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Original article

A possible role of troponin I, copeptin and ischemia-modified albumin in prognosis of the development of post-hypoxic myocardial damage in newborns

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Abstract

Aim: The aim was to improve the prediction of the development of post-hypoxic myocardial damage in newborns with asphyxia of varying degrees, by comparing the levels of troponin I, copeptin, and ischemia-modified albumin (IMA), to study their correlation with Holter monitoring and echocardiography.

Materials and methods: The study involved the examination of 94 neonates in the early neonatal period, assessment of history data, umbilical blood sampling with determination of levels of troponin I, copeptin, IMA, Holter monitoring, echocardiography.

Results: Depending on the diagnosis of asphyxia at birth, infants were divided into 2 main groups: Group 1 (n = 50) included newborns with asphyxia and Group 2 (n = 44) without asphyxia. Depending on the severity of asphyxia, Group 1 newborns were divided into subgroups: Group 1a (n = 16) with severe asphyxia, Group 1b (n = 34) with moderate asphyxia. The study showed that the risk factors for the development of hypoxic events in Group 1 infants (RR = 4.787, 95% CI [1.650; 13.887], $\chi^2 = 18.049$; $p < 0.001$) were obstetric and gynecological complications in newborn's mothers. Daily ECG monitoring, conducted at the age of 1-2 days, determined that 84% of Group 1 newborns had different variants of cardiac rhythm and conduction abnormalities. Assessment of the levels of biochemical markers in the umbilical cord blood showed that the levels of copeptin (p1a,2 = 0.0008; p1a,1b = 0.0093) and IMA (p1,2 = 0.00000039; p1a,2 = 0.000025; p1a,1b = 0.022769; p1b,2 = 0.001870) were the highest in newborns with severe asphyxia, as compared both to the comparison group, and the group of infants with moderate asphyxia. Increased levels of troponin I were also found in the group of infants with asphyxia,

especially severe, but no statistically significant difference in the rates among the groups was obtained. Interconnections between the levels of biochemical markers and a range of Holter monitoring and echocardiography parameters in newborns with asphyxia of different degrees at birth were obtained.

Conclusions: Assessment of laboratory-instrumental findings concerning the levels of troponin I, copeptin, and IMA in newborns showed that determination of copeptin levels and IMA could provide an opportunity for early prediction of the development of post-hypoxic disorders of the cardiovascular system in newborns.

Keywords

Neonatal post-hypoxic myocardium, neonatal myocardial damage, asphyxia effects, copeptin, ischemia-modified albumin, troponin I.

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Introduction

Perinatal asphyxia is a pathological condition that occurs in the absence of blood flow or gas exchange in a child immediately before, during labor or after birth and is caused by different perinatal events [1]. The incidence of asphyxia in developed countries is about 2:1,000 births, but this figure is 10 times higher in developing countries. Asphyxia and ischemia are responsible for disruption of the functions of different organs and systems (central nervous system – 28%, cardiovascular system – 25%, kidney – 50%, lungs – 23%). Taking into account multifactorial dependence, severity and consequences of this disease, it is relevant to search for useful tools for assessing and identifying complications associated with post-event events [2-7].

Violation of acid-alkaline state, electrolyte imbalance, hypercatecholaminemia, hypoglycemia, deterioration of rheological properties of blood due to hypoxic events are determining factors in the development of systemically cellular damage [8].

Cardiomyocytes of the sinus node and other parts of the conduction system have signs of degeneration, apoptosis, dystrophy, critical changes in electrical activity, which, in turn, cause the development of arrhythmias [9].

Cardiac troponin I, copeptin, and ischemia-modified albumin (IMA) are biomarkers of myocardial damage widely used in clinical practice in adult patients [10-12].

Troponin's isoforms are widely used in neonatology [7], but troponin isoforms are released from cardiomyocytes approximately 4 hours after the onset of myocardial damage and reach peak values only after 12 hours [13]. It becomes clear that their diagnostic value is limited in neonatal practice – immediately after the birth of the baby.

Copeptin is a 39-amino acid glycopeptide, a C-terminal fragment of the precursor of arginine-vasopressin (AV), which directly reflects the concentration of AV as a marker of endogenous stress, including in hypoxic events; it is synthesized after 5-20 minutes of acute myocardial damage and remains stable for several days [5, 14]. There are data on the use of copeptin as a marker for the development of various pathological conditions in newborns [15-18]; however, its role in predicting post-hypoxic cardiac events in newborns as compared with troponin I and IMA has not been studied.

IMA is a form of human serum albumin, in which N-terminal amino acids cannot bind to transition metals, since this protein is subject to modification in ischemia, including in cases of myocardial ischemia. Its concentration rises already in 6-10 minutes from the moment of development of ischemia, and returns to the initial level in 6-24 hours, that is, even before the increase of levels of cardiac troponin, creatine kinase isoform (CK-MB) or myoglobin [19, 20].

Current studies illustrate the usefulness of using IMA as a marker of oxidative stress [20], for the diagnosis of cardiac events [19, 21] and other pathological conditions, in neonatal practice [22-26]. However, the available literature does not provide data on the role and use of IMA in predicting post-hypoxic myocardial damage in newborns as compared to troponin I and copeptin.

The aim of the study was to improve the prediction of the development of post-hypoxic myocardial damage in newborns with asphyxia of varying degrees by comparing the levels of troponin I, copeptin, and IMA and studying their correlation with Holter monitoring and echocardiography.

Materials and methods

Blood samples and study population

Within the framework of the research work of the Department of Pediatrics No. 1 and Neonatology of Kharkiv National Medical University "Stratification of cardio-vascular risk in newborns and children of Kharkiv region", which was conducted during 2017-2018, in those years a prospective study of 94 newborns was conducted at the regional perinatal center in Kharkiv. The ethical committee of Kharkiv National Medical University approved the study protocol and written informed consent was obtained from the parents before enrolment in the study. The reagents of biochemical markers used in the study were bought by the university only for scientific activities according to the price of the manufacturer. Researchers did not spend personal funds on the purchase of equipment, supplies, etc., and have no commercial interest.

The gender distribution of the sample was dominated by the proportion of male children (53.2%). The mean gestational age of the newborn surveyed was 33.6 ± 4.1 weeks, with a birth weight of 2,112.9 ± 848.0 grams. Babies were born at 2.5 ± 1.8 pregnancies and 1.7 ± 0.9 births. The share of vaginal birth was 47.9%. At birth, on the Apgar scale in the 1st minute, infants had a score of 5.3 ± 1.6 points, in the 5th minute a score of 6.8 ± 1.4 points. The exclusion criteria were congenital malformations and/or organic disorders of the cardiovascular system.

The study implied assessment of history data, cord blood sampling with determination of levels of troponin I, copeptin and IMA, Holter analyses in first 2 days of life, echocardiography in early neonatal period followed by static analysis.

Blood was taken for testing right after the baby's birth from the umbilical cord's vein by gravity to a sterile 2 ml vials. Then the samples were left in the refrigerator for 1-3 hours at a temperature of +4°C. The plasma was taken from the upper layer without touching the platelet and leukocyte layers. The received plasma was immediately frozen and stored at a temperature of -20°C until the study.

Metabolic analysis

Enzyme-linked immunosorbent assay

The quantitative determination of the content of biochemical analytes was carried out by immunoassay methods based on sandwich technology, using the principle of double binding of the biotinized antibodies with the analyte under investigation.

Determination of the troponin I's level in the patient's blood serum was carried out by enzyme-linked immunosorbent assay using a commercial test system of the company "Biomerica" (USA) on the analyzer "Labline-90" (Austria) according to the instructions attached to the kit. 100 µl of prepared standards, controls and serum samples were added to the plate's wells that were part of the kit. After this, 100 µl of enzyme conjugate was added. Samples were incubated for 90 minutes at 180°C without a shaker. Then the wells were washed 5 times with distilled water and 100 µl of substrate solution was added to each well. The plate was incubated for 20 minutes at a temperature of 180°C in the dark. The reaction was stopped by adding 100 µl of stop reagent to each well. After 10 minutes, the optical density of each sample was determined at a wavelength of 450 nm. The amount of troponin I in the sample was determined by the calibration graph, which was built in parallel with the determination in the samples using the standards from the kit. The amount of troponin I was expressed in nanograms per milliliter of serum (ng/ml).

Determination of the copeptin's level in the patient's blood serum was carried out by enzyme-linked immunosorbent assay using a commercial test system of the company "Phoenix Pharmaceutical" (USA) on the analyzer "Labline-90" (Austria) according to the instructions attached to the kit. 50 µl of controls, standards and serum samples were added to the wells of the plate from the kit, and then 25 µl of the dissolved first antibodies and 25 µl of the dissolved biotinylated peptide were added to each well. The plate was incubated for 2 hours at a temperature of 180°C on an orbital shaker. Then the wells were washed 4 times with a special wash buffer. 100 µl of the prepared streptavidin reagent SA-HRP was added to each well. The microplate was incubated for 1 hour at a temperature of 180°C

on an orbital shaker and then the wells were washed again 4 times with a special washing buffer. After that, 100 µl of substrate solution was added to each well and the plate was incubated for 1 hour at a temperature of 180°C. After this, the reaction was stopped by adding 100 µl stop reagent (2 N HCl) to each well. After 20 minutes, the optical density of all samples was determined at a wavelength of 450 nm. The amount of copeptin in the sample was determined by the calibration graph, which was built in parallel with the determination in the samples, using the standards that were in the kit. The amount of copeptin was expressed in nanograms per milliliter of serum (ng/ml).

Determination of the IMA level in the patient's blood serum was carried out by enzyme-linked immunosorbent assay using a commercial test system of the company "Elisa" (USA), on the analyzer "Labline-90" (Austria) according to the instructions attached to the kit.

Before testing, serum samples were gradually diluted with a 0.9% NaCl solution 5,000 times. 50 µl of each standard, diluted serum sample and working solution, which reagent A detects, were added to the wells of the plate included in the kit. The plate was incubated for 1 hour at a temperature of 37°C. After this, the wells were washed 3 times with a special washing buffer and 100 µl of working solution, which detects reagent B, was added. The plate was incubated for 30 minutes at a temperature of 37°C. Then the wells were washed again 5 times with special washing buffer and 90 µl of substrate solution was added to each well. The plate was incubated for 20 minutes at 37°C in the dark and the reaction was stopped by adding 50 µl of stop reagent to each well. The optical density of each sample was immediately determined at a wavelength of 450 nm. The amount of IMA in the sample was determined by the calibration graph, which was built in parallel with the determination in the samples using the standards from the kit. The amount of IMA was expressed in nanograms per milliliter of serum (ng/ml).

Cardiac dimensions

Echocardiography was performed using the "Gray Scale" ultrasound device "My Lab 25 Gold" from the company "Esaote" (Italy), color and spectral Doppler imaging by a phase sensor with a frequency range of 3-8 MHz [27].

Cardiac dimensions were obtained in systole and end-diastole from the apical 4-chamber view, the long axis parasternal view, and the short axis parasternal view. The ventricular diameters, interventricular septum thickness, left ventricle (LV) posterior wall thickness, aortic diameter were determined using M-mode of the long and short axis parasternal views at the level of the mitral valve (MV) leaflet tips.

Conventional echocardiography functional assessment

The following conventional LV function measurements were made: ejection fraction (EF) using Simpson Biplane Method from the apical 4- and 2-chamber views. Mean gradient, stroke volume, cardiac outputs were calculated by echocardiography. Systemic vascular resistance corrected by BSA was calculated with a standard formula [27-29]. Mean pulmonary gradient and mean pulmonary acceleration (peak pulmonary velocity/acceleration time) were calculated by echocardiography.

Holter analyses

The recording and analysis of daily electrocardiogram (ECG) monitoring were performed using the electrocardiographic "ECG pro" device (Holter monitor "EP810"), IMESC, in the first 2 days of life. The data received were interpreted using ECGproHolter v.7.44.7-S12 software [30]. The daily ECG was carried out comprehensively using Holter monitoring with analysis of heart rate variability. The method was based on recording the bioelectric activity of the heart using an electrocardiographic hardware-software complex during the day in physiological conditions, without creating additional stress on the patient, using hydrogel electrodes that were directly fixed on the chest of a newborn. 3 ECG channels with a system of typical modified leads – V3mod, IImod, V5mod were recorded. The electrodes were positioned in the center of the sternum grip, in V intercostal space along the mid-clavicular line and in VI intercostal space along the anterior axillary line on the left, in the V intercostal space along the mid-clavicular line and in VI intercostal space along the anterior axillary line on the right.

Statistical analysis

The statistical analysis was conducted using STATISTICA 10 (developed by StatSoft.Inc). In

the case of quantitative indicators having a normal distribution, the obtained data were combined into variations in which the arithmetic mean values (M) and standard deviations (SD) were calculated.

The aggregate of quantitative indicators, the distribution of which differed from normal, were described using median (Me) values, minimum and maximum values, interquartile range (25% Q/75% Q). The Mann-Whitney U-criterion was used to compare independent populations. The relationship between the signs was calculated by Spearman rank correlation coefficient and the possibility of ROC analysis with the calculation of the plane under the curve, the cutoff points with sensitivity and specificity, the relation of likelihood and prediction of the result. A comparison of nominal data was performed using the Chi-Square Test. Relative risk indicator (RR) was used as a quantitative measure of the effect in comparing relative indicators. In order to design the obtained values of RR for the general population, the limits of 95% confidence interval (95% CI) were calculated. Based on the data obtained, the significance of the relationship between the result and the factor was considered proven in the case of finding the confidence interval outside the boundary of the absence of the effect taken as 1. P-value < 0.05 was considered significant.

Results

Depending on the presence of asphyxia at birth, infants were divided into 2 main groups: Group 1 (n = 50) included newborns with asphyxia and Group 2 (n = 44) newborns without asphyxia. Depending on the severity of asphyxia, Group 1 newborns were divided into subgroups: Group 1a (n = 16) included children with severe asphyxia, Group 1b (n = 34) with moderate asphyxia.

By gender distribution, among newborns of Group 1 there were more children of male sex (60%), among children of Group 2 – babies of female sex (54.5%). Newborns of Group 1 were born at a gestational age of 31.2 ± 3.8 weeks with a birth weight of 1,804.5 ± 758.2 grams. Newborns of Group 2 had indicators of average gestation period of 35.3 ± 3.7 weeks with a weight of 2,463.5 ± 814.7 grams at birth. Thus, children in Group 1 had lower rates of both gestational maturity (p = 0.0004) and birth weight (p = 0.0002) compared with infants in Group 2.

Newborns of Group 1 were born from 2.3 ± 1.7 pregnancies and 1.7 ± 0.8 births; infants of Group 2 – from 2.6 ± 1.9 pregnancies and 1.7 ± 0.9 births. The proportion of vaginal births was slightly lower in infants in Group 1 (46%) than in Group 2 (52.3%).

The study showed that the risk factors for the development of hypoxic events in Group 1 infants (RR = 4.787, 95% CI [1.650; 13.887], $\chi^2 = 18.049$; $p < 0.001$) were obstetric and gynecological complications in newborns' mothers.

In assessing the state of adaptation of the child's body at birth by Apgar score during the 1st and 5th minutes (**Fig. 1** and **Fig. 2**), the rates in the group of newborns with asphyxia were lower than in the group without asphyxia (during the 1st minute 5 [4; 5] points and 6 [5; 8] points respectively, p <

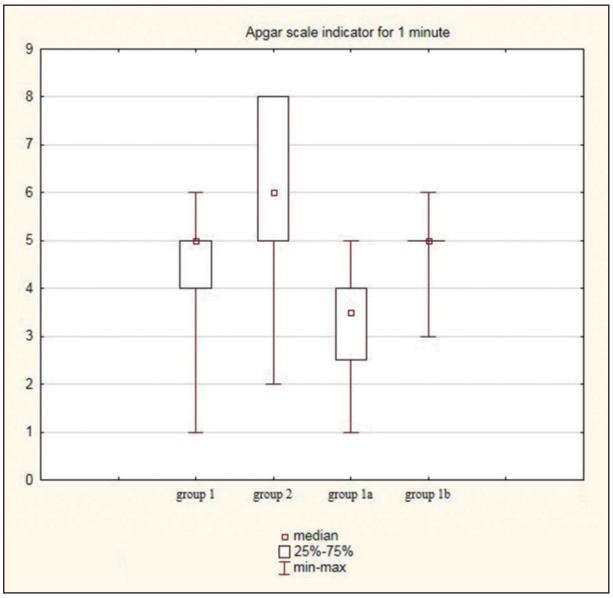

Figure 1. Apgar score during the 1st minute.

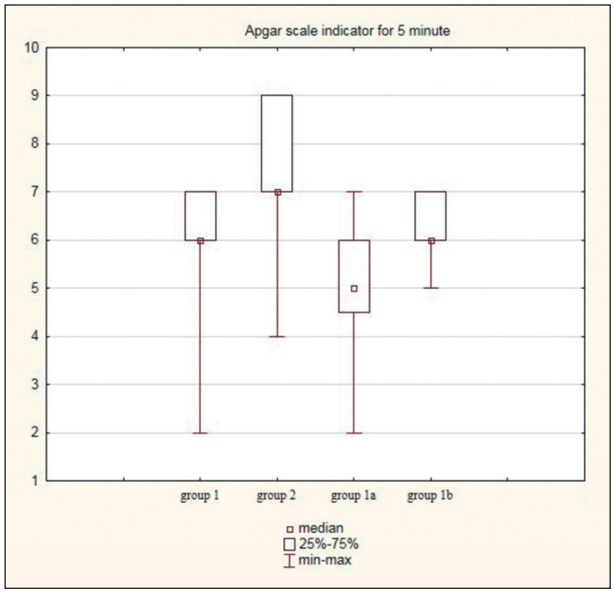

Figure 2. Apgar score during the 5th minute.

0.001; during the 5th minute 6 [6; 7] points and 7.8 [7; 8] points, respectively, p < 0.001). Moreover, during the 1st and the 5th minute, the lowest rates were registered in Group 1a, i.e. infants with severe asphyxia (during the 1st minute: p1a,2 < 0.001, p1a,1b = 0.00002; during the 5th minute: p1a,2 < 0.001, p1a,1b = 0.00002).

Assessment of acid-alkaline state of the umbilical cord blood (**Fig. 3**) showed that the levels of pH in newborns with asphyxia (7.3 [7.15; 7.35]) were lower than in the group without asphyxia (7.35 [7.3; 7.4], p1,2 = 0.001896). The lowest rates were recorded in the subgroup of infants with severe degrees of asphyxia (p1a,2 = 0.00000074, p1a,1b = 0.004573).

According to Holter monitoring performed at the age of 1-2 days of life, 84% of Group 1 newborns had different variants of cardiac rhythm and conduction disruption: violation of autonomous sinus node in the form of tachy-/bradyarrhythmias in 86%, supraventricular extrasystole in 29%, ventricular extrasystole in 5%, 2nd-degree sinoatrial block in 7%, 2nd-degree atrioventricular block in 10%, migration of pacemaker from the sinus node to the lower atria in 36%, transient long QT interval in 24% of newborns.

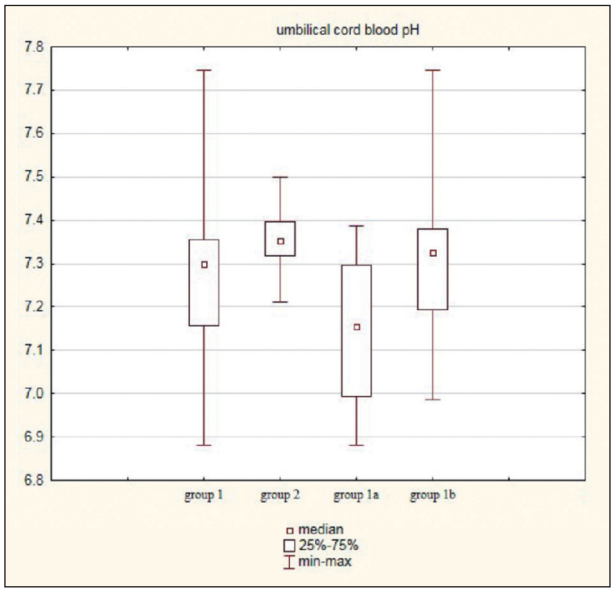

Figure 3. The levels of pH of the umbilical cord blood.

Assessment of the levels of biochemical markers in the umbilical cord blood are shown in **Tab. 1**. Copeptin levels were the highest in newborns with severe asphyxia, in comparison both with the control group and with the group of infants with moderate asphyxia (p1a,2 = 0.0008; p1a,1b = 0.0093). Increased levels of troponin I were also found in the group of infants with asphyxia, especially of severe type, but no statistically significant difference in the rates among the groups was obtained. The study of IMA levels in the umbilical cord blood showed that the indicators of this biological marker of myocardial damage had the most variability depending on the presence of asphyxia at birth and the degree of its severity. The highest IMA levels were registered in newborns with asphyxia at birth (p1,2 = 0.000039), with maximum values in the subgroup of children with severe asphyxia (p1a,2 = 0.000025). A statistically significant difference in rates was also recorded among subgroups of newborns with asphyxia of varying severity (p1a,1b = 0.022769) and in the group of infants with moderate asphyxia as compared to the group of newborns without asphyxia (p1b,2 = 0.001870).

A comparison of the areas of ROC curves was performed in order to provide unbiased assessment of the diagnostic significance of troponin I, copeptin, IMA.

Table 1. The levels of biochemical markers in the umbilical cord blood.

	Troponin I, ng/ml	Copeptin, ng/ml	IMA, ng/ml
Group 1 (n = 50)	1.15 (0.38; 4.75) [0.69; 1.55]	0.24 (0.04; 1.92) [0.13; 0.6]	3,145.6 (1,062.8; 10,392.6) [2,195.7; 4,670.9]
	p1,2 = 0.066	**p1,2 = 0.02**	**p1,2 = 0.000039**
Group 2 (n = 44)	0.8 (0.35; 8.96) [0.57; 1.33]	0.16 (0.03; 0.73) [0.06;0.32]	1,907.7 (665.7; 8,045.9) [1,087.1; 2,832.8]
	p1a,2 = 0.08	**p1a,2 = 0.0008**	**p1a,2 = 0.000025**
Group 1a (n = 16)	1.32 (0.38; 4.75) [0.66; 2.26]	0.51 (0.12; 1.92) [0.17; 1.19]	4,376.6 (1,363.7; 10,392.6) [2,788.8; 5,564.3]
	p1a,1b = 0.3	**p1a,1b = 0.0093**	**p1a,1b = 0.022769**
Group 1b (n = 34)	1.10 (0.38; 4.18) [0.69; 1.3]	0.21 (0.04; 1.17) [0.1; 0.35]	2,871.6 (1,062.8; 9,069.9) [1872.6; 4178.0]
	p1b,2 = 0.16	p1b,2 = 0.27	**p1b,2 = 0.001870**

IMA: ischemia-modified albumin.

According to the constructed curves, an informative evaluation was performed by analyzing the critical thresholds of an unfavorable prognosis for the development of post-hypoxic myocardial damage in newborns with asphyxia at birth. IMA and copeptin showed larger areas under ROC curves (AUCs) than troponin I.

At values of IMA (**Fig. 4**) above 2,279.96 ng/ml, the sensitivity (Se) was 72.9% (95% CI 58.2-84.7), the specificity (Sp) was 70.7% (95% CI 54.5-83.9), AUC was 0.793 (95% CI 0.694-0.871), the positive predictive value (PPV) was 71%, negative predictive value (NPV) was 72%, positive likelihood ratio (+ LR) was 2.38, negative likelihood ratio (-LR) was 0.49.

For copeptin (**Fig. 5**) at values above 0.155 ng/ml, the corresponding indices were: Se = 87.2% (95% CI 72.6-95.7), Sp = 41.7% (95% CI 25.5-59.2), AUC = 0.679 (95% CI 0.562-0.783), PPV = 60%, NPV = 77%, (+ LR) = 1.49, (-LR) = 0.31.

For troponin I (**Fig. 6**) at values higher than 1.07 ng/ml: Se = 58.0% (95% CI 43.2-71.8), Sp = 63.6% (95% CI 47.8-77.6), AUC = 0.611 (95% CI 0.505-0.710), PPV = 62%, NPV = 60%, (+ LR) = 1.59, (-LR) = 0.66.

In predicting the development of post-hypoxic myocardial damage in newborns with severe asphyxia (**Figures 7-9**), IMA levels had the highest diagnostic significance (with IMA values

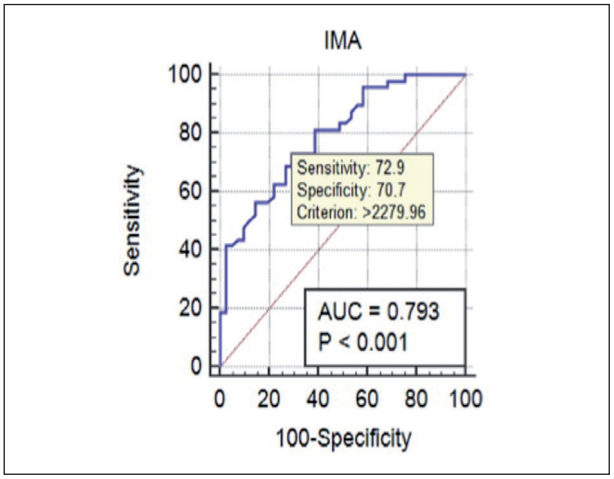

Figure 4. The levels of ischemia-modified albumin (IMA) and development of myocardial damage.

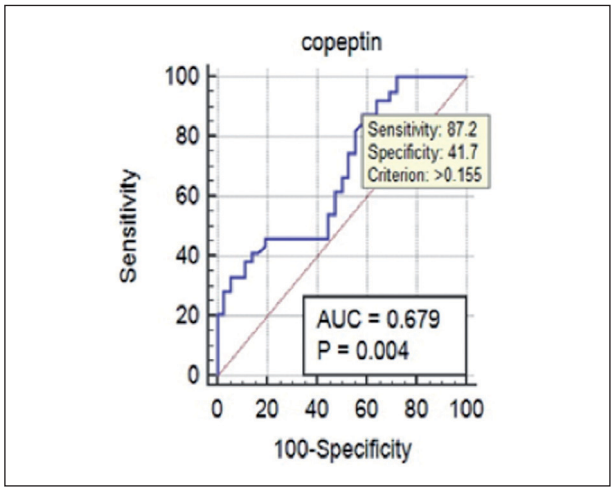

Figure 5. The levels of copeptin and development of myocardial damage.

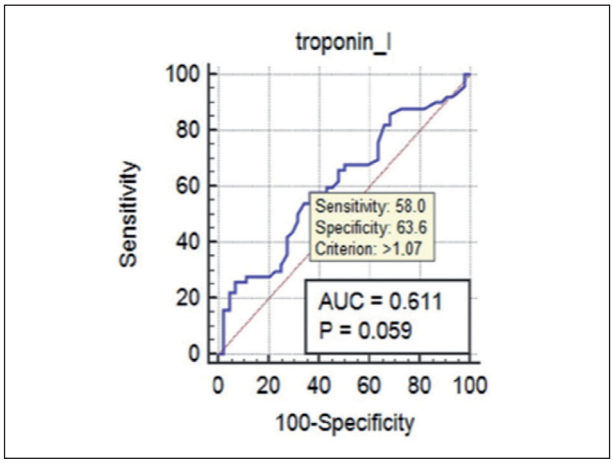

Figure 6. The levels of troponin I and development of myocardial damage.

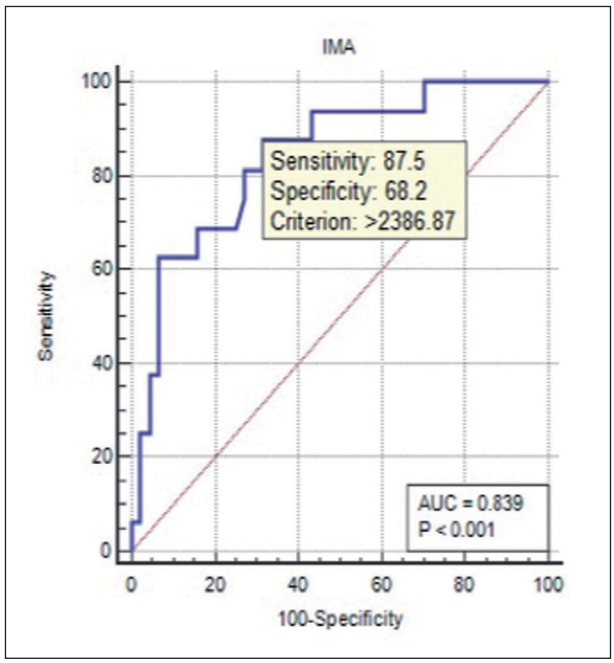

Figure 7. The levels of ischemia-modified albumin (IMA) and development of post-hypoxic myocardial damage in newborns with severe asphyxia.

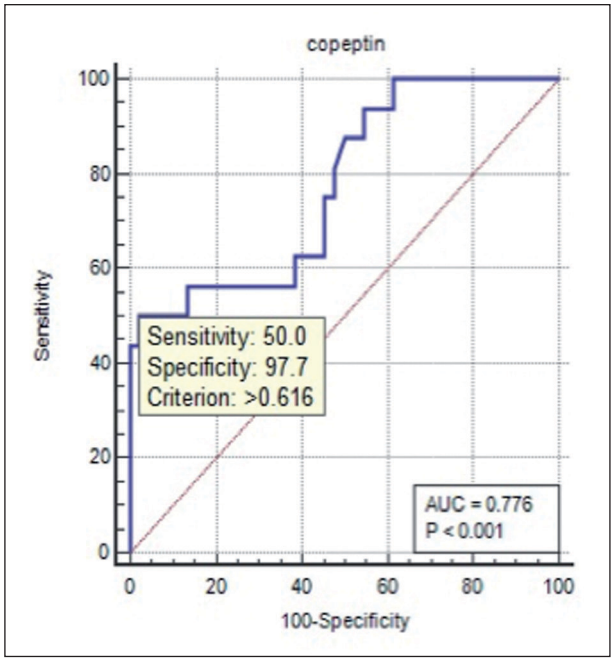

Figure 8. The levels of copeptin and development of post-hypoxic myocardial damage in newborns with severe asphyxia.

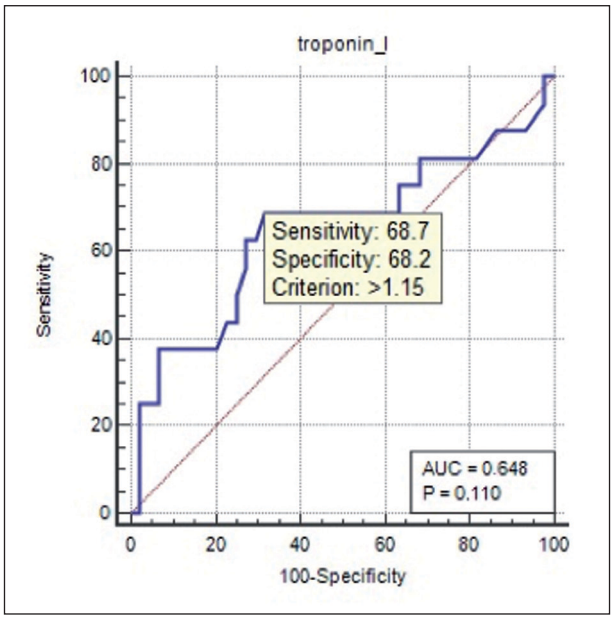

Figure 9. The levels of troponin I and development of post-hypoxic myocardial damage in newborns with severe asphyxia.

higher than 2,386.87 ng/ml: Se = 87.5% [95% CI 61.7-98.4], Sp = 68.2% [95% CI 52.4-81.4], AUC = 0.839 [95% CI 0.721-0.921], PPV = 73%, NPV = 85%, [+ LR] = 2.75, [-LR] = 0.18) in comparison with copeptin (with copeptin values higher than 0.616 ng/ml: Se = 50.0% [95% CI 24.7-75.3], Sp = 97.7% [95% CI 88.0-99.9], AUC = 0.776 [95% CI 0.650-0.874], PPV = 96%, NPV = 66%, [+ LR] = 22.0, [-LR] = 0.51) and troponin I (with values of troponin I above 1.15 ng/ml: Se = 68.7% [95% CI 41.3-89.0], Sp = 68.2% [95% CI 52.4-81.4], AUC = 0.648 [95% CI 0.514-0.776], PPV = 68%, NPV = 69%, [+ LR] = 2.16, [-LR] = 0.46).

The findings suggest that the determination of copeptin and IMA levels may be a useful tool for predicting the risk of development of post-hypoxic myocardial damage in newborns with asphyxia at birth and the most severe degree of asphyxia and can be used for early diagnosis of cardiovascular post-hypoxic origin in newborns.

We performed laboratory-instrumental comparisons with the pH values of the umbilical cord blood, the indicators of the Apgar score during the 1st and 5th minutes of life, parameters of blood pressure (systolic [SBP], diastolic [DBP], mean blood pressure [mBP]), the level of oxygenation of capillary blood (Sat O_2), the main parameters of Holter monitoring, echocardiography in newborns. As a result, the comparison of the levels of biomarkers with the parameters of Holter monitoring, namely, the circadian index (CI), the length of the minimum and maximum sinus RR intervals (min RR and max RR), the length of the corrected QT interval (QTc), parameters of cardiac variability rhythm (SDNN, SDANN, SDNN index, rMSSD, pNN50), as well as echocardiography parameters (LV internal diastolic diameter and LV internal systolic diameter [LVDd and LVDs], left atrial diastolic volume index [LAd] and right atrial diastolic volume index [RAd], right ventricle internal diastolic diameter [RVDd], EF, aortic peak gradient and pulmonary peak gradient, pulmonary peak mean [P pm]), showed the following data.

Assessment of correlation dependencies in the group of newborns with asphyxia (Group 1) showed a direct correlation between the levels of troponin I (**Fig. 10**) and SBP (r = 0.305122, p = 0.034962).

Copeptin index in this group of infants had a direct relation to the diameter of the left atrium (r = 0.359095, p = 0.040138) and a negative correlation with Apgar score during the 1st (r = -0.317798, p = 0.024513) and 5th (r = -0.294227, p = 0.038076) minutes, indices of umbilical cord blood pH (r = -0.352782, p = 0.01291) and the parameters of min RR (r = -0.327685, p = 0.020178). Thus, the levels of this biomarker can be one of the indicators of the severity of the child's condition at birth and act as a signaling criterion for the development of tachyarrhythmias.

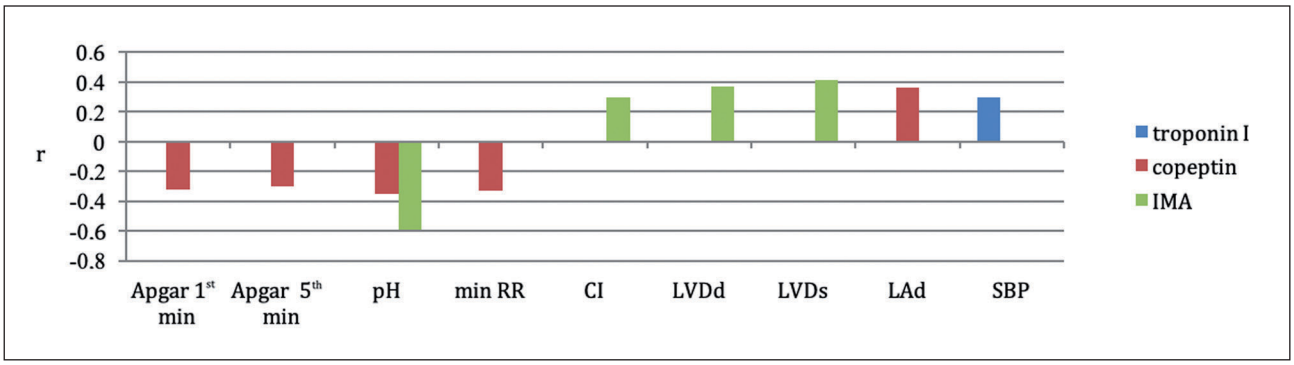

Figure 10. The analysis of laboratory-instrumental comparisons of cardiac biomarkers in Group 1.
min RR: minimum sinus RR intervals; CI: circadian index; LVDd: left ventricle internal diastolic diameter; LVDs: left ventricle internal systolic diameter; LAd: left atrial diastolic volume index; SBP: systolic blood pressure.

In view of the presence of a direct relationship between IMA and CI (r = 0.296297, p = 0.036680) and feedback with the indices of the umbilical cord blood pH (r = -0.584167, p = 0.000011), it can be assumed that its high levels in newborns secondary to severe metabolic disturbances indicate an imbalance in the functioning of the regulatory mechanisms of the heart rate with increased sympathetic regulation or parasympathetic damage. Also, IMA showed a direct relationship with the linear size of LV, namely LVDd (r = 0.369635, p = 0.008950) and LVDs (r = 0.412582, p = 0.00438).

In the subgroup of newborns with severe asphyxia (Group 1a), troponin I level had a negative correlation with the mBP (r = -0.518146, p = 0.039777) and a direct correlation with corrected QT interval (r = 0.591, p = 0.015876). In view of this, an increase in troponin may be used as a marker for the development of violations of the rate of myocardial repolarization and its electrical instability (**Fig. 11**).

Copeptin and IMA had a reverse relationship with the main characteristic index of acid-alkaline state, pH of the umbilical cord blood (r = -0.608824, p = 0.012315 and r = -0.65000, p = 0.006416, respectively).

In the subgroup of newborns with moderate asphyxia (Group 1b), troponin I was shown to have a direct correlations with the linear parameters of echocardiography (**Fig. 12**): LVDd (r = 0.400324, p = 0.020971) and LVDs (r = 0.568580, p = 0.000846). The presence of a direct dependence of this marker with SBP (r = 0.485406, p = 0.004861) and DBP (r = 0.389157, p = 0.027706) suggests that an increase in troponin I may reflect the processes of activation of compensatory cardiovascular responses in the form of increasing peripheral vascular resistance.

The presence of a direct correlation of copeptin with hemodynamic parameters of P pm (r = 0.3367313, p = 0.035481) and LAd (r = 0.405717, p = 0.049179) suggests its prognostic significance for development of dilatation changes in the left atrium and pulmonary hypertension.

The presence of direct correlation of IMA with the values of max RR (r = 0.398103, p = 0.01971) and the reverse correlation with pH levels (r = -0.485168, p = 0.004212) suggest the possibility of employment of these relationships as an

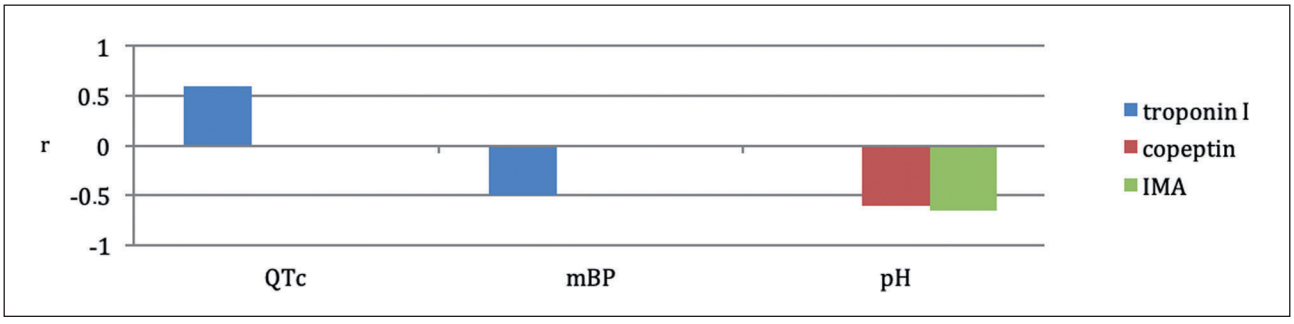

Figure 11. The analysis of laboratory-instrumental comparisons of cardiac biomarkers in Group 1a.
QTc: corrected QT interval; mBP: mean blood pressure.

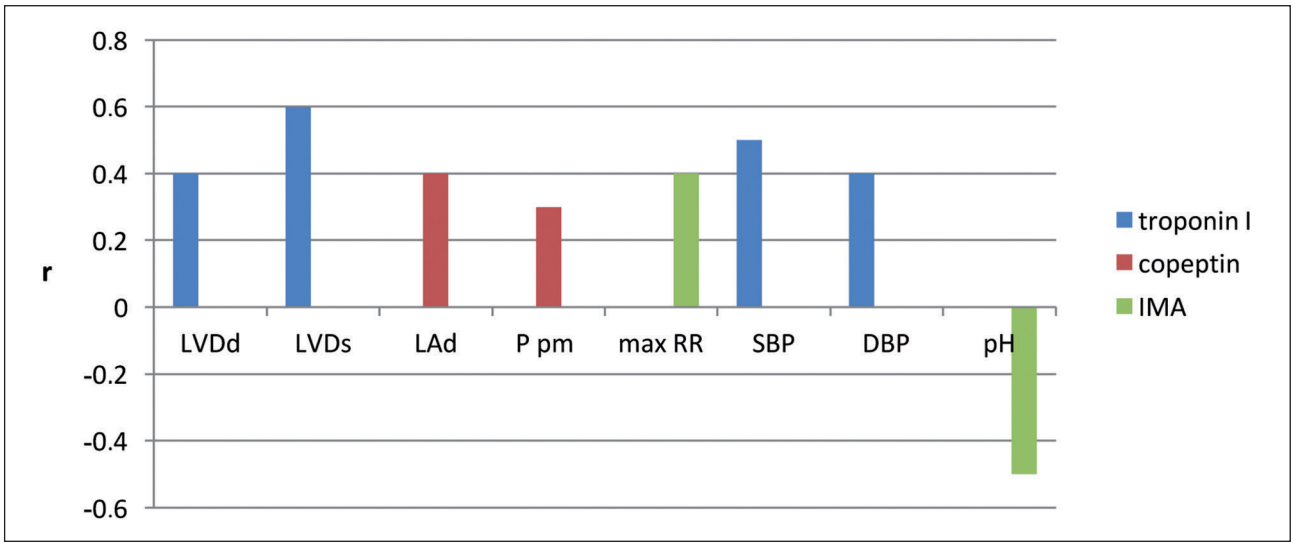

Figure 12. The analysis of laboratory-instrumental comparisons of cardiac biomarkers in Group 1b.
LVDd: left ventricle internal diastolic diameter; LVDs: left ventricle internal systolic diameter; LAd: left atrial diastolic volume index; P pm: pulmonary peak mean; max RR: maximum sinus RR intervals; SBP: systolic blood pressure; DBP: diastolic blood pressure.

additional prognostic factor for the formation of bradyarrhythmias secondary to metabolic disorders.

No correlation was found between the values of troponin I, copeptin, IMA and EF, heart rate variability parameters (SDNN, SDANN, SDNN index, rMSSD, pNN50) in the groups of children examined.

Discussion and conclusions

Copeptin and IMA are active and stable metabolic components which levels collectively reflect the severity of the oxidative component of endogenous stress, which is one of the key components in the pathogenesis of hypoxic cardiac damage.

The results of our study show that copeptin and IMA, taking into account specificity and sensitivity, can be used as informative tests for the development of post-hypoxic myocardial disorders. These markers are significantly increased in newborns with asphyxia and are related to parameters of assessment of the child's adaptation at birth, indices of acid-alkaline state of the umbilical cord blood, a number of indicators of standard instrumental research methods of the cardiovascular system.

Copeptin shows correlations with Apgar scale, pH of the umbilical cord blood, parameters of LAd, P pm, parameters of the length of min RR. These data suggest that the determination of copeptin levels is important in determining the prognosis of newborn post-hypoxic changes in the cardiac muscle in the form of cardiac rhythm disturbances by the type of tachyarrhythmias, elevated pressure in the pulmonary artery and left atrium dilation secondary to metabolic disorders.

The direct relationship of IMA with the indices of the acid-alkaline state of the umbilical cord blood (i.e., pH) in all newborns groups, with a strong correlation precisely in the group of newborns with severe asphyxia, suggests the possibility of using this biomarker as an indicator of the severity of metabolic disorders. The level of IMA was found to correlate with LVDd and LVDs, CI and the parameters of max RR. Taking into account the above, determination of the level of this biomarker can be recommended for predicting the risk of developing heart rhythm disturbances such as bradyarrhythmias, disruption of autonomic regulation of cardiac rhythm, left ventricular dilatation secondary to metabolic disorders.

According to the results of our study, troponin I also has relationship with Holter monitoring and echocardiography. However, the determination of its levels in the umbilical cord blood, at the early stage of the adaptation of the newborn, is not very informative to predict the development of post-hypoxic myocardial disorders, given the rather low specificity and sensitivity rates, compared with the indicators for copeptin and IMA. These markers demonstrate a significant increase in their levels in newborns with asphyxia.

Thus, copeptin and IMA can be used for early prediction of a whole series of adverse events as a result of post-hypoxic myocardial damage. The determination of these biomarkers has a prognostic

value, given that they begin to be produced in the early stages of the post-hypoxic pathogenic process of myocardial damage and have proven connections with a number of parameters of protocol instrumental studies of the cardiovascular system. That is why the determination of these biomarkers is useful in the early prediction of the risk of post-hypoxic damage of the cardiovascular system in newborns with asphyxia at birth and the elaboration of further therapeutic and diagnostic approach for this group of children.

Abbreviations

25% Q/75% Q: interquartile range
95% CI: 95% confidence interval
AUC: area under the ROC curve
CI: circadian index
DBP: diastolic blood pressure
EF: ejection fraction
IMA: ischemia-modified albumin
LAd: left atrial diastolic volume index
+LR: positive likelihood ratio
-LR: negative likelihood ratio
LV: left ventricle
LVDd: left ventricle internal diastolic diameter
LVDs: left ventricle internal systolic diameter
NPV: negative predictive value
max RR: maximum sinus RR intervals
mBP: mean blood pressure
Me: median
min RR: minimum sinus RR intervals
MV: mitral valve
P pm: pulmonary peak mean
PPV: positive predictive value
QTc: corrected QT interval
RAd: right atrial diastolic volume index
RR: relative risk indicator
RVDd: right ventricle internal diastolic diameter
SBP: systolic blood pressure
SD: standard deviation
Se: sensitivity
Sp: specificity

Declaration of interest

The Authors have no conflicts of interest to declare and no specific funding was required for this paper.

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