ENGLISH VERSION: PLATELET-VESSEL WALL INTERACTION COMPONENT AND COAGULATION COMPONENT OF HEMOSTASIS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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This article presents the results of a study of platelet-vessel wall interaction and coagulation disorders in patients with chronic obstructive pulmonary disease (COPD) in the stable phase of the pathological process. Parameters of platelet aggregation properties were measured by using optical platelet aggregation test and aggregation inducers such as adenosine diphosphate (ADP) and collagen. The key markers of coagulation profile were measured to assess the coagulation part of hemostasis. It has been established that patients with COPD in the stable phase and mild bronchial obstruction, treated with standard bronchodilators and showing lower platelet aggregation ability have disorders in the coagulation component of hemostasis towards hypercoagulability in the extrinsic coagulation pathway. In COPD patients with severe airflow obstruction taking inhaled glucocorticosteroids, the measurement of the platelet aggregation test indicated a tendency to hyperaggregation versus healthy subjects. No disorders of the coagulation component of hemostasis have been found. These data can be used for individual adjustment of anti-inflammatory therapy in COPD patients to influence the platelet-vessel wall interaction and coagulation parameters of hemostasis.

Key words: chronic obstructive pulmonary disease, hemostasis, platelet aggregation, coagulation.

It is presently commonly recognised that hemostasis disorders develop in patients with chronic bronchopulmonary disease. A special category are patients with chronic obstructive pulmonary disease (COPD) who often suffer from abnormal hemorheological status — in 35 to 52% of cases [5, 9, 16] — with disorders in both platelet-vessel wall interaction and coagulation parts of hemostasis.

Platelet-vessel wall interaction changes in COPD patients seem to be the result of the inflammatory process in the lung tissue due to increased production of proinflammatory cytokines and inflammatory mediators [4, 9, 14, 15, 17] ultimately contributing to leukocyte and platelet adhesion to vascular endothelium and enhancing their aggregation properties [22]. In the acute phase of COPD, these disorders, on the one hand, are common as they are observed in more than a third of patients, and, on the other hand, they are quite pronounced [17, 21]. However, platelet aggregation properties can be enhanced in the stable phase of the pathological process. According to Ye. O. Merenkova and N. Ye. Monoharova, these disorders are observed in 12.5% of patients [9], and in up to 25% of patients, according to J. D. Maclay et al. [21].

It is noteworthy that some patients with COPD can develop incoagulability disorders in the stable phase of the pathological process. So, Ukrainian researchers have shown that platelet aggregative ability nearly in a quarter of patients is lower than that of healthy individuals [9]. Similar results were obtained by foreign researchers who found that patients with COPD in the stable phase, when assessed in a platelet aggregation test that used adenosine diphosphate (ADP) and collagen as aggregation inducers, had significantly lower platelet aggregation properties than virtually healthy subjects [10].

Coagulation disorders in COPD patients can be caused by bronchial obstruction, a major pathogenetic element of the disease, and can lead to arterial hypoxia resulting in compensatory stimulation of erythropoiesis and increased release of catecholamines into the blood. Hypokalemia and respiratory acidosis that occur in this process along with systemic inflammatory reactions activate coagulation factors which ultimately destabilise coagulation part of hemostasis [1, 2, 7, 13, 18].

It has been found that patients with COPD in the stable phase of the pathological process experience changes in plasma parameters (such as fibrinogen levels, antithrombin III, euglobulin blood fractions activity, etc.) towards hypercoagulability while parameters of the anticoagulation component remain normal [3, 5, 19, 20]. For example, Ya. A. Dzyublyk et al. showed that ethanol test results and soluble fibrin and fibrinogen levels in older and elderly COPD patients were significantly higher than that of virtually healthy individuals [5].

The recent studies indicate that there is a correlation between the degree of coagulation hemostasis disorders and severity of the disease by functional sings and carbon dioxide tension in the blood [20].

Very few studies have been conducted to research both mechanisms of hemostatic disorders in their combination [5, 9], so we aimed to investigate the nature and peculiarities of the platelet-vessel wall interaction and coagulation status of hemostasis in patients with COPD in the stable phase of the pathological process.

Materials and methods

We have examined 30 patients with COPD in the stable phase of the disease (mean age: 62.7±6.4 years; including 27 (90%) men and 3 (10%) women) who were assigned to the study group. All patients had past or present active smoking history with a pack year factor 40.8±24.5. COPD was diagnosed in accordance with Order No.555 of the Ministry of Health of Ukraine dated June 27, 2013 [11].

All patients gave their written informed consent to participate in the study.

The study excluded patients with severe cardiovascular disorders in the past and/or at the time of screening, severe heart failure, a history of thromboembolism, cancer history or obesity at the time of screening.

All patients were divided into 2 subgroups depending upon the severity of their ventilation disorders. Subgroup 1 included 16 people with mild bronchial obstruction, i. e. with the level of forced expiratory volume in one second (FEV1) during the post-test higher than 50% of the predicted value (FEV1 = 65.9±10.6% pred.); there were 15 (93.8%) men, 1 (6.2%) woman; mean age was 61.9±6.4 years; basic therapy was performed according to the stage of the disease (a long-acting β_2-adrenergic agonist and/or a long-acting anti-cholinergic drug and a short-acting β_2-adrenergic agonist, as required). Subgroup 2 included 14 patients with severe bronchial obstruction, i. e. with FEV1 post-test level lower than 50% of the predicted value (FEV1 = 40.8±7.2% pred.); there were 12 (85.7%) men, 2 (14.3%) women; mean age was 63,8±8.8

years; patients received an inhaled glucocorticosteroid (IGCC) and a long-acting β_2-adrenergic agonist and/or a long-acting anti-cholinergic drug and a short-acting β_2-adrenergic agonist, as required.

The control group included 10 healthy subjects of comparable age and sex (average age was 55.3±4.0 years; there were 8 (80.0%) men and 2 (20.0%) women).

The severity of ventilation disorders was assessed by FEV1 percentage of the predicted value and FEV1/forced vital capacity (FVC) ratio in morning fasting computer-based spirometry. For spirometry we used Master Screen Body/Diff system (Jager, Germany). Reversibility of bronchial obstruction was evaluated by a post-test change in the absolute FEV1 value (mL) by using a short-acting β_2-adrenergic agonist (400 μg of inhaled salbutamol).

To assess the platelet-vessel wall interaction component of hemostasis we performed optical platelet aggregation test that measures the extent (%), time (s) and rate (%/min in the first 30 seconds) of platelet aggregation.

Venous blood samples were collected in a closed vacuum system with a 3.8% solution of sodium citrate by following standard methodology. To prevent the influence of the pre-analitycal stage on the test results patients rested for at least 30 minutes prior to blood sample collection. Measurements were done within two hours after sampling.

Optical platelet aggregation testing by using AR 2110 (Solar, Belarus) system, a weak aggregation inducer (ADP at a concentration of 2.0 mcIU) and a strong aggregation inducer (collagen at a concentration of 2.0 mcIU), which allowed us to estimate the total platelet aggregation ability. The inductor dose was selected by the need to assess platelets ability to secrete granule contents for involvement in the coagulation cascade. A comprehensive assessment of the platelet aggregation measurements was made by all indicators.

To assess the coagulation part of hemostasis the following key indicators of coagulation profile were measured: prothrombin index (PI) which, if increased, indicates disorders of coagulation properties of the blood towards hypercoagulability in the extrinsic coagulation pathway [4]; prothrombin ratio (PR), which abnormal measurements also describe the extrinsic pathway of the coagulation cascade; international normalised ratio (INR), an important parameter of the state of the coagulation system and the extrinsic pathway of blood clotting, and a standard factor of coagulation efficiency of the blood clotting system; activated partial thromboplastin time (aPTT) that characterises the intrinsic and common pathways of the coagulation cascade [4].

Since the levels of platelets can affect coagulation properties of the blood this parameter was also measured in all patients.

Statistical analysis of the results was performed by using biometric methods of analysis implemented in EXCEL-2003 and STATISTICA 6.0 software packages [8, 12]. The difference between comparable values was deemed reliable where $p<0.05$.

Results and discussion

The comprehensive assessment of platelet aggregation measurements with ADP as an aggregation inducer allowed finding out that abnormal platelet aggregation properties were present in both subgroups of COPD patients. However, while the majority of patients in subgroup 1 (14 (87.5%)) did not show any disorders of platelet aggregation, and 2 (12.5%) patients had decreased platelet aggregation properties, the majority of patients in subgroup 2 (8 (57.1%)) had hyperaggregation, 5 (35.7%) patients had normal aggregation, and 1 (7.2%) patient had hypoaggregation of platelets.

As to the platelet aggregation measurements recorded by using collagen as an aggregation inducer, most patients in subgroup 1 (11 (68.8%)) had hypoaggregation, and for a third of patients (5 (31.2%)) the values were within normal limits. In subgroup 2 only 3 (21.4%) patients had no aggregation disorders while 11 (78.6%) patients showed hyperaggregation of platelets.

It should be remembered that the platelet aggregation test with ADP as an aggregation inductor enables to study in detail the first (reversible) wave of aggregation when changes occur in the form of platelet without any release reaction, as well as the second (irreversible) wave aggregation when active substances are released from platelet granules, and under the influence of strong inducers such as collagen cell aggregation occurs at high speed, which makes it possible to assess the total platelet aggregation capacity [4]. Patients in subgroup 1 had no disorders at the early stage of aggregation, however, the total platelet aggregation capacity changed towards hyperaggregation probably at the later stages of the aggregation cascade, while patients in subgroup 2 had disorders at all stages of platelet aggregation.

The levels of all parameters of platelet aggregation with both inducers for patients of the study and control group were not significantly different, but patients in subgroup 2 changes in these parameters indicated the tendency of platelets to increase their ability to aggregate, while the results of the test for patients in subgroup 1 did not show any presence of such tendency (Table 1).

Table 1
Parameters of Platelet Aggregation in COPD Patients (M±m)

Groups and subgroups of patients	Inducers					
	ADP			Collagen		
	degree (%)	time (s)	rate (%/min)	degree (%)	time (s)	rate (%/min)
Study group (n=30)	64.2±21.8	557.9±45.9	33.2±21.4	56.2±29.8	542.1±119.7	39.6±26.8
subgroup 1 (n=16)	52.5±14.1#	562.5±29.5	24.1±10.6	34.2±17.3*#	517.5±157.3	23.6±15.9*
subgroup 2 (n=14)	77.8±7.6#	552.6±60.3	43.6±26.0	81.6±18.6*#	570.5±41.9	58.0±25.3
Control group (n=10)	65.3±9.9	526.0±75.1	38.4±11.9	65.9±6.9	459.3±84.6	56.5±10.5

*Note: * — parameter significant difference ($p<0.05$) vs the control group;*
 # — parameter significant difference ($p<0.05$) between the patient subgroups

The degree of ADP-induced platelet aggregation in patients from subgroup 1 was significantly lower ($p<0.05$) than that observed in patients from subgroup 2 though degrees of aggregation in both subgroups were not significantly different from the control group ($p>0.05$).

The collagen-induced platelet aggregation test allowed establishing that the degree of aggregation in patients from subgroup 1 was significantly lower than that measured in patients from subgroup 2 and the control group while this parameter in patients from subgroup 2 was significantly higher than in patients from the control group. The higher degree of aggregation in subgroup 2 indicates an increase in platelet aggregation properties, while lower parameter in subgroup 1 means that platelets reduced their ability to aggregation. The higher degree of platelet aggregation when induced by collagen suggests that cells tend to be more actively involved in the release reaction and that there is a high potential for release of specific substances contained in platelet granules, while the lower degree of aggregation has the opposite effect [4].

Platelet aggregation time in subgroup 1 was not significantly different from that of subgroup 2 and the control group for both aggregation inducers, which excludes the period break-up from platelet monolayer formation to the beginning of activation of the platelet cascade induced by ADP or collagen [6].

The aggregation rate within the first 30 seconds of measurements recording in subgroup 1 did not significantly differ from that observed in subgroup 2 (for ADP-induced aggregation), and the values of both subgroups were not significantly different from the control group ($p>0.05$).

Collagen-induced platelet aggregation rate in patients from subgroup 1 was significantly lower than the rate measured in the control group but not different from the value recorded in subgroup 2 ($p>0.05$), which was not different from the control group. The decrease in the rate of platelet aggregation indicates that cells have weaker aggregation properties [4].

These data suggest that COPD patients with mild bronchial obstruction experience depletion of platelet secretion and reduction of platelet functional response. These changes were affected by hypercoagulability disorders in the extrinsic coagulation pathway in the coagulation element of hemostasis.

The levels of all coagulation hemostasis parameters in the study group were not significantly different from those of the control group ($p>0.05$) (Table 2).

Table 2

Coagulation Profile Parameters in Examined COPD Patients (M±m)

Groups and Subgroups of patients	Parameters			
	PI (%)	PR	INR	aPTT (s)
Study group (n=30)	107.3±12.4	0.94±0.14	0.94±0.16	21.8±4.3
subgroup 1 (n=16)	113.7±7.4*	0.87±0.06*	0.86±0.07*	20.8±4.0
subgroup 2 (n=14)	99.7±12.8	1.02±0.17	1.02±0.20	22.9±4.5
Control group (n=10)	94.8±2.04	1.05±0.02	1.07±0.03	25.7±2.4

*Note: * — parameter significant difference ($p<0.05$) vs the control group*

However, PI level in subgroup 1 was significantly higher than that of the control group ($p<0.05$) but not statistically different from the level measured in subgroup 2 ($p>0.05$).

PR level in subgroup 1 was significantly lower than in the control group ($p<0.05$) and was not significantly different from the PR level in subgroup 2.

INR level in subgroup 1 was also significantly lower than that in the control group ($p<0.05$) and was not significantly different from that of subgroup 2 ($p>0.05$). The lower PR and INR levels and increased PI value indicate a predisposition to hypercoagulability in patients from subgroup 1.

aPTT levels in the study group or in the subgroups of patients were not significantly different from aPTT levels in the control group.

The low INR level and unchanged aPTT value in subgroup 1 indicate disorders of the coagulation element of hemostasis in the extrinsic coagulation pathway.

The peripheral blood platelet levels in COPD patients in the study group (248.1±47.3x109/L) and in subgroups 1 and 2 (257.3±44.4x109/L and 220.9±38,9x 109/L, respectively) did not differ from the value measured in the control group (236.5±53.1x109/L) ($p>0.05$).

The results indicate a slowdown at the later stages of platelet thrombi formation in patients from subgroup 1, which may be due to either a decrease in the number of platelet granules or abnormal platelet secretion and expression of a compensatory response to changes in hemostasis towards hypercoagulability at the plasma level of hemostasis [10].

Meanwhile, patients from subgroup 2, whose ventilatory function of the lungs was more severely impaired than in patients from subgroup 1 ($p<0.05$) and were taking IGCC, did not have any significant changes in the coagulation element of hemostasis vs the control group though hyperaggregation of platelets with both inducers was observed in most patients.

These changes are likely to indicate normalisation of the coagulation element of hemostasis in patients taking IGCC due to the effects the medication has on severity of the local inflammatory reaction in the bronchopulmonary system, which cause a decrease in the release of substances with high coagulation potential. However, IGCC therapy aimed at improving airway conductance and reducing chronic inflammation cannot directly affect the processes of endothelial damage and repair, as evidenced by the literature [10, 12].

Conclusions

1. In patients with COPD in the stable phase of the pathological process, blood coagulability disorders can develop in both the coagulation and platelet-vessel wall interaction elements of hemostasis.
2. The coagulation component of hemostasis in COPD patients is disturbed in the extrinsic coagulation pathway with a decrease in platelet aggregation properties.
3. Impaired ventilation function of the lungs in COPD patients is associated with increased platelet aggregation activity.
4. IGCCs are likely to affect the coagulation component of hemostasis but have nearly no effect on the platelet-vessel wall interaction part of hemostasis.

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