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PLACENTIAL CAUSES AND OTHER ETIOLOGICAL CORRELATIONS OF HYPOXIC ASPHYXIC SYNDROME AT BIRTH- STUDY ON A LOT OF NEWBORNS FOR A PERIOD OF 3 YEARS

PHD THESIS SUMMARY

Scientific coordinator:

UNIV. PROF. DR GHERGHINA IOAN

PhD student: AVRAMESCU (căs. CALOMFIRESCU)

ANDREEA VALENTINA

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Introduction

Hypoxic-ischemic encephalopathy (HIE) is an important cause of permanent damage of the nervous system that can lead to neonatal death, cerebral paralysis or can later manifest as development (motor, neurological and cognitive) problems. About 20-30% of infants with HIE die during the neonatal period and about 33-50% of the surviving ones remain with permanent neurodevelopmental problems (cerebral paralysis, mental retardation)[1].

The present thesis tries to highlight the placental factors that speed up and maximize the effects of early treatment, to prevent the effects of irreversible neuronal impairment. In newborns with severe acidosis (pH <6.7) the rate of mortality or of permanent impairment is 90%, and a base deficit> 25 mmol/l is associated with a 72% mortality.[2]

In recent years, the incidence of perinatal asphyxia significantly increased, ranging from mild to very severe forms, thus becoming one of the basic problems neonatology faces with nowadays. Perinatal asphyxia complicated with hypoxic-ischemic multiple organ injury (cardiac, renal, digestive, neurological and respiratory) is the most important cause of perinatal morbidity and mortality, especially in the newborn at term. [3].

Being a frequent pathology, it raises a lot of research topics and controversies regarding both etiology and efficient therapeutic strategies. At present, we do not have a clear association between an etiology and the onset of perinatal hypoxic-asphyxia syndrome, as we do not have the most effective therapeutic strategy, although new treatments and molecules developed for neuroprotection.

The placenta was shown to be the organ with the highest impact on the development of the fetus, and a thorough examination can provide us with quite important information on pregnancy progression [4]. Therefore, we propose, through a detailed analysis of a single "key" element such as the placenta, to formulate data on the degree of neurological impairment and, in brief terms, to present the elaboration of positive or negative prognostic factors.

Being such a vast subject, with an acute need to highlight clear etiological correlations and an efficient neuroprotective treatment for establishing a better prognosis, the present research aims at identifying some correlations between the pathological aspects

of the placental histopathological study and the presence of neonatal encephalopathy. Thus, the theme of the present doctoral thesis is based on limited data from the literature. We identified a small number, about 10 studies, highlighting the correlations between the micro and macroscopic aspect of the placenta, as well as the anomalies of the umbilical cord and the presence of perinatal hypoxia.

The first publications on this topic were documented since the 2000s by Redline et al., who presented the placental changes associated with the presence of cerebral paralysis and neurological impairment in term newborns. [5] Since then, the theme became topical in many research centers and more research studies were published, but it was only in 2016 that the first guide of the placenta lesions classification was published [6]. Ever since, the resumption of studies was limited, being identified only 3 international studies regarding this classification. In our country, the studies were focused on the description of hypoxic-ischemic encephalopathy and less on the placental etiological correlations.

The genuine characteristic of this thesis comes from the anatomopathological approach of an extremely versatile syndrome, in terms of etiology. The anatomopathological examination of the placenta is scarcely used for the achievement of etiological correlations within the hypoxic-asphyxia syndrome and quite rarely for the elaboration of prognostic factors. Although, in many cases, the only cases defining the cause of hypoxic-ischemic encephalopathy are placental lesions.

The present paper was structured in two parts: general part with 2 chapters (hypoxic-asphyxia syndrome and the placenta) and the special part with 9 chapters and 3 studies (Study 1- Association of placental changes with hypoxic-ischemic encephalopathy on a group of newborns, Study 2-correlations of placental macroscopic examination, membranes and changes in the umbilical cord in hypoxic-neonatal encephalopathy, Study 3- placental microscopic lesions associated with newborns with hypoxic-ischemic encephalopathy).

The research methodology involved the performance of the anatomopathological (micro and macroscopic) examination of the placenta, membranes and umbilical cord in all pregnancies with newborns that fulfilled the inclusion criteria (diagnosis criteria of hypoxic-ischemic encephalopathy, consent, etc.). The placenta that was sent to the Pathological Anatomy Department was collected in the first hours after birth, obtaining the first results in maximum one day after the informed consent was given. We formed two groups of

patients: the study group (patients with encephalopathy) and the control group (patients from neonatal intensive care unit without encephalopathy). After birth, there was performed the monitoring of newborns and the establishment of the treatment according to the pathology, and for those with neurological impairment, there was initiated a neurological follow-up program. In order to establish the inclusion criteria, there was performed a complete physical examination (which included the neurological examination), there were established the diagnosis encephalopathy criteria and immediately after birth, in severe cases of neurological impairment, an EEG monitoring was also implemented.

The database was performed in Excel, and the statistical analysis of the obtained data was carried out with current medical software (IBM SPSS 25), thus allowing us to obtain quantifiable results to support the initial research hypotheses.

After the completion of the group of patients, chapters 6, 7 and 8 describe the performed studies, namely observational prospective studies with a statistically representative impact. The results obtained were detailed in every chapter and were divided on every performed study. The first results showed that there is an association between placental changes and the presence of ischemic-hypoxic syndrome in term newborns, subsequently detailing the associated changes in all forms of encephalopathy. In the second study, we showed that there is a statistically significant association between the weight of the placenta and the the umbilical cord changes and the presence of hypoxic-ischemic encephalopathy. In the last study, we presented the associations of placental microscopic changes in newborns with hypoxic-ischemic encephalopathy.

The performance of this thesis was possible due to an excellent interdisciplinary collaboration (gynecology, neonatology, anatomopathology) within our clinic. Placenta harvesting was possible with the help of gynecology, and the macro and microscopic placental examination with the help of the pathological anatomy department. The neonatologist established the diagnosis of hypoxic-ischemic syndrome, started the treatment, made the correlations between the placental aspect, the disease form and defined the prognostic factors.

The current thesis raises new research topics in the field of prognostic factors elaboration regarding the hypoxic-ischemic syndrome, as well as the identification of new associations in the placental anatomopathological examination with other neonatal or pediatric diseases. It is also important to associate microscopic lesions with the cerebral

imaging investigation of hypoxic-ischemic lesions and subsequent neurological development of newborns. The limits of the current research are the short period of data collection, the lack of imaging identification of the hypoxic-ischemic lesions, the limited equipment of the anatomopathological laboratory, the lack of a long-term neurological follow-up of the included newborns.

I. General Part

1. HYPOXIC ISCHEMIC ENCEPHALOPATHY

Neonatal encephalopathy is mainly correlated with the hypoxic-ischemic injury of the newborn brain occurring during the peripartum period. It is important to admit that not all neonatal encephalopathies are related to hypoxic-ischemic disease. Antepartum factors and postpartum disorders (infectious, metabolic, genetic) can lead to neonatal encephalopathies. [7, 8]

Hypoxic-ischemic encephalopathy associates an APAGAR score 6 or lower after 5 minutes, a base deficit higher than 12 mmol / l or a pH below 7.2 in the umbilical cord blood [9]. The continuous need for ventilation after 10 minutes is also a proof that the APGAR score could not have been higher than 6 after 10 minutes. In the context of a newborn, the tonus, activity and stimulus-response capacity are affected [10]. Clinical seizures are not an essential criterion, still, if they are present, they indicate encephalopathy. The term neonatal encephalopathy is used generically because, initially, the doctor may recognize the brain function impairment, but the etiology requires further investigations.

The hypoxic mechanism affects all the cellular structures of the embryo, the oxygen deprivation and the increase of the partial pressure of the carbon dioxide acts mainly on the blood pH, causing acidosis, with effect on the basal cellular metabolism. Besdies the change of the blood pH, it causes cerebral hypoxemia with long-term severe damage (intraventricular hemorrhage, leukomalacia, porenecephaly) [11]. Metabolic acidosis determines the decrease of ATP, decreases the enzymatic activity and causes pulmonary and intestinal vasoconstriction (trying to mobilize the vascular bed to the heart and brain). [12]. Through the onset of acidosis, the effects are severe on all the important systems:

- heart - myocardial dysfunction, heart failure

- lung- pulmonary bronchodysplasia, persistent pulmonary hypertension (PPH)
- intestine- necrotozing enterocolitis (NEC)

The pathological changes observed in the brain after the hypoxic-ischemic injury reflect a combination of different metabolic disturbances and cerebral hypoperfusion, the most common post-asphyxia disturbance being selective neuronal necrosis. The types of lesions observed following neonatal hypoxic-ischemic injury are: selective neural injury, parasagital brain injury, white matter injury and focal and multifocal brain necrosis [13].

Hypoxic-ischemic encephalopathy is diagnosed on the basis of the complete clinical examination of the newborn (with a Sarnat score inclusion, the only scoring system widely used to evaluate all three degrees of neonatal encephalopathy in the first 6 hours of life), laboratory tests, electroencephalogram and neuroimaging investigations [12].

Neurological impairment is the mark of ischemic hypoxic encephalopathy and the neurological clinical examination should be rigorously and carefully performed in order to identify and record any changes. The encephalopathic newborn will associate changes in the response mediated by the cranial nerves, practically do not respond to stimuli (bright, auditory, tactile or pain) or have changed responses. Also, the posture asymmetry, motility decrease, the presence of hypotonia/hypertonia and changed responses of the reflexes are characteristic to brain dysfunction.[14] During the neurological examination, the presence of seizures supports the diagnosis of encephalopathy. Usually, they occur within the first 12-24 hours after birth and have a delayed response to the anticonvulsant treatment.

The laboratory tests performed presented: high values of creatinine, of liver enzymes and cardiac I troponin; prolonged coagulation times; and thrombocytopenia. Together with the initial markers of: low pH, increased base deficit, low pO2 deficiency and high pCO2, increased lactate.

The electroencephalogram is used to monitor the brain activity and is initiated immediately after birth, the presence of seizures being a marker of brain impairment.[15]

From a neuroimagistic point of view, hypoxic-ischemic encephalopathy is a multi-stage process presenting cerebral edema, parasagital brain injury, subcortical injury, selective neuronal necrosis, hypoperfusion ischemia, neural death and ultimately sequellae stage. With the help of transfontanelle ultrasound, the injury can be identified in any of the stages, but the MRI remains the golden investigation, while CT scan only for the cases where the other investigations would not be sufficient.[16]

The treatment of ischemic hypoxic encephalopathy includes: neuroprotective treatment, supportive treatment for multiple-organ injury and anticonvulsant treatment. Therapeutic hypothermia for the whole (systemic) body or selective cerebral one reduces the mortality or major impairment of long-term neurodevelopment in newborns with HIE.[17]

The prognosis varies depending on the severity of the injury and the treatment. Infants with the initial blood pH <6.7 have a 90% risk of severe neurological development impairment at the age of 18 months old. In addition, newborns with APGAR scores of 0-3 after 5 min, high base deficit (> 20- 25 mmol/l), decerebrate posture, with severe lesions in the basal lymph nodes or thalamus, severe HIE persistence after 72 hours and spontaneous lack of brain activity also present an increased risk of death or severe neurological sequelae. In general, severe encephalopathy, characterized by coma, apnea, soft tonus, absence of reflexes and refractory seizures is associated with a poor prognosis.[18]

2. PLACENTA

Placenta represents the bonding organ between mother and fetus, developing from the ninth week of pregnancy. It presents a fetal part that develops from the blastocyst and a maternal part that derives from the decidua basalis. At its level, there occur changes of nutrients, blood gases and residue elimination. Knowing the development of normal placenta is essential and necessary to identify pathological aberrations. There is evidence that many of the major complications of pregnancy have their roots in defects of early placentation [19].

The types of placenta are classified according to the form and the insertion site, in all cases influencing the pregnancy progression and causing the presence of specific pathologies that can affect the fetus [20].

The umbilical cord begins to form from the fifth week of gestation, at the level of the allantoid, and houses two umbilical arteries and a vein, wrapped in the Wharton gelatin. It is inserted in the center of the placenta. It may have particular features, presenting a marginal, velamentous or membranous insertion, a single umbilical artery, modified aspect, etc.[21]

The histopathological study includes the examination of the membranes, the umbilical cord and the placenta itself. It starts with the macroscopic aspects (form, insertion site, color, smell) and then the samples for the microscopic examination are processed. Due to the multitude of lesions encountered in the placenta and for a more standardized classification, in the studies presented in the following chapters, we used the Amsterdam guide from 2016 to classify the placental changes and to correctly harvest the histopathological samples. [6].

Category	**Characteristics**
Chorioamnionitis	The presence of inflammatory cells in the chorionic layers of membranes and placenta with/without the presence of necrosis
Maternal vascular malperfusion	Includes: placental hypoplasia (weight under the percentile 10 for gestation age), decidual vasculopathy, distal villous hypoplasia, presence of syncytial knots, perivillous fibrin deposits, villous necrosis, fibrin islands in the trophoblast, the presence of giant cells at the implantation, placental infarction
Fetal vascular malperfusion	Presence of thrombosis in the chorion, placental vessels with or without occlusion of vessels and the presence of avascular villi. It may be high-degree (<5 villi/ image) or global (major occlusive or non-occlusive thrombosis areas)
Chronic villitis of unknown etiology	The presence of inflammatory cells and the damaging of vessels due to their obstruction, or in combination with the presence of avascular villi. I can have a low or a high degree.
Late maturation of villi	The presence of villi with a small number of vasculo-syncytial membranes for the gestational age and capillaries centrally placed. It may be focal (<30 % of the parenchyma) or diffuse (30 % of the parenchyma)
Placental abruption	Presence of retroplacental hematoma

Table 2.1. Histopathological characteristics of placental lesions

II. Special Part

3. Aim and objectives

The present thesis mainly aims at identifying etiological correlations between placental lesions and hypoxic-ischemic encephalopathy. In encephalopathy, time is a very important factor that makes the difference between the presence and absence of irreversible brain damage. The faster the treatment is initiated, the more favorable the prognosis. Thus, the present thesis aims at identifying the placental lesions that predispose the newborn to an unfavorable neurological progression, so that the neonatologist can act accurately and promptly in every particular case.

Placental examination is an easily accessible tool, requiring low resources, but which can highlight a case with an unfavorable prognosis even before carrying out other thorough and complex investigations, with a much higher cost.

The present paper has the objectives of identifying both macroscopic and microscopic placental lesions that could be used as predictive markers for the progression of newborns diagnosed with hypoxic-ischemic encephalopathy, regardless of its form (mild, moderate or severe).

By carefully analyzing the data in the literature, we proposed the following as secondary objectives:

- characterization of patients with neonatal encephalopathy according to the Sarnat criteria
- characterization of placental lesions according to a standardized guide
- selecting lesions that can become markers for hypoxic-ischemic encephalopathy
- correlation of lesions observed in the umbilical cord with hypoxic-ischemic encephalopathy
- correlation of microscopic and macroscopic lesions with every type of encephalopathy (mild, moderate and severe)

4. Research Methodology

For a period of 3 years (2016-2019), we performed 3 prospective, non-randomized control studies that included 132 newborns admitted to the Neonatal Intensive Care Unit

within the "Filantropia" Clinical Hospital of Gynecology in Bucharest. The patients included in the study were normal weight newborns with the diagnosis of neonatal encephalopathy (mild, moderate and severe) and newborns admitted to intensive care unit for at least 7 days, with gestation ages over 30 weeks without encephalopathy. We mention that all patients were born in our maternity.

The studies performed was approved by the Medical Ethics Committee of the "Filantropia" Clinical Hospital of Gynecology in Bucharest. The study group was made up only of newborns for whom the written consent of the legal guardian (mother) was received, who was informed regarding the present study, its methodology, aim and objectives.

After obtaining the informed consent of the legal guardian, the clinical, anamnestic and paraclinical data of the newborn from his observation sheet were collected and the database was created. Immediately after birth, the placenta was collected in order to perform the macroscopic and microscopic examination within the Department of Anatomopathology of the hospital.

Newborn Group

The patients included in the study were selected based on well-established inclusion criteria presented in the table below, those who did not meet the requirements being excluded. Newborns with encephalopathy were diagnosed after a general neurological clinical examination and classified according to the Sarnat score (1-mild, 2-moderate, 3-severe). It also included a pH value <7.20 sampled from the blood of the umbilical cord clamped immediately after birth. From the sample blood, it was performed the complete acid-base analysis (pH, PCO2, PO2, base deficit). The control group included newborns who required neonatal intensive care for at least seven days, admitted in the same period without the diagnosis of hypoxic-ischemic encephalopathy.

Placental examination

Within the "Filantropia" Clinical Hospital of Obstetrics and Gynecology, the placentas do not undergo an usual examination process, only in cases selected for interest for the obstetrician or the neonatologist. All placemntas were analyzed by the same anatomopathologist in the hospital, with vast experience in the field of placenta examination. During the studies, there was performed the macroscopic examination in the beginning. It included the aspect of the umbilical cord, membranes and the placenta itself.

The weight of the placenta was obtained in the first hour after birth, after the membranes and the umbilical cord were removed. The examination of the umbilical cord included: the length, the insertion site in relation to the center or the margins of the placenta, the twisted or untwisted aspect, the presence/absence of the single umbilical artery. The description of the membranes included: the opaque aspect and the color, the circumvallate or circummarginate insertion.

For the microscopic examination, 5 samples were collected from the membranes, 1 from the umbilical cord and 3 from the placental parenchyma. The identified lesions were classified into 6 categories: chorioamnionitis, maternal vascular malperfusion (MVM), fetal vascular malperfusion (FVM), chronic villitis of unknown etiology (VUE), delayed villous maturation, placental abruption. The inclusion of placentas in a single category was made according to the surface and degree of the lesion. Other types of lesions were identified but were considered insignificant.

All placental samples were examined with the Zeiss Axioscope 5 Microscope, and the images were captured with an Axiocam 208 Color incorporated camera.

5. Statistical Analysis of Data

The database was made in Excel and included all the variables of interest for the performed studies, while the statistical analysis of the data was carried out with an IBM SPSS 25 (Windows version). The statistical significance was established at a p-value <.05.

6. Study no. 1. Association between Placental Changes and Hypoxic-Ischemic Encephalopathy in a Group of Term Newborns

In the study, there were included 84 patients with the diagnosis of hypoxic encephalopathy in whom the placental examination could also be performed. The subjects included in the study had birth weights between 1700 g and 4390 g, with an average +/- SD of 3030.95 +/- 602.62 g and a median of 3050 g. The gestational age values were between 36 and 42 weeks with an average +/- SD of 37.83 +/- 1.46 weeks and a 38-week median.

The patients were diagnosed with hypoxic-ischemic encephalopathy according to the Sarnat classification, 1-mild, 2-moderate, 3-severe. And the distribution highlighted that

most of them were in class Sarnat 1 (42.9%), the rest in Sarnat 2 (38.1%) and Sarnat 3 (19%).

After establishing the study group, the patients were divided into 2 groups according to the presence of placental changes, presented in the table below. The placental examination included both the macroscopic and microscopic evaluation, being also included the particular aspects of the umbilical cord (single umbilical artery, cord velamentous and marginal insertions, its length and aspect).

Placental changes	No. of cases					
	Valid		Not valid		Total	
	84	100%	0	0%	84	100%
With	53	63.1%	0	0%	53	63.1%
Without	31	36.9%	0	0%	31	36.9%

Table 6.1. Patient distribution in the two groups

In both groups of newborns with encephalopathy there were compared the pH values in the umbilical cord, the value of the APGAR score after 5 minutes and the duration of ventilation. Following the application of a T test for independent samples, we highlighted a statistical significant difference between the two groups and the data were summarized in the table below:

Patient group	**With placental changes**	**Without placental changes**	**T test**	**P value**	**95% Trust interval**	
					Low	**High**
pH	7.03	7.08	.39	0.01	0.00	0.09
AScore after 5 min	4.57	5.52	.92	0.00	0.30	1.59
Duration of ventilation (days)	3.23	1.45	2.96	0.00	2.96	0.58

Table 6.2 T test for independent groups

By analyzing the data from the two groups and the Sarnat classification, there resulted a distribution of data as follows: 41.7 % (n = 15) of those with Sarnat 1 did not

associate placental changes, while in the Sarnat 2 group, 8 % (n = 14) were without changes and in the Sarnat group 3 only 12.5% (n = 2), according to the chart below. Because the Sarnat score 1 involves in a few cases the presence of neurological sequelae, we decided to divide the group into 2 categories: Sarnat 1 and Sarnat 2-3 to highlight the statistical association between the presence of placental changes and the high scores. Applying a t-test for independent samples, it resulted that there is a statistically significant association between Sarnat scores and placental changes (t = -2.07, p = 0.04).

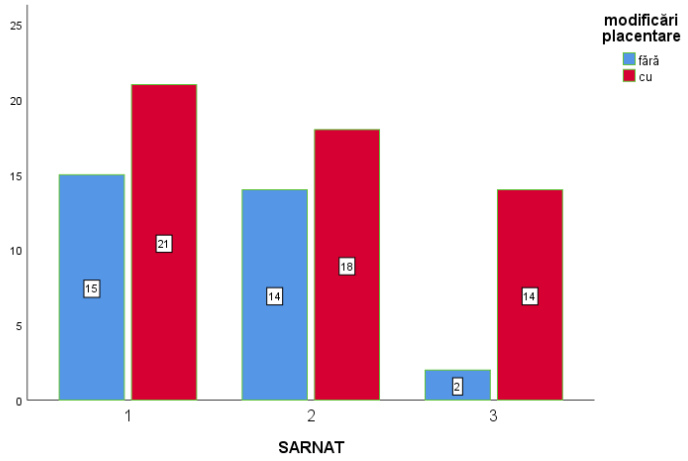

Figure 6.1. Distribution of Sarnat scores according to the presence of placental changes

The originality of our study comes from the inclusion criteria of patients who were strictly diagnosed with neonatal encephalopathy according to the Sarnat classification, as opposed to literature studies that also included term newborns with normal weight.

The main limitation of this study was that the anatomopathologist knew all the details of the case, such as the intrapartum events, the presence/absence of the Caesarean section, the APGAR score, the pH in the blood cord, the Sarnat score and the neurological changes. Another limitation of our study was the relatively small size of the group.

7. Study no. 2. Correlations of the placental macroscopic examination and the umbilical cord changes in the hypoxic-ischemic encephalopathy

The study included 134 newborns admitted to the intensive care unit, where there could be performed the placental macroscopic examination and the umbilical cord examination, divided into two groups:

- 84 newborns with the diagnosis of neonatal encephalopathy
- 48 newborns without encephalopathy (control group)

Analyzing the presence of placental macroscopic changes in both groups, these were present in the control group in 43.75% (n = 21), while in those with hypoxic-ischemic encephalopathy in 63.09% (n = 76) of cases. Applying a Chi-Square Test I, we highlighted a statistically significant association between the presence of placental macroscopic lesions and newborns with hypoxic-ischemic encephalopathy (p= .03).

For a more thorough analysis of the details of patients in the encephalopathy group, diagnosed according to the Sarnat score, we decided to carry out T tests for independent groups on every Sarnat category. Thus, we divided the patients with encephalopathy into two Sarnat 1 and Sarnat 2+3 subgroups. The data obtained were summarized in the tables below.

Variables	**Control**	**Sarnat 1**	**T test**	**P value**	**Trust interval**	
					High	**Low**
Placental weight	429.85	459.58	-1.17	.24	-71.66	18,21
UC anomalies	**101**	**31**	**-2.04**	**.04**	**-.40**	**-.00**
Placental changes	101	31	0.56	.57	-.14	.26

Table 7.1. T test for independent groups according to the Sarnat 1 score (mild encephalopathy)

Variables	**Control**	**Sarnat 2+3**	**T test**	**P value**	**Trust interval**	
					High	**High**
Placental weight	425.56	451.89	-1.34	.18	-65.12	12,46
UC anomalies	79	53	1.82	.07	-.01	.33
Placental changes	**79**	**53**	**-2.65**	**.00**	**-.40**	**.05**

Table 7.2. T test for independent groups according to the Sarnat 2+3 score (moderate and severe encephalopathy)

One of the strengths of our study was that the group of newborns with encephalopathy was well-identified and the diagnosis of encephalopathy was also clearly

defined clinically (according to the universal classification) and paraclinically (by the presence of specific acid-base imbalances). Also, the use of the classification according to the Amsterdam Criteria Guide of placental and umbilical cord lesions represents another strength of this study as it can be used as a reference point for other future meta -analyses.

The limitation of the study was the fact that we did not subdivide the changes identified on subgroups in order to make several statistical correlations between the identified lesions and the newborns with hypoxic-ischemic encephalopathy, but it offers new perspectives for future studies. Also, another limitation was that there was no imaging examination (MRI) to highlight the clear association between extensive brain damage and macroscopic changes of the placenta or the umbilical cord, our study being based on the clinical Sarnat classification.

8. Study no. 3. Placental microscopic lesions in newborns with hypoxic-ischemic encephalopathy

Within the study, 122 newborns, 59 (48%) girls and 63 (52%) of boys, met the inclusion requirements. They had gestation ages between 30 and 42 weeks with an average of +/- 37 weeks. The birth weights varied between a maximum of 4390 grams and a minimum of 1100 grams with an average +/- SD of 2926.36 +/- 730.98 grams.

In all newborns in the study, the placental anatomopathological examination was performed and the presence of placental lesions was identified in 77 % (n = 65) of patients with hypoxic-ischemic encephalopathy, while in the control group only in 47 % (n = 23). A Chi-square test showed that there is a statistically significant association between the presence of placental microscopic lesions and the diagnosis of hypoxic-ischemic encephalopathy (p= .00)

The group of patients was divided according to the presence of placental microscopic changes, as follows:

- 65 newborns with ischemic hypoxic encephalopathy
- 23 newborns without encephalopathy

We analyzed the frequency of placental changes on every Sarnat group (1- mild, 2- moderate, 3-severe) compared to the control group and applied the Chi test for every variable (placental microscopic lesion).

Chi test results for Sarnat 1 group were summarized in the table below:

Placental changes (variables)	**Mild HIE**	**Control**	**Pearson Chi**	**p-value**
MVM	1	0	3.17	.07
FVM	0	1	.32	.57
Chorioamnionitis	1	9	1.23	.26
Delayed villous maturation	**10**	**5**	**17.90**	**.00**
Chronic inflammatory villitis	**8**	**4**	**13.74**	**.00**
Placental abruption	1	3	.00	.96

Table 8.1. Chi test for placental changes in mild encephalopathy (MVM- maternal vascular malperfusion, FVM – fetal vascular malperfusion)

Application of Chi-square test for placental changes in moderate encephalopathy revealed the data in the following table:

Placental changes (variables)	**Moderate HIE**	**Control**	**Pearson Chi**	**p-value**
MVM	**3**	**0**	**7.70**	**.00**
FVM	**9**	**1**	**20.70**	**.00**
Chorioamnionitis	**0**	**9**	**4.04**	**.04**
Delayed villous maturation	4	5	1.21	.27
Chronic inflammatory villitis	5	4	3.52	.06
Placental abruption	4	3	3.00	.08

Table 8.2. Chi test for placental changes in Sarnat 2 forms (MVM- maternal vascular malperfusion, FVM – fetal vascular malperfusion)

In severe encephalopathy, the placental changes were more common and, after applying Chi - Square tests, we identified the statistically significant values presented in the following table:

Placental changes (variables)	Severe HIE	Control	Pearson Chi	p-value
MVM	**7**	**0**	**27.24**	**.00**
FVM	**7**	**1**	**22.52**	**.00**
Chorioamnionitis	2	9	.09	.75
Delayed villous maturation	1	5	.10	.75
Chronic inflammatory villitis	1	4	.01	.91
Placental abruption	1	3	.02	.87

Table 8.3. Chi test for placental changes in Sarnat 3 forms (MVM- maternal vascular malperfusion, FVM – fetal vascular malperfusion)

The first limitation of this study includes a relatively small number of cases of hypoxic-ischemic encephalopathy and a limited control group in newborns hospitalized in the neonatal intensive care unit. The latter fact, however, illustrates the importance of the control group in determining the associations between placental changes and HIE. The second limitation of this study is the lack of a follow-up on the neurological development of the newborns included in the study, because the follow-up programs are not performed in our clinic. Another limitation is the lack of highlighting the hypoxic-ischemic lesions through a thorough imaging investigation (MRI), as our clinic does not have the necessary equipment.

The strengths of this study include the well-defined criteria for encephalopathy (Sarnat classification) and a standardized classification of placental pathology (Amsterdam criteria). Another strength of the study is its prospective population feature, carried out in a tertiary maternity clinic with a high number of complicated births.

9. Summarizing the Etiologies of Hypoxic-Asphyxia Syndrome in Newborns Included in the Study

In all newborns we analyzed the presence of traumatic injuries during pregnancy (abdomen injuries, surgical interventions that required local or general anesthesia, falls or road accidents) and the distribution was according to the following chart:

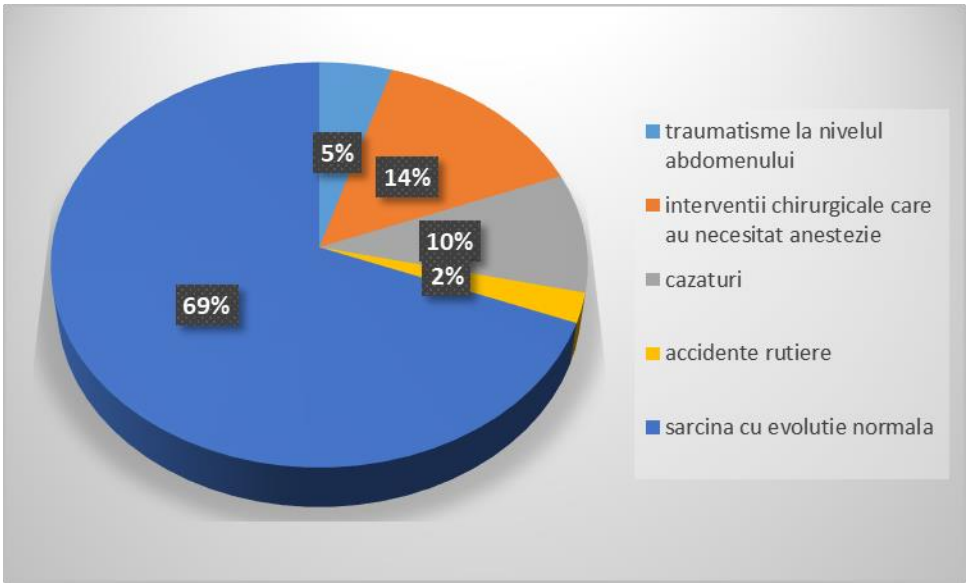

Figure 9.1 Distribution of events during pregnancy

Pathological maternal history identified in HIE pregnancies included autoimmune diseases (systemic lupus erythematosus, thyroiditis), respiratory diseases (COBP, asthma), heart diseases (cardiomyopathy), psychiatric diseases (with treatment). The figure below highlights their presence.

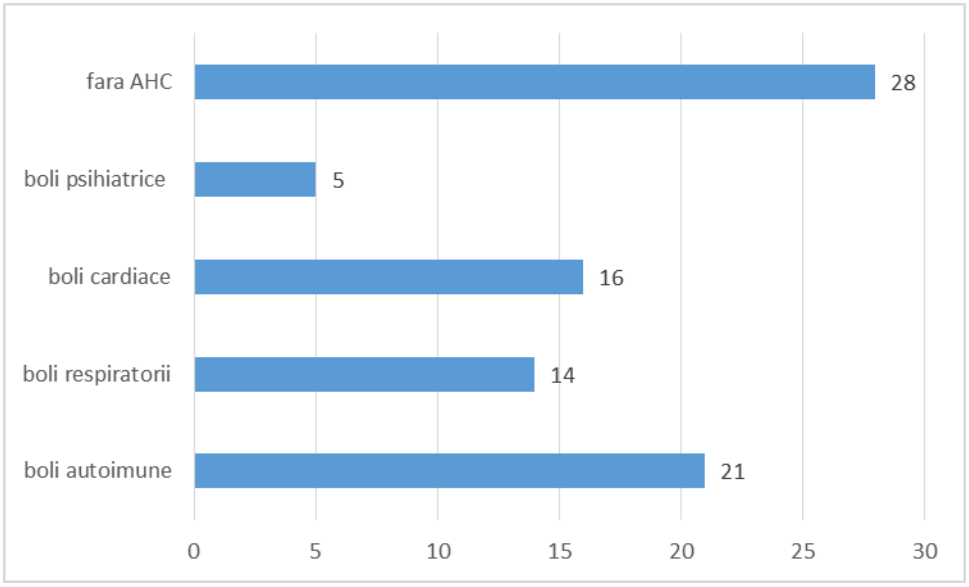

Figure 9.2 Pathological maternal history

The pregnancy pathology included: gestational diabetes (GD), pregnancy induced hypertension (PIH), pregnancy cholestasis, infections (GBS, E.Coli, Klebsiella, Listeria, etc.), amniocentesis and pregnancy anemia. These pathologies were highlighted in the figure below.

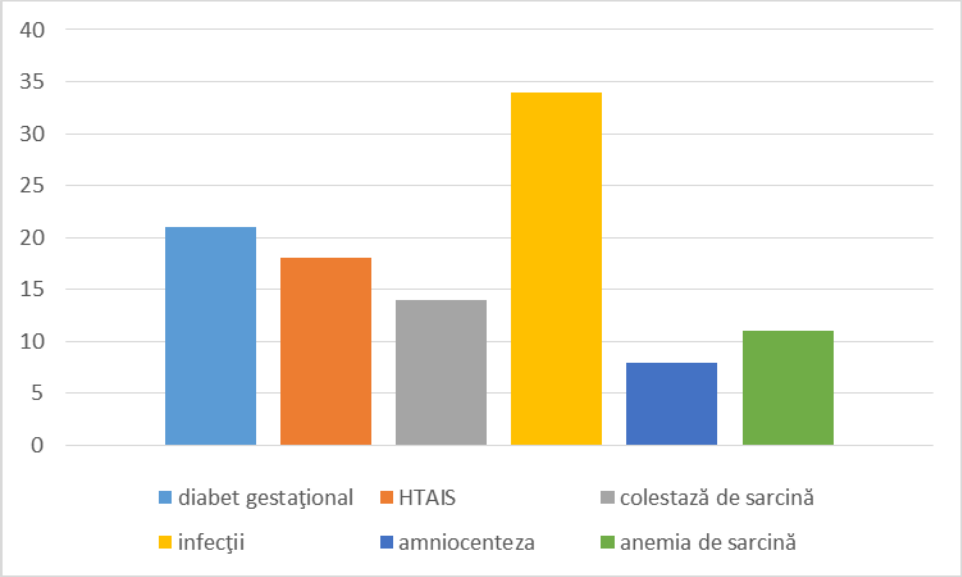

Figure 9.3 Pregnancy pathology

The pathologies of the newborns associated with the hypoxic-ischemic syndrome at birth were sepsis (with GBS, E.Coli, Listeria, unidentified germs), genetic diseases, neurodegenerative diseases, congenital malformations. Their distribution is presented in the figure below.

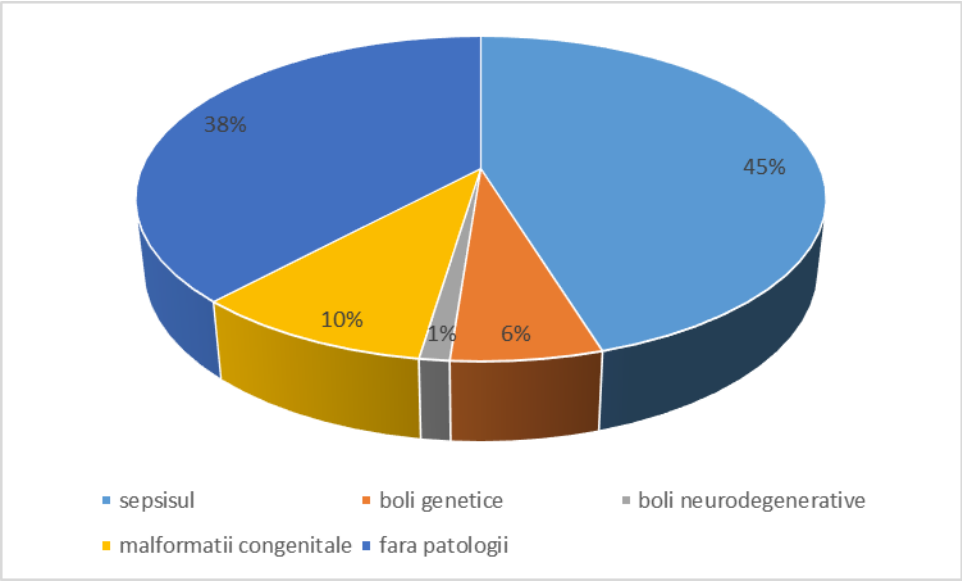

Figure 9.4 Pathologies of newborns associated with HIE

10. Final conclusions and personal contributions

Hypoxic-ischemic encephalopathy remains a syndrome with many attractive elements for research in terms of etiology and treatment, but, according to the data obtained so far in the current thesis and in other literature studies, we can state that placental lesions are certainly an etiological factor. The placental changes was present in 77% of the cases of neonatal encephalopathy. It remains to be highlighted to what extent the placental lesions determine the presence of the encephalopathy degree, considering that in all the analyzed cases the highlighted changes were over 50% from the placental parenchyma.

The placental examination is not commonly performed in all the care centers for newborns, most of them using a protocol in which this is performed only in pregnancies that have maternal-fetal complications: presence of the meconium amniotic fluid, decrease of the fetal heart rate during labor or in newborns who require resuscitation at birth. The association between the presence of placental changes and the type of hypoxic-ischemic encephalopathy should emphasize the importance of performing a placental examination in all newborns with perinatal asphyxia (p= .04).

Also, the placental macroscopic examination does not require any specialized medical staff, it can be done by a midwife, a nurse or a resident doctor. The presence of macroscopic changes, such as increased placental weight, is associated with the presence of hypoxic-ischemic encephalopathy in term newborns. Even if, most of the time, only mild forms seem to be associated with the presence of umbilical cord changes, this must be carefully examined, the details being accessible immediately after birth (p= .04). Moreover, the marginal or velamentous insertions of the umbilical cord can be highlighted during the ultrasound investigation in the second quarter and can raise the suspicion of high-risk pregnancy.

Microscopic placental changes, although they require the presence of an anatomopathologist, can be available before other thorough investigations and the progression of the newborn improved with the help of an individualized treatment. The presence of maternal and fetal vascular malperfusion lesions is associated with the presence of moderate or severe encephalopathy (p= .03). Thus, the identifying these lesions should emphasize a closer pursuit of the newborn to prevent the emergence of irreversible neurological sequelae.

Moreover, the presence of placental inflammatory lesions (chorioamnionitis) and the presence of chronic inflammatory villitis of unidentified cause is associated with the presence of hypoxic-ischemic encephalopathy (p= .00). Therefore, our results underline the contribution of the placental inflammatory processes to the presence of hypoxic-ischemic encephalopathy.

Finally, we can state, according to the obtained results, that the placental changes are associated with the presence of hypoxic-iscemic encephalopathy. Also, severe forms of the disease are associated with placental microscopic changes of maternal, fetal malperfusion and chorioamniotits, while mild forms of the disease are associated with changes in the umbilical cord and microscopic lesions from: delayed villous maturation and chronic inflammation of the chorionic villi.

According to the analysis of the obtained results, the current thesis complied with all the objectives initially established. This was possible after a thorough research of the specialized literature and the choice of the correct and complete criteria for defining the hypoxic-ischemic syndrome and the idenitication of placental lesions. The present thesis represents a foundation stone for future research and can be assimilated into international meta-analyses, as it is the only one in the national literature that used the Amsterdam criteria for classifying the placental lesions.

Also, the personal contributions to the identification of placental lesions specific to each Sarnat score (mild, moderate and severe), place the present thesis as a point of interest and debate within the international societies of Neonatal Neurology and Brain Development.

Selective bibliography

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List of published scientific papers

1. Placental changes in a group of full-term newborns with hypoxic-ischemic encephalopathy
   Andreea Calomfirescu-Avramescu, Luminita Ceauselu, Vlad Dima, Mihaela Demetrian, Anca Balanescu, Paul Balanescu, Ioan Gherghina
   [full text] — Ref: Ro J Pediatr. 2022;71(2). DOI: 10.37897/RJP.2022.2.1
   https://view.publitas.com/amph/rjp_2022_2_art-01/page/1

2. Placental fusion in a dichorionic-diamniotic IVF twin pregnancy – case presentation
   Andreea Calomfirescu-Avramescu, *Mihaela Demetrian, Georgeta Grecu, Vlad Dima, Ioan Gherghina*
   [full text] — Ref: Ro J Med Pract. 2020;XV(1). DOI: 10.37897/RJMP.2020.1.18
   https://view.publitas.com/amph/rjmp_2020_1_art-18/page/1

3. EN. Perinatal asphyxia associated with early neonatal sepsis with *Listeria monocytogenes* –case presentation
   RO. Asfixie perinatala asociata cu sepsis neonatal precoce cu *Listeria monocytogenes* – prezentare de caz
   Andreea Calomfirescu-Avramescu, *Mihaela Demetrian, Andreea Vidru, Georgeta Grecu, Vlad Dima, Ioan Gherghina*
   Ref: Ro J Infect Dis. 2020;XXIII(1). DOI: 10.37897/RJID.2020.1.4
   Ref: Ro J Infect Dis. 2020;XXIII(1). DOI: 10.37897/RJID.2020.1.8
   https://view.publitas.com/amph/rjid_2020_1_ro_art-08/page/1