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## Endothelial Cell Activity in Chronic Obstructive Pulmonary Disease Without Severe Pulmonary Hypertension

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**Summary:** Pulmonary hypertension is common in patients with chronic obstructive pulmonary disease (COPD), but the precise mechanism of vascular impairment in these patients is unknown. We, therefore, decided to investigate whether endothelial cell dysfunction is present in patients with COPD with a wide range of chronic airflow obstruction before the development of severe pulmonary hypertension. Selected plasma markers of endothelial cell activity were studied: nitrate+nitrite (NO<sub>2</sub>/NO<sub>3</sub>), thrombomodulin (TM), tissue factor pathway inhibitor (TFPI), soluble selectins (endothelium sES, leukocyte sLS, platelet sPS), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble platelet endothelial cell adhesion molecule-1 (sPECAM-1). Twenty-five patients with COPD (forced expiratory volume in one second/vital capacity [FEV<sub>1</sub>/VC] < 88% predicted) and 29 healthy control subjects were recruited to the study. Among patients nine had a pulmonary artery systolic pressure (PASP) between 15 and 30 mmHg, 13 between 32 and 38 mmHg, 2 had a PASP of 41 and 42 mmHg, respectively. One patient had severe pul-

monary hypertension with a PASP of 70 mmHg. The average FEV<sub>1</sub> of patients with COPD was 46 ± 4% predicted. As compared to control subjects, patients with COPD showed a significant increase in plasma levels of TM and TFPI, indicating that their endothelial cells are still able to produce potent coagulation inhibitors. Levels of NO<sub>2</sub>/NO<sub>3</sub> were similar in the two groups of subjects examined, further suggesting preserved endothelial function in patients with COPD. In regard to adhesion molecules, patients with COPD showed a reduction in sLS, sPS, and sPECAM-1, and an increase in sICAM-1. This study shows that endothelial cell activity is largely preserved in patients with COPD without severe pulmonary hypertension, suggesting that these patients, despite quite severe airway obstruction, retain reasonably normal endothelial function until they develop severe pulmonary hypertension.

**Key Words:** Chronic obstructive pulmonary disease—Pulmonary hypertension—Nitric oxide—Thrombomodulin—Tissue factor pathway inhibitor—Selectins.

Chronic obstructive pulmonary disease (COPD) is a disorder characterized by progressive airflow obstruction and chronic inflammation. Pulmonary hypertension, cor pulmonale and thrombotic complications are common sequelae to chronic airflow obstruction (1–3). However, the precise mechanisms involved in these pulmonary vascular disorders are not fully understood.

Under normal physiological conditions, the endothelium tends to favor vasodilation, inhibition of thrombosis, and leukocyte adhesion, pro-

ducing a variety of mediators, such as nitric oxide, thrombomodulin, and selectins (4). Therefore, impairment of endothelial function may play a crucial role in the initiation and progression of vascular disorders (5). We have previously demonstrated that in patients with severe pulmonary hypertension, significant endothelial cell dysfunction is present, as shown by a reduction of plasma levels of nitric oxide and thrombomodulin (6).

The aim of the present study was to investigate whether endothelial cell dysfunction is present in patients with COPD with a wide range of chronic airflow obstruction, but without severe pulmonary hypertension. In particular, we examined the plasma levels of thrombomodulin (TM), tissue factor pathway inhibitor (TFPI) (which are potent inhibitors of coagulation pro-

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duced by endothelial cells), nitrate + nitrite ( $\text{NO}_2/\text{NO}_3$ ), and several adhesion molecules (sP, sL, sE selectins, soluble intercellular adhesion molecule-1 [sICAM-1], and soluble platelet cell adhesion molecule-1 [sPECAM-1]).

## PATIENTS, MATERIALS AND METHODS

### Patients

We recruited 21 men and 4 women with COPD. The disease was defined as forced expiratory volume in one second/vital capacity ( $\text{FEV}_1/\text{VC}$ )  $< 88\%$  predicted after 200  $\mu\text{g}$  of inhaled salbutamol, according to the Criteria of the European Respiratory Society Consensus Statement (7). During the month preceding the study, no patient had an acute exacerbation, defined as increased dyspnea associated with a change in quality and quantity of sputum that would have led to seeking medical attention. The patients were nonatopic (i.e., they had negative skin tests for common allergens) and had no past history of asthma or allergic rhinitis. All subjects recruited had no past history of pulmonary neoplasm, severe cardiovascular disease, diabetes mellitus, or interstitial lung disease. None of them had received systemic glucocorticoids. Patients using bronchodilators withdrew the treatment 48 hours before collection of blood samples.

The study conformed to the Declaration of Helsinki, and informed written consent was obtained for each subject. Each patient underwent an interview, echocardiography, arterial blood gas tension, and complete blood count. We used continuous-wave Doppler echocardiographic analysis for estimations of pulmonary artery systolic pressure (PASP). The expected upper limit of PASP, among echocardiographically normal subjects, is dependent on age, gender, and body mass index, and may be as great as 40 mmHg in some older or obese subjects (8). Pulmonary function tests, which were performed as previously described (9), included measurements of vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in one second ( $\text{FEV}_1$ ), total lung capacity (TLC), and carbon monoxide diffusing capacity (Dlco).

A control group of 29 healthy nonsmoking volunteers was selected from blood donors and hospital staff.

### Collection of Blood Samples

Venous blood samples were drawn without stasis from the antecubital vein by using a double

syringe butterfly (20 G needle) technique into 3.8% sodium citrate (9:1, v/v). The collected blood samples were centrifuged at 3000 g for 20 minutes at  $+4^\circ\text{C}$ . The plasma was divided into aliquots and kept frozen at  $-70^\circ\text{C}$  until analysis.

### Laboratory Tests

Soluble adhesion molecules of the selectin class (endothelium sES, leukocyte sLS, platelet sPS), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble platelet endothelial cell adhesion molecule-1 (sPECAM-1) with concentrations expressed in nanograms/mL (ng/mL) were determined by using commercial enzyme-linked immunosorbent sandwich assays (ELISA) supplied by R&D System Minneapolis MN, USA and by Bender MedSystems, Vienna, Austria. Tissue factor pathway inhibitor (TFPI), total and free, and thrombomodulin (TM) antigens expressed in nanograms/mL (ng/mL) were measured by commercially available ELISA kits supplied by Diagnostica Stago Ltd (Asnières sur Seine, France). Nitrate + nitrite ( $\text{NO}_2/\text{NO}_3$ ) in nanomoles/mL (nmol/mL), as a measure of nitric oxide (NO) metabolism, was determined with a quantitative colorimetric commercial kit supplied by Cayman, Ann Arbor, MI, USA (10).

### Statistical Analysis

All results were expressed as a mean  $\pm$  standard error (SE) of the mean. Analysis of the differences was performed by Mann-Whitney U test. A  $p$  value  $< 0.05$  was taken to be significant. Correlation coefficients were determined by the Spearman test and from the linear correlation analysis.

We used a General Linear Model (GLM) to assess the influence of age and current smokers on studied parameters. Since 19 patients stopped the smoking habit at least 6 years (6–20 years) before the study, we considered these subset as nonsmokers.

## RESULTS

### Clinical Findings

Patients with COPD and controls were similar with regard to gender (21 men/4 women vs 23 men/6 women), leukocyte counts (mean  $\pm$  SE:  $7.06 \pm 2 \times 10^9/\text{L}$  vs  $6.35 \pm 1.6 \times 10^9/\text{L}$ ) and platelet counts ( $223.7 \pm 54.4 \times 10^9/\text{L}$  vs  $248.74 \pm 50.97 \times 10^9/\text{L}$ ). Patients with COPD were significantly older than controls ( $69.6 \pm 6.9$  vs  $54.9 \pm 7.4$ ;  $p < 0.05$ ).

As for pulmonary function, patients with COPD showed: FEV<sub>1</sub> 46±4% predicted, FEV<sub>1</sub>/VC 54±3% predicted, TLC 95±3% predicted, DLCO 51±4% predicted, partial pressure of arterial oxygen (PaO<sub>2</sub>) mmHg 71±2, partial pressure of arterial carbon dioxide (PaCO<sub>2</sub>) mmHg 42±1, arterial oxygen saturation (SaO<sub>2</sub>) mmHg 93.04±0.67, and pH 7.40±0.01. Sixteen had severe airway obstruction (FEV<sub>1</sub> < 50% predicted), seven had moderate airway obstruction (50 ≤ FEV<sub>1</sub> < 70% predicted), and two had mild airway obstruction (FEV<sub>1</sub> ≥ 70% predicted). Nineteen subjects had symptoms of chronic bronchitis (cough and sputum production occurring on most days of the month for at least 3 months a year during the 2 years prior to the study).

Twenty-four of 25 patients with COPD had a history of cigarette consumption (19 ex-smokers, and five current smokers with a mean pack-years of 40±5).

Among patients with COPD, nine had a PASP between 15 and 30 mmHg, 13 had a PASP between 32 and 38 mmHg, and two had a PASP of 41 and 42 mmHg, respectively. One patient had severe pulmonary hypertension with PASP of 70 mmHg.

**Thrombomodulin-Tissue Factor Pathway Inhibitor**

Thrombomodulin (controls 31.7 ± 2.46 ng/mL; patients 57.4 ± 4.5 ng/mL, p<0.001), total TFPI (controls 76.2 ± 3.3 ng/mL; patients 96.7 ± 3.7 ng/mL, p<0.001), and free TFPI (controls 10.6 ± 1.0 ng/mL; patients 16.2 ± 0.9 ng/mL, p<0.001) were significantly higher in patients with COPD as compared to controls (Fig. 1).

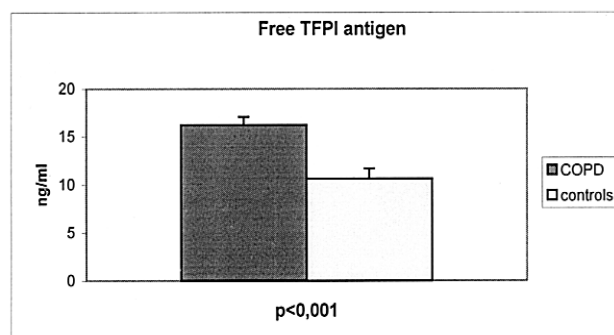
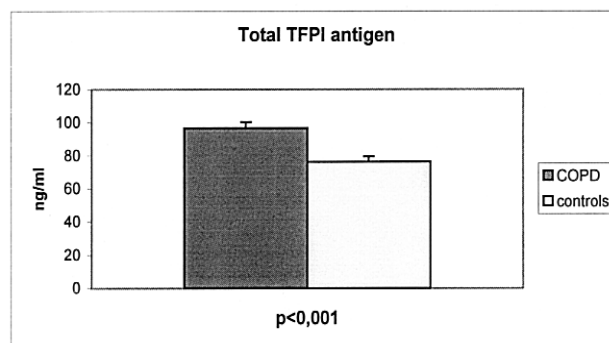
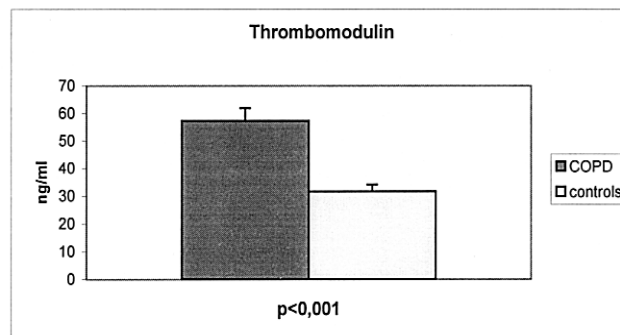
**Nitrate + Nitrite**

NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup>, an expression of nitric oxide (NO) metabolism, was similar in patients and controls (controls: 28.6 ± 3.3 nmol/mL, patients: 35.6 ± 4.0 nmol/mL; p=n.s.).

**Cellular Adhesion Molecules**

The level of sICAM-1 was significantly increased in patients with COPD as compared to controls. sES was similar in the two group of subjects, whereas sLS, sPS, and sPECAM-1 were significantly reduced (Table 1).

The only patient with severely elevated pulmonary artery systolic pressure (PASP) (70 mmHg) exhibited the lowest NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> level (8.8 nmol/L) and high levels of sES (49.2 ng/mL).



**FIG. 1.** Plasma levels of thrombomodulin and tissue factor pathway inhibitor (TFPI) total and free in patients with COPD and controls. Highly statistical differences (p<0.001) were found between patients and controls.

**GLM Analysis**

The GLM analysis showed that COPD was the only factor affecting the plasma levels of TM (F 26.6, p<0.01) and TFPI total (F 17.02, p<0.01) and free (F 16.33, p<0.01) plasma levels. In con-

**TABLE 1.** Soluble Cellular Adhesion Molecules in Patients with COPD and Controls

Adhesion Molecules	COPD	Controls	p
	Nanograms/mL $\pm$ SE		
sE-selectin	22.01 $\pm$ 15.6	22.38 $\pm$ 13.5	n.s.
sL-selectin	764 $\pm$ 21	869.8 $\pm$ 72.9	<0.05
sP-selectin	130 $\pm$ 8.9	186 $\pm$ 134	<0.05
sICAM-1	559 $\pm$ 198	427.2 $\pm$ 118	<0.001
sPECAM-1	26.06 $\pm$ 10.1	34.98 $\pm$ 13.9	<0.05

sICAM-1: Soluble intercellular adhesion molecule-1; sPECAM-1: Soluble platelet endothelial cell adhesion molecule-1.

trast the plasma levels of the adhesion molecules were also influenced by age and smoking habitus.

### Correlations

In patients with COPD, the levels of sPECAM-1 were positively correlated with the levels of sP selectin ( $r$  0.43,  $p$ <0.05), sE selectin ( $r$  0.52,  $p$ <0.01), and sL selectin ( $r$  0.53,  $p$ <0.01). The levels of total TFPI were positively correlated with the levels of free TFPI ( $r$  0.65,  $p$ <0.01). No significant correlations were observed between the degree of airway obstruction (as measured by FEV<sub>1</sub>) and any of the plasma markers tested.

## DISCUSSION

This study shows that patients with COPD without severe pulmonary hypertension have a significant increase in plasma levels of TM and TFPI, indicating that their endothelium is able to produce potent coagulation inhibitors. Levels of NO<sub>2</sub>/NO<sub>3</sub> were similar in COPD patients and controls, suggesting a preserved endothelial function in patients with COPD. In regard to adhesion molecules, patients with COPD showed a reduction in sLS, sPS, and sPECAM-1, but an increase in sICAM-1.

Pulmonary hypertension, in which thrombotic complications in the pulmonary circulation may occur, is a common feature in patients with COPD, in particular when the disease becomes severe (1). The impairment of endothelial function may play a crucial role in the initiation and progression of these vascular disorders (1). Indeed, the pulmonary endothelial dysfunction has been demonstrated in end-stage patients with COPD undergoing lung transplantation (11). Peinado and co-workers (12) have reported

that endothelial dysfunction may also be present in mild COPD, as shown by impaired endothelium-dependent relaxation and by a thickened intimal layer in small pulmonary arteries. As a result, there is an impaired release of endothelium-derived relaxing factors (nitric oxide) that may predispose these patients to pulmonary hypertension. The mechanisms involved in the development of pulmonary vascular abnormalities are not, however, fully understood.

Patients with COPD have an ongoing prothrombotic state, as shown by high levels of prothrombin F 1+2 fragment, which could potentially account for thrombosis occurring in pulmonary vessels and the consequent increase of pulmonary vascular resistance (13,14). Thrombomodulin is the endothelial-surface receptor for binding and inactivating thrombin, leading to subsequent activation of protein C (15). TFPI is a Kunitz-type protease inhibitor of the factor VIIa/tissue factor complex in the presence of factor Xa.; it is also a direct inhibitor of factor Xa (16). Thrombomodulin and TFPI are powerful inhibitors of the coagulation cascade, primarily synthesized by the vascular endothelium of the lung and heart (16,17). In fact, high levels of thrombomodulin are associated with a decreased risk of coronary artery disease (18,19). By contrast, reduced levels may further contribute to the initiation or worsening of "in situ" thrombosis (20).

The high levels of thrombomodulin and TFPI found in the present study indicate that patients with COPD may retain reasonably normal endothelial function until they develop a severe pulmonary hypertension. This is supported by our previous report in which we found that patients with severe pulmonary hypertension have low levels of soluble TM and NO, and high levels of sES, suggesting serious impairment of endothe-

lial function (6). In the present study only the single patient with COPD with severe pulmonary hypertension manifested similar abnormalities.

It is conceivable that the increased expression of thrombomodulin and TFPI in our patients may downregulate thrombin production. This event may result in reduction of the release of specific selectins since thrombin is a strong inducer of platelet, leukocyte, and endothelial cell activation (21–23). The reduced levels of sPS and sLS in patients with COPD observed in the present study support this hypothesis. We cannot, however, completely exclude the alternative hypothesis, that the high levels of TM and TFPI reflect significant endothelial cell activation and injury, leading to exhausted circulating platelets and leukocytes and reduced level of sPS and sLS.

Among its functions, endothelium modulates leukocyte adhesion through adhesion molecules, such as ICAM-1. ICAM-1 can be expressed not only on endothelial cells and leukocytes but also on dendritic cells, fibroblasts, and epithelial cells (24). ICAM-1 serves as the main surface receptor for rhinoviruses and is upregulated by viral infection (25–27). Since its expression is increased in airway epithelial cell of patients with COPD (28,29), it is possible that a chronic, latent viral stimulus may induce increased expression of ICAM-1, suggesting the involvement of this adhesion molecule in the pathogenesis of COPD. Our observation of increased plasma levels of soluble ICAM-1 in COPD, which is in agreement with the previous findings of Riise and colleagues (30), supports this hypothesis. Moreover, it is conceivable that high levels of circulating ICAM-1 may compete with membrane ICAM-1 on vascular endothelium, thus preventing the activation of granulocytes and their interactions with platelets and endothelial cells. The low or normal levels of sPS, sLS, sES, and sPECAM-1 observed in our study seem to support this possibility. At variance with the report of Riise and colleagues (30), we found no increase in serum levels of ES in patients with COPD. This apparent discrepancy may be due to the different COPD populations examined in the two studies. Riise and colleagues (30) examined patients with a milder degree of airway obstruction (average  $FEV_1 = 63\%$  predicted), and they found that the serum level of ES was positively correlated with the values of  $FEV_1$  (the higher the value of  $FEV_1$ , the higher the level of ES). The low value of  $FEV_1$  in our study may, therefore, explain the absence of an increased level of ES.

In conclusion this study shows that endothelial cell activity is largely preserved in patients with COPD without severe pulmonary hypertension, suggesting that these patients, despite quite severe airway obstruction, retain reasonably normal endothelial function until they develop severe pulmonary hypertension.

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