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Tissue factor and tissue factor pathway inhibitor in chronic obstructive pulmonary disease

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ABSTRACT

Background. The inflammatory process in patients with chronic obstructive pulmonary disease (COPD) interferes with the normal function of respiratory lung. There is strong evidence indicating that inflammatory process in patients with COPD may activate blood coagulation. However, little is known about the role of tissue factor (TF) pathway in the coagulation system activation in COPD.

Objectives. The aim of the study has been to evaluate the concentration of selected parameters of coagulation process, including TF and tissue factor pathway inhibitor (TFPI), in plasma of patients with COPD.

Patients and methods. The study included 66 patients with COPD at different stages of disease according to GOLD 2006, mean age of 60.4 years. The control group consisted of 25 healthy volunteers (non-smokers). Total TF and TFPI concentrations and other haemostatic parameters were measured using Enzyme Linked Immunosorbent Assay (ELISA).

Results. Significantly higher concentrations of TF and TFPI ($p = 0.03$; $p = 0.004$, respectively) were observed in patients with COPD. The level of fibrinogen was also higher in the study group than in controls ($p = 0.002$). However, the activated partial thromboplastin time (aPTT) was shortened in COPD patients as compared with the control group ($p = 0.0001$).

Conclusion. The study showed that in patients suffering from COPD, the stimulation of the tissue factor-dependent activation of coagulation is observed.

Key words: COPD, inflammation, haemostasis, TF, TFPI

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Introduction

Chronic obstructive pulmonary disease (COPD) is one of the most common diseases in developed countries. Epidemiology data shows a clear global increase in the death rate as a result of COPD. It is estimated that COPD will be the third leading cause of death worldwide by 2020 and the fifth most common disease causing the incapacity for work [1, 2].

Chronic obstructive pulmonary disease is a new designation connecting diseases so far referred to as chronic bronchial inflammation and pulmonary emphysema. These two diseases (with a few exceptions) are the result of smoking, and both occur most often simultaneously with a different intensity. In 1964, the Americans suggested the new name to combine both diseases [3, 4].

Chronic obstructive pulmonary disease, according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), is a disease which can be prevented and treated effectively. Pulmonary changes are characterized by partially reversible limiting of the air flow through the respiratory tract. This is a progressive process, developed as the effect of impaired inflammatory response of lungs to the action of harmful dusts or gasses [4–6].

Almost 80–90% of patients with COPD are active or former smokers which makes smoking the main etiopathogenetic factor [7, 8]. Besides nicotinism, the following risk factors are present among the environmental factors,: intense occupational exposure to dusts and chemical substances, atmospheric air pollution, infections of the respiratory system in the childhood, reoccurring bronchial-pulmonary infection as well as low

socioeconomic status [9]. The best documented individual factor is rare inborn α1-antitrypsin deficiency of the main circulating inhibitor of serine proteinases [10, 11].

In patients with COPD, the inflammatory process disturbs the correct respiratory lung activity which leads to accumulation of the following inflammatory cells in the lungs: neutrophils, macrophages, lymphocytes (especially CD 8+, Th_1 and Th_2) and B lymphocytes. The activated inflammatory cells release various mediators which damage the structure of lungs and (or) sustain neutrophil inflammation. The inflammatory process disturbs the balance between proteases (enzymes breaking down lung proteins) and antiproteases (enzymes protecting lung proteins from degradation) [12]. The pathomechanism of bronchi-pulmonary changes involves also the participation of oxidants found in the tobacco smoke and released from flowing neutrophils and macrophages [13].

The coagulation process activation is an element of inflammatory reaction in COPD. The crucial role in coagulation cascade is played by platelets, endothelium cells, adhesion molecule and plasma coagulation proteins. The inflammation results in an increased expression of the cytokines leading to blood coagulation activation [14], which, in turn, can cause thromboembolic complications. The research of the last few years demonstrates the primary role of the tissue factor (TF) in the blood coagulation activation. The analysis of available literature shows that only few papers deal with the TF role in the pathogenesis of haemostasis disorders in COPD patients. The aim of the study has been to assess the concentration of tissue factor pathway inhibitor (TFPI), tissue factor (TF), soluble form of thrombomodulin (sTM), thrombin-antithrombin complex (TAT), fibrinogen, and the activity of protein C in patients with COPD.

Patients and methods

Sixty-six patients with chronic obstructive pulmonary disease were recruited at various disease severity stages (GOLD 2006): stage (FEV_1 > 80%) — 8 patients, II stage (FEV_1 > 80 to < 50) — 20 patients, III stage (FEV_1 > 30 to < 50) — 22 patients and IV stage (FEV_1 < 30) — 16 patients, treated in Department of Pulmonary Diseases and Respiratory Failure, Kuyavian-Pomeranian Regional Pulmonology Centre, Bydgoszcz, Poland.

The study group consisted of 18 females and 48 males aged from 40 to 82 (the average age was 60.4 years). disease advancement level was identified on the basis of interviews, the examination of symptoms and spirometry. The disease symptoms lasted 3 to 32 years. The exclusion criteria included the presence of coexisting diseases such as hypertension, diabetes, glomerulonephritis, surgery < 3 months, COPD exacerbation < 3 months, receiving anticoagulants, thrombolytics, and antiplatelet drugs, thromboembolic disease and pulmonary embolism < 6 months. The inclusion criteria were: age > 40 years, current or former smoker (> 10 pack-years), and a stable disease period. The patients were treated according to the GOLD 2006.

The control group consisted of 25 healthy volunteers, non-smokers with normal spirometry, matched for age and sex.

Written informed consent was obtained from each participant before entering the study. The study was approved by the Bioethics Committee of the Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz, Poland.

Venous blood (4.5 mL) for tests of TF, TFPI, fibrinogen, sTM, activity of protein C, PT, INR, aPTT was collected in a fasting state into cooled tubes (Becton Dickinson Vacutainer® System, Plymouth, UK) containing 0.13 mol/L trisodium citrate (the final blood — anticoagulant ratio was 9:1) after 30 min of rest between 7.30 and 9.30 a.m. and after a 12 h overnight fast. The blood samples were immediately mixed and centrifuged at 3000 × *g* at + 4°C for 20 min. The platelet-poor plasma was divided into 200 μL Eppendorf-type tubes and then the samples were frozen at –80°C (according to the manufacturer's procedures) until assayed, however, no longer than six months.

Hemostatic assays

The concentrations of TF, TFPI sTM were determined by the Enzyme Linked Immunosorbent Assay (ELISA) (IMUBIND® total TF, IMUBIND® total TFPI, IMUBIND® Thrombomodulin, respectively; American Diagnostica Inc., Greenwich, USA). The TAT concentration was determined by ENZYGNOST® TAT micro, Behring, Marburg, France. However, the concentration of fibrinogen, activity of protein C, PT, INR, aPTT were measured in an automated coagulometer CC-3003 apparatus and reagents produced by Bio-Ksel Co, Grudziądz, Poland.

Statistical analysis

The statistical analysis was performed using the statistics program Statistica v. 9 StatSoft® software (StatStoft®, Cracow, Poland). The Shapiro–Wilk test was used to assess the distribution normality. ,The arithmetic mean (X) and standard deviations (SD) were determined for the variables with normal distribution. The median (Me), lower quartile (Q1), and upper quartile (Q3) were applied for the values with non-normal distribution. The significance of differences between the groups for the variables analysed with normal distribution was

Table 1. Chosen parameters of haemostasis in the study group with COPD and in the control group

	Study group (N = 66)		Control group (N = 25)		p value
	M/Me	SD/Q1,Q3	M/Me	SD/Q1,Q3	
PT [s]	14.46	1.64	15.00	0.98	0.1289
INR	1.03	0.12	1.07	0.07	0.1193
Protein C (%)	96.89	17.65	103.64	11.69	0.0807
TAT [μg/L]	1.79	1.38, 2.62	2.49	1.42, 5.11	0.0732
sTM [ng/mL]	2.00	1.59, 2.30	1.90	1.52, 2.42	0.9394

M/Me — mean or median; SD/Q1, Q3 — standard deviation or upper and lower quartile

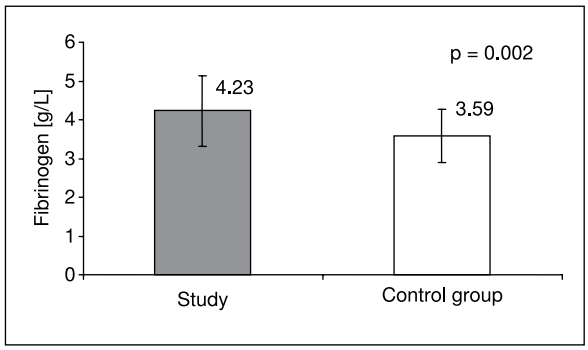

Figure 1. The concentration of fibrinogen in the study group and in the control group

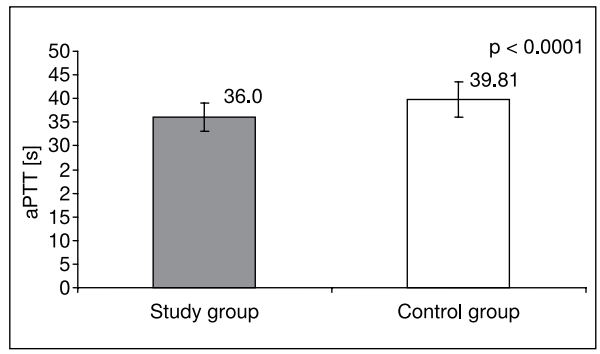

Figure 2. aPTT in the study group and in the control group

examined using the classical t-Student test and for other variables Mann-Whitney U test rank-sum test was used. The p-values < 0.05 were considered statistically significant.

Results

Table 1 shows the selected parameters of haemostasis in patients with COPD and the control group. No significant differences were observed in the concentration of TAT complexes and sTM, also in prothrombin time, INR, and activity of protein C in the study and in the control groups.

Figures 1 and 2 present significantly higher concentration of fibrinogen in the study group as compared with the control group ($p = 0.002$) and shorter aPTT in the study group ($p < 0.0001$). Significantly higher levels of TF and TFPI were noted in the study group as compared with the control group ($p = 0.03$; $p = 0.004$, respectively) (Figs. 3 and 4).

Discussion

The results of the study have shown that the increased concentration of TF, TFPI, fibrinogen and shortened activated partial thromboplastin time were observed in patients with COPD.

Activation of the extrinsic blood coagulation pathway plays a crucial role in the complications of thromboembolism occurring in cancer, atherosclerosis and sepsis [15]. The tissue factor is a fixed component of cell membranes, it also appears in cytosol and the intercellular matrix as well as in plasma and fluids of body cavities. High concentrations of tissue factor are observed in tunica adventitia of blood vessels Moreover, the brain, lungs and placenta are also rich in TF [16]. TF is a glycoprotein; its chain is composed of three domains: submembrane, intramembrane and overmembrane (transmembrane protein) and it is mainly exposed by cells with locations physically separate from the circulating blood. In normal conditions, tissue cells and endothelial cells do not release the tissue factor and, therefore, its concentration in plasma in healthy people is low. However, it plays a key role in the protection against bleeding as a result of tissue damage. Blood vessels injury leads to the initiation of blood coagulation activation via the TF [17].

Present studies have demonstrated that the average value of TF in plasma of healthy people is 83.08 pg/mL. The available literature analysis shows a large discrepancy between test results for TF levels in healthy individuals. Radziwon et al. noted that the average

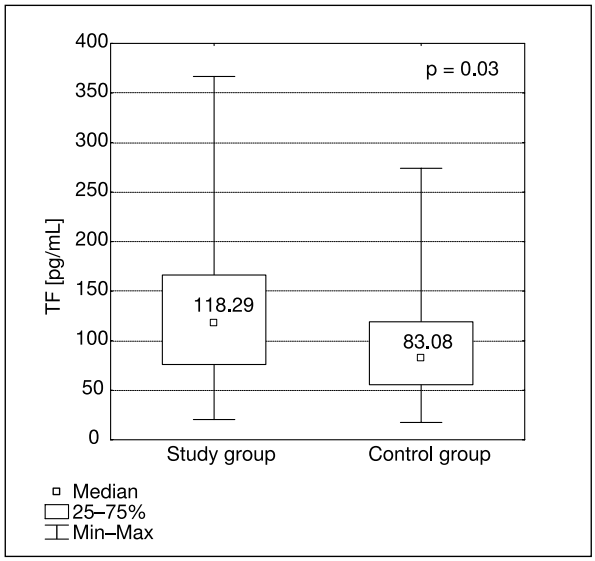

Figure 3. TF in the study and in the control group

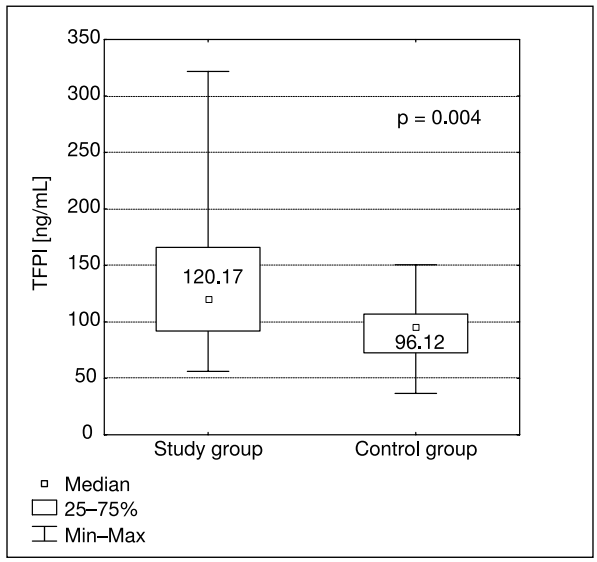

Figure 4. TFPI in the study group and in the control group

concentration of TF amounted to 226 ± 86.19 pg/mL in the group of 100 healthy people. Pawlak et al. observed that the TF concentration was 112 pg/mL among 20 healthy people. However, Bronowicz et al., in the group of 35 healthy blood donors, found that the average concentration of TF was equal to 87.58 pg/mL, most similar to the TF concentration recorded in the present research [18–20].

Blood coagulation disorders, particularly TF-dependent hypercoagulability, are the cause of many serious diseases such as: atherosclerosis, sepsis, malignancies, acute respiratory distress syndrome (ARDS) and glomerulonephritis. The TF source starting blood coagulation can cover: cancer cells, tissues damaged by injury or surgery, tissues of dead foetus, procoagulating material from placenta. Bacterial endotoxins and cytokines, such as TNF-α, IL-1 cause endothelial cell damage and the TF exposure located in the subendothelial layer. Mucopolysaccharides of bacterial cell membranes are capable of activating blood coagulation in a similar way as endotoxin. In addition, tissue factor may be present on tumour cells or macrophages, which are activated by endotoxin or cytokines [17, 21].

Papers on the activation of the coagulation process occurring in lung diseases have been found in the available literature. An increased fibrin accumulation in the pulmonary interstitial space was observed in patients with idiopathic pulmonary fibrosis, in scleroderma, acute lung injury and ARDS. In most of these syndromes increased local production of thrombin was also observed. An elevated TF level, observed in bronchoalveolar lavage fluid (BALF), recorded in this group of diseases, indicates at least a partial participation of the extrinsic coagulation pathway in the activation of prothrombin to thrombin [22, 23].

In the available literature there seem to be few studies about TF in patients with COPD.

Vaidyula and colleagues found elevated activity of tissue factor (TF — PCA, TF — procoagulant activity) among 11 patients with COPD as compared with the control group (52.3 U/mL vs. 20.7 U/mL) [24]. In our study, elevated levels of the total pool of TF in patients prove an increased blood coagulation activation by extrinsic pathway.

The key role played by the TF in initiating the blood coagulation cascade with subsequent thrombin generation, platelet activation and fibrin deposition is controlled by the tissue factor pathway inhibitor. Endothelium produces TFPI, which is a Kunitz-type protein with molecular weight of 42 kD with three inhibitor domains. The first of them is bonded with a complex of TF and factor VII, the second one involves an active factor X, while the third one is responsible for binding of TFPI to heparin and lipoproteins. TFPI associated with endothelium accounts for 50–90% of the total pool and it is released into plasma after heparin injection. The plasma concentration of inhibitor increases 2 to 10 times after intravenous and subcutaneous injections of heparin and other glycosaminoglycans [20].

According to Radziwon, the concentration and activity of TFPI in plasma of healthy people varies in a fairly wide range: 50–170 ng/mL [21]. In Kłoczko's opinion, the average concentration of TFPI in the plasma of healthy people is approximately 100 ng/mL, which corresponds to the level of about 2.5 nmol/L [25]. Similarly, according to Naumnik et al., the physiological concentration of TFPI in plasma is low and amounts to approximately 2.5 nmol/L [17]. The concentration of TFPI found in the present study in healthy people amounts to 96.12 ng/mL and is similar to the results of most studies available in the literature.

Literature reports on TFPI concentration being elevated in many clinical states: it increases during pregnancy by 29%, in bacteremia by 70%, in metastatic cancer by 83%, in ARDS by 47%, in hypercholesterolemia from 30 to 53%, after t-PA injection by 60%, after exercise from 20 to 40% [17].

An increased TFPI content was observed in patients with advanced malignancy of colorectal, pancreatic, gastric, breast and prostate. Increasing concentrations of TFPI occur with disease progression, however, so far TFPI production by tumor cells has not been found [20, 26]. The largest TFPI concentration has been noted by the authors in acute and chronic myeloid leukaemia. Significantly increased TFPI levels were also observed in patients with urinary tract cancer (kidney and bladder cancer) as compared with healthy subjects [21].

Many researches indicate the importance of the TFPI as a risk marker for cardiovascular disease. Ott et al. noticed increased TFPI concentrations in patients with acute myocardial infarction undergoing angioplasty with stent placement as compared with the patients with stable angina following the same procedure [27]. He et al. noted an elevated TFPI level correlating with an increasing TF level in patients with acute myocardial infarction, and lower TFPI concentration, despite an increasing TF, in patients with acute ischemic stroke [28].

The authors of the majority of papers about TFPI in various thrombotic states are of the opinion that high TFPI concentration is a symptom of strong inhibition of TF activating blood coagulation, while reduced TFPI concentration is an expression of inhibitor consumption in this mechanism and the evidence of prothrombotic risk. Different attempts have been made to use of TFPI recombinant as an antithrombotic drug [28].

Our study demonstrates significantly higher concentrations of this inhibitor in patients with COPD. The research on TFPI in patients with COPD from only one centre has been found in the literature. Cella et al. reported elevated TFPI concentration in patients with COPD suggesting that endothelial cells are potentially able to produce natural inhibitors of blood coagulation [5, 29].

As far as endothelial TFPI origin is concerned, its high concentration in patients' blood indicates endothelial vascular damage. On the other hand, it is also a defence mechanism against the development of blood clots. In patients with COPD, hypoxemia, which can cause endothelial damage, is a factor increasing prothrombotic processes. Lewczuk et al. suggest that the coexistence of COPD and chronic thromboembolic pulmonary hypertension increased their 18-month risk of death twice [30].

The literature analysis of the TFPI role in blood coagulation process disorders shows that increased TFPI level plays an important part in inhibiting blood coagulation process initiated by the tissue factor.

Our research demonstrated higher concentration of fibrinogen in general group of patients with COPD. High concentration of fibrinogen in COPD patients undoubtedly increases the blood viscosity, platelets activation, and it is an important factor increasing blood coagulability.

The aPTT value was significantly shortened as compared with healthy people, indicating a coagulation activation in the patients with COPD and the intrinsic pathway, which, in the light of current trends, is less effective in creating thrombin.

In conclusion, it should be noted that among patients with COPD, regardless of a greater intensity of the disease, the risk of thrombotic complication occurs, expressed by increased concentration of TF and fibrinogen. This process is limited by effective action of the natural coagulation inhibitors (TFPI). However, as it comes from the analysed literature, the acute inflammation can activate the coagulation process, which creates a significant risk of thromboembolic complications in patients with COPD during the disease exacerbation.

Conclusions

The study demonstrates the coagulation activation (higher TF and fibrinogen, shortened aPTT) in patients with COPD in a stable disease phase. However, inhibiting the extrinsic coagulation pathway is expressed by elevated levels of TFPI in patients with COPD.

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