Diagnostic Significance of S100 Protein and Phospholipase A2 in Bacterial Injures of CNS in Premature Infants

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Abstract

The level of S100 and phospholipase A2 were analyzed in the blood serum of 34 newborns with pathology of CNS. Depending on the nature of CNS lesions, two groups of newborns were identified: the study group - 14 premature newborns with bacterial meningitis (mean gestation age is 31,7±0,9 weeks), and the comparison group - 10 premature newborns (mean gestation age is 32,3±0,4 weeks) and 10 full-term newborns (gestational age is 38,7 ± 0,5 weeks) with severe hypoxic-ischemic injury of CNS. The study of children in the study group was conducted twice: in the acute period of the disease and in the period of convalescence on reaching their post-conceptual age of 38-40 weeks. The control group consisted of 18 full-term newborns with a gestational age of 38,6±0,3 weeks without signs of CNS injury and inflammatory diseases. It was found that content of S100 protein in the blood serum of premature newborns has a diagnostic value in relation to assessing the risk of meningitis. With its value equal to 104,1 ng/l or more, meningitis is diagnosed with a sensitivity of 85,7% and a specificity of 75,0%.

The determination of this protein in blood serum can also be used as an additional biochemical marker in premature newborns with meningitis to monitor the effectiveness of therapeutic measures. At the same time, increased serum phospholipase A2 activity is not a specific diagnostic marker of meningitis in preterm newborns, but reflects the processes of CNS damage regardless of the etiology of the disease.

Keywords: meningitis, S100 protein, phospholipase A2, premature newborns.

Introduction

The results of the studies showed that pathologies of the perinatal period are often found in premature babies, playing a leading role in increasing mortality, social and biological maladaptation. Moreover, as a result of both infectious and non-infectious pathological processes, the brain is most often exposed to pathological effects [25]. Perinatal pathology of CNS is represented by various etiopathogenetic causes: hypoxic-ischemic disorders of the central nervous system (cerebral ischemia), intracranial hemorrhages (traumatic and hypoxic etiology), toxic-metabolic disorders, congenital malformations, infectious diseases, etc. [33, 10].

In premature infants, any exogenous and endogenous factors may contribute to the development of hypoxia or cerebral ischemia [35].

Hypoxia leads to a violation of oxidative processes and the development of acidosis, a decrease in the energy balance of the cell, an excess of exciting neurotransmitters, and a disruption in the metabolism of glia and neurons. Under hypoxia, lipid peroxidation is disrupted with the accumulation of aggressive free radicals, hydroperoxides, which destructively act on the membranes of neurons [26].

Under the action of the end products of lipid peroxidation, activated the phospholipase A2 enzyme, the substrate for which are cell membrane phospholipids [27, 12].

Phospholipase A contains a supergroup of esterase enzymes those present in all human cells and play a key role in ensuring the production of free fatty acids and lysophospholipids from glycerophospholipids [3]. These enzymes are important for the regulation of homeostasis and the pathogenesis of many diseases. Phospholipase A2 plays several important physiological functions, including the production of inflammatory eicosanoid compounds from arachidonic acid [14].

On phospholipase activity can influence many factors. So, it was found that corticosteroids can inhibit phospholipase A2 [31].

Timely diagnosis of brain lesions, especially of inflammatory origin, will ensure timely etiopathogenic treatment and improve survival of newborns. In this regard,

there is a need to develop new biomarkers for early detection of children with this disorder.

Among the biochemical markers of brain lesions, the determination of the neurospecific proteins level is being actively studied [32]. The participation of proteins specific for neurons and glia in the pathogenesis of acute neuroinfectious diseases is confirmed by published data. It was found that their excessive synthesis with access to the intrathecal space can enhance inflammatory reactions, damaging brain tissue [21].

Protein S100B is a glial biomarker, the most studied and included in laboratory diagnostics due to its neurospecificity [30]. S100 is a paracrine neurotrophic factor in the central nervous system that affects brain formation, glial cell proliferation and neuronal maturation, promoting surviving of the cells in stressful situations, and counteracts the effects of neurotoxins. At the same time, its concentration in serum does not depend on age and gender [32]. This protein is believed to be a marker of not only isolated glia damage, but also generalized damage to the blood-brain barrier [36]. S100B is used as a diagnostic marker for various diseases, in particular hypoxic brain damage [11, 15]. Furthermore, S100 family of proteins associated with activation of innate immunity [7]. Several studies have shown promise in determining the S100B as an early biomarker of meningitis due to its increased concentration in astrocytes and glial [20]. At the same time, studies conducted by Gazzolo D. et al, (2004) showed that S100B in serum or cerebrospinal fluid exhibits suboptimal sensitivity and specificity for bacterial meningitis [18].

Objective: to determine the protein content of S-100 and phospholipase A2 in the serum of premature infants with purulent meningitis and to determine their diagnostic value.

Methods

Were monitored 34 infants with pathology of CNS. Depending on the character of CNS lesions, two groups of newborns were identified: the main group - 14 premature infants (mean gestational age is $31,7 \pm 0,9$ weeks) with bacterial meningitis and the comparison group - patients with severe hypoxic-ischemic CNS lesions, among which 10 were preterm (average gestational age is $32,3 \pm 0,4$ weeks) and 10 full-term infants (gestational age is $38,7 \pm 0,5$ weeks). The examination of the children of the main group was conducted twice: in the acute period of the disease and in the period of convalescence after reaching their post-conceptual age of 38-40 weeks. The diagnosis of "meningitis" was established on the basis of clinical and laboratory criteria. The control group consisted of 18 full-term infants with a gestational age of $38,6 \pm 0,3$ weeks without signs of CNS and inflammatory diseases.

Level of protein S-100 and phospholipase A2 in the serum of examined children were investigated using a CanAg S100 EIA kit, (Fujirebio Diagnotics AB, Sweden) and Lipoprotein Associated (LpPLA2) Phospholipase A2 ELISA Kit by enzyme immunoassay.

Statistical Analyses

The obtained results were processed by the method of variation statistics using Statistica 13.0 analysis package (StatSoftInc, No. JPZ8041382130ARCN10-J) and IBM SPSS Statistics 23 with the calculation of arithmetic mean (M), standard deviation (σ) and mean errors (m). The median and the interquartile interval were calculated. The relationship between the individual factors was estimated using the Pearson correlation coefficient. The degree of significance of differences in characteristics between groups was determined using the Mann-Whitney test.

The significance level for testing statistical hypotheses on the significance of differences was taken to be 0,05. The significance of protein S100 and phospholipase A2 serum levels in the diagnosis of meningitis in preterm infants was assessed using the ROC analysis method (Receiving Operating Characteristics) by constructing ROC curves with the determination of AUC (Area under ROC curve). AUC in the range of 0,5-0,6 was considered as a criterion of unsatisfactory information content of the studied indicator, 0,6-0,7 - poor, 0,7-0,8 - average, 0,8-0,9 - good, 0,9 -1,0 - excellent informativeness of indicator [4].

To analyze the quality of diagnostic test determined the sensitivity and specificity of the assay.

Sensitivity - the proportion of truly positive cases, calculated by the formula:
$Se = (TP/(TP+FN)) \times 100\%$,
Specificity - the proportion of truly negative cases, calculated by the formula:
$Sp = TN/(TN+FP) \times 100\%$, where
Se - sensitivity
Sp - specificity
TP (True Positives) - truly positive cases;
TN (True Negatives) - true negative cases;
FN (False Negatives) - positive cases classified as negative (false negative cases);
FP (False Positives) - negative examples classified as positive (false positive cases).

As a criterion for finding the optimal threshold point (optimal cut-off value) used Judena index (J), which determines the optimal (maximum for a given point) ratio of sensitivity and specificity values [29].

Results and discussion

According to the results of the observation, it was found that in the acute period of meningitis in the newborns of the main group, the course of the disease was characterized by the abrasion of the clinical picture with negative meningeal symptoms and the absence of fever. Clinically, according to the assessment of neurological status in the group of newborns suffering from bacterial meningitis prevailed CNS depression syndrome (92,8%) and convulsions (64,3%).

In the period of convalescence in premature infants with meningitis, the main syndromes were hypertension-hydrocephalic (71,4%) and vegeto-visceral disorders (50,0%).

According to the results of the assessment of neurological status, in the clinical picture of preterm infants of the comparison group also prevailed oppression syndrome (60,0%). Among full-term newborns of the comparison group, the most often syndromes were: hyper-excitability syndrome (60,0%) and hypertension-hydrocephalus syndrome (30,0%).

Taking into account the attrition of the clinical picture of meningitis in premature infants in order to search early diagnostic markers of the disease, we studied the content of glial protein S100 and phospholipase A2 in the blood serum of newborns from observation groups.

Analysis of the results showed increased levels of protein S-100 in infants with perinatal CNS lesions compared with control group (Table 1, Fig. 1).

Table 1. Content of S100 protein and phospholipase A in serum of newborn in observation groups (Me [Q25; Q75])

Characteristics	Study group, n=14		Comparison group, n=20		Control group, n=18
	Acute period	Period of convalescence	Preterm newborns n=10	Full-term newborns n=10	
S100, ng/l	$120,0^{123}$ [107,5; 200,0]	$63,0^{234}$ [58,0; 75,8]	$90,0^{1}$ [85,0; 90,0]	$102,0^{1}$ [92,5; 109,0]	65,0 [60,0; 66,0]
Phospholipase A2, ng/ml,	$3,2^{13}$ [2,4; 4,0]	$3,0^{13}$ [2,0; 3,5]	$2,2^{13}$ [1,8; 2,9]	$5,1^{1}$ [3,95; 5,9]	1,9 [1,8; 2,05]

Note:
[1] – p<0,05 - compared to the control group,
[2] – p<0,05 - compared to premature newborns of the comparison group,
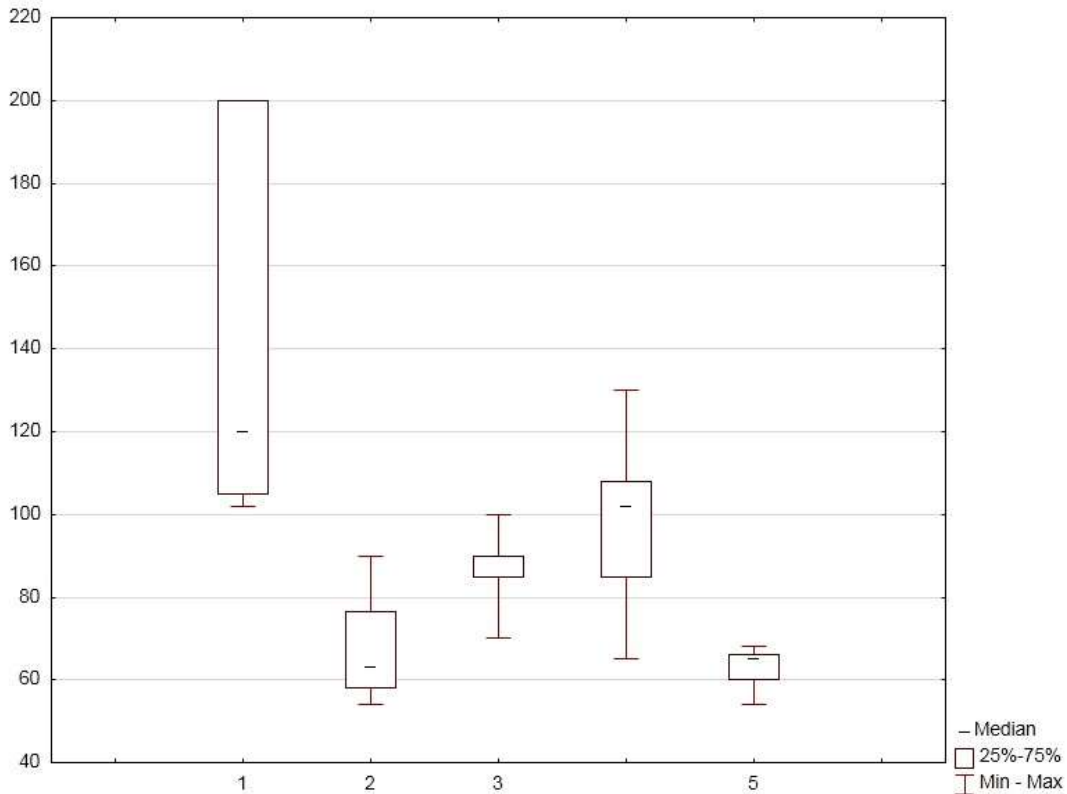[3] – p<0,05 - in comparison with full-term newborns of the comparison group,
[4] – p<0,05 - compared with preterm newborns of study group in acute period

Fig. 1 The content of protein S100 (ng/l) in serum of newborn in observation groups
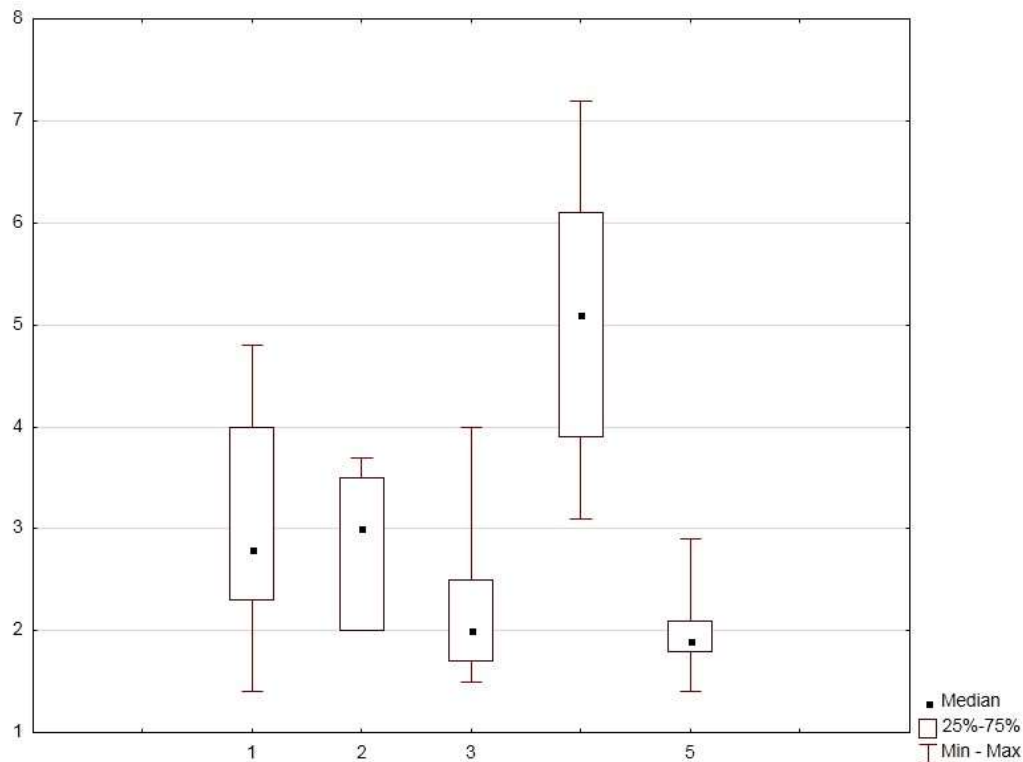
Note:
1 - preterm newborns with meningitis (acute period);
2 - preterm newborns with meningitis (period of convalescence),
3 - preterm newborns with perinatal lesions of the CNS;
4 - full-term newborns with perinatal lesions of CNS;
5 - control group.

It is known that perinatal hypoxia initiates processes leading to increased permeability of cell membranes, death of neurons and glial cells [23, 8], therefore, an increase of S100 protein level in newborns can be considered as a marker of brain damage. Moreover, the highest values of the S100 protein were observed in children after acute asphyxiation, confirmed by the presence of a negative correlation between the S100 protein parameters in the blood serum and the Apgar score ($r=-0,51$, $p <0,05$).

At the same time, the highest levels of S-100 protein in preterm infants were observed in the acute period of meningitis, which was 2,3 times higher than in the control group and 1,5 times higher than in the group of premature infants with perinatal CNS lesions ($p<0,05$). During the period of convalescence of meningitis the protein level of S-100 decreased and was not statistically different from that of the control group ($p>0,05$).

Analysis of the obtained phospholipase A2 level in the serum of newborns in the comparison group, depending on the gestational age, found that in full-term infants its content was 2,5 times higher than in children of the control group, while among premature infants with perinatal lesions of CNS observed no significant increase of phospholipase A2 content in serum (Table 1, Fig. 2).

Fig. 2 The content of phospholipase A2 (ng/ml) in serum of newborn in observation groups

Note:
1 - preterm newborns with meningitis (acute period);
2 - preterm newborns with meningitis (period of convalescence),
3 - preterm newborns with perinatal lesions of the CNS;
4 - full-term newborns with perinatal lesions of CNS;
5 - control group.

During the development of premature meningitis, although there was an increase in the production of phospholipase A2, the level of which was significantly higher than in the control group ($p<0,05$), but its indicators did not reach the values of the term infants of the comparison group ($p<0,05$). At the same time, in the period of convalescence of meningitis, unlike the content of protein S100, there was not sufficient reduction of phospholipase A2, which may indicate the persistence of lipid peroxidation and phospholipase A2, in particular, even when the inflammatory process was reduced.

Considering the initially different concentration of S100 protein and phospholipase A2 in the serum of preterm newborns in the acute period of meningitis and in preterm newborns with perinatal CNS lesions of non-infectious genesis, we analyzed these indicators as an independent diagnostic factors.

It was found that the area under the ROC curve for the S100 protein level was found to be 0,888±0,06 (CI 95% 0,771-1,0, p<0,05) with model sensitivity of 85,7% and specificity of 75,0% , which corresponds to a good diagnostic accuracy of the test (Fig. 3).

AUC=0,888±0,06
[0,771-1,0]
Cut-point 104,1 ng/l
Sensitivity 85,7%
Specificity 75,0%
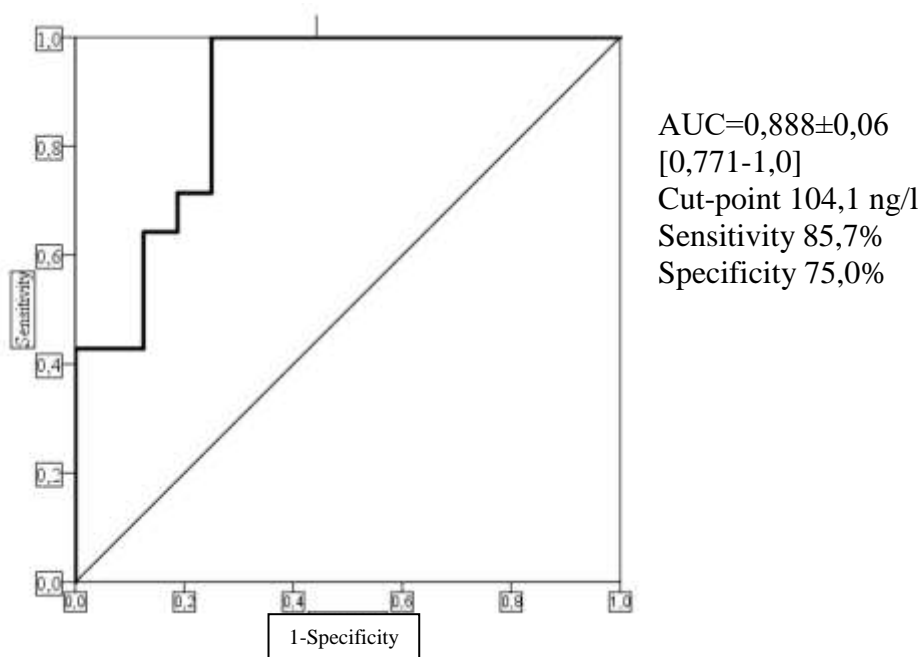
Fig. 3 ROC curve of serum S100 protein diagnostic value in meningitis in premature newborns

The optimum cut-point was 104,1 ng/l. That is, in premature infants, increased S100 protein serum level above 104,1 ng/l was associated with the development of meningitis. Confirmation of the above is the fact that against the background of antibiotic therapy during the further study, the level of S100 significantly decreased.

At the same time, according to the ROC analysis, the diagnostic value of serum phospholipase A2 in preterm infants with meningitis was in the range of unsatisfactory information content (AUC=0,533±0,128, 95% CI 0,702-0,962, p>0,05) with a sensitivity of 53,3% and specificity of 40,0%, which does not allow the use of this indicator as an additional diagnostic criterion for meningitis (Fig. 4).

AUC=0,533±0,128
[0,283-0,784]
Cut-point 4,1
Sensitivity 53,3%
Specificity – 40,0%
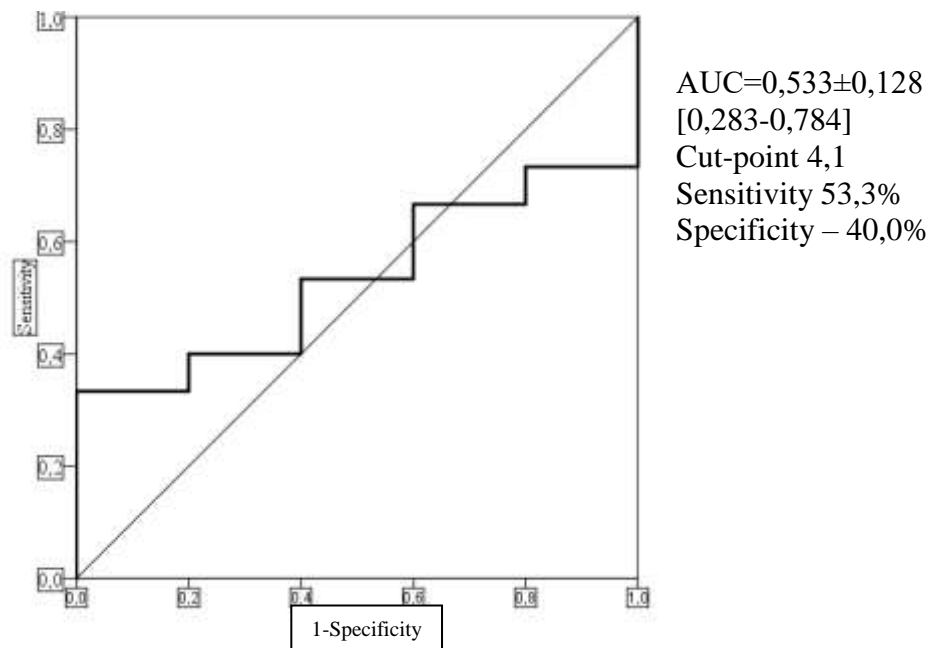
Fig. 4 ROC-curve of the diagnostic value of determining serum phospholipase A2 in meningitis in premature newborns

Subsequent ROC analysis showed that the determination of phospholipase A2 can be used as a diagnostic criterion for perinatal damage of the central nervous system, regardless of the cause of its occurrence, it once again confirms that activation of phospholipase A2 in newborns occurs in response to various damaging factors.

Figure 5 demonstrates a graph of the ROC curve for serum phospholipase A2 in the study group of children with perinatal CNS lesions, the area under which was 0,832±0,066 (95% CI 0,702-0,962, $p < 0,05$), which indicates a good quality of model.

Discussion

Meningitis is a life-threatening disease affecting 0,1–0,4 newborns per 1000 live births, with a higher incidence in premature babies [17]. Every tenth infant dies from meningitis, and up to half of the survivors have serious life-long complications, including convulsions, hearing and visual impairment, and psychomotor retardation [9].
One of the main links in the pathogenesis of fetal and neonatal diseases, including meningitis, is oxidative stress [2, 19]. oxygen deficiency affects all organs and tissues of the body, but the most sensitive to hypoxia is the brain, which is called the "critical organ" [24].

AUC=0,832±0,066
[0,702-0,962]
Cut-point of 2,1
Sensitivity – 79,2%
Specificity – 78,6%
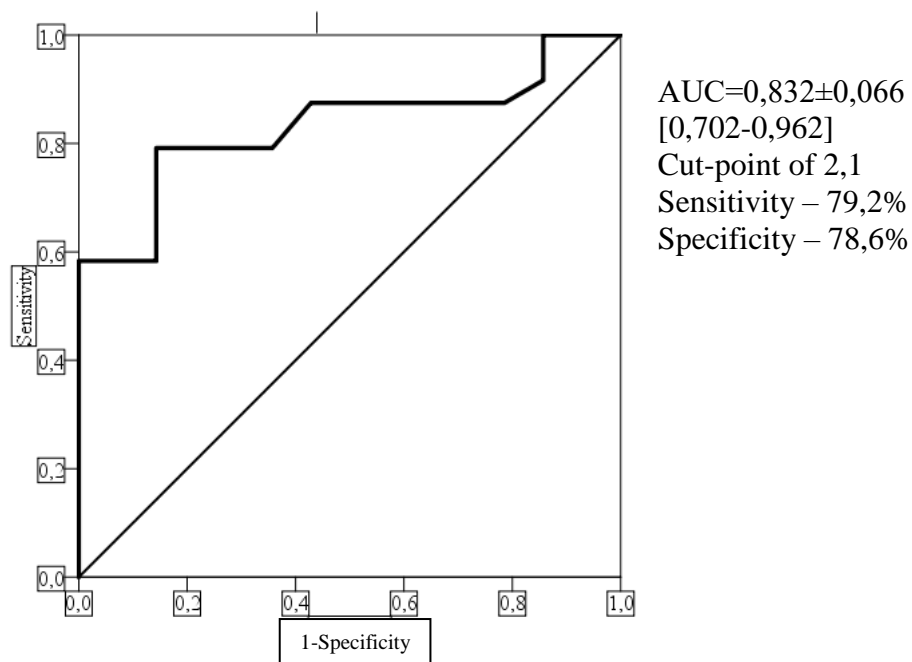
Fig. 5 ROC-curve of the diagnostic value of phospholipase A2 serum level in newborns with perinatal CNS lesions

Protein S100, involved in numerous intra- and extracellular processes, including enhancing the survival of neuronal cells and regulates energy metabolism seen as an antihypoxant [15]. In addition, at low concentrations, S100β exhibits neuroprotective properties, blocking NMDA receptors and acting as a growth and differentiation factor for neurons and glia, while at high concentrations it triggers the synthesis of pro-inflammatory cytokines and leads to apoptosis of neurons [28]. It has been experimentally proved that increasing the concentration of S-100 can lead to generalization of inflammation, neurodegeneration and the release of other neurotoxic molecules [13]. On the other hand, it is possible that increased S-100 expression during injury can contribute to sanogenesis, repair of damaged neurons, and increase resistance to possible subsequent damage [16].

That is, in premature newborns with meningitis, increased serum level of S100 protein in the acute period of the disease, on the one hand, can contribute to a more severe course of the inflammatory process, and on the other, it can be a protective-compensatory reaction aimed at restoring damaged brain structures. It seems important that with a reducing of in the inflammatory process, that is, in the stage of convalescence of meningitis, this protein decreases to the level of healthy full-term newborns.

In our opinion, taking into account the obtained findings and the results of the ROC analysis, the determination of the S100 protein in the blood serum of prema-

ture newborns can be used as an additional biochemical marker both for making diagnosis of meningitis and for monitoring the effectiveness of therapeutic measures. According to modern concepts, hypoxia of any origin realizes its pathophysiological effect by changing the structure and function of cell membranes. At the same time the mechanisms of cerebral ischemia in term and preterm newborns relatively different and have their own characteristics. In full-term newborns who have undergone hypoxia, the formation of cerebral ischemia is the result of a cascade of pathobiochemical reactions due to the development of energy deficiency and metabolic acidemia. But in premature infants, a high level of brain damage is associated primarily with its immaturity, features of vascularization, increased capillary permeability and the dependence of cerebral blood flow on general hemodynamics [26].

Nevertheless, regardless of the type of hypoxia, it is based on the insufficiency of the cellular energy-generating system of mitochondrial oxidative phosphorylation, which leads to metabolic and structural changes in various organs and tissues, accumulation of lactate, development of acidosis, which activates some phospholipases and proteases, including phospholipase A2 [24]. Activation of phospholipase A2, in turn, causes the breakdown of phospholipids and other lipid-containing cell structures with the formation of a large amount of FFA, which enhances tissue acidosis [22]. At the same time, the products of the hydrolysis of phospholipids with phospholipase A2 (lysophosphatidylcholine and arachidonic acid) can participate directly or indirectly in the synthesis of a significant number of various biologically active substances with a pro-inflammatory character - prostaglandins, thromboxanes, leukotrienes [1]. Lysophosphatidylcholine has chemoattractive properties for circulating monocytes; it is capable of causing lysis in the plasma membrane of endothelial cells, causing their death by type of apoptosis [34].

In the studies of F.M. El-Gendy (2018) has demonstrated that A2 phospholipase acts as a diagnostic and prognostic factor in neonates with sepsis [5]. Our study demonstrated that an increased level of phospholipase A2 was detected not only in newborns with meningitis, but also in children with perinatal CNS lesions of non-infectious genesis. It should be noted that in newborns with meningitis during the period of convalescence there was no proper decrease in serum phospholipase A2, that reflects not only continuing activity of lipid peroxidation, but also indicates development of neurodegeneration [6]. The highest levels of phospholipase A2 were observed in full-term infants with perinatal lesions of the central nervous system, which reflects the activity of lipid peroxidation. Moreover, the content of phospholipase A2 in the blood serum of preterm newborns of both the study group and the comparison group exceeded the values of the control group, but were significantly lower than in full-term newborns with perinatal CNS lesions (p <0,05). Detected lower concentrations of this marker in preterm newborns with CNS pathology compared to full-term ones, in our view, may be due to the use of a number of drugs that inhibit the synthesis of this enzyme, including glucocorticosteroids, in the treatment of premature newborns [31]. Thus, an incre-

ase in serum phospholipase A2 activity was not a specific diagnostic marker of meningitis in premature newborns, but reflected processes of damage to the central nervous system regardless of the etiology of the disease.

Conclusions:

1. The indicator of protein S100 content in serum of preterm newborns has a diagnostic value for assessing the risk of meningitis. With its value equal to 104,1 ng/l or more, meningitis is diagnosed with a sensitivity of 85,7% and a specificity of 75,0%.
2. Increase phospholipase A2 levels in the serum of newborns is a non-specific diagnostic marker of perinatal damage to the central nervous system. The degree of expression in the serum content of phospholipase A2 in newborns with CNS pathology depends on the gestational age and is not associated with the development of meningitis.

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