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PREDICTION OF UNFAVORABLE COURSE OF BRONCHOPULMONARY DYSPLASIA IN CHILDREN AT THE PRESENT STAGE

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Abstract. *The article provides the results of vasoconstrictive and adaptogenic proteinases level analysis in children with bronchopulmonary dysplasia. It was shown that proteinase-proteinase inhibitor system plays a significant role in the development of cardiovascular complications in bronchopulmonary dysplasia and it can be regarded as markers of unfavorable course of bronchopulmonary dysplasia.*
Key words: *bronchopulmonary dysplasia, unfavorable course, components of proteinase-proteinase inhibitor system.*

With the development of the technologies in special care nursery and respiratory support of premature newborns there has been noted a reduction in mortality along with an increase in frequency of bronchopulmonary dysplasia (BPD) in children [1, 2, 3].

One of the most important conditions which determine peculiarity of pathologic processes in the lungs of newborns is that they develop in one of the most critical period of a child's life during rearrangement of functional systems, first of all, respiration and blood circulation and their gradual maturation [4]. Over the period of many years bronchopulmonary dysplasia was considered to be a condition which affects newborns, premature newborns in particular. Subsequently it has been shown that the significance of BPD falls beyond the scope of neonatology [1, 5].

Due to close morphofunctional interrelation, obstructive or restrictive changes in chronic diseases of the respiratory system lead to early disorders of cardiopulmonary ratio, which result in hypoxic pulmonary vasoconstriction, mechanic narrowing of vessels and obstruction of vascular pulmonary bed, which in

its turn result in pulmonary hypertension and development of chronic cardiac insufficiency [6]. It is critical for the disease prognosis both in children and in adults.

The search for the means which could inhibit the progression of chronic pulmonary disease is at the current moment one of the main problems of pulmonology and children pulmonology in particular. That is why the search for more sensitive, delicate markers of further damage of cells and gradual decrease in lung function is still in progress. Due to this the scientists' interest to proteinases has been growing recently as its level determination is of significant clinicodiagnostic information value in many pathologic processes [7, 8].

The aim is to determine the activity of proteinases of vasoconstrictive and adaptogenic action and their participation in the development of unfavorable course of bronchopulmonary dysplasia in children.

Materials and methods. The research was carried out at the department of Pediatrics No.1and Neonatology of KhNMU (the head of the department is Doctor of Medical Science, professor G. S. Senatorova) in the Regional Center for diagnostics and treatment of bronchopulmonary dysplasia in children Public Health Care Institution "Kharkov Regional Clinical Children Hospital" (head doctor – Candidate of Medical Science, associate professor G. R. Muratov; administrative manager – Candidate of Medical Science O. L. Logvinova).

60 children aged from 1 month to 3 years were examined, among them 29 patients were found to have classical form of BPD (1^{st} group), 16 patients had new form of BPD (2^{nd} group), 15 patients had BPD of mature newborns (3^{rd} group). Bronchopulmonary dysplasia was diagnosed according to international classification of diseases 10^{th} edition (code P27.0). Severity criteria were determined according to classification of clinical forms of bronchopulmonary diseases in children of Russian Respiratory Society (2009) [9].

The employment of high-sensitivity (10^{-9} – 10^{-10} g) enzyme method, which was elaborated in State Institution «L. T. Malaya Institute of Therapy at the Academy of Medical Science of Ukraine» (L. M. Samokhina, 1997, 2001, 2002, 2004) [10] allowed to study trypsin-inhibitory activity of α-1-inhibitor of proteinases (TIA α-1-

IP) and the activity of proteinases of vasoconstrictive action (non-trypsin-like proteinases (NTLP), chymase, tonin) and adaptogenic action (calpain) in blood serum, and also of their inhibitors – α-2-macroglobulin (α-2-MG) and trypsin-inhibitory activity. The research was carried out according to ethical principles of medical human trials stipulated by the Declaration of Helsinki.

Statistical processing of the received data was carried out with the help of statistical package of the program Statistica 7.0. Median (Me) and interquartile range (Lq – lower quartile; Uq – upper quartile) were determined for samples with distribution which doesn't correspond to normal law of errors. Non-parametric Mann-Whitney U-test (MW) was used to compare two samples. The difference in values, which was compared by two points, was considered to be statistically significant in $p < 0,05$. Kruskal–Wallis variance analysis was used in correlation of criteria which were characterized by comparison of more than 2 points and discrepancies were considered probable on the basis of Bonferonni adjustment (in p^=p/k, where k is the number of paired comparisons).

Results and their discussion. During the analysis of vasoconstrictive proteinases changes in the 1st group children it was determined that NTLP and tonin activity didn't significantly differ from the indices in healthy children (all $p>0,05$), however chymase activity was definitely more significant in comparison with the control group ($p<0,05$) (Table 1). The significant increase in α-2-MG level ($p<0,01$) against the background of absence of changes in TIA α-1-IP ($p>0,05$) activity can be regarded as protection, in particular, from an increase in chymase activity as this inhibitor plays a more important role in chymase inhibition than α-1-IP [7]. The presence of statistically significant positive correlation relationship between α-2-MG level and activity of tonin ($r = + 0,43$, $p=0,017$) and chymase ($r= + 0,78$, $p=0,0000$) against the background of absence of an increase in tonin level can be indicative of a sufficient role of this inhibitor in down-regulation of the specified vasoconstrictive enzyme. An increase in chymase activity in blood serum of children with classic form of BPD demonstrates the activation of tissue pathway of angiotensin II formation,

which can contribute to the development of vasoconstrictive mechanisms of formation and progression of cardiovascular complications [7].

Table 1

Activity of vasoconstrictive proteinases in the blood serum of the examined children

Index	1st group (n=29)	2nd group (n=16)	3rd group (n=15)	Control group (n=12)
	Me (Lq; Uq)	Me (Lq; Uq)	Me (Lq; Uq)	Me (Lq; Uq)
NTLP, mg/l/h.	0,095 (0,060; 0,190)	0,090 (0,071; 0,155)	0,070 (0,055; 0,160)	0,110 (0,090; 0,145)
Tonin, E mcM substr/min.	0,594 (0,078; 2,673)	2,231 (0,259; 3,426)	0,344 (0,000; 2,409)^	2,012 (1,175; 3,107)
Chymase, $Ex10^{-3}$nM substr/min	3,104 (0,753; 4,264)^	2,184 (1,384; 2,902)	0,000 (0,000; 0,001)	0,851 (0,047;2,869)
Calpains, g/l h.	0,107 (0,024; 0,253)^^	0,308 (0,101; 0,508)	0,063 (0,004; 0,354)	0,238 (0,161; 0,290)
α-2-MG, g/l h	0,25 (0,22; 0,43)^^	0,30 (0,23; 0,49)^^	0,28 (0,20; 0,30)	0,20 (0,17; 0,29)
TIA α-1-IP, g/l h	7,75 (7,30; 7,90)	7,65 (7,32; 7,71)	7,80 (7,30; 7,89)	7,67 (7,53; 7,83)

Note. ^ - in comparison with control $p<0,05$; ^^ - in comparison with control $p<0,01$

Blood serum of the 2nd group children demonstrated a statistically significant increase in only α-2-MG ($p<0,01$) level. The increase in α-2-MG activity, absence of an increase in TIA α-1-IP in blood serum of children with the new form of BPD, presence of statistically significant positive correlation relationship between α-2-MG and NTLP ($r= + 0,48$, $p=0,05$) and chymase ($r= + 0,79$, $p=0,0002$) levels in blood serum leaves open the possibility of inhibitors participation in down-regulation of excessive proteinase activity.

The 3rd group children demonstrated a statistically significant decrease in tonin level ($p<0,05$), a vasoconstrictive proteinase which takes part in alternative route of angiotensin II formation directly of angiotensin, without substantial changes

in the level of inhibitors as comped to the control (all p>0,05). There has also been determined a statistically significant positive correlation relationship between the activity of chymase and α-2-MG (r = +0,50; p=0,05) which, against the background of absence of an increase in vasoconstrictive proteinases activity, can be indicative of a sufficient participation of this inhibitor in down-regulation of vasoconstrictive enzymes. Besides, there has been detected a statistically significant negative correlation relationship between the activity of α-2-MG and TIA α-1-IP (r = - 0,53; p=0,05). Such a character of changes in proteinase inhibitors contributes to proteolysis inhibition, especially with the participation of trypsin-like enzymes. That is, in full-term children with BPD α-2-MG inhibits proteolysis in great measure without having to involve α-1-IP, which is indicative of natural protection of the organism from proteolysis activation. The absence of an increase in this inhibitor activity reflects a higher possibility of NTLP participation in vasoconstrictive effects development. It is confirmed by statistically significant strong positive correlation relationship between the level of NTLP and the activity of chymase (r = + 0,73; p=0,001),which can suggest that the specified proteinases were spent on angiotensin II formation and that the possibility of their synthesis was depleted and/or tissue vasoconstrictive effects developed and released due to prolonged formation of pathologic process.

Multiple comparison of the statistical characteristics of proteinases and their inhibitors activity determined that Kruskall-Wallis test was highly significant by such indices as chymase (H = 9,09; p=0,0280) and α-2-MG (H = 8,61; p=0,0349). This provides evidence that statistical characteristics of the corresponding indices of the different groups fairly differ against each other and chymase and α-2-MG activity level in blood serum depends on the group to which the patients belong.

Multiple logistic regression analysis was performed to determine which factors should certainly be considered when predicting complications and unfavorable outcome of BPD. Clinical material of 63patients has been randomly selected to develop a mathematical model for the prediction of BPD outcome in

children, among them 27 patients were found to have favorable BPD course; its criteria were chosen as follows: prolonged remission of the disease, absence of severe accompanying abnormalities and satisfactory life quality. 36 patients were found to have unfavorable BPD course, its criteria were chosen as follows: presence of severe accompanying abnormalities, frequent relapses of pneumonia or obstructive bronchitis, disability or untimely death.

Favorable (y=1) or unfavorable BPD outcome (y=0) was correspondingly chosen as binary dependent variable (y). Both quantitative and qualitative factors were chosen as independent variables. Duration of artificial lung ventilation, gestation term, body mass at birth, activity of proteinase-proteinase inhibitor system components, blood pH level, CO_2 partial pressure, mean pulmonary arterial pressure and heartbeat rate were chosen to be quantitative factors. Clinical and anamnestic data, namely perinatal anamnesis and physical examination data, presence of accompanying abnormality, duration of the disease were considered to be independent qualitative variables. Every qualitative factor was coded as “1” if the child was noted to have this factor or “0” if this factor was not detected. Statistical significance of the obtained results (possibility that the patient will be included in the certain study group according to the factor being estimated) was determined by Wald criterion (the higher the module of this criterion (coefficient), the stronger its influence on the dependent variable). The quality of the developed model was checked by percent concordant (PC). This index is equal to the part of observations which were correctly reclassified to separate groups of dependent factors by logistic regression equation. The closer this factor to 100%, the higher the quality of the obtained model. The possibility of event development for certain case was calculated by the formula:

$$P = \frac{1}{1 + e^{Z}},$$

where $z = a + b_1 * X_1 + b_2 * X_2 + ... + b_n * X_n$,

X_1, X_2, X_n — independent variables values, b_1, b_2, b_n — coefficients which should be calculated by binary logistic regression, a — certain constant. $p>0,5$ is indicative of a significant possibility that the event will take place (y), in $p<0,5$ this possibility is insignificant.

The procedure of multiple logistic regression allowed to separate a group of factors, which can help to predict BPD outcome with a sufficient level of statistical significance. The results of statistical analysis of multiple logistic regression are summarized in Table 2.

Table 2

Statistical characteristics of multiple logistic regression of factors, which are potentially able to influence bronchopulmonary dysplasia outcome in children

Variable	Coefficient	Standard error	Wald criterion	Statistical significance, p	Confidence range 95% for correlation of chances
Constant	-178,894	75,30	5,643	0,01	[1,610; 2,556]
Duration of artificial lung ventilation	- 0,0819	0,04	3,846	0,05	[0,849; 1,000]
Gestation term	0,254	0,18	1,870	0,05	[0,896; 1,855]
Body mass at birth	-0,00204	0,001	3,614	0,049	[0,996; 1,000]
α-2MG activity	-10,614	5,51	3,710	0,048	[0,000005; 1,205]
Tonin activity	1,821	0,75	5,839	0,01	[1,410; 27,036]
Calpains activity	-5,816	2,84	4,171	0,044	[0,00004; 0,791]
Blood pH	24,105	9,93	5,887	0,01	[215,337; 1,084]
pCO_2	0,142	0,05	6,227	0,01	[1,031; 1,289]
Heartbeat rate	-0,0342	0,01	3,325	0,047	[0,931; 1,003]
P Pulmonary artery	-0,0745	0,06	1,292	0,043	[0,816; 1,055]

The quality of the developed model was checked by percent concordant (presenting factors of belonging to a subgroup (1=favorable course, 0=unfavorable course) were matched and stipulated on the basis of the developed model)).

The results of the performed analysis allowed to develop multiple regression equation:

$$\text{Logit P (z)} = -178{,}894 - (0{,}0819 * \text{duration of ALV}) + (0{,}254 * \text{gestation term}) - (0{,}00204 * \text{body mass at birth}) - (10{,}614 * \alpha\text{-2MG}) + (1{,}821 * \text{tonin}) - (5{,}816 * \text{calpains}) + (24{,}105 * \text{blood pH}) + (0{,}142 * pCO_2) - (0{,}0342 * \text{heartbeat}) - (0{,}0745 * \text{P pulmonary artery}).$$

Specified mathematical models have 94,8% of specificity and 92,2% of sensitivity.

It is commonly known that BPD outcome can be influenced by a number of factors, among them is, first of all, immaturity of lung tissue, gestation term and low body mass at birth in particular. This, in its turn, results in inability of proper spontaneous respiration and children require long-term artificial lung ventilation. An increase in calpains activity in blood serum of patients with BPD can be connected with a transition to the development of structural and functional changes in lung tissue or vessels. Imbalance between proteinases of adaptogenic action and their inhibitors creates conditions for intensification of destructive processes providing other proteinases are involved and this creates conditions for pathologic process progression. The development of vasoconstrictive mechanisms of the formation and progression of cardiovascular complications in children with BPD can be associated with an increased tonin activity. An increased level of mean pulmonary artery pressure can be considered to be a manifestation of secondary pulmonary hypertension, which plays an important role in fast development of cardiovascular complications. The cause of acceleration of cardiovascular disorder development and its progression in patients with BPD can be conditioned by tissue hypoxia, which promotes activation of vasoconstrictive factors and apoptosis which by all means

should be considered when planning individual clinical monitoring for children with BPD.

Conclusion.

1. Children with bronchopulmonary dysplasia have been found to have presenting imbalance of proteinase-proteinases inhibitor system, which at the first stages, performing the “program of natural protection from excessive proteolysis activity”, creates structural (histologic) conditions for further intensification of pathologic process.

2. Such constituents as the duration of artificial lung ventilation, gestation term and body mass at birth, activity of proteinase-proteinases inhibitors system components, acid-base balance of blood, heartbeat rate and mean pulmonary artery pressure influence the prediction of outcome. It is possible to draw a conclusion that it could be expedient to use these factors for comprehensive estimation when predicting favorable outcome of BPD.

The study of proteinase-proteinases inhibitor system in children with bronchopulmonary dysplasia is of great potential for prevention of complications and unfavorable course, which should be considered at clinical management of this patient population.

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