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PhD THESIS

BASIC BIOCHEMICAL AND HEMATOLOGICAL INVESTIGATIONS IN PERINATAL ASPHYXIA AND THEIR CORRELATION WITH HYPOXIC ISCHEMIC ENCEPHALOPATHY

A B S T R A C T

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ABSTRACT

1. INTRODUCTION:

Hypoxic-ischemic encephalopathy represents a worldwide diagnostic and therapeutic challenge, both for gynecologists, neonatologists and pediatricians. A quick approach in order to establish a prompt diagnosis led me to start this study to analyze the less studied markers in this condition, but which are part of the routine investigations, namely the hematological markers: hemoglobin (HB), leukocytes (LE), neutrophils (NE), lymphocytes (LI) platelets (TR), enzyme values: lactate dehydrogenase (LDH), aspartate aminotransferase (TGO), alanine aminotransferase (TGP) and also inflammatory markers such as C reactive protein (CRP) and procalcitonin (PCT), which could bring quick information about both the acute stage and the long-term neurological prognosis.

2. GENERAL PART:

Neonatal encephalopathy associated with perinatal asphyxia is called hypoxic-ischemic encephalopathy (HIE). Involvement of multiorgan systems is a hallmark of hypoxic-ischemic encephalopathy. Apart from brain damage, organs affected by hypoxic-ischemic events include the following: heart (43-78%), lungs (71-86%), kidneys (46-72%), liver (80-85%), hematological (32-54%). Asphyxia-induced redistribution of cerebral blood flow is the main post-asphyxia change. More than a million children who survive birth asphyxia develop problems such as cerebral palsy, mental retardation, learning difficulties, integration disorders, later school, social adjustment and other disabilities.

Brain damage results from hypoxia and ischemia. As a result of asphyxiation, cardiac output is compensated by redistribution, thus increasing cerebral blood flow. If hypoxia persists, this self-regulatory mechanism is no longer effective, resulting in decreased heart rate, with systemic hypotension and decreased cerebral blood flow leading to brain damage. At the cellular level, oxygen depletion blocks oxidative phosphorylation resulting in anaerobic metabolism, which is energy inefficient, resulting in:

i) rapid depletion of phosphate reserves, including adenosine triphosphate

ii) lactic acid accumulation and

iii) inability to maintain cellular functions.

There is a high correlation between the severity of hypoxic-ischemic encephalopathy and multiple organ dysfunction (MOD) in the first 3 days of life. Studies show that the severity of MOD correlates with the severity of HIE. A positive correlation was found between the number of affected organ systems and the severity of HIE. Measurement of LDH at 72 hours can differentiate between neonatal asphyxia and other non-asphyxial etiologies, particularly in neonates with nonspecific signs of disease. Cerebral ischemia induces an inflammatory response in both the parenchyma and the systemic circulation. Within hours of an insult to the brain, cytokines are produced in large quantities and leukocytes are activated and migrate into the injured brain. There are few studies investigating the role of lymphocytes in HIE. It is possible that a lymphocytic response is involved in the activation and exacerbation of chronic immuno-inflammatories after HIE. It is not yet clear whether this lymphocyte response improves or, conversely, worsens healing after cerebral ischemia.

We want the fastest and most accurate diagnosis of the hypoxic-ischemic event and the subsequent HIE certification, it has been proven that the use of various serological parameters together with the latest imaging methods are helpful in the diagnosis and prediction of the severity of asphyxia, as well as the prognosis on long term.

The changes in the biological parameters have implications both on the therapeutic behavior and the duration of hospitalization, with socio-economic impact and also with multiple implications related to the increase in the number of days of hospitalization in the intensive care unit, increasing the risks related to both the invasive maneuvers performed, the treatments medicines and biological products, administered parenteral nutrition. The risks of nosocomial infections increase with the increase in the number of days of hospitalization and the number of implanted medical devices.

Early monitoring of hematological, inflammatory and enzymatic parameters with the establishment of the diagnosis and the application of individualized therapeutic behavior can prevent the risks associated with these changes (anemia, infections, bleeding).

This thesis was based on 3 published scientific articles:

1. Basic biochemical and hematological parameters in perinatal asphyxia and their correlation with hypoxic ischemic encephalopathy.

2. Clinical and biological evolution of the newborn with thrombocytopenia in the neonatal intensive care unit.
3. Interdisciplinary Approach and the Value of a Scoring System for Hypoxic Ischemic Encephalopathy in Predicting Newborn Neurodevelopmental Outcome

3. SPECIAL PART:

3.1 PURPOSE AND OBJECTIVES:

The current thesis aims to make correlations between the biological values obtained in newborns included in the study, obtained in the first 24-96 hours after birth and predictive power, as a marker of their severity in hypoxic-ischemic encephalopathy of the newborn.

Relatively few studies have been conducted on this topic that use blood samples collected at different times, from birth to 96 hours postpartum and that aim to track both the results of various investigations and the possibility of developing hypoxic-ischemic encephalopathy. Knowing that hypoxic-ischemic encephalopathy is a common pathology with potentially serious complications in a full-term and premature newborn; it was wanted to identify possible predictive factors for its evolution.

We have outlined the objectives of this study as it follows:

1. Investigating biological changes and interpreting in the context of the particularities of newborns, as appropriate.
2. Monitoring the evolution of biological parameters that demonstrate the presence of neonatal hypoxia accompanied by brain damage.
3. The correlation between hematological (HB, LE, NE, LI, TR), inflammatory (CRP, PCT) and enzymatic (LDH, TGO, TGP) parameters to see if there is a sustainable correlation between HIE severity and hematological and biochemical disorders in full-term and premature babies.
4. Classification in stages of severity of the disease in order to establish the immediate and distant prognosis.
5. Early monitoring of hematological, inflammatory and enzymatic parameters with the diagnosis and the establishment of individualized therapeutic conduct can prevent the risks associated with these changes (anemia, infections, bleeding).

6. Optimizing the diagnosis for a prompt and efficient treatment in the case of newborns with cerebral hypoxia, regardless of gestational age.
7. Few studies have been performed to find a relationship between platelet count and the degree of hypoxic-ischemic encephalopathy, most studies have been performed in animals.
8. Analyzing and interpreting the results obtained in dynamics.
9. Identification of clinical and paraclinical prognostic factors to allow the stratification of patients into risk groups, in order to choose a multidisciplinary treatment strategy.
10. Establishing strategies-follow-up programs in order to interdisciplinary dispensary of these children so as to improve the prognosis of these common pathologies among full-term and premature newborns.

3.2 MATERIAL AND METHOD:

We performed two retrospective, cross-sectional cohort studies. The study was carried out in the Neonatology Department of the "Louis Turcanu" Children's Emergency Hospital in Timișoara. This study was conducted over a period of 3 years, from January 1, 2016 to December 31, 2018. The first study included 78 newborns weighing between 1 kg and 3.8 kg at birth. The second study: included 97 newborns with a birth weight between 1kg and 4,9 Kg. The research methodology assumed the collection of peripheral venous blood and the performance of the following hematological parameters: HB, LE, NE, LI and TR dosage; enzyme parameters: liver transaminases (AST, ALT) and LDH; as well as inflammatory markers: CRP and PCT.

An amount of up to 2 ml of peripheral venous blood was collected, and the collected samples were sent to the laboratory, obtaining the results within a maximum of one hour after collection. The newborns were classified according to gestational age, sex, biological values obtained, degree of severity and according to prognosis. Newborns included in the studies were selected based on strict inclusion and exclusion criteria. Demographic, gestational, and perinatal data for the neonates included in the study were reviewed, including the presence of prenatal risk factors for HIE, congenital anomalies, and infections of any kind. A database was compiled by computerized search of medical records in the medical unit's online database, which was analyzed using the statistical package (SPSS), version 23.0 (IBM, Corp.).

Comparisons between group means were analyzed using the ANOVA test. Pearson's Chi-square test was used for each separate variable. If Pearson's Chi-square test could not be used, Fisher's test was used. A p value <0.05 was considered to indicate a statistically significant difference.

3.3 RESULTS:

3.3.1. The first study aimed to identify potential biomarkers for the mechanisms underlying hypoxic-ischemic lesions and the early neuroinflammatory response, focusing on the levels of inflammatory mediators in the blood and comparing their levels.

The analyzed biomarkers were collected at time 1 (t1), meaning blood samples were collected in the first hours after birth, and time 2 (t2), where blood samples were collected within 24–96 hours. Classification of patients was done according to prognosis. The prognosis was based on the following criteria: birth weight, biological markers obtained, the evolution of the patient during hospitalization and also the days of hospitalization in which they needed therapy. Depending on these 4 criteria, patients were considered to have a good or poor prognosis. In this study it was observed that:

The vast majority of patients had a favorable prognosis and subsequent evolution, even if this evolution was slowly favorable, requiring a greater number of days of hospitalization, compared to patients with unfavorable evolution who in a relatively short period evolved to death .

The severity of HIE has a negative impact on the proliferation and maturation of red cells. HB decreased from time t1 to time t2 both in patients with a favorable prognosis and those with an unfavorable prognosis, but the decrease was more pronounced in those with an unfavorable prognosis, which is in accordance with the literature reports. We understand that the low values of Hb detected at 96 hours correlates with the unfavorable evolution of patients with HIE.

We have a statistically significant increase in the evolution of white cell values, which correlates both with the increase in inflammatory samples and with the unfavorable evolution of the patients.

Regarding the inflammatory samples, we can say that they were increased in both study groups, but in the group with favorable evolution the values tend to

decrease in the first 96 hours and in the one with unfavorable evolution they increase, obtaining statistically significant values between the two groups For study.

LDH is a specific enzyme and a good early predictor, in the first 12/24 hours of HIE, being able to be used together with the rest of the mentioned samples, as a predictive and severity marker of HIE.

3.3.2 In the second study, we studied the clinical and biological evolution of the newborn with thrombocytopenia in the neonatal intensive care unit. Thrombocytopenia is one of the most common hematological disorders detected in the neonatal period, especially in neonates admitted to intensive care units, and usually indicates an underlying pathological process.

For this study, serial analyzes were performed both during the first 72 hours and after, with the key time point being 72 hours (3 days). After this time, retesting was performed until thrombocytopenia resolved. Depending on the value of the platelets, the patients were classified in degrees of severity. We know that the onset of thrombocytopenia is more frequent in the first 3 days of life in hypoxic patients and after 3 days in patients with infections or necrotic ulcerative enterocolitis, based on this information the patients were classified into two groups. In this study it was observed that:

The vast majority of patients included in the study developed thrombocytopenia with onset in the first 3 days of life. The most common cause of early-onset thrombocytopenia is fetal hypoxia which is most often self-limited and rarely severe. After 72 hours, the most common cause of thrombocytopenia in newborns admitted to the intensive care unit is infectious pathology.

Like the duration of evolution, thrombocytopenia was self-limited to approximately 5 days on average. Those who developed more frequent thrombocytopenia with evolution over 3 days were premature newborns. Most of the newborns were classified in the first degree of severity.

A connection between HIE and thrombocytopenia was revealed. A significant percentage of patients with hypoxic distress developing thrombocytopenia - grade I. In the case of hypoxic patients, thrombocytopenia started earlier, had a longer duration and a greater degree of severity compared to newborns without hypoxic distress.

Regarding hemorrhagic accidents, we can say that the longer the duration of thrombocytopenia, the higher the risk of hemorrhagic accidents. Almost half of the newborns with HIE had intraventricular hemorrhage (IVH) during hospitalization. It needs to be mentioned that the vast majority of hemorrhagic accidents occurred in patients classified in the first degree of severity of thrombocytopenia, thus being able to deduce that there is no close connection between the severity of thrombocytopenia and the incidence of IVH.

Thrombocytopenia occurring in newborns admitted to the neonatal intensive care unit is not a negative prognostic factor, but rather a marker of the severity of the underlying pathology.

3.3.3. Identification of early predictors for mortality and neurological prognosis in infants with HIE is particularly important in predicting relevant clinical outcomes and rapid decision making. Ideal predictors for neonates with HIE should be sensitive, specific, rapid, and easy to perform.

In the third study, the need for clinical interpretation of the data obtained from this study led to the development of an easy and fast method for assessing the severity of the disease based on biological investigations. Thus, depending on the severity of the changes in the biological samples, the patients received an indicator - marked from 1 to 5.

The procedure entitled "EVOLUTION SCORE" included scoring with an indicator from 1 to 5 for each investigation performed, both at the moment t1 as well as at time t2, following the biological evolution and the degree of modification of the biological parameters during the 96 hours of follow-up. Also, an indication was given to the patients depending on the number of days of hospitalization in which they required intensive therapy. Practically, the purpose of this biological differentiation scale was to observe if, in patients with EHIP, the clinical evolution and average days of hospitalization correlate with the score given at the moment (t1) and (t2). It was analyzed whether the non-interpretation/non-performance of one or more investigations at time t2 can predictably negatively influence the evolution of these patients.

FIGURE 1. Results "EVOLUTIVE SCORE"

In the first category in which all the analyzes were interpreted, it can be seen that the higher the score, the lower the average number of days of hospitalization and the lower the death rate. In the case of patients with a score of around 30 points, the average number of days of hospitalization is low because all patients died early due to the underlying pathology (Image 4.63).

The same can be said for study groups in which one or more analyzes were not interpreted; The higher the score, the shorter the average hospital stay and the lower the mortality. In the case of those with low scores and short hospitalization, it can be seen that mortality is 100%.

In conclusion, we can say that performing this score is really helpful and we realized that if at the time of t1 the newborn is collected a complete set of tests and a score> 40 is obtained accompanied by a good clinical evolution, then at time t2 may waive biological investigations because they do not add to the patient's clinical and therapeutic management.

The results obtained from the study can contribute to the consolidation of the scientific basis for the evaluation and monitoring of newborns with neonatal hypoxia and hypoxic ischemic encephalopathy (EHIP) with the possible final goal of recommending in a local diagnostic guide, a standard and mandatory package of investigations in newborns with suspected EHIP.

4. CONCLUSIONS:

The various blood markers studied provided us with important information about the early evolution of the newborn in the intensive care unit, but no proven biomarker can determine the cause of neonatal encephalopathy, identify the moment of injury or predict the long-term neurological outcome.

Multi-factor screening is the best way to evaluate newborns with encephalopathy. These readily available laboratory results, along with maternal history, fetal heart rate monitoring, placental pathology, blood gas, Apgar score, neonatal physical examination, and neuroimaging, provide information to support or rule out the suspicion of neonatal encephalopathy.

Hematological investigations in newborns with HIE provide additional information to approximate the time and duration of the lesion. In our study in patients with an unfavorable prognosis, the mean hemoglobin values were lower or had a more pronounced decrease in the first days of life.

The values of leukocytes and neutrophils increased in the first 96 hours in patients with unfavorable evolution, being optimal indicators of the evolutionary prediction.

High sensitivity of PCT has been observed in newborns with hypoxic distress and HIE and LDH is a good predictor of HIE in the first 12/24 hours after birth.

Measuring the enzymes released after a hypoxic event in the first 12 hours after birth can be a useful predictor of EHIP severity and can improve the evolution of the newborn when included in an intervention window window of opportunity algorithm.

The concomitant socio-economic conclusions draw attention to the size and implications of hypoxic ischemic pathology; from the increase in the number of days of hospitalization in intensive care units that substantially increase the costs of hospitalization to the short, medium and long term implications on the health of newborns and subsequent neurodevelopment. That is why the medical education of the future parents is very important, in order to increase the addressability to the specialized medical service where the pregnancies can be followed and the fetal development can be monitored.

Although the prevalence of asphyxia is in line with figures reported by other European countries, given the seriousness of the possible consequences, the need

for careful monitoring of pregnancy and birth is clearly outlined, with early identification of risk factors.

We believe that the widespread use of the "EVOLUTIVE SCORE" in maternity wards of any level would provide data of practical importance regarding immediate evolution and the risk of sequelae or death. That is why I believe that a score lower than 40 should alert the neonatologist, considering the transfer of the patient to a level 3 maternity hospital.

Last but not least, the development of markers accessible on a large scale, at low determination costs, short lead times and easy methods of determination, and also with possibilities of collection from various places (serum, CSF, urine, saliva) to facilitate rapid collection and increase the sensitivity and specificity of investigations. Early determination of specific markers and multidisciplinary therapeutic approach are essential to ensure the favorable development of the newborn with hypoxic injury.

The introduction of lactate dehydrogenase into the laboratory algorithm, as a routine determination together with the liver enzymes ALT and AST, may be a factor in the progress of the diagnosis. With lower sensitivity and specificity, ALT and AST enzymes have a lower predictive value than LDH, but can still be used as a surrogate marker of EHI.

Both the reduction in the average length of hospital stay and the prevention of complications (which would involve a long hospital stay and additional costs for specific treatment) will have a positive financial impact on health facilities.

Also, the increased comfort of the mother, once she arrive in the family, will increase the likelihood of exclusively breastfeeding for a longer period, with clear benefits for both the newborn and the mother.

Reducing neonatal morbidity and mortality from asphyxiation at birth should be a goal of public health policies, including appropriate maternal education.