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Morphofunctional features of platelets at comorbidity at the chronic obstructive pulmonary disease and hypertension

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Abstract
Background: Cardiovascular diseases (CVD) are the most commonly associated ailment with patients with chronic obstructive pulmonary disease (COPD). Arterial hypertension (H) is an important and widespread risk factor for CVD and is recorded with high frequency in patients with COPD. Activation of vessel endothelium and platelet hemostasis is the most important factor in the development of CVD and their complications.
Objective: To study the morphofunctional characteristics of platelets (pl) in patients with COPD and H and compare them to patients with H in the stable stage of diseases.
Methods: Anthropometry, spirometry, questionnaires "The Modified Medical Risearch Council Dispnia Scale" (mMRC), "COPD Assessment Test" (CAT), defining of blood pressure, pl functional activity study by estimation of pl adhesion (Adh) degree, induced aggregation (Agr) of pl with adenosine diphosphate (ADP), collagen and thrombin (optical turbidimetric method), count of activated, degranulated pl, packing density (PD) pl granules (transmission electron microscopy (TEM)).
Clinical characteristics: The study included 87 persons. The I group (I gr.) included 39 patients with COPD A-D category with H stage I-II, II group (II gr.) – 38 patients with H stage I-II, control group (C gr.)-10 healthy persons age-matched category. When included in the study, the patients had a stable course of disease for at least the last 2 months.
Results: Functional indices of platelet activity, namely, the degree of Adh pl, induced by ADP, collagen and thrombin pl Agr in patients of both I and II groups exceed those in C gr. In turn, a higher than in II gr. degree of pl Adh, induced by ADP rate of Agr, thrombin inuced degree of pl Agr, as well as the activity of the pl von Willebrand factor (vWf) was found in patients I gr. The total number of pl in I gr. is greater than in II gr. and C gr. Analysis of the TEM data in I gr. showed a greater number of activated and degranulated pl than in II gr. PD α- and λ-granules in I gr. was lower than in II gr. PD pl-granules in I and II are lower than in C. ($p < 0.05$).
Conclusions: In patients with H and in comorbid patients with COPD and H the functional activity of pl is increased in comparison with healthy persons age - matched category. In patients with COPD and H the Adh degree, induced Agr of pl, ultra structural characteristic of pl intravascular activation, increases. In COPD patients and concomitant H these changes are more pronounced than in patients with H. Complex influence of factors which take part in the development and maintenance of increased activity of the vascular-thrombocyte part of hemostasis in comorbid patients with COPD and H, even in the absence of exacerbations, leads to increased thrombogenic potential and the risks associated cardiovascular events.

Keywords: chronic obstructive pulmonary disease**,** hypertension, platelet adhesion, platelet aggregation, ultra structural characteristics of platelet activation

Introduction
COPD is a widespread disease, which, in the vast majority, is diagnosed over the age of 40 years, has a high comorbidity, which is influenced by the frequency and severity of exacerbations and the presence of concomitant diseases [1]. In patients with COPD, the detection of such significant cardiovascular risk factors as H, diabetes, adherence to smoking is higher than in the general population. In this the chances of developing coronary heart disease, arrhythmia, heart failure, disease of peripheral arterial are 2-5 times higher than in general populations [2]. CVD are the most commonly associated ailment with COPD patients. a[3, 4, 5] It is known that only 40% of deaths of patients with COPD are directly or indirectly caused by the progression of respiratory insufficiency. 50% of lethal sources are associated with the presence of concomitant pathology, and first of all with cardiovascular one [3, 5].

The most important aspect in the formation of pathological changes leading to the development of atherosclerosis and atherothrombosis, as a morphological substrate in the vast majority of cardiovascular and cerebrovascular pathology, is a systemic violation of the vessel endothelial function [6, 7]. The cumulative effect of vasopressor, metabolic, immunological factors negatively affects the state of the endothelium. Tobacco smoking is a leading factor in the development of COPD [8], and at once is undeniably proved risk factor for CVD [9]. Numerous compounds formed from tobacco resins contribute directly to the damage of endothelial cells, activating the processes of lipid peroxidation, enhancing vasoconstrictor mechanisms [10]. Factors of persistent systemic inflammation in COPD have additional negative impact on the function of the endothelial system [6, 7, 11].

Vessel endothelium has a close and multifactorial connection with the hemostasis system. First of all, dis function of the endothelium leads to activation of the platelet link of hemostasis. Platelets, anuclear cells, due to the rich receptor apparatus, the presence of a large number of active substances that are secreted from platelet granules and synthesized "de novo" by activation, play an important role not only in the process of clotting, but also in the implementation and support of inflammatory and proliferative processes [6, 7, 11, 12]. Thus, the study of the function of platelets as a marker of activation of the endothelial system under various conditions, including comorbidity, remains a subject of interest for researchers.

The aim is to study the morphofunctional characteristics of platelets (pl) in patients with COPD and H and compare them to patients with H in the stable stage of diseases.

Materials and methods of the research

87 persons have been examined. The I gr. included 39 patients with combined pathology – COPD A-D category concomitant with H I – II stages, 33 males (m), 6 females (f), average age was (56,7 ± 6,26) years, body mass index (BMI) (26,78 ± 3,89) kg/m^2. The COPD was diagnosed based on clinical and anamnestic data (dyspnea, cough, sputum secretion, data on the status of smoking, the effect of harmful factors related to professional activity, data on the exacerbation of the process throughout the year) and confirmed during spirography post-bronchodilation test (forced expiratory volume in 1st s (FEV1), forced expiratory volume in 1st second / forced vital capacity ratio (FEV1)/ FVC) < 0.7). The II gr. included 38 patients with H I–II stages (m –29, f – 9), in age (57,9 ± 6,73) years, BMI was (27,4 ± 3,44) kg/m^2. The C gr. Included 10 healthy persons (m – 6, f – 4) age-matched category (55.3 ± 2.34) years and BMI of (25.3 ± 2.47) kg/m^2. The patients of I gr. and II gr. received standard baseline therapy in accordance with the recommendations of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) and the Clinical Guidelines for Arterial Hypertension of the European Society for Hypertension (ESH) and the European Society of Cardiology (ESC) 2013. At the time of inclusion in the study all patients of these groups had a stable condition for at least two months. Any antithrombotic agents were not used for at least 2 weeks prior to the start of the study. Written informed consent for participation in the study was obtained from all participants.

The study did not include patients who had an anamnesis of acute cardiovascular events (myocardial infarction, stroke), diagnosed diabetes mellitus, oncological pathology, surgical interventions conducted during the past year.

All persons included in the study were examined for the following: anthropometry, measurements of blood pressure, count of pl (pl), pl functional activity study by estimation of pl adhesion degree, induced aggregation of pl with adenosine diphosphate (ADP), collagen and thrombin as inductors (all in concentration 2.0 μM) using an optical turbidimetric method (Aggregation Analyzer "Solar 2110", Belarus). Additionally, transmitted electron microscopy of thrombocytes was carried out among 20 patients (I gr. - 10, II gr. - 10) and among 5 healthy persons (Transmission electron microscope PEM 100-01 "SELMI", Ukraine).

The obtained data with the definition of the median (Me [25%; 75%], the Mann-Whitney test, Kruskal-Wallis test at a p-value < 0.05 was statistically processed using the Statistica 6.1 program.

Results and discussion

The degree of pl Adh in comorbid patients of I gr. was 40.0 [34.0;52.0] % and was significantly higher than in patients of II gr., where it reached 35.0 [26.0;40.0] %, and in the C gr. (32[23.0; 36.0] %). In turn, in II gr. degree of pl Adh exceeded the indicator of the C gr. also (Table 1.) During the study, it was found that the amount of pl in patients from the I gr. (260.0 [230.0; 295.0] x 109 / l; was significantly higher than in patients from the II gr. (217.5 [205.0; 270.0] x 109 / l) and from thr C gr. (213.5 [198.0; 238.0] x109 / l.) This is a background that may promotes an increase the thrombogenic potential in patients with comorbid pathology.

The process of pl adhesion is the initial stage of activation of hemostasis system and is directly connected with damage of vessel intima, since in absence of contact with dysfunctional and damaged endothelial cells and sub endothelial matrix, platelets remain intact [12]. Adhesion of pl is caused by the interaction of constitutive thrombocytes glycoprotein receptors of Ib / IX / V class with ligands of adhesive proteins presented by activated endothelial cells and sub endothelium (von Willebrand factor (vWF), collagen, fibronectin, laminin, trombospondin).

Table 1: Adhesive activity of platelets in patients with COPD and hypertension.

Group	I gr. n=39	II gr. n=38	C gr. n=10	p_H *
Degree of platelet adhesion, %	40.00 [34.0; 52.0] p II, C**	35.0 [26.0; 40.0]* p I, C	32 [23.0; 36.0] p I, II	0.041
Amount of platelets, 10^9/l	260.0 [230.0; 295.0] p II, C	217.5 [205,0; 270.0] p I	213.5 [198.0; 238.0] p I	0.043

* - the level of the Kruskal-Wallis test

**p I, II, C - when comparing the indices of I gr., II gr., C gr. according to the Mann-Whitney test p <0.05

The process of pl adhesion is primarily mediated and stabilized by the vWF – multimer, which is released from the Weibel-Palade bodies of the damaged endothelium, coming from the plasma, secreted from the α-granules of activated pl. The vWF stabilizes the connection between thrombocytes GPIbα integrin receptors and sub endothelial collagen fibers. The activity of the vWF is increased in vessels with high shift rate (arteries, arterioles, especially at vasoconstriction), under which even a weak linkage of GP Ibα with ligands of adhesive proteins causes primary activation, change of form,

appearance of pseudopodies, effective "rolling" of pl by vesel intima and an additional contact of collagen molecules with the GPVI immunoglobulin superfamily receptors. These receptors stimulate the release of secondary inductors of pl activation and aggregation Agr, an increase in the affinity of inducible integrin αIIb/β3 (IIв/IIIa) thrombocytes receptors to the fibrinogen, the vWF and other subendothelial matrix adhesive proteins [12, 13, 14].

A sufficiently large number of factors, which are secreted by activated pl, are exhibited by sub endothelial structures at endothelium damage and come from plasma, are thrombocytes Agr agonists (inductors) [12, 13, 14]. So adrenaline, serotonin, ADP (in low concentration) are considered as weak aggregate inductors and lead to reverse pl Agr. High-potency agonists that cause irreversible pl Agr include ADP in high concentrations, collagen, thrombin, thromboxane-A2, tissue factor of platelets activation. In our study, natural inductors were used as pl Agr agonists: ADP, collagen and thrombin (all inductors at a concentration of 2.0 μM were acting as strong ones) [14].

Table 2: Induced aggregation of platelets in patients with COPD and hypertension**.**

Induc-tor of Agr	Group	Degree of Agr, %	Rate of Agr, %/30s	Count of pl, 10^9/l	vWF activity, %
ADP	I gr. (n=39)	63.7* [52.1; 74.1] p C	46.0* [37.2; 60.2] P II, C	248.7* [204.2; 269.0] p C	180.25 [162.6; 194.8]
	II gr. (n=38)	63.7* [51.5; 66.9] p C	37.4* [25.8; 46.2] P I	228.3 [194.3; 289.5]	180.2 [162.0;183.7]
	C gr. (n=10)	53.9* [49.8; 53.3]p I, II	33.8* [26.9; 41.1] p I	201.9* [196.4; 210.8] p I	171.5 [167.8;176.3]
Collagen	I gr. (n=39)	61.65* [54.9; 75.9] p C	36.3 [27.80; 50.0]	239.9 [198.30; 267.8]	177.25 [167.0;197.2]
	II gr. (n=38)	61.9* [50.3; 85.7] p C	36.40 [25.60; 43.4]	216.50 [192.4; 257.10]	167.05 [150.85;184.50]
	C gr. (n=10)	49.35* [45.6; 55.3]p I, II	30.55 [27.3; 34.5]	247.45 [226.0; 280.2]	171.75 [168.3;173.9]
Thrombin	I gr. (n=39)	71.05* [61.9; 82.0] p II, C	47.8* [38.0; 57.2]p C	245.0* [211.3; 273.0] p C	192.9* [177.6; 205.2] p II, C
	II gr. (n=38)	61.1* [52.6; 73.4] p I, C	44.0 [33.4; 51.4]	212.7 [196.1; 269.7]	176.4* [163.4;193.8] p I
	C gr. (n=10)	56.25* [53.6; 58.4] p I,II	42.5* [38.9; 45.1] p I	201.1* [193.0; 212.7] p I	169.85* [164.8;173.0] p I

* - the level of the Kruskal-Wallis test < 0.05; p I, II, C - when comparing the indices of I gr., II gr., C gr. according to the Mann-Whitney test p <0.05

It was found (Table 2) that when stimulating with ADP, in I gr. (63.7 [52.1; 74,1] %) and II gr. (63.9[51.5; 66.9] %) the degree of pl Agr was significantly higher than in the C gr. (53.9[49.80; 53.3] %), the rate of pl Agr in I gr. (46.0 [37.2; 60.2] %/30s) was higher than in C gr. (33.8[26.9; 41.1] % / 30s) and in II gr. (37.4 [25.8; 46.2] %/30s) with significantly higher amount of pl than in the C gr. (248.7 [204.2; 269.0] x10^9/l and 201.9 [196.4;210.8] x10^9/l). Induction with collagen showed a significant excess of the degree pl Agr compared to C gr. (49.35 [45.6, 55.3] %) as in I gr. (61.65 [54.9, 75.9] %) and in II gr. (61.9 [50.3, 85.7] %), while at the same time this figure in I gr. and II gr. almost did not differ. When using thrombin, all the studied parameters in I gr., namely: the degree of pl Agr (71.05[61.9; 82.0] %), the rate of pl Agr (47.8 [38.0; 57.2] %/30s), amount of pl (245.0[211.3;273.0] x10^9/l), vWF activity (192.9 [177,6; 205.2] %) significantly exceeded the indicators of C gr. (respectively: 56.25[53.0; 58.4] %, 42.5[38.9; 45.1] %/30s, 201.1[193.0; 212.7] x10^9/l, 169.85[164.80; 173.0] %). In II gr. the degree of pl Agr (61.1[52.6; 73.4] %) also was higher than in the C gr., but significantly lower than in the I gr. The vWF activity has increased in I gr. compared with this indicator in II gr. (176.4[163.4; 193.8] %), indicating an increase paracrin pl activity due to additional thrombin-induced release of thrombocytic vWF. It is known that thrombin interacts with thrombocytes PAR1 receptors that are cofactorly activated by GP Ibα receptors, which have an initial role in the process of interaction with adhesion molecules, primarily with vWF [12, 13, 14]. Increased intravascular pl activation in the presence of comorbidity of COPD and H was confirmed in the data obtained during the TME of pl (Table 3).

Table 3: Ultra structural characteristics of intravascular platelet activation in patients with COPD and H.

Group	Activated pl, %	Aggregated pl, %	Degranulated pl, %
I gr. (n=10)	45.0 [43.0; 48.0]	11.5 [10.0; 14.0]	16.5 [16.0; 20.0]
II gr. (n=10)	35.0 [32.0; 39.0]	16.5 [15.0; 17.0]	9.0 [9.0; 11.0]
U*	p = 0.0082	p = 0.0546	p = 0.0051
C gr. (n = 5)	25 [24.0; 27.0]	7 [6.0; 9.0]	6 [6.0; 8.0]
H*	p < 0,05	p < 0,05	p < 0,05

*- the Mann-Whitney test when comparing the indices of I, II groups.
** - the Kruskal-Wallis test when comparing the indices of I, II, C groups.

During activation of pl due to the influence of adhesive molecules on the pl para- and autocrine receptors, morphological changes occur in the platelets (change in shape, appearance of pseudopodia, redistribution of cytoplasmic granules) and release of secondary mediators from numerous granules (Fig. 1, 2) Indicators of intravascular pl activation exceed C gr. (p < 0.05) in both groups, but the amount of activated pl in I gr. (45.0 [43.0; 48.0] %) significantly exceeded count in II gr. (35 [32.0; 39.0] %). At the same time, there was a tendency to increase the number of aggregated platelets in II gr. (16.5 [15.0; 17.0] %) compared to 1 gr. (11.5 [10.0; 14.0] %). Under the influence of agonists, pl granules secrete their content through the system of open channels of the cytoskeleton, which leads to irreversible adhesion and aggregation of pl and the further promotion of thrombogenesis with the involvement of a cascade of coagulation plasma proteins [12, 14, 14].

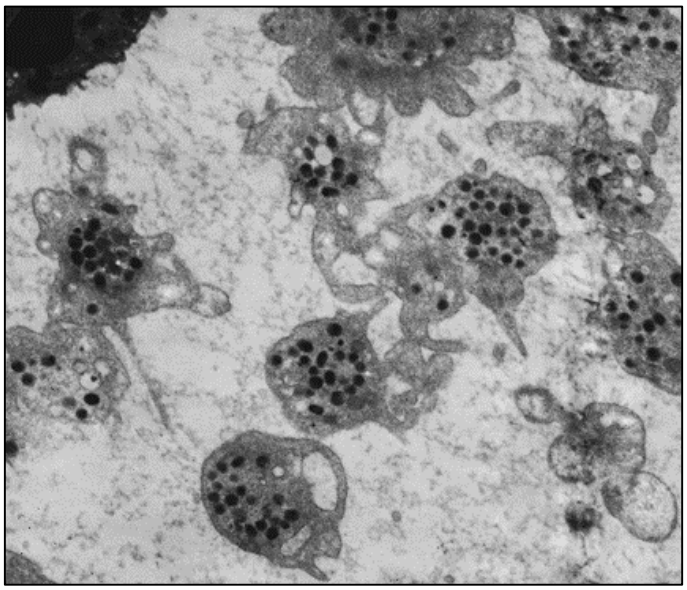

Fig 1: Activated platelets in patient with COPD and H. Electron microphotography x 10000

The number of degranulated pl, those, which under the influence of inductors released the content of their granules, was significantly ($p < 0.05$) higher in I gr. (16.5 [16.0; 20.0] %) than in II gr. (9.0 [9.0; 11.0] %), Pl granules deposite the vWf, thrombospondin, P-selectin, fibronectin, fibrinogen, a number of coagulation and anticoagulation proteins, growth factors, some cytokines and chemokines (α – granules), ADP, adenosine triphosphate, calcium, serotonin (δ – granules), lysosomes with the lysosomal protein LIMP-1 (λ– granules) [12, 14, 15]. According to the data presented in Table 4, the amount (density of packaging) of cytoplasmic granular structures in both I and II groups is lower than C gr. ($p < 0.05$). This is due to the fact that in the absence of stimulating factors, primarily of endothelial and sub endothelial origin, the platelets are in a relatively stable state, the redistribution of granules and their release have minimal activity. With the increase in thrombocyte activity, which was revealed in both study groups of our patients, the process of platelet degranulation is significantly enhanced. This is confirmed by a decrease in the packing density of platelet granules (Fig. 3).

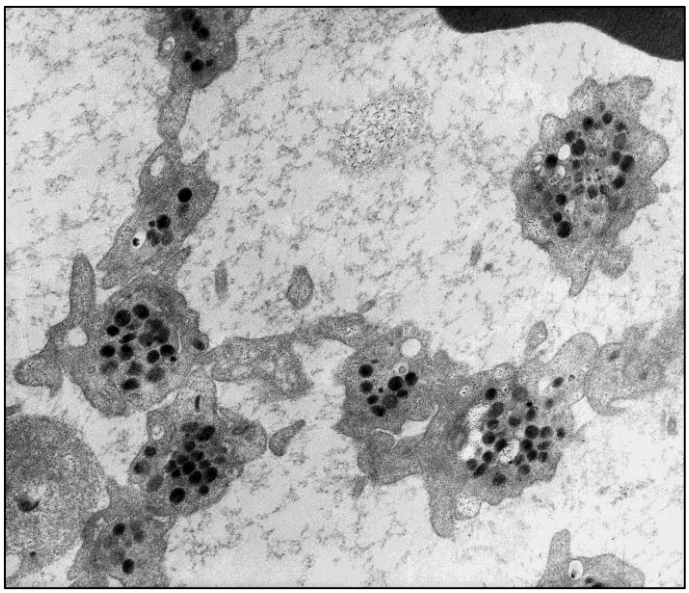

Fig 2: Activated platelets in patient with H. Electron microphotography x 10000

Table 4: The density of platelets granules in patients with COPD and hypertension.

Group	Density of packaging of α- granules, μm³ / μm³	Density of packaging of δ- granules, μm³ / μm³	Density of packaging of λ- granules, μm³ / μm³	Density of packaging of mitochondria, μm³ / μm³
I gr.	0.073 [0.071;0.078]	0.028 [0.023;0.030]	0.089 [0.07; 0.096]	0.025 [0.021;0.028]
II gr.	0.093 [0.088;0.096]	0.020 [0.014;0.025]	0.107 [0.095;0.112]	0.018 [0.012;0.029]
U*	p = 0.0051	p = 0.1093	p = 0.0306	p = 0.5218
C gr.	0,110 [0.98; 0.114]	0,037 [0,035; 0.39]	0,140 [0.130; 0.148]	0,030 [0.028; 0.028]
H**	p < 0,05	p < 0,05	p < 0,05	p < 0,05

*- the Mann-Whitney test when comparing the indices of I, II groups.

** - the Kruskal-Wallis test when comparing the indices of I, II, C groups.

The density of the packaging of pl α-granules and λ-granules is significantly lower in I gr. (0.073[0.071; 0.078] μm3 / μm3 and 0.089 [0.070; 0.096] μm3 / μm3) than in II gr. (0.093 [0.088; 0.096] and 0.107 [0.095; 0.112] μm3/μm3). This can be explained by more pronounced intravascular effects of strong inducers, those that induce irreversible activation of pl and a stable release reaction [11, 14] in comorbid patients with COPD and H than in patients with H without COPD.

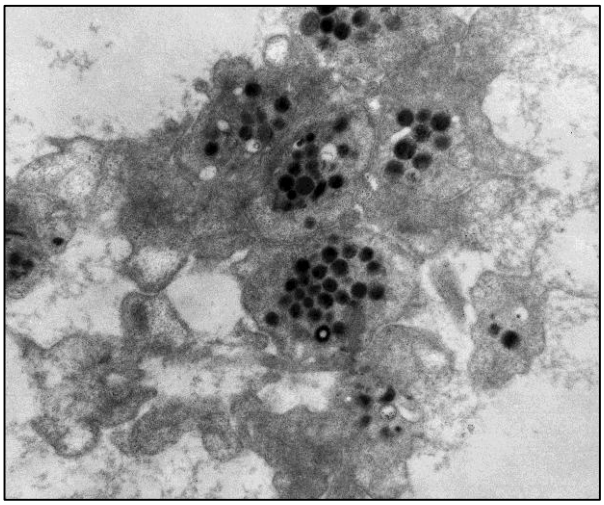

Fig 3: Aggregated and de granulated platelets **i**n patient with COPD and H. Electron microphotography x 10000

Conclusions

1. In patients with H and in comorbid patients with COPD and H, the functional activity of platelets is increased in comparison with healthy persons age - matched category.
2. In patients with COPD and H the adhesion degree, induced aggregation of platelets, ultrastructural characteristic of platelets intravascular activation, increases.
3. In COPD patients and concomitant H these changes are more pronounced than in patients with H.
4. Complex influence of factors which take part in the development and maintenance of increased activity of the vascular-thrombocyte part of hemostasis in comorbid patients with COPD and H, even in the absence of exacerbations, leads to increased thrombogenic potential and risks associated with cardiovascular events.

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