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PhD Thesis Abstract

**THE EFFICIENCY OF THE EXTENSIVE  
PROTOCOLS FOR ULTRASOUND  
EXAMINATION IN THE PRENATAL  
DIAGNOSIS OF CENTRAL NERVOUS  
SYSTEM ANOMALIES**

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## **I. CURRENT KNOWLEDGE**

Ultrasonography marked a huge progress in obstetrical practice, becoming an indispensable tool in prenatal care, since its introduction in Obstetrics, about 40 years ago. The modern technology and highly equipped machines nowadays provide the possibility to obtain information regarding the pregnancy even since embryonic age. If in the beginning, ultrasonography was used to obtain data for pregnancy ascertainment, fetal viability, placental localization or fetal biometry, now it is possible to detect ultrasonographically subtle anatomic disorders of the fetus at early gestational ages and even to predict obstetrical complications and fetal anemia.

The importance of materno-fetal medicine lead to the elaboration of various examination protocols by ultrasound societies like ACOG (The American Congress of Obstetrician and Gynecologist, ISUOG (International Society of Ultrasound in Obstetrics and Gynecology), AIUM (American Institute of Ultrasound in Medicine) and ACR (American College of Radiology), in order to standardize the medical language and offer an equal healthcare for everybody. These societies agreed to offer an ultrasound examination as a mandatory standard procedure in the second trimester of pregnancy. Unfortunately, not all healthcare systems can provide such assessment due to logistic reasons on one hand and to the lack of trained healthcare providers on the other.

Although the role of mid-trimester ultrasonography in fetal anatomy assessment is self-evident, efforts have been made to lower the gestational age for fetal anomalies detection. Consequently, the 11-13<sup>+6</sup>weeks ultrasound evaluation has started to acquire more and more advocates. Thank to professor K.Nicolaides and his team, a screening programme for genetical anomalies detection in the first trimester of pregnancy was established successfully. It consists in nuchal translucency assessment as a screening tool for aneuploidies detection.

The central nervous system represents the most complex system of the human body. Therefore, the anomalies occurred at this level are associated with a high degree of individual handicap, with limited therapeutic resources and modest recovery possibilities. On account of

that, social integration of such persons is extremely difficult due to neurological and psychiatric disorders associated.

The development of the nervous system has been studied for many years. Human embryology has been a theme of interest during ages, the development of the human embryo being studied ever since the beginning of the 20th century.

This study tries to prove the efficiency of the ultrasonographic protocols of examination in early detection of fetal CNS disorders and the implementation of certain markers as screening tools in order to increase the number of CNS anomalies detected in the first trimester and to decrease the gestational age of the antenatal diagnosis.

Fetal and neonatal neurosonography is an extremely informative mean of noninvasive exploration of the central nervous system. The prenatal ultrasonographic assessment of the fetal brain is made by using either a transabdominal approach or a transvaginal one. Nowadays, the fetal neurosonography is recommended for the patients with high risk of fetal CNS malformations or who were suspected to have a fetal CNS malformation at the previous examinations. Therefore, this kind of assessment is not universally used or accessible in any circumstances. It is conditioned by the examiner's grade of expertise and the need of a highly performant equipment.

One of the most disputed questions regarding the prenatal assessment of the fetus is "who is qualified to perform an ultrasound assessment?". The suitable answer is: "the healthcare providers specially trained to perform such assessment, who own an Obstetrical Ultrasonography certificate." In spite of a permanent debates between radiologists and obstetricians, the actual guidelines claim that as far as the examiner is trained adequately, his specialty doesn't matter".

## **II. ORIGINAL CONTRIBUTIONS**

### **1. OBJECTIVES**

The main objective of my study was the establishment of the opportunity of an extended ultrasonographic assessment of the central nervous system starting with the first trimester of pregnancy with the purpose to increase the percentage of CNS detected

anomalies and to lower the gestational age of the antenatal diagnosis. The study was centered on diagnostic efficiency in the first trimester and early second trimester of pregnancy (15-19 weeks) in order to answer a number of questions and to check a few working hypothesis initiated by our team or by other international centers:

1. Can we diagnose acrania/anencephaly at 8-10 weeks? How about holoprosencephaly?
2. Can we diagnose spina bifida at 11-14 weeks in a reasonable percentage (above 70%)? In this case, on our study population, we analysed the efficiency of many screening tools: intracranial translucency, BS/BSOB ratio, the direct assessment of the fetal spine, or of the parallelism of the cerebral peduncles, the CP/HA (choroid plexus/head area) ratio, a ratio proposed by our department.
3. Can we lower the gestational age for the diagnosis of the agenesis of corpus callosum at 15-19 weeks? How about 11-14 weeks?
  - What percentage of fetuses with absent CSP at 11-14 weeks have agenesis of corpus callosum (ACC)?
  - What percentage of fetuses with present CSP at 15-19 have ACC?
  - What is the rate of satisfactory visualization of corpus callosum at 15-19 weeks?
4. How early can we detect ventriculomegaly? Are there any signs to suggest an increased risk to develop ventriculomegaly or hydrocephaly?
5. How early can we detect Dandy-Walker syndrome? Is it possible to detect it in the first trimester of pregnancy?

## **2. METHODS**

The study included a number of 3276 pregnant women who addressed to our unit for various reasons with a gestational age of 8-10<sup>+6</sup> weeks during the research period – 01.10.2015 – 01.09.2018. . The patients underwent serial examinations according to some protocols concentrated on four groups of gestational age.

The groups were:

- **1st group: early first trimester:** in this group there were examined pregnant women with gestational ages between 8 and 10<sup>+6</sup> weeks, with the purpose of detecting acrania and anencephaly and to analyse early signs of some fetal malformations like holoprosencephaly and spina bifida
- **2nd group: first trimester:** in this group we examined pregnant women with gestational ages between 11 and 14 weeks, in order to detect spina bifida, encephalocele, posterior cerebral fossa anomalies and early signs of agenesis of corpus callosum,
- **3rd group: early second trimester:** there were examined patients with gestational ages between 15-19 weeks, with the purpose of detecting spina bifida, posterior cerebral fossa anomalies, ventriculomegaly and signs of total or partial agenesis of corpus callosum
- **4th group: second trimester:** we examined patients with gestational ages between 20-24 weeks. The target of the examination was detecting the anomalies that hadn't been diagnosed during the previous assessments and to affirm or disaffirm the suspicion of a certain anomaly in case of hazy diagnosis.

All the patients were informed regarding the study, inclusion being done, only after obtaining their written consent.

In the second group of the study, we analysed a few markers, in order to detect some indirect signs of spina bifida, agenesis of corpus callosum and Dandy-Walker malformation. In this group, we conducted a prospective study, where we assessed the cases detected during the research period and a series of retrospective studies, where we included cases detected in our department before the research period, in order to evaluate new or already acknowledged diagnostic markers, on narrowed groups of patients, so we could assess the detection rates and the interobserver differences for each marker of study.

The statistical data was processed by using the Microsoft Excel(Microsoft Corp., Redmond, WA, USA) programme, together with IBM SPSS Statistics 20.0 (IBM Corporation, Armonk, NY, USA) and XLSTAT series for MS Excel (Addinsoft SARL, Paris, France). The data processing supposed a descriptive analyze of the study population

according to a few parameters. By using the Excel programme, Pivot Tables, Chart, Functions-Statistical commands and Data Analysis mode, we calculated fundamental statistical parameters like Average and Standard Deviation, Coefficient of variation and the Coefficient of regression. After the calculation of these statistical indicator, we made a chart representation.

### **3. RESULTS**

There were included in the study a number of 3276 pregnant women, who adressed to our unit, for various reasons with 8-10<sup>+6</sup> weeks of amenorheea. The second stage of the study involved 3093 patients, and the third stage involved 2912 patients. Due to objective reasons, the whole protocol was applied to a number of 2775 patients, the other patients being excluded for not presenting to all the four stages of examination.

During the study we managed to detect a number of 28 fetal anomalies of the CNS: 5 cases of acrania/anencephaly, 5 cases of spina bifida, 2 cases of holoprosencephaly, 2 cases of hydrocephaly, 1 case of hydranencephaly, 4 cases of agenesis of corpus callosum, 3 cases of mega cisterna magna, 5 cases of ventriculomegaly ( the final diagnosis was established at 20-24 weeks of gestation), 1 case of Dandy Walker malformation. All these malformations were detected during different stages of the study. In case of lethal anomalies, the possibility of termination of pregnancy (TOP) was offered to the patients only in accordance with the legal provisions of our country.

Regarding the distribution of the study population, we detected: insignifiant statistical distribution differences of some parameters like: *age* (p Chi square=0,121), *parity rank* (p Chi square=0,240), *the origin enviornment* (p Chi square=0,517), *tobacco consumption* (p Chi square=0,679), signifiant statistical distribution differences of parameters like: *level of education* (p Chi square=0,008), *Body Mass Index (BMI)* (p Chi square=0,003), highly signifiant statistical distribution differences of parameters like: *the reason for seeking medical care* (p Chi square=0,000006), or *marital status* (p Chi square=0,00078). Due to the heterogenity of the patients some of the statistical distribution differences were in concerdance with the literature but other not.

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**In the first stage of examination**, we detected 4 cases of acrania/anencephaly, resulting in a 14,28% of the fetal anomalies detected during the whole study. All the patients chose for TOP, after adequate counseling and only one opted for cell-free fetal DNA (cffDNA) before termination of pregnancy, which proved to be normal.

**The second stage of examination**, performed to a number of 3093 patients, detected one case of acrania, which was missed during the previous examination stage, two cases of alobar holoprosencephaly, and four cases of spina bifida. One case of spina bifida was missed at this evaluation stage but diagnosed in the third group of assessment. The detection of the acrania was straightforward, due to its classical sonographical signs and the patient opted for TOP without obtaining the genetic diagnosis due to personal reasons. The two cases of holoprosencephaly proved to be alobar forms, one being a recurrent genetic disorder (trisomy 13) in a couple with an already trisomy 13 detection at the previous pregnancy. All patients opted for TOP.

In this group we conducted two studies, one prospective and one retrospective, where we assessed the value of some indirect signs of spina bifida as screening tools in the diagnosis of such anomaly, concentrating our attention on a ratio proposed by our department CP/HA ratio.

The markers' analysis from the prospective group (intracranial translucency, the assessment of the spine, BS/BSOB ratio, CP/HA (choroid plexus/head area) ratio) was:

<b>Table 1 – The markers for spina bifida analyse – prospective study</b>			
<b>Marker of diagnosis</b>	<b>Normal fetuses (average measurement)</b>	<b>Fetuses with spina bifida</b>	<b>Detection rate</b>
<b>Intracranial translucency</b>	2,023	Abnormal  The missed case had an IT of 1,51 mm	80%
<b>Spine assessment</b>	Normal	Apparently normal for 2 cases Abnormal for 3 cases The missed case had	40%

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<b>BS/BSOB ratio</b>		normal aspect of the spine	
	0,755	1,618	80%
<b>CP/HA ratio</b>		The missed case had a BS/BSOB ratio of 0,98	
	0,3021	0,3826	80%
		The missed case had a CP/HA ratio of 0,339	

We noticed a very interesting aspect: none of the markers generated false-positive results, the same case was missed by all the diagnostic markers at 11 weeks of gestation. It is likely that a closer to 13<sup>+6</sup> weeks of gestational age assessment to have had another outcome.

The retrospective study: We created a particular populational group consisting of 100 cases, where we included seven cases of fetal spina bifida diagnosed at 11-14 weeks in our department foregoing our study, in order to widen the study lot to 12 cases of spina bifida (88 – normal fetuses, 12 with spina bifida). To analyse the interobserver differences, all cases were assessed by two examiners. Both examiners were offered static 2D images and 3D volumes of the fetal brain for assessment. In our retrospective study, we assessed the following markers: intracranial translucency, BS/BSOB ratio, the paralelism of the cerebral peduncles, CP/HA ratio, Octopus sign. For these markers, we scrutinized the detection rate, the sensibility, the positive or negative predictive value; for the measureable markers we also scrutinized the interobserver differences. The value of the statistical indicators of the evaluated markes are listed below (table 2). What is worth mentioning is the fact that de undiagnosed case in the prospective study was detected by two markers by the second examiner, a measurable marker BS/BSOB and a subjective one, the „octopus sign”.

<b>Table 2 – The value of the statistical indicators of the assessed markers</b>						
<b>Marker</b>	<b>Examiner</b>	<b>Sensibility</b>	<b>Specificity</b>	<b>Positive predictive value</b>	<b>Negative predictive value</b>	<b>Relative risk (95% CI)</b>
<b>Intracranial translucency</b>	<b>EX1</b>	91.67%	98.86%	91.67%	98.86%	80.67 (4.70 -

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<b>BS/BSOB ratio</b>	<b>EX2</b>	91.67%	98.86%	91.67%	98.86%	80.67 (4.70 - 1383.35)
	<b>EX1</b>	91.67%	98.86%	91.67%	98.86%	80.67 (4.70 - 1383.35)
<b>The paralelism of the cerebral peduncles</b>	<b>EX2</b>	100.00%	98.86%	92.31%	100.00%	-
	<b>EX1</b>	75.00%	97.73%	81.82%	96.63%	24.27 (3.57 - 164.98)
<b>CP/HA ratio</b>	<b>EX2</b>	66.67%	100.00%	100.00%	95.65%	-
	<b>EX1</b>	91.67%	98.86%	91.67%	98.86%	80.67 (4.70 - 1383.35)
<b>The “octopus sign”</b>	<b>EX2</b>	91.67%	98.86%	91.67%	98.86%	80.67 (4.70 - 1383.35)
	<b>EX1</b>	83.33%	96.59%	76.92%	97.70%	33.46 (4.98 - 224.94)
	<b>EX2</b>	91.67%	97.73%	84.62%	98.85%	73.62 (6.16 - 880.11)

Regarding the interobserver differences for the BS/BSOB ratio, the Student test results found a  $p=0,215>0,05$ , therefore a number above the threshold allowed that shows statistical significance. By using the student test, we found there is a highly significant statistical difference between the values of the BS/BSOB of the spina bifida fetuses versus the normal ones, the first ones having significant higher values in comparison with the latter.

Concerning the CP/HA ratio, the differences between the two observers were even lower,

without a significant difference, with a Student test result of  $p=0,426 > 0,05$ . By using the Student test we demonstrated that there is a highly significant difference, CP/HA range of the spina bifida cases and the normal fetuses ( $p=3.08 \times 10^{-15} < 0.001$ ).

Within this stage of assessment we suspected a case of Dandy Walker by using the „octopus sign” and a intracranial translucency measurement above the normal ranges, but we had the confirmation of the diagnose and the TOP only in the third group of study, at 18 weeks of gestational age.

We also tried to detect the agenesis of corpus callosum at 11-14 weeks. The method proposed by our department was an adaptation of a second trimester sign – the absence of the cavum septum pellucidi. We tried to appraise how many of these cases had agenesis of corpus callosum. The diagnostic rate was 75% (3 cases out of 4), with a false positive rate of 14 cases and one false negative case. Retrospectively, we arbitrarily chose images of 96 cases sonographically normal. Together with the 4 cases of ACC diagnosed during our study, all the images were assessed by two experienced sonographers. The two sonographers had to ascertain whether the fetuses assessed had ACC or not only by evaluating one marker: one sonographer, the CSP aspect and the other the M/FC ratio. The only thing the examiners knew was that among the 100 fetuses an unknown number of cases were confirmed with ACC. In the retrospective study, the detection rate of ACC by using the CSP aspect assesment was of 100% with a extremely high rate of false positive diagnosis (12 FP cases for 4 positive cases), while the M/FC ratio detected only 75% of the cases with 5 false positive cases. The high rate of positive cases, as well as false positive ones are due to the high suspicion rate, as the examiners knew that among these cases of study there are cases with ACC. Both methods had great limitations in their usage as a screening tool, but they can be a warning sign for a further detailed evaluation or for a refferal to a superior forum.

**The third group of examination** offered the diagnostic confirmation of the Dandy Walker malformation case, detected a new case of spina bifida, 3 cases of ventriculomegaly (two mild and 1 moderate), 4 cases of borderline ranges (9-10 mm), 2 dangling plexus and 3 cases of mega cisterna magna. We also suspected 4 cases of agenesis of corpus callosum, but the diagnostic confirmation was obtained at 22 weeks with the help of some indirect signs like the absence of CSP and colpocephaly, the direct visualisation of the corpus callosum

being difficult. The final classification of the cases of ventriculomegaly was obtained at 20-24 week group. Some of these anomalies, like mega cisterna magna and ventriculomegaly, benefited of additional imagistical explorations like fetal RMN in the third trimester of pregnancy.

**The fourth group** detected 3 cases of mild ventriculomegaly, all suspected since the 15-19 week assessment, 2 cases of moderate ventriculomegaly, one with normal aspect at the previous examination, and two cases of hydrocephaly, one suspected and one with normal aspect at 15-19 week evaluation. Both patients with fetal hydrocephaly opted for TOP. We also detected one case of hydranencephaly, in a patient diagnosed with chicken pox, a week before the evaluation, who admitted alcohol and ethnobotanical abuse during pregnancy. The TOP was performed without obstetrical events.

We also detected during the study, **anomalies that could not be included in the study lot** due to the failure to comply with the study terms. In spite of these impediment, these cases are worth mentioning. We detected one case of encephalocele at 16<sup>+5</sup> weeks, one case of acrania at 29<sup>+3</sup> weeks and one case of lobar holoprosencephaly at 32 weeks of gestational age. Unlike these cases that were not included in the study, we diagnosed a case of aneurysm of the vein of Galen at 34 weeks, in a patient who participated, with negative assessments during all the four stages of study. This scenario, emphasizes once more the dynamics of the fetal CNS development, and the importance of an extended protocol of the CNS examination inclusively in the third trimester.

#### **4. CONCLUSIONS**

The CNS anomalies represents an important health issue, with a major medical, social and economical impact. Therefore, the elaboration of more extended protocols of pregnancy care and the obedience to these protocols are mandatory.

The objective of our study was to establish the opportunity of a comprehensive assessment of the fetal central nervous system starting with the first trimester of pregnancy, with the purpose to increase the number of anomalies detected antenatally and to decrease the gestational age of the prenatal diagnosis.

During the study, we managed to outline a few real **advantages** of such broad protocol of assesement.

Therefore, a broad protocol of assesement offers:

- An early prenatal diagnosis of the CNS malformations;
- An elaboration of a scientific basis for an upgrading of the standard protocols.
- A therapeutic abortion at an early age, with minimal side effects.
- An improvement of the epidemiological indicators like incidence and prevalence regarding the prenatal diagnosis of fetal malformations of the CNS.
- A spending decrease originated from the medical and social assistance of such persons concerning the parents and society.

Particularily, this study disclosed:

- We can detect acrania at 8-10 weeks in 80% of cases;
- We can detect spina bifida at 11-14weeks in 80% of cases;
- We can diagnose the complete form of agenesis of corpus callosum at 15-19 weeks;
- We can enclose in a high risk group, most of the the pregnant women who will develop ventriculomegaly during pregnancy since the 15-19 week assesement;
- We can suspect Dandy-Walker malformation since 11-14 weeks.

Like all screening methods, this type of evaluation has its disadvantages, which have to be taken into consideration:

- The high costs of such assesement in the daily practice.
- The prolongation of the evaluation time with an increase of the examination costs.
- The need of an extremely expensive high performance equipment.
- The need of an appropriate training of the sonographers with a constant updating of the medical information.

In our country, in the vast majority of cases, the fetal anomalies screening is performed at 11-13<sup>+6</sup> weeks, at the time of the nuchal translucency assesement. Still, the the

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low level of sanitary education of the patients, the low adresability and accessibility to medical care, makes this assesement to be suboptimal. Accordingly, such patients are in the care of the primary health providers throughout the whole pregnancy. Therefore, the need of an appropriate training and equipment of these health providers is pressing. Despite of not dettected sophisticated anomalies, they can report a deviation from normal and send the patient for further assesements in highly equiped departments.

The implementation of such screening programmes and their obedience by the doctors in the territory on one hand and the pregnant women on the other, the development of new health education strategies, human training and equip of the sanitary units represent the key for prenatal diagnosis effectiveness regardless of the anatomical structure.