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INFLUENCE OF CONTROLLING THE COURSE OF BRONCHIAL ASTHMA ON BIOPHYSICAL CHARACTERISTICS OF ERYTROCYTES WITH LONG-TERM DYNAMIC OBSERVATION

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INFLUENCE OF CONTROLLING THE COURSE OF BRONCHIAL ASTHMA ON BIOPHYSICAL CHARACTERISTICS OF ERYTROCYTES

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Summary. *The aim* of the work: to study and evaluate the influence of the control of the course of bronchial asthma on the biophysical characteristics of red blood cells.
Materials and methods: 165 patients with asthma in the phase of remission of the disease were examined. The diagnosis was made according to the medical history, clinical symptoms, parameters of external respiration, reversibility of airway obstruction to bronchodilatators. The selection of patients was conducted in accordance with the Order No. 128 of the Ministry of Health of Ukraine dated March 19, 2007 "On the Approval of Clinical Protocols for the Provision of Medical Aid in the Specialty "Pulmonology". As a control, 30 healthy volunteers were examined, but not those who had a severe, clinically significant pathology. All patients received standard basal therapy of the remission period, including inhaled corticosteroids, as well as a short-acting β_2-agonist to reduce asthma symptoms. In the follow-up, patients were divided into 3 groups: 50 (30.3 ± 3.8) % 60 (37.5 ± 3.5) % — patients with partially controlled asthma and 55 (33.3 ± 3.8) % — with severe uncontrolled asthma. The study of the degree of deformation of erythrocytes was carried out with using the method of boundary dehydration of biological fluids and using the electron microscope "NU 2" of "VEB Carl Zeiss" company with photographic system MRS 60 and using BioVision program, the study of the ventilation function of the lungs was performed in all patients according to the spirographic analysis of the flow-volume curve and general plethysmography of the body on the Master Scope and MasterScreen BodyDiff from Erich Jaeger (Germany). Dynamic observation was conducted for 10 years. Statistical processing of the received data was performed using licensed software products included in the Microsoft Office Professional 2000 software package on the IBM Celeron personal computer in Excel. After 10 years in patients with asthma the most significant change was observed in patients with moderate BA: normocytes decreased (47.1 ± 1.0) %, echinocytes increased (16.8 ± 0.3) %, target cells increased (14.5 ± 0.6) %, the number of generative forms of erythrocytes increased (21.3 ± 1.2) %. In patients with severe asthma level of normocytes decreased (36.7 ± 0.5) % echinocytes increased (18.4 ± 0.8) %, target cells — to (21.8 ± 0.9) %, the number of degenerative erythrocytes increased to (23.0 ± 1.2) %.
Conclusions. As a result of this work, for the first time changes in the morphological structure of red blood cells in patients with bronchial asthma were studied and compared, depending on the control of the course of the disease with standard treatment for a long ten-year dynamic observation. Our studies confirm that the course of the BA is accompanied by a staggering change in the morphological profile of the erythrocytes, depending on the degree of disease control. The compensatory reaction of the part of the red blood in the form of rounding cells in response to the persistence of bronchial obstruction led to a certain extent to the leveling of hypoxic and circulatory shifts in the body, while creating prerequisites for strain, depletion of the erythrocytic system in the regulation of intercellular co-operative interactions, maximally expressed with severe course of asthma. In addition, the erythrocytic membrane is a sensitive factor, indicating that it may be an indicator of the risk of loss control even in the absence of clinical symptoms in the remission of the disease. Taking into account the received data, in the future, it is necessary to improve the methods of diagnosing the risk factors of loss control of asthma in view of the results obtained.
Key words: bronchial asthma in adults, blood rheology, morphological and functional characteristics of red blood cells.

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ВПЛИВ КОНТРОЛЬОВАНОСТІ ПЕРЕБІГУ БРОНХІАЛЬНОЇ АСТМИ НА БІОФІЗИЧНІ ХАРАКТЕРИСТИКИ ЕРИТРОЦИТІВ КРОВІ ПРИ ТРИВАЛОМУ ДИНАМІЧНОМУ СПОСТЕРЕЖЕННІ

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Резюме. *Мета проведеної роботи:* дослідження та оцінка впливу контрольованості перебігу бронхіальної астми (БА) на біофізичні характеристики еритроцитів крові.

Матеріали та методи. Було обстежено 165 хворих на бронхіальну астму в фазі ремісії захворювання. При постановці діагнозу враховувався анамнез, клінічні симптоми, показники функції зовнішнього дихання, Повернення обструкції в пробі з бронхолітиками. Відбір хворих проводився відповідно Наказу № 128 МОЗ України від 19.03.2007 р "Про затвердження клінічних протоколів надання медичної допомоги за спеціальністю" пульмонологія". В якості контролю були обстежені 30 здорових добровольців, а не мали важкої клінічно значущої патології. Всі хворі отримували стандартну базисну терапію періоду ремісії, включала застосування інгаляційної ГКС, а також β2-агоніст короткої дії для зменшення симптомів астми. У процесі спостереження хворі були розділені на 3 групи: 50 (30,3 ± 3,8) % — хворі з контрольованим перебігом захворювання, 60 (37,5 ± 3,5) % — хворі з частково контрольованим перебігом БА і 55 (33,3 ± 3,8) % — із важким не контролював перебігом. Дослідження ступеня деформації еритроцитів крові проводились із використанням методу крайової дегідратації біологічних рідин і за допомогою електронного мікроскопа "NU 2" фірми "VEB Carl Zeiss" з фотосистемою MPS 60 і з застосуванням програми «BioVision». Дослідження вентиляційної функції легень проводилося всім хворим за даними спірограми аналізу кривої "потік-об'єм" форсованого видиху і загальної плетизмографії тіла на апараті «Master Scope» і «MasterScreen BodyDiff» фірми "Erich Jaeger" (Німеччина). Динамічне спостереження проводилося протягом 10 років. Статистична обробка отриманих даних виконувалася за допомогою ліцензійних програмних продуктів, що входять в програмний пакет Microsoft Office Professional 2000 року на персональному комп'ютері IBM Celeron в Excel.

Висновки. В результаті проведеної роботи вперше були досліджені та порівняні зміни морфологічної структури еритроцитів крові у хворих на бронхіальну астму, в залежності від контрольованості перебігу захворювання на тлі стандартного лікування протягом тривалого десятирічного динамічного спостереження. Наші дослідження показали, що перебіг БА супроводжується стадійними змінами морфологічного профілю еритроцитів, в залежності від ступеня контролю захворювання. Компенсаторна реакція з боку червоної крові у вигляді сферуляції клітин у відповідь на персистирування бронхіальної обструкції призводила до нівелювання гіпоксемічних і циркуляторних зрушень в організмі, створюючи при цьому передумови до перенапруги та виснаження еритроцитарної системи в регуляції міжклітинних взаємодій, максимально виражених при тяжкому перебігу БА. Крім того, еритроцитарна мембрана є чутливим фактором та показує, що може бути показником ризику втрати контролю навіть при відсутності клінічної симптоматики в ремісії захворювання. З огляду на отримані дані, в подальшому, необхідно удосконалювати способи діагностики факторів ризику втрати контрольованості БА з урахуванням отриманих результатів.

Ключові слова: бронхіальна астма, біофізичні характеристики еритроцитів крові, контрольованість.

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Bronchial asthma (BA) still remains not only incurable, but also a complexly controlled disease, a real challenge for modern medicine. It is worth recognizing that asthma remains one of the most urgent medical and social problems of the present, due primarily to the extremely high prevalence of this disease. So, according to The Global Asthma Report, there are at least 334 million people affected by asthma, which is about 5 % of the inhabitants of our planet [4].

In clinical practice, the concept of a phenotype and an endotype of BA is being increasingly implemented, which should be taken into account when choosing a treatment regimen to achieve control of the course of the disease. Key characteristics in the selection of certain BA's phenotypes are the clinical picture (age disease onset, triggers, severity, concomitant pathology) and the type of respiratory inflammation (eosinophilic, neutrophilic, or paucigranulocytic.)

All these criteria together may be estimated by so-called cluster analysis. There are 5 clusters of BA in adults, and each patient can be attributed to only one of them. For the determination of clusters, it is necessary to consider both clinical and biological parameters. Among the BA biomarkers used for quite a long time, the content of nitrogen oxide in exhaled air, the number of inflammation cells (eosinophils, neutrophils) in induced sputum, the level of eosinophils and IgE in serum should be noted. Relatively new and interesting bio-

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marker of eosinophilic inflammation is periostin — a component of the extracellular matrix released by respiratory fibroblasts in response to the effects of IL-13 and IL-4 [1].

An endotype is a subtype of a disease characterized by a unique or distinct pathophysiological (pathogenetic) mechanism, which largely determines the response of patients to therapy [16, 18, 19]. Thus, there are:

1) allergic asthma: eosinophilic, Th2-induced inflammation, with glucocorticosteroid (GCS) sensitivity, with anti-IgE sensitivity, with interleukin-5 (IL-5) sensitivity, with anti-IL-4 / IL-13 sensitivity, with sensitivity to allergen-specific immunotherapy (ASIT);

2) endogenous BA: eosinophilic, neutrophilic, associated with autoantibodies / superantigens, with susceptibility to GCS, with resistance to GCS;

3) neutrophilic BA: with the activation of congenital immunity, with increased survival of neutrophils, with resistance to GCS, with anti-oxidant / macrolide susceptibility, with anti-TNF-α sensitivity (tumor necrosis factor-α);

4) aspirin-induced BA: eosinophilic, with a violation of the metabolism of eicosanoids / sensitivity to leukotrienes C4, D4, E4, with sensitivity to GCS, with sensitivity to anti-leukotriene drugs;

5) asthma with respiratory remodeling: with diffuse remodeling, with activation of endothelial-mesenchymal transformation (EMT), etc. [1, 2].

According to GINA criteria (2014), achievement of complete control of asthma implies:

- absence of daily symptoms or occurrence of attacks twice or less per week;
- absence of restriction of physical activity during the day; absence of asthma symptoms, which make you wake up at night;
- absence (or ≤ 2 times a week) of the need for symptomatic treatment;
- normal or close to normal pulmonary function;
- no exacerbations.

However, despite all the advances in modern pharmacotherapy, even in the USA and Western Europe, the level of adequate asthma control is low (only 8 to 30 %), and the percentage of patients with uncontrolled asthma varies from 40 to 67 % in different countries [3].

The role of erythrocytes in the pathogenesis of asthma is actively studied for many years. The huge pathogenetic value of the state of the erythrocytic membrane is shown. The erythrocyte model is generally accepted, well-tested and widely used to study the membrane-receptor characteristics of cells, not only in pulmonology. In essence, the lungs are the largest biological membrane of the body. Its outer surface is represented by the alveolar epithelium and surfactant, and the inner surface of this membrane is lined with endothelial cells of the pulmonary capillaries. The aero-hematic barrier of this membrane is provided by the structural organization of membrane lipids. The cells of the alveolar epithelium and the endothelium of the pulmonary capillaries, as well as the erythrocyte membrane, are among the first to perceive information on changes in external conditions and the state of the internal environment of the organism. Depending on the control of the disease, the patient's body produces a different adaptive response (increased heart rate, cardiac output, respiratory rate and respiratory volume, ejection of catecholamines, cortisol and other biologically active substances, changes in temperature, pH of blood and mucous membranes), actively changes not only microenvironment of alveolocytes, endothelial cells and red blood cells, but also, first of all, the state of their membranes **[5]**.

Thus, the key role in the perception of changes, depending on the controllability of the course of bronchial asthma, as well as in the formation of an adequate adaptive response, belongs to cell membranes of red blood cells, endothelial cells, and cells of the alveolar epithelium [6].

The universal defect of the perception and transmission of an information signal by the cell leads to pathological changes at different levels of the functioning of the broncho-pulmonary system, depending on the control of the course of the disease, which is connected to the relevance of the study [7, 8, 9].

The main purpose of the work was to determine and assess the effect of controlling the course of bronchial asthma on the biophysical characteristics of red blood cells [10-13].

Materials and methods

165 patients with asthma were examined in the phase of remission of the disease, middle age (36.8 ± 5.8 years). The diagnosis was made according to the medical history, clinical symptoms, parameters of external respiration, reversibility of airway obstruction to bronchodilatators. The selection of patients was conducted in accordance with No. 128 of the Ministry of Health of Ukraine dated March 19, 2007 «On Approval of Clinical Protocols for the Provision of Medical Aid in the Specialty "Pulmonology"» [19]. As a control, 30 healthy volunteers who had no serious clinically significant pathology were examined, with an average age of 38.5 ± 6.5 years. All patients received standard basal therapy of the remission period, which included the use of an inhaled corticosteroids, as well as a short-acting β_2 agonist to reduce asthma symptoms.

During the observation, the patients were divided into 3 groups: 50 patients (30.3 ± 3.8) % — with controlled course of the disease, 60 patients (37.5 ± 3.5) % — with partially controlled course of asthma and 55 (33.3 ± 3.8) % — with severe uncontrolling course. All patients were at the stage of remission of the disease. As a control, 30 healthy volunteers with no clinically significant pathology were examined, with an average age of (52.3 ± 6.2) years. All patients received standard basal therapy of the remission period, which included the use of an inhaled corticosteroids, as well as a short-acting β_2-agonist to reduce asthma symptoms.

In the course of the study, the relative charge of the membrane (VZME) and the relative gradient membrane potential (VGMP) of the erythrocytes (ER), the total number of erythrocytes * 10 / l, Hb (g/l), the character of

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the liquid crystal lattice of blood serum were evaluated, and the count of pathologically altered morphostructures erythrocytes in peripheral blood with determination of erythrocyte deformation degree (EDD), taking into account the level of blood saturation with the definition of SaO_2. The study of relative gradient membrane potential of erythrocytes was carried out with the help of the ionometer "OR-264/1" (Hungary). Determination of the relative charge of red blood cells was carried out with the help of mathematical calculations [19, 20]. Sorption capacity of erythrocytes (SCE) was determined using a dye — methylene blue. Units of measurement — percentages (%). The amount of methylene blue, bound to the cells (Cb), the amount of remaining dye in the solution (Cbs) was determined, which allowed to calculate the distribution of methylene blue between the cell and medium (Q). The values — of the coefficient Q were determined by the formula: Q = Cb / Cbs = (Vc / Ve) (Do-Dk) / Dk, where Do and Dk are the optical density of the methylene blue solution before and after the red blood cells incubation. Investigation of the degree of red blood cell deformation was carried out using the method of boundary dehydration of biological fluids and using the electron microscope "NU 2" of VEB Carl Zeiss with photosystem MRS 60 [20]. The degree of deformation of erythrocytes was determined according to the scale: 0 — no deformation; 1 — 10 %–29 % of erythrocytes deformed; 2 — deformed 30 %–69 % of red blood cells; 3 — deformed 70 % or more red blood cells [25].

All patients underwent a spirography with an analysis of the flow-volume curve of forced exhalation and a total plethysmogram of the body in the kit for the study of the "Master Screen Pneumo" respiratory system, SN 511263, 2007, and Master Screen PFT apparatus "Cardinal's Health" (Germany). The following parameters were studied: vital capacity of the lungs (VC), forced vital capacity of the lungs (FVC), volume of forced exhalation for 1 sec (FEV_1), maximum volumetric expiratory rate at 25 %, 50 %, 75 % of vital capacity of the lung (MEF_{25} %, MEF_{50} %, MEF_{75} %), peak expiratory flow (PEF), total bronchial resistance (Rtot), total lung capacity (TLC), residual volume of lungs (RV), expiratory reserve volume (ERV), Capacity of inspiration (IC). The study was conducted in the morning, after a 12–14 hour break in the administration of drugs [15]. The spirometric indices were reflected in percentages to the proper values and assessed the dynamics of obstructive violations over a ten-year observation period (FOP). Additionally, the index of FEV_1 decline (ID) during the observation period by the formula: ((absolute index of FEV_1 beginning of observation) — (absolute index of FEV_1 end of observation)) × 100 % / absolute index of FEV_1 beginning of observation. The statistical processing of the data obtained was performed using the

Table 1. Indicators of pulmonary volumes, capacities, bronchial patency in patients with asthma with varying degrees of control of the course of the disease at the beginning of the observation (M ± m)

Parameters	Healthy	Controlled BA (Group I)	Partially controlled asthma (group II)	Uncontrolled BA
	(group III)	(n = 50)	(n = 60)	(n = 55)
R tot. %	102.2 ± 19.2	104.8 ± 15.2	144.6 ± 17.2	198.2 ± 12.5*♦
IC. %	118.8 ± 6.4	103.6 ± 5.2	93.7 ± 5.4#	91.1 ± 3.1
VC_{MAX}. %	102.3 ± 5.2	100.4 ± 6.9	96.1 ± 4.5	85.2 ± 4.4
ERV. %	102.2 ± 7.5	102.2 ± 7.5	98.7 ± 5.2	78.3 ± 4.1*♦
RV. %	91.8 ± 8.2	96.2 ± 7.1	88.2 ± 6.5	130.2 ± 7.2*♦
ITGV. %	97.5 ± 6.2	98.3 ± 8.2	101.5 ± 4.2	113.4 ± 5.2*
TLC. %	102.3 ± 8.2	101.4 ± 7.3	106.3 ± 5.2	108.3 ± 5.3
FEV_1. %	89.7 ± 3.5	85.6 ± 5.5	68.2 ± 3.6#	54.9 ± 4.2*
FVC. %	92.3 ± 2.5	93.1 ± 2.4	78.2 ± 1.3#	72.9 ± 2.0*
FVC. абс.	2.3 ± 1.2	2.9 ± 1.4	2.1 ± 1.2	3.1 ± 1.2
FEV_6. л	2.9 ± 1.2	2.8 ± 1.5	2.2 ± 0.1	2.6 ± 0.2
FEV_1 / VC_{MAX}. %	88.6 ± 4.5	86.9 ± 4.3	83.6 ± 2.8	67.6 ± 4.8*♦
FEV_1 / FEV_6. %	87.9 ± 6.6	86.7 ± 5.9	72.1 ± 4.8	69.1 ± 4.8*
MEF_{75}. %	72.4 ± 4.2	73.2 ± 4.2	51.3 ± 3.1#	32.3 ± 2.1*♦
MEF_{50}. %	63.2 ± 4.9	59.4 ± 4.2	41.2±24.2#	28.1 ± 2.2*♦
MEF_{25}. %	41.4 ± 2.2	39.8 ± 1.3	28.3 ± 1.2#	22.4 ± 1.3*
PEF. %	83.5 ± 3.6	83.7 ± 3.6	69.8 ± 1.5#	55.3 ± 2.1*♦
DLCO. %	85.9 ± 4.1	86.5 ± 4.1	78.3 ± 2.1#	68.3 ± 2.2*♦
KCO. %	87.3 ± 6.2	85.8 ± 5.2	72.1 ± 5.6#	66.3 ± 2.2*
VA. %	103.5 ± 4.6	101.8 ± 4.7	96.4 ± 3.2	88.3 ± 2.2*
V_{IN}. %	111.8 ± 3.5	110.3 ± 3.2	97.3 ±3.2#	93.5 ± 3.2*
FRC. %	106.4 ± 5.3	104.8 ± 4.4	96.2 ± 3.2	84.4 ± 2.6*

Notes: # — statistically significant difference between the I and II groups ($p < 0.05$); * — statistically significant differences between I and III groups ($p < 0.05$); ♦ — statistically significant differences between II and III groups ($p < 0.05$).

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T a b l e 2. Indicators of pulmonary volumes, capacities, bronchial patency in patients with asthma with varying degrees of control of the course of the disease in dynamics (M ± m)

Indices	Controlled BA (Group I)		Partially controlled asthma (group II)		Uncontrolled BA	
	The first year of observation	10 years of observation	The first year of observation	10 years of observation	The first year of observation	10 years of observation
	(n = 50)		(n = 60)		(n = 55)	
R tot. %	104.8 ± 15.2	103.4 ± 14.2	144.6 ± 17.2	138.4 ± 16.2	198.2 ± 12.5*♦	202.4 ± 13.8*♦&
IC. %	103.6 ± 5.2	101.8 ± 5.2	93.7 ± 5.4#	90.7 ± 3.2#	91.1 ± 3.1	89.7 ± 3.2
VC_{MAX}. %	100.4 ± 6.9	102.2 ± 5.3	96.1 ± 4.5	94.1 ± 3.8	85.2 ± 4.4	81.1 ± 3.2
ERV. %	102.2 ± 7.5	103.2 ± 7.2	98.7 ± 5.2	93.9 ± 6.1	78.3 ± 4.1*♦	72.6 ± 2.3*♦&
RV. %	96.2 ± 7.1	97.3 ± 6.2	88.2 ± 6.5	95.5 ± 5.9	130.2 ± 7.2*♦	168.3 ±7.8*♦
ITGV. %	98.3 ± 8.2	97.5 ± 6.3	101.5 ± 4.2	102.5 ± 4.3	113.4 ± 5.2*	121.6 ± 5.1*
TLC. %	101.4 ± 7.3	102.6 ± 8.6	106.3 ± 5.2	108.8 ± 6.1	108.3 ± 5.3	115.3 ±52.3
FEV_1. %	85.6 ± 5.5	87.3 ± 6.6	68.2 ± 3.6#	65.2 ± 4.2#	54.9 ± 4.2*	50.2 ± 2.5&*
FVC. %	93.1 ± 2.4	95.2 ± 2.3	78.2 ± 1.3#	74.2 ± 1.5#&	72.9 ± 2.0*	70.3 ± 2.0&*
FEV_6. л	2.9 ± 1.4	2.6 ± 1.3	2.1 ± 1.2	2.0 ± 0.2	3.1 ± 1.2	2.2 ± 032
FEV_1 / VC_{MAX}. %	86.9 ± 4.3	85.3 ± 4.1	83.6 ± 2.8	82.2 ± 2.5	67.6 ± 4.8*♦	60.5 ± 2.3*♦&
FEV_1 / FEV_6. %	86.9 ± 4.3	85.6 ± 4.8	83.6 ± 2.8	78.3 ± 4.5	67.6 ± 4.8*♦	53.4 ± 613*
MEF_{75}. %	86.7 ± 5.9	82.6 ± 5.2	72.1 ± 4.8	69.3 ± 4.2	69.1 ± 4.8*	54.3 ± 3.7*♦
MEF_{50}. %	59.4 ± 4.2	60.8 ± 5.1	41.2±24.2#	39.5 ± 3.5	28.1 ± 2.2*♦	19.1 ± 1.1*♦&
MEF_{25}. %	39.8 ± 1.3	41.2 ± 1.3	28.3 ± 1.2#	26.8 ± 1.1#	22.4 ± 1.3*	10.5 ± 1.1*&
PEF. %	83.7 ± 3.6	84.3 ± 4.2	69.8 ± 1.5#	64.5 ± 2.2#	55.3 ± 2.1*♦	43.2 ± 2.3*♦&
DLCO. %	86.5 ± 4.1	90.5 ± 4.5	78.3 ± 2.1#	71.3 ± 1.8#&	68.3 ± 2.2*♦	62.2± 3.1*♦&
KCO. %	85.8 ± 5.2	88.7 ± 7.1	72.1 ± 5.6#	65.4 ± 4.3#	66.3 ± 2.2*	65.8 ± 2.1*
VA. %	101.8 ± 4.7	103.5 ± 5.5	96.4 ± 3.2	91.3 ± 3.6	88.3 ± 2.2*	74.1 ± 2.1*
V_{IN}. %	110.3 ± 3.2	115.8 ± 6.9	97.3 ±3.2#	96.4 ± 3.3#	93.5 ± 3.2*	91.8 ± 5.1*
FRC. %	104.8 ± 4.4	106.7 ± 5.5	96.2 ± 3.2	95.4 ± 2.1	84.4 ± 2.6*	75.9 ± 2.2*&

Notes: # — статистично достовірна відмінність показників між I та II групами ($p < 0.05$); 2.* — statistically significant differences between I and III groups ($p < 0.05$) ♦ — statistically significant differences between II and III groups ($p < 0.05$); & — statistically significant difference between the start and the end of the observation ($p < 0.05$).

licensed software products included in the Microsoft Office Professional 2000 software package on the personal computer of the Celeron IVM in the Excel program [16, 17]. The work is done at public expense.

Results

The results of long-term dynamic observation have shown that spirometric abnormalities in patients with bronchial asthma depend on the control of the course of the disease. Indicators are presented in table 1. The obtained data show that in patients with controlled course of disease, the speed characteristics of the spirography in the remission phase are within the normal range. In patients with partially controlled asthma, FEV_1 in comparison with the control group is generally decreased, there is a lack of patency of the distal bronchial units: MEF_{25}%, MEF_{50}%, MEF_{75}%. In severe uncontrolled course of the disease, respiratory failure is the most severe. Such patients have marked transient disturbances in the distal respiratory tract, which creates favorable conditions for the development of chronic hypoxia in the body. The general diffusion capacity of the lungs does not differ between patients with bronchial asthma with controlled course of the disease and healthy, but already in patients with partially controlled, and especially with uncontrolled course, even in the absence of significant decrease of speed spirographic indicators, the value of the membrane component of the diffusion capacity of the lungs is reduced, which cannot be explained only by a decrease in the surface area of gas exchange.

During a ten-year period in a controlled course of disease, there is no significant change in pulmonary volume and spirometric parameters. In partially controlled course, the reduction of practically all speed indicators of the spirogram appears. In case of the severe course of the disease, the identified changes and respiratory failure are the most severe. The general diffusion capacity of the lungs in patients with mild asthma is stable and practically does not differ with the indicators in the healthy group, and in patients with a moderate course, and especially with severe course, the value of the membrane component of the diffusion capacity of the lungs is reduced, which can be explained not only by reducing the surface area of gas exchange: persistent inflammation in the respiratory tract causes hypertrophy of the smooth muscle elements, thickening of the basement membrane, reducing the elastic properties of the vessels, accumulation of collagen fibers [17]. These pathogenetic changes are maintained in

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T a b l e 3. Biophysical parameters of blood in patients with bronchial asthma with varying degrees of control of the disease (compared with the healthy group) (M ± m)

Parameters	Healthy	Controlled BA (Group I)	Partially controlled asthma (group II)	Uncontrolled BA (group III)
	(n = 30)	(n = 50)	(n = 60)	(n = 55)
VGMPE. relative units	0.013 ± 0.001	0.028 ± 0.019	0.257 ± 0.022*&	0.321 ± 0.021#&
VZME. relative units	0.31 ± 0.005	0.301 ± 0.004	0.083 ± 0.008*&	0.046 ± 0.007#&
Degree of deformation of membranes of red blood cells (points)	1.3 ± 0.1	1.4 ± 0.0	2.1 ± 0.0*&	3.3 ± 0.2#&
Degree of hypoxic anisotropy (points)	0.1 ± 0.0	0.1 ± 0.0	1.7 ± 0.0*&	2.3 ± 0.2#&
Sorption capacity of red blood cells (%)	31.0 ± 4.6	42.3 ± 6.3	79.2 ± 12.4*#&	81.7 ± 12.8#&
The coefficient of distribution of methylene blue between the cell and the medium (Q). relative units	0.73 ± 0.02	0.76 ± 0.06	1.32 ± 0.09*#&	1.29 ± 0.07#&

Notes: * — statistically significant difference between the 1st and 2nd groups ($p < 0.05$); # — statistically significant distinction between groups II and III ($p < 0.05$); & — a statistically significant difference with a group of healthy individuals ($p < 0.05$).

intervals between exacerbations. Remodeling of the bronchi is due to damage of the epithelium of the bronchial mucosa, a violation of its recovery, the active production of growth factors by inflammatory cells, even in the early stages of the disease. More detailed information is presented in table 2.

The index of FEV_1 decline (ID) during the observation period was the lowest in a controlled BA — 1.3 %, a partially controlled course — 12.8 %, an uncontrolled course — 25.6 %.

It has been established that in BA there are morphological changes in the erythrocyte itself, reducing the rate of transition of one structure of hemoglobin to another and its affinity to oxygen. If in patients with controlled course of the disease there will be no significant changes in the percentage of pathologically deformed erythrocytes of peripheral blood as compared to healthy ones, then in patients with asthma with a partially controlled course there is a tendency to decreased aggregation of erythrocytes, in comparison with healthy, which is the result of microcytosis, because reduced size of erythrocytes and reduction of

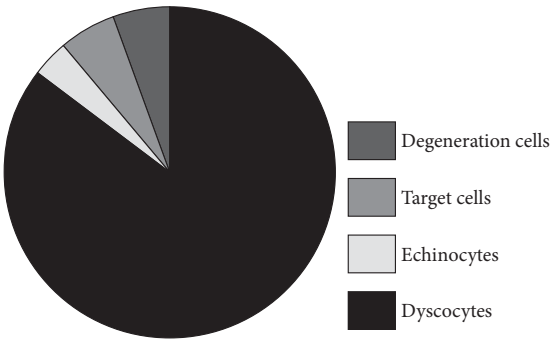

Fig. 1. The morphological structure of erythrocytes in patients with asthma with controlled course of the disease is the first year of observation.

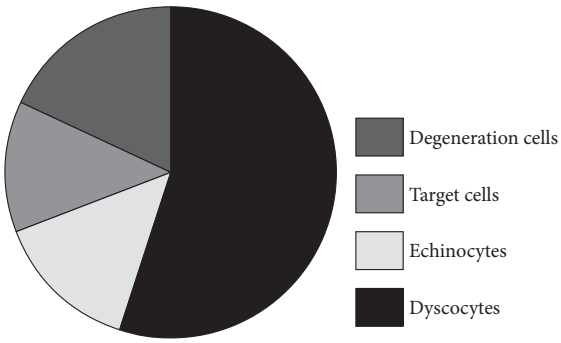

Fig. 3. Morphological structure of erythrocytes in patients with asthma with uncontrolled course of the first year of observation.

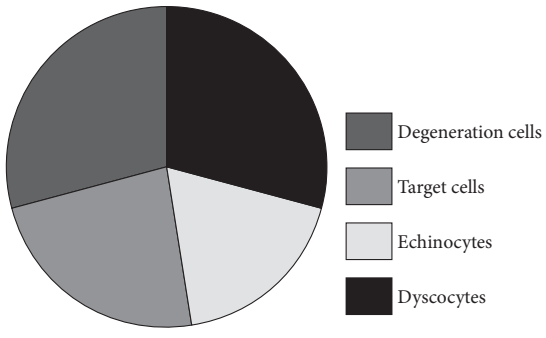

Fig. 2. Morphological structure of erythrocytes in patients with asthma with partially con-trolled disease during the first year of observation.

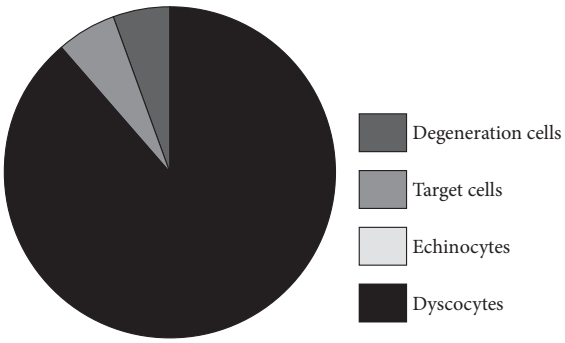

Fig. 4. Morphological structure of erythrocytes in patients with asthma with controlled course of the tenth year of observation.

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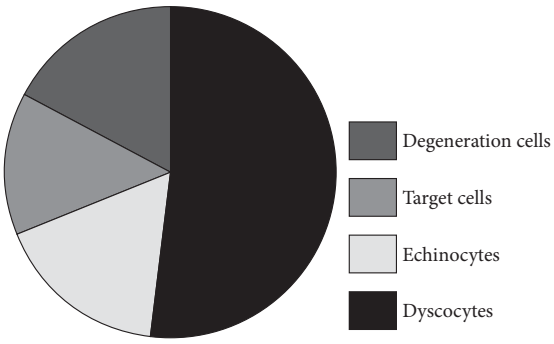

Fig. 5. Morphological structure of erythrocytes in patients with asthma with a partially controlled course of the tenth year of observation.

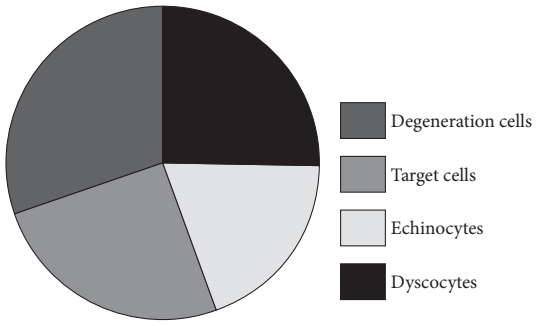

Fig. 6. Morphological structure of erythrocytes in patients with uncontrolled course of BA is the tenth year of observation.

aggregation are adaptive mechanisms that create conditions for optimization of microcirculation in patients. In patients with uncontrolled course of disease, the magnitude of aggregation of erythrocytes is elevated, which indicates a significant damage of erythrocyte with the formation of pathologically deformed morphoforms that are unable to maintain their functions in the rheological properties of blood. At a more detailed examination, it was found that in patients with controlled asthma, the morphological picture of the erythrocytic unit is practically no different from the healthy indexes: normocytes (diskocytes) — (85.3 ± 1.7) %, echinocytes — (4.1 ± 1.2) %, target cell — (5.4 ± 0.5) %, number of degenerative erythrocytes — (5.2 ± 0.8) %. In patients with partially controlled asthma there is a decrease in normocytes (52.2 ± 1.3) % (healthy — (95.2 ± 2.2) %; the number of deformed forms (echinocytes) increases to (14.5 ± 0.4) %, (healthy — 2.3 ± 0.8) %; target cell — to (13.6 ± 0.5) % (healthy — (1.5 ± 0.6) %); the number of degenerative erythrocytes increases to (19.7 ± 1.3) % (healthy — (1.5 ± 0.2) %). In BA, uncontrolled course, the percentage of normal forms decreases to (29.3 ± 1.8) %, echinocites — (18.2 ± 1.1) %, target cells — to (23.6 ± 0.6) %, degenerative forms — (28.9 ± 2.1) % (see fig. 1, 2, 3). In this case, no significant changes in the number of reticulocytes are observed in any of the groups. This testifies to the presence of directly pathological changes in red blood cells of peripheral blood.

A completely different picture is observed in patients with bronchial asthma, and, as the results of the studies showed, depends on the control of the course of the disease. In patients with bronchial asthma with controlled course, the indicators of VGMP and VZME and the degree of hypoxic anisotropy are changed, but not reliable. In patients with a partially controlled course of the disease, all of the above characteristics are significantly different from both the healthy group and the group of patients with controlled course. The most severe changes in the estimated rates were observed in patients with uncontrolled course of the disease (Fig. 3).

Biophysical characteristics of deformation ability and osmotic stability of erythrocytes confirm all of the above. In a healthy person, the biophysical parameters of the deformation properties of red blood cells are stable, unchanged. The relative gradient membrane potential of erythrocytes (VGMPE) is in average (0.011 ± 0.01) relative units, VZME in average is (0.29 ± 0.005) relative units, the degree of deformation of membranes of erythrocytes is (1.1 ± 0.1) points. More detailed information is presented in table 3.

T a b l e 4. Biophysical parameters of blood in patients with bronchial asthma with varying degrees of controllability of the disease after 10 years of follow-up (compared with healthy group) (M ± m)

Parameters	Healthy	Controlled BA (Group I)	Partially controlled asthma (group II)	Uncontrolled BA (group III)
	(n = 30)	(n = 50)	(n = 60)	(n = 55)
VGZME. relative units	0.010 ± 0.001	0.032 ± 0.018	0.272 ± 0.025*&	0.331 ± .021#&♦
VZME. relative units	0.33 ± 0.005	0.298 ± 0.004	0.075 ± 0.006*&	0.039 ±0.006#&♦
Degree of deformation of membranes of red blood cells (points)	1.5 ± 0.1	1.5 ± 0.0	2.4 ± 0.0*&	3.3 ± 0.2#&
Degree of hypoxic anisotropy (points)	0.1 ± 0.0	0.5 ± 0.0	1.5 ± 0.0*&	2.5 ± 0.2#&
Sorption capacity of erythrocytes (%)	31.5 ± 4.2	44.6 ± 6.5	79.5 ± 12.2*#&	82.5 ± 12.9#&
Coefficient of distribution of methylene blue between cell and medium (Q). relative units	0.72 ± 0.02	0.85 ± 0.08	1.30 ± 0.09*#&	1.35 ± 0.09#&

Notes: * — statistically significant difference between the 1st and 2nd groups ($p < 0.05$); # — statistically significant distinction between groups II and III ($p < 0.05$); & — a statistically significant difference with a group of healthy individuals ($p < 0.05$); ♦ — statistically significant difference. the first year of observation ($p < 0.05$).

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Table 5. Correlation between the biophysical parameters of the erythrocytic membrane and FEV_1 in patients with asthma (n = 165)

Показники	FEV_1 (%)	
	r	p
VGZME. relative units	- 0.68 ± 0.12	p < 0.001
VZME. relative units	0.65 ± 0.13	p < 0.001
Degree of deformation of membranes of red blood cells	- 0.52 ± 0.10	p < 0.001
Degree of hypoxic anisotropy (points)	- 0.54 ± 0.08	p < 0.001
Sorption capacity of erythrocytes (%)	- 0.57 ± 0.09	p < 0.001
Coefficient of distribution of methylene blue between cell and medium (Q). relative units	- 0.49 ± 0.10	p < 0.001

After 10 years of observation in patients with asthma with varying degrees of control, a different dynamics of the biophysical parameters of the erythrocytes layer of blood was observed: more uncontrolled was the course of the disease, the more pronounced was the loss of charge on the membrane, the increase in its gradient potential, increased permeability of the erythrocytes membrane and sorption capacity of erythrocytes, deepening of hypoxic anisotropy of blood serum, increase of percentage of deformed forms. The obtained results are presented in table 4. The morphological picture of the erythrocytes layer also changed: more uncontrolled was the course of asthma, the more pronounced was the reaction of the erythrocytes link to physical activity in time.

In patients with a controlled course of asthma for a ten-year observation period, the percentage of normocytes (discocytes) practically did not change: from (85.1 ± 1.7) % to (85.8 ± 1.5) %, echinocytes from (3.6 ± 2.1) % to (3.2 ± 1.5) %, target cells from (5.6 ± 0.5) % to (5.7 ± 0.7) %, the number of degenerative erythrocytes from (5. 3 ± 0.8) % to (5.2 ± 0.8) %. In patients with asthma with a partially controlled course, there was a decrease in the normal forms from (55.2 ± 1.2) % to (51.5 ± 1.3) %; percentage of echinocytes from (14.2 ± 0.4) % to (16.9 ± 0.5) %, target cells — from (12.6 ± 0.5) % to (14.3 ± 1.1) % ; the number of degenerative erythrocytes from (18.0 ± 1.3) % to (17.3 ± 1.2) %. In uncontrolled BA the percentage of standard forms after ten years of observation changed from (29.3 ± 1.8) % to (25.3 ± 1.1) %, echinocytes — from (18.2 ± 1.1) % to (19.4 ± 1.2) %, target cells — from (23.6 ± 0.6) % to (25.2 ± 0.5) %, degenerative forms from (28.9 ± 2.1) % to (30.1 ± 2.0) % (see fig. 5, 6).

This was confirmed by the reaction of the erythrocytic membrane in time, which also depended on the degree of control of the course of asthma. The more uncontrolled flow, the more pronounced over time will be changes in the biophysical parameters of the erythrocytic membrane. More detailed information is presented in table 4.

As a result of this work, a correlation analysis was carried out between the biophysical parameters of the erythrocytes membrane and FEV_1 in patients with bronchial asthma, which showed that the degree of deformation of the erythrocyte, its membrane biopotential and charge is a sensitive method for determining and predicting the control of the course of bronchial asthma (table 5).

Conclusions

As a result of this work, for the first time, changes in the morphological structure of red blood cells in patients with bronchial asthma were investigated and compared, depending on the control of the course of the disease with basic standard treatment for a long-term ten-year dynamic observation. Our studies have shown that the course of asthma is accompanied by a stage change in the morphological profile of erythrocytes, depending on the degree of disease control. The compensatory reaction from the red blood cells in the form of cellular rounding in response to the persistence of bronchial obstruction led in a certain extent to the leveling of hypoxic and circulatory changes in the organism, thus creating preconditions for strain, the depletion of the erythrocytes system in the regulation of intercellular co-operative interactions, maximal expressed in severe course of asthma. In addition, the erythrocyte membrane is a sensitive factor indicating that it may be an indicator of the risk of loss of control even in the absence of clinical symptoms in the remission of the disease. Taking into account the received data, in the future, it is necessary to improve the methods of diagnosing the risk factors for loss of asthma control based on the results.

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