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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

THE STATUS OF ERYTHROPOIESIS IN THE NEONATAL PERIOD AND CATAMNESIS IN PRETERM INFANTS WITH PERINATAL ASPHYXIA

Speciality: 3220.01 – Pediatrics

Field of science: Medicine

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GENERAL CHARACTERISTICS OF THE WORK

Relevance. Perinatal asphyxia (PA) has a high specific gravity in morbidity and mortality and is more common in premature infants. At present, perinatology states that changes in the blood of premature infants are due to the immaturity of their organs and systems, including the hematopoietic system[1].

Changes in peripheral blood are always referred to the study of the clinical picture of various pathological conditions in newborns, especially in premature infants, and in their differential diagnosis[2].

Recently, perceptions of the development of early anemia in premature infants and views on its pathogenesis have changed significantly. It was found that the deficiency of erythropoietin, which is a humoral factor, plays an important role in this process[3].

Erythropoietin (EPO) is a physiological stimulator of erythropoiesis and is a glycoprotein-containing hormone synthesized by the kidneys. Erythropoietin is synthesized in the liver during the intrauterine period and in the kidneys after birth. The transfer of this process from the liver to the kidneys begins at 32 weeks of gestation and ends in the second month of life. Oxygen receptors of peritubular cells are very sensitive to hypoxia and protect the fetus from hypoxia by maintaining erythrocyte balance[4].

Erythropoietin is an important factor in fetal and neonatal erythropoiesis and plays a major role in the development of anemia. It is known that blood production in the fetus and the newborn occurs due to iron reserves in the mother's blood, and in children born to moth-

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[1]Kawakami, M.D. Neonatal mortality associated with perinatal asphyxia: a population-based study in a middle-income country / M.D. Kawakami, A. Sanudo, M.L.P. Teixeira [et al.] // BMC Pregnancy Childbirth., - 2021. vol. 21, no 1, - p. 169.

[2]Workineh, Y. Prevalence of perinatal asphyxia in East and Central Africa: systematic review and meta-analysis / Y. Workineh, A. Semachew, E. Ayalew [et al.] // Heliyoniç – 2020. vol. 6, no 4, - e03793.

[3]Cassady, G. Anemia of Prematurity / G. Cassady, C.F Potter**,** T. Rosenkrantz , - Medscape Updated: Jan 08, - 2016.

[4]Алексеев, Н.А. Гематология и иммунология детского возраста / Н.А.Алексеев, - Москва: «Гиппократ», - 2009. – 1040 с.

ers with iron deficiency, anemia is difficult to treat.[5].

It has been established that anemia in pregnant women is accompanied by changes in the mother-twin-fetus complex, which results in late gestoses (preeclampsia), chronic fetal hypoxia and intrauterine growth retardation syndrome.[6]

According to the American Academy of Pediatrics, PA is manifested by perinatal encephalopathy along with organ damage. Given the interaction of these systems, it dictates the importance of determining erythropoietin as a biochemical marker of hematoencephalic barrier and nerve tissue damage[7]. At the same time, the number of studies related to the S100 protein, as a specific indicator of brain damage, in pediatrics and neonatology is also growing. There is a small amount of the comparative researches of these markers in premature infants[8].

Recently, it has been claimed that the S100 protein is as informative in the detection of neonatal brain damageas as Apgar score, lactate and pH in the umbilical vein.

In pediatrics and neonatology, it is recommended to routinely check this marker for emergencies. However, it has not been fully investigated which of the EPO and S100 proteins are the most sensitive biochemical markers of nerve tissue damage[9].

Thus, the study of erythropoiesis in premature infants with perinatal asphyxia is of particular importance. At the same time, along

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[5]Figueiredo, A.C.M.G. Maternal anemia and low birth weight: a systematic review and meta-analysis / A.C.M.G. Figueiredo, I.S. Gomes-Filho, R.B. Silva [et al.] // Nutrients, - 2018. vol. 12, no 5, - p. 601.

[6] Kanata, M. Clinical outcomes of hypertensive disorders in pregnancy in the offspring during perinatal period, childhood, and adolescence / M. Kanata, E. Liazou, A. Chainoglou [et al.] // J Hum Hypertens, - 2021. vol. 35, no 12, - p. 1063-1073.

[7]Castillo, C. Neuroprotective effects of erythropoietin on neurodegenerative and ischemic brain diseases: the role of erythropoietin receptor / C. Castillo, C.F. Burgos, A. Hidalgo [et al.] // Neural Regen Res., - 2017. vol. 12, no 9, - p. 1381-1389.

[8]Bersani, I. Early predictors of perinatal brain damage: the role of neurobiomarkers / Bersani, I. F. Pluchinotta, A. Dotta [et al.] // Clinical Chemistry and Laboratory Medicine (CCLM), - 2020. vol. 58, no 4, - p. 471-486.

[9]Beharier, O. S100B - a potential biomarker for early detection of neonatal brain damage following asphyxia / O. Beharier, J. Kahn, E. Shusterman [et al.] // J Matern Fetal Neonatal Med., - 2012. vol. 25, - p. 1523-1528.

with hematopoietic factors in the development of anemia in premature infants born to mothers with complications of anemia and preeclampsia, the study of EPO has not been resolved in modern neonatology.

Object of research. 132 newborns aged 28-37 weeks were involved in clinical, laboratory and functional examinations to carry out research work.

The purpose of the study status of erythropoiesis in children born prematurely with perinatal asphyxia from mothers whose pregnancy was complicated by anemia and preeclampsia.

Research objectives:

1. To determine the criteria for the clinical evaluation of the early adaptation in premature infants from mothers with complications of anemia and preeclampsia during pregnancy;
2. Assess the state of erythropoiesis in the neonatal period in premature infants with perinatal asphyxia;
3. Comparative study of the role of erythropoietin and the S100 protein in CNS disorders during the early adaptation in premature infants with perinatal asphyxia;
4. To identify informative criteria for predicting the course and outcome of perinatal hypoxic CNS damage in premature infants with perinatal asphyxia;
5. To assess the somatic and neurological status of premature infants from mothers with complications during pregnancy with anemia and preeclampsia in the catamnesis.

Research methods. Premature infants were assessed during the early adaptation period on days 1-3, 5-7 of their lives, and functional examinations (neurosonography and echocardiography) were performed along with clinical and laboratory findings.

The main provisions of the defense:

– The study of important components of erythropoiesis (hemogram, iron, transferrin, total iron binding ability, erythropoietin) in children born to mothers with complications during the pregnancy in the early neonatal period allows to assess the frequency of the development of anemia.

– By identifying more informative and sensitive prognostic in-

dicators (EPO, S100) reflecting central nervous system damage in the early neonatal period, provides a basis for preventive measures in children at risk of central nervous system delay in catamnesis.

– Premature infants born to mothers with complications of anemia and preeclampsia were characterized by the predominance of somatic diseases developing in the catamnesis: iron deficiency anemia, hypovitaminosis D and atopic dermatitis.

Scientific novelty of the research:

– Important components of erythropoiesis (hemogram, iron, transferrin, total iron binding ability, erythropoietin) in infants born prematurely from mothers with complications of pregnancy anemia and preeclampsia were studied;

– The role of erythropoietin and the S100 protein in the adaptation of the CNS in the early neonatal period in premature infants with PA was determined;

– The somatic and neurological status of premature infants born to mothers with anemia and preeclampsia was studied in catamnesis (up to 1 year of age);

Practical significance of the research:

– As a result of the study of complex clinical, neurosonographic, hematological and immunoenzymatic examinations, it may be possible to determine the course of the early adaptation and the state of erythropoiesis in premature infants who underwent PA;

– Hemorrhagic damage to the central nervous system in infants born with perinatal asphyxia based on EPO and S100 protein levels in the early neonatal period can be predicted.

– Predicting the pathology of children born prematurely from mothers with pregnancy anemia and preeclampsia in later life from an early neonatal period and the application of the algorithm may be important for perinatal prophylaxis.

Approbation and application of the research work. The initial discussion of the dissertation was held at the extended meeting of the II Department of Pediatrics of the Azerbaijan Medical University (protocol №15, date 01 June 2021). Approbation of the case was discussed at the meeting of the Approbation Council (protocol №10, 02.03.2022), which conducts scientific seminars under the Disserta-

tion Council ED 2.27 on the specialty 3220.01 - "Pediatrics" of the Azerbaijan Medical University. Separate fragments of the dissertation was presented at the I World Congress on Maternal fetal neonatal medicine (London 2017), 7th Congress of the European Society of Pediatricians (EAPS) (Paris, 2018), 3rd Congress of European Neonatal Societies (JENS) (Maastricht, 2019) , was discussed and reported at the 5th International Scientific-Practical Conference of the Caspian Coastline Countries (Astrakhan, 2020) dedicated to "Actual problems of modern medicine".

The results of the scientific research were applied in the daily practical work of the Department of Neonatal Anesthesiology and Intensive Care of the Educational Surgical Clinic of AMU and the Research Pediatrics Institute. The results obtained during the implementation of scientific work were used in the teaching process of the II Department of Pediatrics, Faculty of Treatment and Prevention, Azerbaijan Medical University.

Published works. 7 scientific articles on the topic of the dissertation, including 2 in journals included in the International citation database and 5 theses were published, 1 additional speech was made.

Name of the organization where the dissertation is performed. The research was carried out in the neonatology departments of the Educational Surgical Clinic and the Scientific Research Pediatrics Institute named after K. Farajova, which is the clinical base of the 2nd Department of Pediatrics of the Azerbaijan Medical University, and catamnestic examinations were carried out mainly in the polyclinic department of the Educational Surgical Clinic in 2014-2016.

The volume and the structure of the dissertation. The dissertation consists of 170 printed pages (199.600 characters), 30 graphics, 45 tables and 2 interpreted clinical examples. The dissertation consists of an introductory part (8.700 characters), 5 chapters (51.300 + 13.000 + 34.100 + 24.800 + 22.800 characters), a conclusion, results, practical recommendations (44.600 characters) and a list of references. The bibliography covers 198 sources in Azerbaijani, Turkish, Russian and English

MATERIALS AND METHODS OF RESEARCH

132 newborns aged 28-37 weeks of gestational age were involved in clinical, laboratory and functional examinations to carry out the research work. Of the 132 newborns, 102 were born to women whose pregnancies were made complicated by anemia and preeclampsia, 30 of the children were exposed to hypoxia, and 30 were healthy children who did not report any perinatal damage to the CNS from mothers with a physiological course of pregnancy and childbirth. The infants involved in the study were divided into 3 groups: 46 infants born prematurely with PA from mothers with complications of pregnancy anemia (1A) and preeclampsia (1B) in the 1st main group, and anemia (2A) and preeclampsia (2B) in the 2nd comparison group. 56 infants born prematurely (2B) from mothers with complications, 30 healthy children born from physiological pregnancy were included in the 3rd group.

Since 9 children out of the ones that were under our control were lost and 22 children were not regularly examined and monitored for various reasons, only 101 infants were followed up to 1 year of age.

Clinical and laboratory examinations were carried out at the entrance to the maternity hospital in pregnant women, their obstetric and gynecological anamnesis was studied in detail, the course of the current pregnancy and its complications were investigated. Depending on the level of hemoglobin in pregnant women, the diagnosis of anemia was confirmed according to a classification developed by the WHO / UNICEF. A Goecke scale modified by Savelyeva GM was used to diagnose mothers with preeclampsia.

Early adaptation, physical development, and neurosonography, echocardiography, and clinical and laboratory examinations were performed in infants born prematurely with anemia and preeclampsia on days 1-3 and 5-7 of life.

Apgar, Silverman, Ballard scale and Sarnat scale were used to assess the condition of newborns. In catamnesis, children's psychomotor development was assessed on the Denver II scale, and the diagnosis was confirmed by clinical and laboratory examinations. The examinations were evaluated by a neurologist.

In children, the following parameters were determined in the peripheral blood: erythrocyte count (RBC); Hemoglobin (HGB); Mean Corpuscular Hemoglobin (MCH); Mean Corpuscular Volume (MCV); Mean Corpuscular Hemoglobin concentration in erythrocytes (MCHC); Serum iron (SI); Total Iron Binding Capacity (TIBC); The level of transferrin has been studied.

Concentrations of EPO and S100 proteins as markers sensitive to hypoxia were determined in the dynamics of the neonatal period using an appropriate set of enzyme-linked immunosorbent assays. 1-2 ml of blood was taken for the serum, after centrifugation it was given to the laboratory of AMU TCK for examination.

Erythropoietin was produced by Peninsula laboratories inc. and was assessed by the enzyme-linked immunosorbent assay.

S100 protein was studied by the solid-phase immunoenzyme. For this purpose, a jet kit belonging to the company Can Aq (Switzerland) was used.

Statistical examination methods. All figures obtained in the course of the study were performed using the methods of variation, discrimination, variance, correlation, regression, ROC-analysis.

RESEARCH RESULTS AND THEIR DISCUSSION

Clinical criteria for the early adaptation and features of erythropoiesis in premature infants with asphyxia. 46 infants with asphyxia and premature birth were examined and compared in 2 subgroups. Group 1A included 18 infants born to mothers with anemia, and group 1B included infants born to mothers with preeclampsia (PE). The results were also compared with those in the control group and those without asphyxia. There was no difference in subgroups of gestational age and the child's body weight, height, head and chest circumference ($p > 0.05$).

In infants born to mothers with anemia, recovery of vital functions is delayed due to the depletion of the body's compensatory responses to hypoxia. Thus, although infants born in subgroup I were assessed on approximately the same Apgar scale, 12 children in subgroup 1A received less than 5 points in the 5th minute, and 9

children in subgroup 1B. There was no statistically significant difference between the subgroups in terms of the volume of resuscitation measures performed in the delivery room ($p > 0.05$). Thus, 14 infants (77.8%) in subgroup 1A and 22 infants (78.6%) in subgroup 1B were subjected to full resuscitation. Resuscitation measures continued until the 5th minute in 6 (21.4%) infants in subgroup 1A and (22.2%) in 4 infants in subgroup 1B.

Premature infants had a higher risk of developing respiratory distress syndrome due to atelectasis caused by a surfactant deficiency. In infants with asphyxia, the breakdown of surfactants is accelerated by acidosis, which increases the incidence of the respiratory distress syndrome. The type of respiratory therapy in this group of infants was based on the severity of the respiratory failure. Thus, during the study of ALV and CPAP therapy according to the age of gestation, we see that in this case, 6 infants receiving ALV therapy in subgroup 1A were 28-32, 2 were 33-34, and 1 was 35-37 weeks of gestational age ($\chi^2 = 5{,}513$, $p<0.05$), 1 of the infants receiving CPAP therapy was 28-32, 6 were 33-34, and 1 was 35-37 weeks of gestational age ($\chi^2 = 6.356$, $p<0.05$) .

Surfactant therapy was performed in 7 (38.9%) infants in subgroup 1A and (17.9%) in 5 infants in subgroup 1B. As can be seen, infants born to anemic mothers received twice as many surfactant insulations ($\chi^2 = 4{,}570$, $p > 0.05$).

No distinction was made between the subgroups according to the neurological symptoms noted during the examination of the nervous system. Thus, in subgroup 1A, 16 infants (88.9%) had hyporeflexia, 2 infants (11.1%) had reflexes, and in subgroup 1B, 24 cases (85.7%) had hyporeflexia, and in 4 (14.3%) cases areflexia was noted ($\chi^2 = 0.095$, $p = 0.758$). All infants underwent neurosonography on the third and seventh days of life. Third-degree hemorrhage was observed in two infants of subgroup 1A, which further aggravated the condition of the infants ($p > 0.05$).

On echocardiography, ongoing fetal blood flow was detected in these infants (50% in subgroup 1A, 28.6% in subgroup 1B, PFO, 11.1% in 1A, and 21.4% in 1B).

Changes in the nervous and cardiovascular systems were clini-

cally manifested by perioral and acrocyanosis, low or high blood pressure, signs of tachycardia or bradycardia (p> 0.05).

Comparing hematological indicators in children, we see that the number of erythrocytes in infants of subgroup 1A was statistically significantly lower than in infants of subgroup 1B on the first day (p <0.05).

It was noted that the concentration of erythropoietin in 5-7 days was statistically significantly higher in infants of subgroup 1B compared to other subgroup (14,2 ±1,5, p<0,020).

Although iron concentrations did not differ statistically between the subgroups, transferrin levels were statistically high in subgroup 1B (p = 0.03).

One of the objectives of our study was to compare the role of EPO and the S100 protein in CNS disorders during the early adaptation. Comparison of S100 between premature infants with S100 was higher than normal in children with asphyxia and increased in dynamics, but there was no statistically significant difference between half-groups due to perinatal factors (p>0.05) (table 1).

Table 1

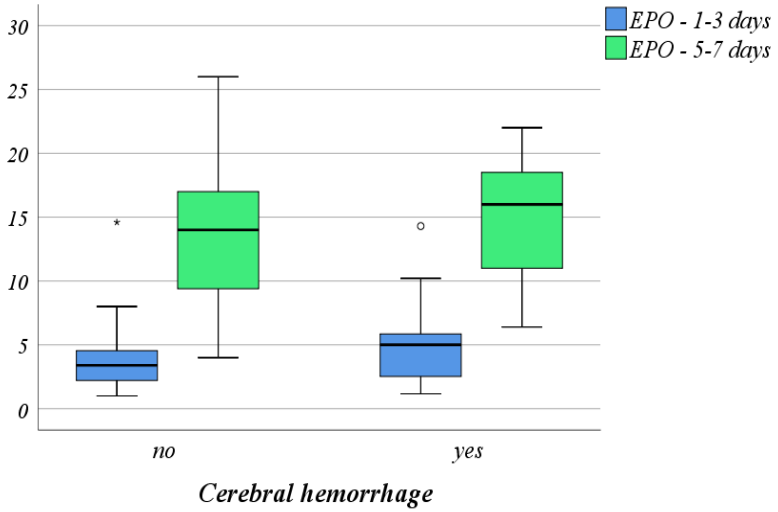
Comparative characteristics of S100, EPO, Fe and transferrin levels in infants with cerebral hemorrhage

Indicators		Hemorrhage	N	M	±m	min	max	P
S100	1-3 days	no	34	6.0	0.3	4.0	12.0	0.005
		yes	12	12.9	1.4	6.0	25.0	
	5-7 days	no	34	8.4	0.2	7.0	14.0	0.003
		yes	12	14.9	1.4	8.0	26.0	
EPO	1-3 days	no	34	3.5	0.4	1.0	14.6	0.067
		yes	12	8.2	3.1	1,2	41.1	
	5-7 days	no	34	12.9	0.8	5.6	22.0	0.024
		yes	12	16.0	2.8	6.4	44.0	
Fe		no	34	90.7	4.0	29.8	145.0	0.032
		yes	12	74.0	4.8	50.0	105.0	
Transferrin		no	34	279.9	4.1	240.0	325.0	0.042
		yes	12	298.3	4.5	265.0	315.0	

A comparative study of S100, EPO, Fe, and transferrin values in

infants with intraventricular hemorrhage showed that S100 was higher in children with hemorrhage on both the first day and in the dynamics, and EPO was not statistically significant on the first day, but increased in dynamics and became statistically significant. Fe levels were lower in children with hemorrhage on the first day.

The study confirmed which EPO and S100 proteins were more sensitive and specific for prognostic purposes in cerebral hemorrhage. Using the ROC statistical analysis, we determined the cut-off level for EPO and the S100 protein due to cerebral hemorrhage. Thus, high EPO levels on the first day were accompanied by cerebral hemorrhage ($p < 0.05$) (graphic 1).



Graphic 1. Comparison of EPO levels in days 1-3 and 5-7 depending on the hemorrhage

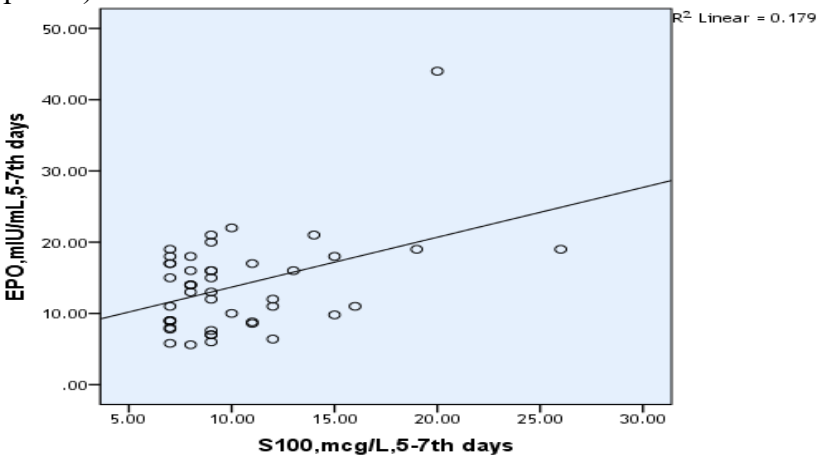
According to the ROC analysis, the cut-off level of EPO for the first day of bleeding was higher than 4.26 mlU / ml, with a sensitivity of 66% and a specificity of 74% ($p < 0.05$). In the case of cerebral hemorrhage in children with PA, the cut-off level of the S100 protein on the first day was 8.4 mIU / ml, in which case the sensitivity was 90% and the specificity was 60% ($p < 0.05$) (table 2).

Table 2

Sensitivity and specificity of EPO and the S100 protein for hemorrhage in the early neonatal period

Indicators	Sensitivity	Specificity	Cut of point
S100 in 1-3 days	60%	90%	8.4
S100 in 5-7 days	92%	70%	10.5
EPO in 1-3 days	66%	74%	4.26
EPO in 5-7 days	77%	85%	19

It was also noted that there was a direct correlation between EPO and S100 both on the first day and on the dynamics. This is most likely due to increased levels of both EPO and S100 due to hypoxia (graphic 2).



Graphic 2. Correlation between EPO and S100

There is a direct correlation between the gestational age and Apgar, and inversely, resuscitation measures in the 1st and 5th minutes ($r = -0.357$, $p < 0.05$, $r = -0.262$, $p < 0.05$), CPAP ($r = -0.326$, $p<0.05$, $r = -0.503$, $p<0.05$) and surfactant therapy ($r = -0.326$, $p<0.05$) were found to be inversely correlated. At the same time, the openness of the oval hole and the borehole were inversely correlated with the age of gestation. ($r = -0.319$, $p<0.05$, $r = -0.249$, $p<0.05$)

As for the correlation between hematological indicators and the

gestational age, a direct correlation ($r = 0.251$, $p<0.05$) was found between 5-7 days of life and Hb. On the first day and in the dynamics, a direct correlation was observed between Fe levels and an inverse correlation between transferrin levels ($r = -0.566$, $p<0.001$, $r = 0.320$, $p<0.001$, $r = 0.483$, $p<0.001$).

An interesting correlation was observed on days 5-7 of life between the level of EPO and the gestational age ($r = -0.463$, $p<0.001$). Thus, the decrease in the dynamic level of EPO with the increasing age of gestation, ie, the inverse correlation ($r = -0.563$, $p<0.001$) once again reflects the high synthesis of EPO as a compensator in premature infants with asphyxia.

An interesting correlation was also observed between the EPO level and the Apgar indicators and the first-minute resuscitation measures. A direct correlation was found with Apgar scores in the 1st and 5th minutes ($r = 0.287$, $p<0.001$); an inverse correlation was found with resuscitation measures. ($r = -0.295$, $p<0.05$). On the first day, only physical mass was directly correlated to the level of EPO, ($r = 0.236$, $p<0.05$), and in the dynamics there was an inverse correlation between EPO and body weight ($r = -0.458$, $p<0.001$).

When studying the correlation between EPO and hematological parameters, we see that there is a direct correlation between EPO and the erythrocyte count on the first day ($r = 0.230$, $p<0.05$), there is an inverse correlation with the levels of Fe on both the first day and the dynamics ($r = -0.428$, $p<0,001$ – on the first day, $r = -0,557$, $p<0,001$ in dynamics). An inverse correlation was observed with Hb both on the first day and in the dynamics ($r = -0,242$, $p<0,05$, $r = -0,339$, $p<0,05$).

In infants receiving respiratory therapy, a direct correlation of ALV, CPAP and surfactant therapy with EPO levels was found in the dynamics ($r = 0.484$, $p<0.001$, $r = 0.314$, $p<0.001$, $r = 0.457$, $p<0.01$, respectively).

Among the subgroups, infants did not differ in terms of physical development and the volume of resuscitation in the delivery room. Type of respiratory therapy ALV and CPAP therapy were performed at the same frequency in every 2 subgroups. However, surfactant therapy is more commonly used in infants born to mothers with ane-

mia (χ^2 = 4,570, p <0.05). At the same time, according to the results of neurosonography and echocardiography, no difference between the groups was noted. High concentrations of EPO on the first day and in the dynamics were observed in infants born to mothers with PE. Infants born to PE mothers had lower concentrations of Fe on the first day and higher concentrations of transferrin compared to other subgroups.

Hematological parameters and the erythropoietin status were studied in premature infants from mothers with anemia and preeclampsia who did not undergo PA. Newborns were compared in two subgroups based on complications during pregnancy. Subgroup 2A included 36 infants born to mothers with anemia, and subgroup 2B included 20 infants born to mothers with preeclampsia. Hence, in this group of children there was no statistically significant difference between the physical development indicators of the groups.

Due to the development of RDS, ALV was administered to 5 (13.9%) infants in the 2A subgroup, 1 (5%) in the 2B subgroup, and CPAP therapy was administered to 18 (50%) and 9 (45%) infants, respectively. Nasal oxygen therapy was performed in 32 (88.9%) infants in subgroup 2A and 14 (70%) in subgroup 2B. There was no statistically significant difference between subgroups according to the type of respiratory therapy. Surfactant therapy is more commonly used in infants born to mothers with anemia. Thus, surfactant therapy was performed in 10 cases (27.8%) (χ^2 = 8.8; p <0.01) in this subgroup of infants, and in 4 (20%) cases in subgroup 2B. The increased use of surfactant therapy in infants born to anemic mothers was associated with the faster breakdown of the surfactant in the setting of severe morphofunctional insufficiency or hypoxia.

Microcirculatory disorders are more common in infants born to mothers with a history of complicated pregnancies, as there are more signs of dysadaptation. Acrocyanosis and perioral cyanosis were observed in 27 (75%) and 29 (80.6%) in subgroup 2A, and in 14 (70%) and 15 (75%) cases in subgroup 2B in the examined infants. There was no statistical difference between the subroups (χ^2 = 0.16, p>

0.05).

According to the clinical course of CNS lesions, mild, moderate, and severe lesions were found at approximately the same frequency among the subgroups. Looking at the clinical signs separately, we see that 3 (8.3%) infants in the subgroup 2A did not show oral AFR, and 3 (8.3%) did not show spinal AFR. In subgroup 2B, 3 (15%) oral reflexes and 3 (15%) spinal reflexes were not acquired in the infant. Muscle hypotension was reported in 7 (19.4%) infants in subgroup 2A and in 2 (10%) infants in subgroup 2B. Based on these symptoms, general lethargy syndrome was assessed in 4 (11.1%) cases in subgroup 2A and in 3 (15%) in subgroup 2B.

As can be seen from the results of the neurosonographic examination performed on day 3, grade I hemorrhage was very common in infants of subgroup 2A and was statistically significant ($p<0.05$). Based on the results of the echocardiography, PFO was noted in 14 cases (38.9%) and PDA in 6 cases (16.7%) in subgroup 2A. Appropriate changes were observed in 5 (25%) and 3 (15%) individuals in subgroup 2B . In premature infants, the opening of the oval hole may be due to pulmonary hypertension in the presence of surfactant deficiency in the lungs or retardation of fetal fluid.

One of the main objectives of the study was to study erythropoiesis in premature infants, but no significant differences were observed between the subgroups.

Comparing the level of EPO by subgroups, we see that the level of EPO increased dynamically in both subgroups, but although it was higher in 2A subgroups, it was not statistically significant ($p>0.05$).

Because of the high risk of developing anemia in premature infants, anemia in the first week of life in this group of infants was assessed and diagnosed based on hemoglobin and erythrocyte values. Thus, the incidence of anemia did not differ between the groups. The odds ratio was calculated to study the mother's chances of developing anemia in these infants. The mother's anemia plays a role in the first day of anemia, and the presence of anemia in the child increases the mother's chances of having anemia by 2.6 times ($\chi^2 = 4.15$, $p = 0.04$).

When comparing Fe, transferrin, and TIBC levels across groups, Fe concentrations were lower in subgroup 2A ($p> 0.05$). Transferrin

and TIBC were high (p> 0.05), which is found in Fe-deficient anemias.

Hematological parameters, the value of EPO and ferrokinetic parameters were also studied comparatively based on the presence of hemorrhage in this group of infants. In children with hemorrhage, the level of EPO was higher on days 1-3 ($p < 0.05$), and in infants with hemorrhage, the increase was even greater ($p > 0.05$). In hemorrhagic infants, MCH and MCHC values were lower ($p < 0.05$) in the first day than in hematological indicators, and in dynamics, MCHC values continued to decrease ($p < 0.05$).

Based on the level of EPO in days 1-3 and 5-7 the cut-off levels, for the ROC curve did not reflect the statistical accuracy due to a hemorrhage.

The direct correlation of erythropoietin levels at days 1-3 and at days 5-7 with ALV therapy was of interest. This once again indicates that the high EPO level in the dynamics, as well as on the first day, is directly correlated with the severity of the child's condition. Inversely, a direct correlation was noted between Fe levels and transferrin, ALV, surfactant, and oxygen therapy. The inverse correlation between the level of Fe on 5-7 days and CPAP therapy is also noteworthy. An inverse ($r = -0.228$, $p = 0.042$) correlation was noted between the number of erythrocytes in days 5-7 and transferrin. On days 1-3, a direct correlation was observed between Hb levels with MCV, MCH, MCHC, as well as Fe, and an inverse correlation with transferrin levels ($r = 0.332$, $p = 0.003$; $r = 0.426$, $p = 0.000$; $r = 0.222$, $p = 0.047$; $r = 0.238$, $p = 0.005$; $r = -0.257$, $p = 0.021$).

Thus, no statistically significant difference in hematological parameters between infants in both 2A and 2B subgroups was noted. When we look at the adaptive responses of the early neonatal period, it is clear that infants in group 2A were more likely to be resuscitated. This group of infants received more surfactant therapy, but the ongoing fetal blood flow, especially the oval hole, remained predominant in this group of infants. Also, according to the results of neurosonographic examinations, cerebral hemorrhage was more common in subgroup 2A and was statistically significant ($\chi 2 = 4.64$; $p < 0.05$, $\chi 2 = 4.09$; $p < 0.05$)

In infants with and without asphyxia in the early adaptation period and comparative characteristics of hematological parameters. Features of the early adaptation of groups and the results of the complete blood count tests, which are indicators of erythropoiesis, were compared. In the control group - 30, in the main group - 46 (I) children with asphyxia, in the comparison group - 56 (II) infants without asphyxia were compared.

According to the results of our study, in groups 1 and 2, those with asphyxia were more likely to be born to preeclampsia mothers and differed statistically significantly ($p = 0.0001$).

The earlier the preterm birth, the more pronounced the signs of immaturity are, and the more children are resuscitated in the delivery room during the post-adaptation period. However, among the groups, resuscitation was more common in the first and subsequent minutes in accordance with the ABCD protocol in the delivery room for group I children; 78.3% ($p = 0.001$) in the first minute and 21.7% in the following minutes were resuscitated, the indicators differed statistically significantly. Also, children with asphyxia received more support from the respiratory system. Thus, 50% of children were connected to the mechanical ventilators and 58.7% to the CPAP apparatus, and there was a statistically significant difference ($\chi 2=19,2$, $p = 0.001$). Surfactant therapy was used in almost the same number in both groups and did not show a significant difference.

In assessing the early adaptation period, in children of group I, along with oral and spinal reflexes, microcirculatory disorders, skin rash ($p = 0.024$) and general edema ($p = 0.033$) were noted, and statistically significantly differentiated from other groups.

The results of echocardiography and neurosonography showed the same number of changes in both groups, but they were more pronounced in group I due to the degree of cerebral hemorrhage. Grade III hemorrhage was more common in group I.

During the study of hematological parameters, it was found that the number of erythrocytes in group I was lower than the control group on the first day, the dynamics of the number of erythrocytes in all 3 groups decreased, and in group I this decrease was more pronounced. The level of HGBs also changed in the same year. Accord-

ing to these parameters, changes in MCV and MCHC indicators were also noted. According to MCV, the groups did not differ on the first day, and in the dynamics, a decrease was observed in all groups ($p < 0.05$), and in group II, the decrease was more pronounced than in group III ($p < 0.05$). On the first day, the concentration of Fe was lower in groups I and II compared to the control group, and the difference was greater in group I. In the dynamics, the concentration of Fe increased in group III ($p < 0.05$), and decreased in group II ($p < 0.05$). In group I, although the dynamic decrease was not statistically significant, statistical accuracy ($p < 0.01$) was noted compared to group III. Significant dynamic changes were noted in the comparison of erythropoietin concentrations by groups. Thus, the concentration of erythropoietin increased more dynamically in groups I and II ($p < 0.05$).

Although EPO levels in infants born at 28–32 weeks of gestational age were higher on the first day and in dynamics than in other infants, statistical accuracy was noted in the dynamics. This is due to the fact that these children are more exposed to hypoxia and have a higher EPO due to lower iron reserves.

7 (15.2%) infants in group I died during the first 7 days. Of these infants, 3 (43%) were born with anemia and 4 (57%) were born with preeclampsia. In accordance with the purpose of our study, we studied the state of erythropoiesis in these infants and obtained interesting results in comparison with other infants. The gestational age and body weight of the deceased infants differed statistically significantly. Although there was no difference in HGB levels among infants, there was a statistically significant difference in Fe concentrations, and EPO was higher in infants who died on the first day, with an increase in dynamics compared to survivors. High EPO in infants with asphyxia on the first day indicates an increase in the erythropoietin synthesis from the intrauterine period($p < 0.001$).

An Hb below 150 mg / dL was assessed as anemia. It was revealed that,53.6% of infants with anemia on the first day were born to mothers with anemia ($\chi^2 = 12.5$, $p = 0.002$). Infants born to mothers with anemia also had an advantage in the dynamics. Thus, 46 (44.7) mothers had anemia, 42 (40.8%) had preeclampsia, and 15

(51.7%) had children born to healthy mothers ($\chi^2 = 17.8$). , p = 0,000)

When studying the role of hypoxia in the development of anemia, it was found that 59 infants were not exposed to asphyxia (57.3%). This number meant that 68.6% of infants did not have asphyxia. Of these, 15 infants were born to healthy mothers. Anemia was reported in 78.6% (44 infants) of infants born to mothers with a history of complicated pregnancies and exposure to asphyxia (χ^2 = 22, p = 0,000).

Transferrin levels in the infants we examined were statistically high in infants with anemia. The dynamic increase in EPO occurred faster in infants with anemia. Although the EPO level was low on the first day, the dynamics also increased sharply, but did not statistically significantly differ from the other group. Dynamic levels of EPO have also been studied in infants who develop anemia during neonatal asphyxia. In these infants, EPO levels were also low on the first day, but increased 3-fold in infants with anemia and 1.8-fold in infants without anemia ($p<0.05$). The probability coefficient of maternal anemia in newborns with anemia was 0.497 and the risk value was 2 ($p = 0.03$). In the logistic regression analysis, the following equation was obtained, taking into account maternal anemia (F1-anemia), multiple fertility (F2-polycystic ovary) and gestational age (32-36 weeks-F3) as risk factors for the prognosis of neonatal anemia (P):

P = 1-0,15xF1-0,94xF2-0,935xF3

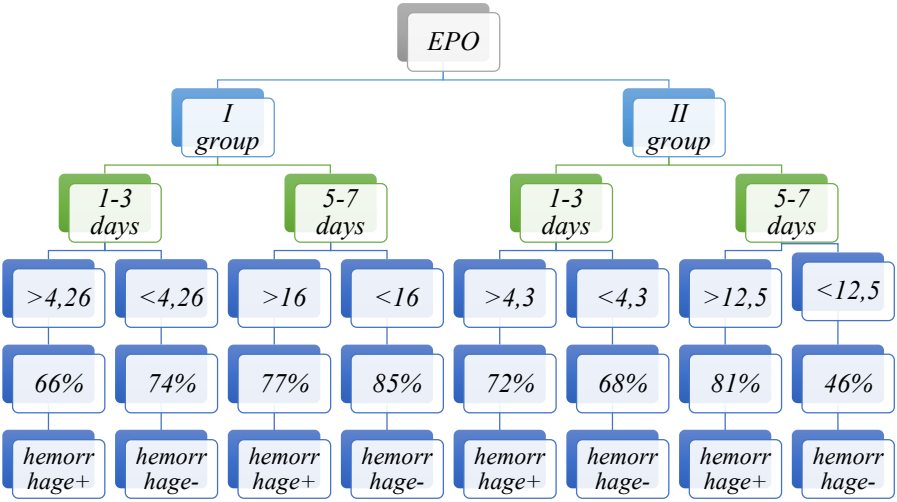
Here, the presence of anemia in the mother was more important ($p = 0.013$).

In infants born to mothers with preeclampsia and anemia, EPO determined the cut-off level for cerebral hemorrhage based on ROC curves on days 1-3 and 5-7. It turns out that the value for infants born to PE mothers is 4.8 U / mL, with specificity of 70% and sensitivity of 79% ($p<0.05$) For mothers born with anemia, the sensitivity was 9.1 U / mL, with 30% sensitivity and 74% specificity ($p>0.05$). An algorithm has also been developed.

In all the children we examined, regardless of the complication of pregnancy, the cut-off level of EPO was more sensitive and specific for days 5–7 ($p<0.05$) according to the ROC analysis on days 1–

3 and 5–7 of the EPO value for bleeding in all infants. For bleeding, the EPO level was 4.3 (sensitivity 72%, specificity 68%) on days 1-3 ($p>0.05$), but the EPO level was 12.5 (sensitivity 81%) on days 5-7 and specificity was 46% ($p<0.05$). According to the results, an algorithm of hemorrhage in line with the level of EPO, depending on the complication of pregnancy and depending on the level of EPO in both groups of children was developed.

One of the main tasks was to develop an algorithm that predicts the occurrence of hemorrhage in the group of patients we examined on the basis of cut levels in the ROC curves (graphic 3).



Graphic 3. The algorithm of hemorrhage formation depending on the EPO level

Thus, anemia is more common in infants with asphyxia. Although the risk of developing anemia in premature infants is high, exposure to asphyxia in these infants increases Fe-deficient anemia and accelerates erythropoiesis. This process is reflected in the blood erythrocyte, hemoglobin, MCV, MCH, MCHC, as well as changes in serum concentrations of Fe, TIBC, transferrin. The increase in the dynamics of EPO reflects the fact that in these infants, the compensa-

tory capacity is still not exhausted and erythropoiesis is stimulated.

Catamnestic control. In the next phase of the study, catamnestic control was performed on 101 children under thee age of 1 year. Children to be examined under catamnestic control are divided into 3 groups:

23 children belonged to the 1st main group, 48 to the 2nd comparison group, and 30 healthy children to the 3rd control group.

Special attention is paid to neurological and somatic pathologies: pathologies of the central nervous system, anemia, hypovitaminosis D and the formation of allergic diseases.

When observing the rate of physical development of premature infants, a decrease in body weight and breast circumference was observed compared to other indicators ($p < 0.05$).

In catamnestic observations of preterm infants under 1 year of age under our supervision, minimal brain dysfunction was found to be twice as common as other neurological pathologies. Gastroentestinal disorders, intolerance to enteral nutrition, and abdominal distension were also reported in both groups of preterm infants. Acrocyanosis, which reflects peripheral blood circulation, and marbling of the skin were more pronounced in group I, and its longevity was more accurate ($p < 0.01$) than in other groups.

Comparison between somatic diseases in 19 children with iron deficiency anemia, 6 with hypovitaminosis D, 13 children with hypotrophy I, 10 children with atopic dermatitis, 14 children with bronchiolitis, in the main group of 20 children with iron deficiency anemia, 7 with hypovitaminosis D, 16 children with II malnutrition, 9 children with atopic dermatitis and 16 children with bronchiolitis.

Iron deficiency anemia was reported in 87% of children born to mothers with anemia ($p < 0.001$). Children born to preeclampsia mothers were found to be deficient not only in iron but also in ferritin and folic acid. This is most likely due to an imbalance of micro and macro elements.

Given that 35% of children with high EPO and S100 protein in the neonatal period have perinatal hypoxic ischemic injury, there was great interest in examining their neurological status. It was found that 19 children developed minimal brain dysfunction, 1 child developed

cerebral palsy, and 7 children developed hypertension syndrome.

CNS developmental delay was observed in 11 children in the main group, 15 children in the comparison group, and 5 children in the control group.

The dynamic increase in EPO and S100 levels in the early neonatal period was directly correlated with neurological developmental delay at 1 year of age ($r = 0.532$, $p<0.01$; r=0,644, p<0,01), but the cut-off level of dynamic increase in EPO (3.2 ± 0.3) was more sensitive. (sensitivity = 82%, specificity = 77%) (Table 3).

Table 3

Dynamic changes in EPO and S100 protein levels in the early neonatal period in infants with CNS delay symptoms and anemia at 1 year of age

Indicators	Day	There is a delay in the CNS	There is no CNS delay	With anemia	No anemia
S100	1-3	16 ± 1.7	5.2 ± 1.4	12.2 ± 1.6	6.5 ± 1.3
	5-7	18 ± 1.9	8.4 ± 1.5	19.4 ± 2.1	10.2 ± 1.5
EPO IU / mL	1-3	7.6 ± 1.5	4.8 ± 1.2	6.8 ± 1.4	8.2 ± 1.5
	5-7	22 ± 2.3	9 ± 1.6	16 ± 1.8	14.7 ± 1.6

The assessment of psychomotor development in these children under 1 year of age was also examined with the Denver 2 test.

During the early adaptation period in children of group 1 with high EPO concentration social adaptation was noted in 5 (17.85%), fine motor in 6 (21.4%), speech retardation in 6 (21.4%), coarse motor in 6 (21.4%) cildren, in group II social adaptation was noted in 4 (28.6%), fine motor in3 (21.4%), speech delay in 4 (28.6%), coarse motor in 2 (14.3%) children, and statistically correct correlation found.

Thus, the organization of early diagnosis, adequate assessment of perinatal pathologies in premature births is one of the main issues of perinatal medicine. As a result, it can reduce perinatal morbidity and mortality, improve health at an early age, and improve quality of life. Namely, the perinatal period is a strategy aimed at reducing these complications.

RESULTS

1. Premature infants with asphyxia from mothers with complications of anemia and preeclampsia were more likely to be exposed to artificial lung ventilation associated with respiratory distress syndrome in the early neonatal period ($\chi 2 = 19.2$, $p < 0.001$). In the early neonatal period, signs of microcirculation and the delay of unconditioned reflexes were more pronounced ($p < 0.05$) [4].

2. Although EPO levels in children with perinatal asphyxia did not differ statistically from other groups on the first day, they increased approximately 3-fold in dynamics (14,2 ±1,5, p<0,020), and were statistically significantly different from those of the infants of the control group ($p < 0.05$). In children born to preeclampsia mothers, a 4-fold increase was observed ($p < 0.05$). Also, on the first day, there was a direct correlation between the EPO and erythrocyte count ($r = 0.230$, $p<0.05$), and an inverse correlation with the Fe level on both the first day and in the dynamics (ρ= –0,428, p<0,001 - on the first day,ρ= –0,557, p<0,001 – in dynamics) [2, 6, 11].

3. High levels of erythropoietin and S100 protein in the first day of birth in infants with perinatal asphyxia from mothers with a history of complicated pregnancies were associated with cerebral hemorrhage ($p < 0.05$). In dynamics, a further increase in EPO was statistically significant (16 ± 2.8; $p<0.024$) [7].

4. According to the ROC analysis, the cut-off level of erythropoietin for the first day of bleeding was higher than 4.26 IU / ml, with a sensitivity of 66% and a specificity of 74% ($p < 0.05$). In children with perinatal asphyxia with cerebral hemorrhage, the cut-off level of S100 protein in the first day was 8.4 mIU / ml, in which case the sensitivity was 90% and the specificity was 60% ($p < 0.05$) [7].

5. The dynamic increase in erythropoietin and S100 levels in the early neonatal period was directly correlated with neurological developmental delay at 1 year of age, ($r = 0.532$, $p<0.01$; r=0,644, p<0,01), but the cut-off point (3.2 ± 0.3) of the level of erythropoietin in the dynamics was more sensitive (sensitivity = 82%, specificity = 77%). Along with psychomotor developmemtal delay (47%), iron deficiency anemia (87%), vitamin D deficiency (91%) and atopic

dermatitis (39%) are more common than other somatic pathologies in children under 1 year of age who were born prematurely with perinatal asphyxia from mothers with complications of pregnancy anemia and preeclampsia [3, 5].

PRACTICAL RECOMMENDATIONS

1. High concentrations of EPO in children born to mothers with preeclampsia are considered an early diagnostic criterion for CNS damage and it is recommended that these children be monitored dynamically.

2. Iron deficiency anemia has been reported in infants born to mothers with a history of complicated pregnancy and asphyxia, which necessitates a physician's examination and treatment plan to prevent anemia in this group of infants, as well as other developing pathologies (hypovitaminosis D, atopic dermatitis). A timely appointment of preventive measures is recommended.

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List of abbreviations

ALV	– artificial lung ventilation
CNS	– central nervous system
EPO	– erythropoietin
Fe	– iron
HCT	– hematocrit
HGB	– hemoglobin
IFA	– immunoenzyme analysis
MCH	– Mean Corpuscular Hemoglobin in the erythrocyte
MCHC	– Mean Corpuscular Hemoglobin Concentration in the erythrocyte
MCV	– Mean Corpuscular Volume of erythrocytes
PA	– perinatal asphyxia
PDA	– Patent Ductus arteriosus
PFO	– Patent Foramen Ovale
PLT	– platelets
RBC	– erythrocytes
RDS	– respiratory distress syndrome
RDW CV	– anisocytosis of erythrocytes
RDWSD	– erythrocytespolykilocytosis
TF	– transferrin
TIBC	– total iron binding capacity
WBC	– leukocytes

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