Elevated Plasma Procoagulant and Fibrinolytic Markers in Patients with Chronic Obstructive Pulmonary Disease

Jun-ichi Ashitani, Hiroshi Mukae, Yasuji Arimura and Shigeru Matsukura

Abstract

Objective There is clinical and pathological evidence of thrombosis in pulmonary vessels of patients with chronic obstructive pulmonary disease (COPD). The purpose of this study was to investigate the presence of hypercoagulability and determine the extent of this abnormality in COPD patients.

Patients and Methods We measured plasma levels of thrombin antithrombin III complex (TAT), fibrinopeptide A (FPA), tissue plasminogen activator-plasminogen activator inhibitor (tPA-PAI): markers of coagulation-fibrinolysis-system, and also β-thromboglobulin (β-TG): a marker of platelet activation, in 40 COPD patients and in 20 control subjects. Measurements were also repeated 12 months after entry in all patients.

Results TAT, FPA, tPA-PAI, and β-TG concentrations were significantly higher in COPD than in control subjects. At 12 months follow-up, ΔA-aDO2 and Δ%FEV1 were significantly higher in patients with high TAT or tPA-PAI levels than in patients with low levels and TAT, FPA and tPA-PAI levels remained elevated, although β-TG levels decreased after domiciliary O2 therapy.

Conclusion Our results showed an enhanced prothrombotic process in COPD patients, which could potentially account for the increased thrombosis in pulmonary vessels in these patients.

Key words: TAT, FPA, tPA-PAI, β-TG

Introduction

Chronic obstructive pulmonary disease (COPD), a condition characterized by a slowly progressive and irreversible airflow obstruction, is a major worldwide health problem with increasing prevalence and mortality (1, 2). Pathological studies have indicated that microthrombosis may occur in the pulmonary vessels of patients with COPD (3), and that such changes might be a cause of disease exacerbation (4). Thrombosis could be due to platelet activation or the existence of prothrombotic condition in patients with vascular and alveolar lesions (5, 6) but this possibility has never been thoroughly investigated in COPD patients. Previous studies indirectly suggested that the presence of a prothrombotic condition in COPD patients based on changes in the activities of platelets and clotting system (7–9). Nenci et al (10) demonstrated platelet activation in COPD patients by detection of high plasma levels of β-thromboglobulin (TG), a substance released from activated platelets. Alessandri et al (11) demonstrated hypercoagulability in COPD patients by measurement of F1+2 fragments and D-dimers. However, previous studies did not examine in detail the relationship between the extent of the prothrombotic state and pulmonary function or disease evolution in patients with COPD.

Because thrombosis is a multifactorial state and involves various processes such as platelet activation, endothelial cell injury, and activation of coagulation-fibrinolysis system, a hypercoagulative state cannot be evaluated with a single marker. Although almost all markers of the coagulation-fibrinolysis system may be abnormal in advanced derangements of the coagulation-fibrinolysis system such as DIC, levels of most markers are within the normal range in underlying hypercoagulative and/or hyperfibrinolytic states. Thrombin antithrombin III complexes (TAT) and fibrinopeptide A (FPA) are sensitive parameters in underlying hypercoagulative states. Tissue plasminogen activator-plasminogen activator inhibitor complex (tPA-PAI) is a marker of secondary fibrinolysis following thrombosis, while β-TG is a highly sensitive marker of platelet activation. These parameters may therefore be suitable for evaluation of microthrombosis.

In the present study, we prospectively measured the plasma concentrations of TAT, FPA, tPA-PAI, and β-TG, spirometric indices and arterial blood gases over a period of one year in patients with COPD. Furthermore, we measured the level of thrombomodulin (TM) to test whether the hypercoagulative state was triggered by COPD-associated endothelial cell injury.

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Methods

Patients
Forty male patients with an average age of 72.6±5.2 years [expressed as mean±standard deviation (SD)] gave their informed consent for participation in the study. All patients were current smokers. Diagnosis of COPD was based on a combination of clinical history, physical examination, pulmonary function tests, and chest X-rays as defined by the American Thoracic Society (12, 13). Clinical conditions were stable in all patients in the month prior to the examination and no patient was on antithrombotic treatment before commencement of the study. We excluded patients with a suspected diagnosis of acute inflammatory disease, episodes of previous pulmonary embolism, immunological disease, cancer, venous or arterial thrombosis, hypertension, peripheral vascular disease, renal disease, and/or diabetes mellitus. The control group consisted of 20 healthy male volunteers with an average age of 71.4±8.0 years and all had never smoked. Laboratory tests of COPD patients at study entry showed a mean leucocyte count of 6,160±275/μl and C-reactive protein of 0.51±0.08 mg/dl.

Spirometry was performed in all patients and control subjects. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) were determined using a spirometer. After practice blows, recording was repeated until three satisfactory tracings were obtained or when subjects could not perform the technique adequately. Analysis was based on the highest FEV₁ and FVC from these tracings corrected for body temperature, air pressure, and water saturation. The predicted values for each subject, based on sex, age, and height, were obtained from standard tables. Data were expressed as percentages of the predicted values. Patients were strongly advised to quit smoking and all declared they never smoked at entry to the study. All subjects were followed closely for a period of one year; patients with COPD were seen once every month in an outpatient clinic and then contacted to reinforce refraining from smoking. There were no dropouts during the study. The usual treatment consisted of aminophylline, expectorant, and anticholinergic inhalant. During this study, 32 of 40 COPD patients were prescribed domiciliary oxygen therapy (DOT) up to 12 hours per day in order to improve hypoxemia at rest, exercise or sleeping time.

Sample preparation
The selected patients had not treated previously with oxygen therapy. After a rest of at least 20 minutes, between 8:00 and 10:00 AM, two blood samples were taken from each patient. The first sample was drawn from the radial artery via a heparinized syringe and immediately used for blood gas analysis. The second sample was taken, without stasis, from the antecubital vein, using a 20 G needle and used for measurement of the procoagulant and fibrinolytic markers, β-TG, and TM. Samples were drawn from a well-flushed, indwelling venous catheter into a plastic syringe and transferred to tubes with the following anticoagulants: sodium citrate for TAT and tPA-PAI, sodium citrate, heparin, and aprotinin for FPA, and sodium citrate, theophylline, adenosine, and dipyridamole for β-TG. Samples were placed on ice and centrifuged immediately at 3,000xg for 20 minutes. Plasma was stored at −70°C until subsequent hemostatic marker analyses. All markers were analyzed in a blinded fashion. At the 12 month sample collection, blood samples were withdrawn after 20 minutes of room air breathing without supplemental oxygen.

Procoagulant and fibrinolytic markers
TAT complexes were measured by enzyme-linked immunosorbent assay (ELISA) (Behring Diagnostics Inc., Somerville, NJ, USA). Plasma FPA levels were measured by radiomunoassay (Byk-Sangtec, Dietzenbacht, Germany). Plasma TPA-PAI complexes were measured by enzyme immunoassay (EIA) by the capture/tag antibody technique using polystyrene beads (TDC-88, Teijin, Tokyo). The above assays were performed as described previously (14–16).

β-thromboglobulin and thrombomodulin
Plasma levels of β-TG and TM were measured by ELISA (Diagnostica Stago, Asnieres, France) and EIA (Zymed Lab. Inc., South San Francisco, CA, USA), respectively. These assays were performed as described previously (17, 18).

Statistical analyses
Data were expressed as mean±standard deviation (SD). The Mann-Whitney U-test was used for comparison of results between COPD patients and control subjects. Differences between initial results and results at 12 months were analyzed using the Student’s t-test for paired data. Significance was accepted at p<0.05.

Results
Clinical characteristics of patients with COPD at entry to the study
The clinical characteristics of patients with COPD at entry are shown in Table 1. Smoking habits were documented using pack-years. Body mass index (BMI) was significantly lower in COPD patients than control subjects. Arterial blood gases in patients with COPD showed hypoxemia and hypercapnia associated with a significantly high alveolar-arterial oxygen gradient (A-aDO₂) relative to controls. On the other hand, spirometry showed reduced VC, %VC, FEV₁, %FEV₁, and FEV/FVC ratio in COPD patients (Table 1).

Markers of procoagulant, fibrinolytic, platelet activation, and endothelial cell injury
At study entry, TAT, FPA, and tPA-PAI were significantly higher in COPD patients than in control subjects (Table 2). In 20% of COPD patients, the concentration of TAT was >3.4 ng/ml (mean±2 SD of control subjects). In 38% of patients, the concentration of FPA was >2.4 ng/ml (mean±2 SD of control subjects). In 60% of COPD patients, the concentration of tPA-PAI was >18 ng/ml (mean±2 SD of control subjects). There
Procoagulant and Fibrinolytic Markers in COPD

Table 1. Clinical Characteristics of Normal Subjects and Patients with COPD at Study Entry

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=20)</th>
<th>COPD group (n=40)</th>
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</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>70.4±8.0</td>
<td>72.6±5.2</td>
</tr>
<tr>
<td><strong>Smoking history (pack-years)</strong></td>
<td>0</td>
<td>63.5±10.2</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>21.7±5.5</td>
<td>18.2±2.4*</td>
</tr>
<tr>
<td><strong>PaO₂ (mmHg)</strong></td>
<td>88±6.0</td>
<td>68.2±6.3*</td>
</tr>
<tr>
<td><strong>PaCO₂ (mmHg)</strong></td>
<td>41.3±2.8</td>
<td>43.7±3.9*</td>
</tr>
<tr>
<td><strong>A-aDO₂ (mmHg)</strong></td>
<td>10.2±2.2</td>
<td>27.2±8.5*</td>
</tr>
<tr>
<td><strong>VC (l)</strong></td>
<td>2.90±0.60</td>
<td>2.50±0.72*</td>
</tr>
<tr>
<td><strong>VC (% predicted)</strong></td>
<td>96.3±18.0</td>
<td>82.8±23.0*</td>
</tr>
<tr>
<td><strong>FEV₁ (l)</strong></td>
<td>2.20±0.40</td>
<td>1.06±0.48*</td>
</tr>
<tr>
<td><strong>FEV₁ (% predicted)</strong></td>
<td>84.3±9.8</td>
<td>41.1±11.7*</td>
</tr>
<tr>
<td><strong>FEV₁/FVC (%)</strong></td>
<td>76.5±9.9</td>
<td>49.1±13.4*</td>
</tr>
</tbody>
</table>

Data are mean±SD. *p<0.05, compared with the control group.

Table 2. Comparison of Markers of Procoagulation, Platelet Activation and Endothelial Cell Injury in Patients with COPD and Normal Subjects (Control)

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=20)</th>
<th>COPD group (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TAT (ng/ml)</strong></td>
<td>1.8±0.8</td>
<td>2.9±1.6*</td>
</tr>
<tr>
<td><strong>FPA (ng/ml)</strong></td>
<td>1.2±0.6</td>
<td>2.7±0.9*</td>
</tr>
<tr>
<td><strong>tPA-PAI (ng/ml)</strong></td>
<td>13.8±2.1</td>
<td>20.2±9.3*</td>
</tr>
<tr>
<td><strong>β-TG (ng/ml)</strong></td>
<td>46.3±8.5</td>
<td>120.0±61.1*</td>
</tr>
<tr>
<td><strong>TM (ng/ml)</strong></td>
<td>3.0±0.5</td>
<td>3.1±0.9</td>
</tr>
</tbody>
</table>

Data are mean±SD. *p<0.05, compared with the control group.

Figure 1. Comparison of TAT, FPA, tPA-PAI, and β-TG before and after 12 months of domiciliary oxygen therapy (DOT). DOT (+): COPD patients treated with DOT during a period of this study. DOT had no effect on TAT, FPA, and tPA-PAI but significantly reduced β-TG levels. Significant difference: *: p<0.01.

Effects of DOT on procoagulant and fibrinolytic markers

Twenty of 32 patients prescribed DOT commenced such treatment soon after study entry, while the remaining 12 patients were prescribed DOT sometime during the study. The use of DOT in 32 of 40 COPD patients was not associated with changes in the plasma concentrations of TAT, FPA, and tPA-PAI. However, β-TG decreased significantly after the use of DOT (Fig. 1). Based on the levels of each procoagulant and fibrinolytic marker, patients with COPD were divided into low and high groups. The low group was defined as patients with TAT, FPA, or tPA-PAI levels below the mean+2SD of control, and the high group as those with values above the mean+2SD of control. Δ-A-aDO₂ (increase in A-aDO₂ during the 12 month period) was significantly greater in the high TAT or tPA-PAI group than in the low group (Fig. 2). In contrast, Δ%FEV₁ (decrease in %FEV₁ during the 12 month period) was significantly greater in the high TAT or tPA-PAI group than in the low groups (Fig. 3), whereas there was not a significant difference in Δ%VC (change in FVC during the 12 month period) between the two groups (data not shown).

Discussion

Our study provides evidence for the presence of a hypercoagulative state in COPD patients. Thrombin plays a primary role in the coagulation-fibrinolytic cascade, activates platelets, combines with antithrombin III, transforms fibrinogen to fibrin, and stimulates endothelial cells to release tPA following tPA-PAI formation. Because thrombin cannot be measured directly, measurements of thrombin-associated products such as TAT,
FPA, tPA-PAI, and β-TG are necessary to evaluate the coagulation-fibrinolytic condition. High levels of TAT and FPA indicate excessive thrombin formation in patients with COPD. Because tPA-PAI is associated with secondary fibrinolysis following activation of the coagulation system, the high tPA-PAI levels indicate indirectly that COPD patients may be in a hypercoagulative state. Arboix et al. studied 1,473 consecutive ischemic stroke patients and showed that the presence of COPD was a strong predictor of lacunar stroke (19). Other studies reported that COPD are also at high risk of ischemic stroke after general surgery (20). These studies suggest the presence of a hypercoagulative state in systemic circulation in COPD patients. Recently, Wedzicha et al. (21) reported that acute exacerbations of COPD are often associated with increased serum IL-6 levels and a rise in plasma fibrinogen. Taken together, the above findings and our results suggest that exacerbation of COPD induces an enhanced procoagulative state, though we could not clarify the mechanism how a decrease in FEV₁ results in the enhanced procoagulative state.

To our knowledge, there are no reports on the relationship between changes in lung function and the coagulation-fibrinolysis system in COPD patients. In the present study, changes in A-aDO₂ or %FEV₁ at the 12 month follow-up period were significantly greater in COPD patients with high TAT or tPA-PAI levels. Because high levels of TAT and tPA-PAI are thought to reflect excessive intravascular thrombin formation, microthrombosis may be present in the COPD patients with high TAT and tPA-PAI levels. If so, pulmonary vessel thrombosis may result in ventilation/perfusion inequalities followed by a greater A-aDO₂ gradient. Our short-term follow-up study of lung function and gas exchange indicated that the extent of the hypercoagulative state might result in deterioration of the ventilatory capacity after one year.

Our study also demonstrated that β-TG levels in COPD patients are high compared with those of control subjects and that TM levels in COPD patients are not significantly different from those of control subjects. High plasma levels of β-TG have been reported in pulmonary hypertension due to COPD (10, 22), suggesting a relationship between increased pulmonary vascular resistance and platelet activation. Platelet activation in COPD patients could actually occur in the systemic circulation as a result of hypoxemia, acidosis or hyperviscosity (23). As shown in Fig. 1, prolonged oxygen treatment reduced β-TG levels in COPD patients, suggesting that DOT could suppress excess platelet activation. However, our results could not establish whether DOT induces normalization of procoagulant markers in COPD patients, because none of our 32 COPD patients was prescribed 24-hour DOT.

TM is a highly sensitive marker of endothelial cells injury (24). High TM levels are present in patients with chronic diseases such as diabetes mellitus and cancer (25), and are associated with a hypercoagulative state. In our study, TM levels in patients with COPD were not significantly different from those of controls. The hypercoagulative state in patients with COPD is therefore unlikely to be caused by pulmonary endothelial cell injury.

In the present study, BMI was lower in the COPD patients than in control subjects. Di Francia et al (26) reported that 30 to 50% of COPD patients had weight loss and high plasma levels of tumor necrosis factor (TNF). Previous reports showed that TNF may be involved in the disturbance of the procoagulant-fibrinolytic balance, leading to microvascular thrombosis (27),...
and that cytokines released from activated platelets may play a role to modulate hemostasis and thrombosis (28). Based on the above findings, we postulate that certain cytokines, such as TNF, may participate in the hypercoagulative and hyperfibrinolytic state in COPD patients. Further studies are necessary to measure changes in cytokines in patients with COPD.

In conclusion, we have demonstrated in the present study the presence of a hypercoagulative state in COPD patients, which may be associated with the development of disease and may result in deterioration of pulmonary function. Further studies are needed to identify the exact mechanisms underlying the hypercoagulative state in COPD, and to determine whether anticoagulant therapy is clinically useful in COPD.

References


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