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Primary hemostatic system condition in mucoviscidosis in children

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The article presents results of a study of platelet hemostasis in children with mucoviscidosis. Motionless and viscous mucus becomes infected with various pathogenic and opportunistic microbes in the setting of disturbed mucociliary clearance; when inflammation becomes chronic, endothelial dysfunction and platelet aggregation disorder may provoke development of bloodstream complications. Early diagnostics of pathologic changes in primary hemostasis is important for therapy and disease prognosis.
***Key words**: platelet aggregate function, mucoviscidosis, inflammation, hemostasis, children.*

According to the genetic trials, the spread of mucoviscidosis in Russia is ca. 1:10,000 neonates [1]. Mucoviscidosis (MV) is a monogenic disease caused by gene *CFTR* (cystic fibrosis transmembrane conductance regulator) mutation characterized by lesions of all exocrine glands of vital organs. Pathogenesis of lesions of different organs and systems at MV is associated with hyperviscosity secretion by mucinous glands, whereas mucociliary clearance disturbance contributes to pathogenic flora growth and development of recurrent chronic respiratory tract infections. Such bacteria as *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia* cause the severest and the most prognostically unfavorable course of chronic bronchopulmonary infection in patients with mucoviscidosis. The inflammation process always affects microvasculature [2, 3]. High vascular penetrability in the inflammation focus, rapid increase in the number of immunocompetent cells and general of various anti-inflammatory cytokines thereby comprise the list of the main phenomena discussed by the scientists studying inflammatory processes. A range of pathological alterations at inflammation also affects pulmonary vascular endothelium, which, in its turn, inactivates serotonin, prostaglandins and bradykinin and synthesizes a range of blood coagulation and anticoagulation system factors. Endothelial dysfunction may later lead to development of vascular complications. It is common knowledge that patients with mucoviscidosis often develop pulmonary hemorrhages and recurrent emptysis, which aggravate prognosis for the disease [4-8]. Study of thrombocytic hemostasis may, in our view, may help to diagnose microvascular disorders early and correct them in time.
Research objective: analysis of thrombocytic hemostasis changes at mucoviscidosis in children.

PATIENTS AND METHODS

We examined 85 children (45 – with mucoviscidosis) from 8 months to 17 years of age (7.57±4.66). The average Shwachman-Brasfield score was 52.3+1.1. Diagnosis "Mucoviscidosis" was verified with positive sweat tests and confirmed by the genetic trial results. All patients were examined in the period of bronchopulmonary process exacerbation

(cough intensification, abundance of diverse rales and viscous purulent sputum, negative X-ray pattern dynamics) on the basis whereof they underwent the baseline therapy: antibiotics (generations III and IV cephalosporins, carbapenems) in the maximum doses, N-acetylcysteine and ambroxol hydrochloride drugs and dornase alfa (mucolytic with anti-inflammatory properties). The control group was comprised of 40 virtually healthy children of the same age undergoing medical examination at the consultative-diagnostic center of the Scientific Center of Children's Health.

All patients underwent blood tests with stabilized ethylenediaminetetraacetic acid (EDTA) on hematology analyzer Sysmex 200i (Japan). Along with standard general blood analysis parameters, we determined thrombocytic indices: PDW – relative platelet distribution width by volume (this index shows heterogeneity of platelets by size (anisocytosis degree)); MPV – mean platelet volume; P-LCR – platelet large cell ratio (out of all platelets); PCT – platelet crit (this parameter shows the share of whole blood volume occupied by platelets. We studied whole blood platelet aggregation in children with aggregometer Multiplate – Verum Diagnostics (VD, Germany). We used adenosine diphosphoric acid (ADP) solution, soluble thrombin receptor activators for peptide 6 (TRAP test) and arachidonic acid (ASPI test) as aggregation inductors. We determined C-reactive protein (CRP) level in blood serum turbidimetrically with biochemical analyzer UniCel DxC 600 (BD, USA). We determined the level of the main anti-inflammatory cytokine – interleukin (IL) 6 immunochemiluminometrically with analyzer Accesses 2 (BD, USA). We used non-parametric variance analysis methods for statistical data manipulation and Mann-Whitney test for group comparison (program SPSS 20.0). Analysis of dependences was performed with Spearman's rank correlation coefficient.

STUDY RESULTS

Analysis of primary hemostasis parameters revealed statistically significant (in comparison with virtually healthy children) platelet aggregation malfunctions in 2/3 of the patients with MV. Aggregation analysis with ADP and arachidonic acid revealed tendency to platelet hypoaggregation in 40% of the patients. Analysis of platelet surface thrombin receptors' condition revealed increase in rate and degree of thrombin-induced aggregation in 22% of the children with MV. Complex platelet aggregation malfunctions were revealed in 1/3 of the patients. The best marked platelet function changes were thrombin-induced hyperaggregation and ADP-hypoaggregation in patients with MV in the period of bronchopulmonary process exacerbation characterized by increase in the blood serum IL 6 level (pic. 1). Hyperaggregation with thrombin receptor activator (pic. 2) in children with MV may indicate primary hemostasis disorders with tendency to thrombogenesis. Hypoaggregation with ADP in children with MV demonstrates poor reaction of platelet ADP release; this indicates reduced platelet aggregation capacity and risk of hemorrhage (pic. 3). We revealed direct correlation between the following tests: TRAP and IL 6 ($r = 0.76$, $p < 0.05$); and moderate negative correlation between platelet aggregation with ADP and IL 6 ($r = -0.57$, $p < 0.05$).

Along with platelet aggregation activity parameters, we analyzed parameters of leukocyte and platelet counts, thrombocytic indices and levels of inflammatory markers CRP and IL 6 (in blood serum). We revealed statistically significant differences between two platelet aggregation tests in patients with MV: TRAP test and ADP; and in the levels of IL 6 and CRP ($p < 0.05$) (tb. 1). Parameters of thrombocytic indices, platelet count and platelet aggregation test with arachidonic acid were not significantly different from the parameters of healthy children ($p > 0.05$).

We analyzed ROC-curves (receiver operating characteristic) at a later stage. Quantitative ROC analysis interpretation in AUC (area under ROC-curve) characterizes clinical relevance of tests (tb. 2).

CONCLUSION

Platelet aggregation function changes in the setting of mucoviscidosis exacerbation may manifest themselves with platelet activation with further hyperaggregation and thrombogenesis and disturbed arachidonic acid and ADP metabolism, which may result in development of hemorrhagic complications. Primary hemostasis disorders in children with MV require further research with other aggregation inductors in order to prevent development of hemorrhagic and thrombotic complications and provide adequate therapeutic correction of primary hemostasis disorders.

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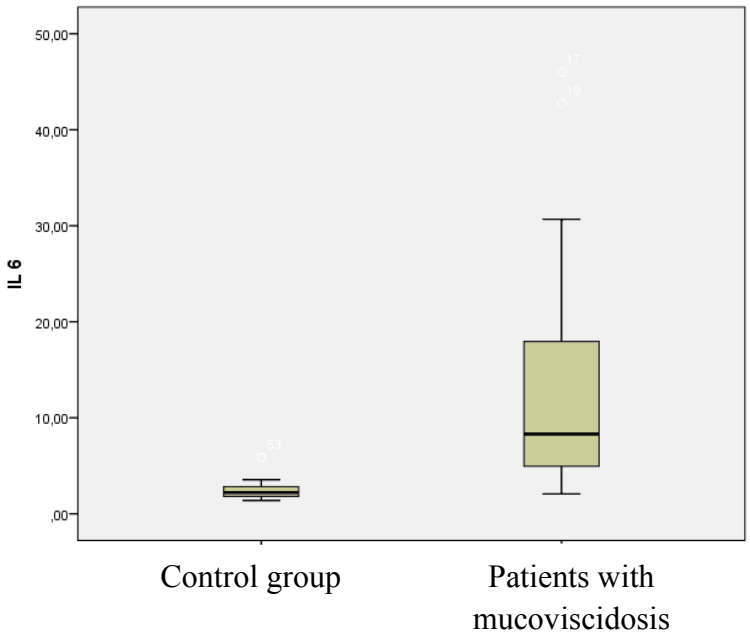
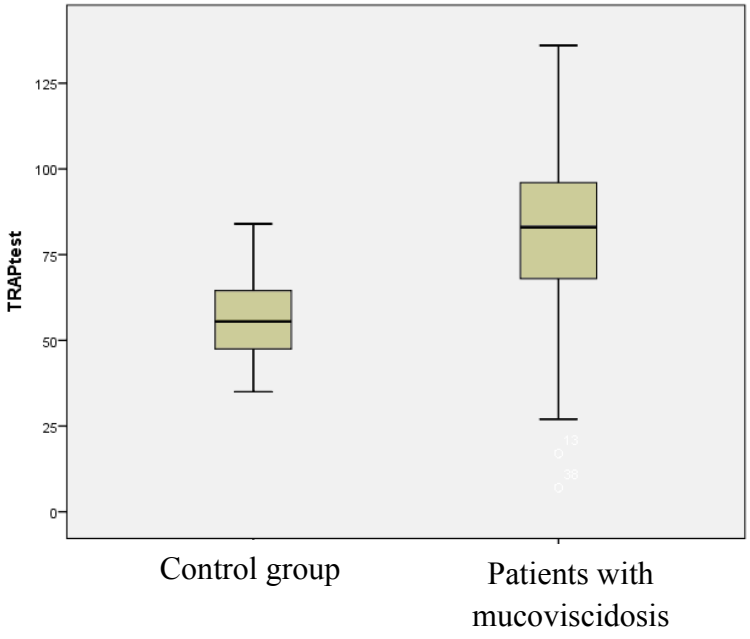
Pic. 1. Serum Il 6 (pg/ml) values



Pic. 2. Platelet aggregation with thrombin receptor activator (TRAP test)



Pic. 3. Platelet aggregation with adenosine diphosphoric acid (ADP test)

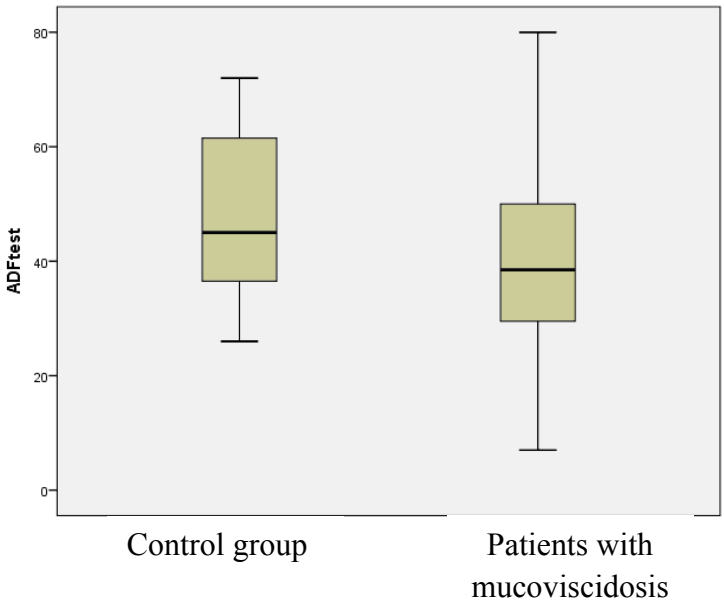


Table 1. Medians of blood parameters (confidence interval 95%)

Laboratory blood parameters	**Children with mucoviscidosis. n=45**	**Virtually healthy children. n=40**	***p***
TRAP test (U)	83 (8.5−132.85)	55 (35−82.7)	**0.001**
ASPI test (U)	42.5 (10−102.35)	51.5 (31−80.75)	0.235
ADP test (U)	38.5 (7.25−80)	45 (26−71.3)	**0.042**
IL 6 (pg/ml)	8.3 (2.07−45.18)	2.2 (1.38−4.59)	**0.001**
C-reactive protein (mg/l)	0.1 (0.1−27.36)	0.1 (0.1−1.28)	**0.011**
Leukocytes ($x10^9$/l)	7.91 (3.41−14.85)	8.2 (4−11.7)	0.973

Platelets (x10^9/l)	342 (139−549)	322 (204−527.4)	0.189
MPV	9.3 (8.3−10.7)	9.5 (8.2−11.2)	0.469
P-LCR (%)	20.7 (13.5−30.9)	21.8 (12.3−35.3)	0.715
PCT (%)	0.29 (0.21−0.44)	0.3 (0.22−0.48)	0.678

Table 2. Parameters of clinical relevance of tests (ROC-analysis)

Test variables	**Area under curve (AUC)**	**Standard deviation**	**Asymptotic confidence interval 95%**	
			Lower limit	**Upper limit**
C-reactive protein	**0.726**	0.109	0.512	0.940
IL 6	**0.908**	0.061	0.789	1.000
TRAP test	**0.812**	0.107	0.602	1.000
ASPI test	0.522	0.137	0.253	0.791
ADP test	0.330	0.094	0.146	0.514