenatal diagnosis must inform the parents in an accurate and complete manner, according to their understanding.

The thesis describes the demographic characteristics of the enrolled patients. They show that a large proportion of pregnant women address both the general practitioner and the specialized obstetricians - late in pregnancy, the situation being more common in rural area patients.

In many cases of pregnancies with congenital abnormalities the conventional karyotyping testing has provided additional information having clinical significance. We hope that these results will support the ongoing subsidizing process for cytogenetic testing in this subgroup of high-risk patients.

The study provided biological samples (obtained by both prenatal invasive maneuvers and using abortion specimens), and the fetal DNA was extracted in the Human Genomic Laboratory in all cases. The samples will be used for further research studies.

Postnatal clinical examination has proved to be particularly valuable: it provided clinical significant additional information in 28% of the cases (7.8% of all cases enrolled). The results are arguments for the continuous improvement of clinical practice in the neonatal period.

From an ethical perspective, prenatal testing of any kind, but especially the study of fetal anatomy by the ultrasound method – fulfill the parental right "to know". Continuous auditing of the prenatal diagnosis is required. The information provided to parents must be scientifically evidence-based.

The US – autopsy data concordance was obtained in 95.2% of the autopsied cases. No statistically significant association was found between the ultrasound-autopsy concordance – and the gestational age: the obtained results are comparable in the first and second trimester. These results reflect both the expertise of the examiners in the Prenatal Diagnostic Unit in the first trimester US, and the quality of conventional autopsies on very small specimens.

The value of perinatal autopsy is influenced by many factors, some of which are difficult or impossible to quantify. Therefore, it is difficult to gain generally valid conclusions. However, the results of this research show that the autopsy provides valuable clinical information. They support the assumption that the perinatal autopsy should be routinely recommended in prenatal diagnostic centers. The clinical-pathological concordance rates should be systematically reported.

The results of this research are critically influenced by the local settings and the operators' skills (ultrasound specialists and pathologists). They may be completely different in other centers. Clinicians should use their center specific figures in detection rates when counseling couples. Moreover, reporting the factors influencing the performance of perinatal autopsy and the characteristics of the fetal / neonatal / parental population involved should be promoted. This would make possible the comparison of the results between different centers.

The thesis, through its design and its results, provides arguments for a good collaboration between obstetricians and fetal pathologists.

The management of cases with congenital abnormalities should be performed involving multidisciplinary teams: medical genetics, imaging, pathology.

The US prenatal information may be a delicate area. This information may be used against the fetus, with a particularly higher risk in prenatally diagnosed minor anomalies.

My goal is to continue this research until reaching a sufficiently large study size, enough powered to confirm and validate the preliminary conclusions above. This will eventually raise the physicians' and patients' confidence in the accuracy of the prenatal diagnosis within PDU Craiova.

Key words: prenatal ultrasound diagnosis, fetal anomalies, perinatal autopsy, multidisciplinary team.