

# Decreased Levels of Protein C and Protein S in Patients with COPD and Pulmonary Embolism

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**Abstract:** *Introduction:* Pulmonary embolism (PE) appears to be a major threat in patients who suffer of chronic obstructive pulmonary disease (COPD) and 13% of these patients suffer of embolism events. There are several causes for the development of a thrombophilic condition in patients with COPD, with abnormalities of the coagulation pathway being one of them. The aim of this study was to research the frequency of abnormalities in the anti-clotting proteins in COPD patients who have had a documented event of PE.

*Methods:* Forty-three COPD/PE patients were studied and their levels of anti-clotting proteins were compared with those of 40 patients diagnosed only with COPD.

*Results:* A reduction in anti-clotting proteins was identified in 23% (10/43) of COPD/PE patients, but in none of the COPD patients showed this condition. In the COPD/PE group, 8 patients showed a significant decrease in protein C (mean  $27 \pm 8\%$ ), and also 5 patients showed decrease levels of protein S (mean  $28 \pm 7\%$ ). Interestingly, decreased levels in both protein C and protein S were observed in 3 of them. None of the COPD/PE patients showed evidence of alterations in the values of activated protein C resistance.

*Conclusion:* Our data shows a higher frequency of alterations in the endogenous anticoagulant protein system in COPD/PE patients. More studies are needed to identify a high risk for patients suffer from this disease.

**Keywords:** Activated protein C resistance, antithrombin III, chronic obstructive pulmonary disease, pulmonary embolism, protein S, protein C.

## INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a worldwide health problem with increased morbidity and mortality [1, 2]. The clinical course of COPD may be complicated by pulmonary embolism (PE), accounting from 10 to 27 % [3, 4] of the patients, and seems to be a relatively frequent cause of death in these patients [5-7].

It is well known that patients who are admitted for acute exacerbations of COPD are generally considered to be at a moderate risk of developing PE, because of the presence of concomitant risk factors, such as immobilization, bronchial superinfection, right ventricular failure and venous stasis. Also, the thrombotic event could be a consequence of vascular and alveolar lesions [8, 9]. Additionally, some reports have indirectly suggested that changes in platelets or abnormalities in some of the components of the coagulation pathway [10-13] lead patients with COPD to a prothrombotic condition and increase the risk of thromboembolic disease [14]. For example, the exacerbation of COPD was greater in patients with high thrombin-antithrombin III complex (TAT)

or tissue plasminogen activator-plasminogen activator inhibitor complex (tPA-PAI) levels [15]. Others [16, 17] reported that acute exacerbations of COPD are associated with the rise in the levels of plasma fibrinogen.

Given the importance of knowing more about the factors related to the development of the thrombotic condition in COPD patients, and the little information about this subject, we investigated the frequency of abnormalities in the anti-clotting proteins in COPD patients who have had a documented PE.

## METHODS

We studied 43 patients with stable COPD and evidence of having developed an event of PE, all of them referred by the *Thrombophilic Research Group* from the National Institute of Respiratory Disease in Mexico. The definition of COPD was consistent with that of the American Thoracic Society consensus statement [18]. The patients had a history of chronic or recurrent productive cough for >2 years and decreased maximum expiratory flow, which had been slowly progressive and irreversible. The presence of others lung or cardiac diseases as the cause of patients' symptoms was excluded by clinical and radiographic examination. The criteria for selection were as follows: (i) all COPD patients were in stable phase: blood gas values and FEV<sub>1</sub>/FVC ratio did not show any significant change in the last 6 month prior to the study, and, (ii) patients with PE (diagnosed by

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perfusion lung scan or angiography, confirming a total occlusion of the right pulmonary artery [19]).

Patients with acute inflammatory disease, metabolic acidosis, immunological disease, cancer, hypertension, diabetes mellitus, surgery, immobilization, pregnancy, postpartum trauma, oral contraception, renal diseases or those who were receiving anti-platelet or anti-coagulant drugs (warfarin) during the previous 20 days, were excluded. The patient's name, age, sex, the duration of the diseases and chest radiographic findings were recorded. All patients were current smokers. While COPD/PE patients were being recruited, 40 stable COPD patients were also being selected using the same criteria as the previous group and their levels of protein coagulation markers were used as comparison.

### Blood Collection and Coagulation Tests

Blood samples were collected into vacuum tubes containing 0.129 mol/L trisodium citrate and were centrifuged at 2,000 g for 15 min to obtain platelet-poor plasma. This was frozen and stored at -20°C until assayed. Plasma levels of selected coagulometric parameters were determined as follows: Protein C (STACLOT® PROTEIN C); protein S (STACLOT® PROTEIN S); prothrombin time (PT): (Start®- NEOPLASTINE® CL); kaolin-activated partial thromboplastin time (APTT): (C.K. PREST®); and activated protein C resistance (APC resistance) (STACLOT® APC-R) were measured by clotting assays. Clotting assays were performed on a coagulometer (STACompact® Hemostasis System, Diagnostica, Stargo, France). Antithrombin III (AT-III) was determined by a colorimetric assay (STACROM® AT III). All the determination system kits were obtained from Diagnostica Stago, Asnieres, France. To reduce measurement error, all assays were performed in duplicate and the average value was calculated for each person. Intra-assay and inter-assay coefficients of variation

were estimated between 2 and 6%.

### Statistical Analysis

Data analyses were performed with the personal computer software (*Sigma Stat for Windows Version 3.5*). Data was expressed as mean  $\pm$  SD. Differences in proportions were tested by the student t test, as appropriate. A two-sided  $p < 0.001$  was regarded as significant.

### RESULTS

Forty-three patient with diagnosis of COPD/PE and 40 COPD patients were studied. After analysis, the following results were obtained: In the COPD/PE patients, 26 were female and 17 male, whereas in the group of COPD patients, there was a predominance of male gender.

Table 1 shows the main characteristics of both groups: thus, the median age for COPD/PE patients was  $56 \pm 12$  (range 40-83 years of age), whereas for COPD patients was  $69 \pm 8$  (range 53-90 years of age). Interestingly, the COPD/PE group was slightly younger than patients with only COPD and also showed higher number of patients with obesity, most of them being female.

In our study, both subgroups showed similar frequency of deep vein thrombosis (DVT), 80 and 79%, respectively. It is known that patients with COPD have a higher risk for DVT and in different studies; it has reported that the incidence of DVT in patients with COPD ranges from 20 to 60% [20]. In addition, pulmonary embolism is also a complication of DVT. The underlying mechanism for this increased risk of venous thromboembolism (VTE) is unknown, but is likely to be associated with changes in endothelial function and integrity, and with induction of a state of hypercoagulability resulting from changes in pro- and anti-coagulant factors associated with inflammation and its therapy.

**Table 1. General Characteristics and Plasma Levels of Coagulations Factors in Patients with COPD vs COPD/PE**

	COPD (n=40)	COPD/PE (n=43)		
<b>General Characteristics</b>				
Gender (M/F)	26/14	17/26		
Age (range)	$69 \pm 8$ (53-90) yrs	$56 \pm 12$ (40-83) yrs		
DVT	32 (80%)	34 (79 %)		
Ulcers in lower limbs	0 (0%)	8 (18.6 %)		
Obesity	16 (40 %)	31(72 %)		
Smokes	40 (100%)	43 (100%)		
<b>Plasma Clotting Proteins</b>			<i>Normal Reference Values</i>	<i>P-Value</i>
Prot C (%)	$117.8 \pm 21.6$	$91.4 \pm 34.3$	70-130	*0.0001
Prot S (%)	$115.2 \pm 26.2$	$83.5 \pm 24.2$	65-140	*0.0001
AT III (%)	$97.6 \pm 13.4$	$94.0 \pm 8.0$	80-120	NS
APCR (%) <sup>§</sup>	$161.8 \pm 18.4$	$165.8 \pm 23.9$		NS
PT (INR)	$1.3 \pm 0.1$	$1.2 \pm 0.2$	1.0-1.3	NS
APTT (sec)	$35.8 \pm 4.1$	$38.1 \pm 4.4$	26.5-40	NS

<sup>§</sup>Clotting time equal to or higher than 120 seconds are considered to be negative for APC resistance. Data are mean  $\pm$  SD. \*  $p < 0.001$ , compared with the COPD group. NS: statistically non significant.

The determination of levels of anti-clotting proteins showed some interesting differences. Thus, for example, the median percent levels of protein C in COPD/PE patients was lower than the obtained in patients with COPD only ( $91.4 \pm 34.3$  vs  $117.8 \pm 21.6\%$ ). The COPD/PE patients also showed a highly significant decrease in the levels of protein S in comparison to the COPD group ( $83.5 \pm 24.2$  vs  $115.2 \pm 26.2\%$ ) (Table 1).

Although the levels of anti-clotting proteins in COPD/PE were still into the normal values; the decrease is *per se* a remarkable phenomenon.

Of 43 patients with COPD/PE, 23% (10/43) showed decrease in levels of anti-clotting system. Eight patients showed a significant decrease in protein C levels whereas another 5 patients showed protein S decrease levels (mean levels of  $27 \pm 8$  and  $28 \pm 7\%$ , respectively). Interestingly, 3 patients showed evidence of decrease in both protein C and protein S (N8, N27 and N41) (Table 2). It is striking that all of them also showed slight a prolongation of prothrombin time, and 2 of them also showed prolongation in the activated partial thromboplastin time.

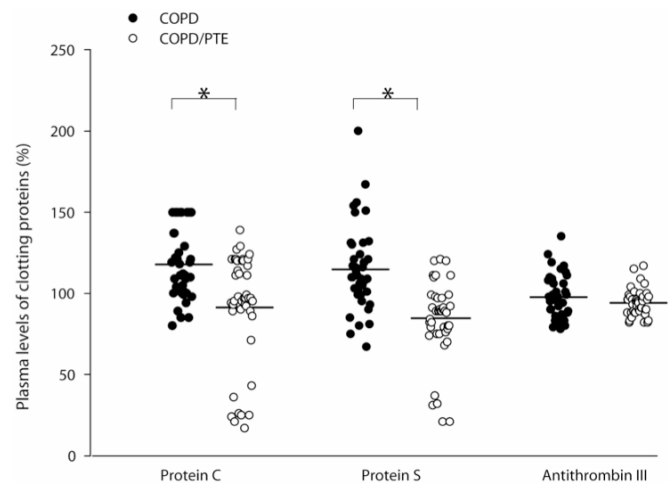
Fig. (1) shows clear evidence that some COPD/PE patients exhibit a decline in 2 endogenous anticoagulant proteins (PC and PS), but no differences could be seen for the AT-III anti-clotting protein. Despite the lower levels of protein C and protein S found in some COPD/PE patients, the overall determination of the PT and APTT in COPS/PE group did not seem to be statistically different from the clotting times observed for COPD patients (Table 1). Likewise, no evidence of APC resistance could be found in any of the groups of COPD patients.

**DISCUSSION**

PE may precipitate an exacerbation of COPD causing additional diagnostic uncertainty with overlapping symptoms from both disorders. Risk factors for PE include conditions that impair venous return, endothelial injury or dysfunction, and underlying hypercoagulability disorders.

In this study, we found that 23% of the patients with

stable COPD and PE showed decrease in either protein C or protein S, but none of the COPD patients showed this condition.



**Fig. (1).** Levels of protein C, protein S and antithrombin III in COPD/PE and COPD patients. Line represents the average for all data. \* Statistic difference with  $p < 0.001$ .

The occurrence of decrease in protein C in COPD/PE patients was of 18% (8/43), this % is higher compared with the prevalence of heterozygous protein C deficiency reported for healthy blood donors (0.1 to 0.4%) [21], unselected patients (3.7%) or thrombotic patients (4.8%) [22-25]. In addition, a protein S decrease was also found in 11% (5/43) of our COPD/PE patients. Once again, protein S decrease was higher than the prevalence reported for unselected patients (2.3%) or thrombosis patients (4.3%), but close to that reported for symptomatic protein S deficient relatives of Italian families (14%) and 1-13% of thrombophilic patients with VTE, respectively [26, 27].

The molecular genetic background of Protein C and Protein S alterations is heterogeneous. Thus, the diagnosis of PC and PS deficiencies is challenging; functional tests are influenced by several pre-analytical and analytical factors, and the diagnosis using molecular genetics also has special

**Table 2. Individual Values of Ten Selected Patients COPD/PE with Alterations in Coagulation System**

No. of Patient	Gender	Age	Prot-C (%)	Prot-S (%)	AT-III (%)	PT (INR)	APTT (sec)
N2	M	73	24	82	96	1.4	35
N6	F	62	36	82	83	1.3	41
N8	F	40	21	31	104	1.5	41
N12	F	56	127	37	93	1.3	35
N16	F	44	26	75	88	1.3	40
N17	M	66	112	32	88	1.3	40
N21	F	48	25	75	95	1.3	40
N27	M	55	17	21	109	1.5	46
N36	F	72	25	70	87	1.3	42
N41	F	68	43	21	82	2.7	54
Reference Values			70-130	65-140	80-120	1.0-1.3	26.5-40

difficulties [28]. For example, in Protein C Type II deficiency a dysfunctional molecule is diagnosed in approximately 5-15% of cases. In our study, the low levels of anti-clotting proteins suggest a decrease activity but not a protein decrease. Therefore, the frequency of PC and PS abnormalities in COPD/PE patients must be considered with caution.

It is well known that the frequency of alterations in clotting protein system is significantly higher in unselected patients with thrombotic disease than in healthy subjects. This difference is striking in selected patients with thrombotic diseases that are also likely, on clinical grounds, to have thrombophilia. Our elevated frequencies for Protein C and Protein S alterations in COPD/PE could be also interpreted as part of the inclusion criteria (i.e. pulmonary thromboembolism) used to join the COPD patients. However, since the unfeasibility to obtain a genetic characterization of each patient and their first degree relatives, the diagnostic of thrombophilia cannot be addressed for the moment.

In most patients with alterations in anti-clotting proteins, especially in those with protein C and protein S deficiency, the median age for the first thrombotic event occurs before the age of 45 [29, 30]. In our study, the age of onset for thrombotic symptoms in the group of COPD/PE patients is unknown. However, this group showed a median age of  $56 \pm 12$  years, which is younger than the median age of COPD patients ( $69 \pm 8$  years [ $p < 0.001$ ]) and similar to age reported for patients with either protein C or protein S deficiency [29, 30].

Among patients with thrombotic disease, the expression of at least a double-deficiency is frequently observed [31]. In many reports, these deficiencies are been often associated with alterations in the proteins of the Protein C pathway: i.e. APC resistance and Protein C or Protein S deficiencies and as well mutations in AT-III [32, 33]. In our study, 7% of COPD/PE patients showed double, protein C and protein S decrease activity.

It is also well known that in younger people, the combined defects in the coagulation system confer a higher risk of thrombosis than either of them alone. Symptomatic patients with Protein S deficiency also carry the FV Leiden mutation; for these patients, the median age of onset is 27 (10-60 years of age), which is similar to the one found in subjects with both protein C deficiency and FV Leiden mutation [30]. In our study, 3 COPD/PE patients with both Protein C and Protein S decrease activity were older ( $54 \pm 14$  years). One possible reason for this difference may be, COPD develops slowly over years, so most people are at least 40 years old when symptoms begin.

APC resistance is the most common defect of the coagulation system known to date, affecting about 21 to 60% of the thrombotic patients, and 3 to 7% of the healthy controls [34]. In addition, among patients with deficiencies of either Protein C or Protein S who have suffered venous thrombosis, the prevalence of FV Leiden is higher (40%) [26, 29, 30]. When we analyzed our COPD/PE patients, we did not find evidence of functional alterations in APC resistance. However, our data seems to be in accordance with some reports that mentioned a lower prevalence of APC resistance or FV Leiden mutation in patients with pulmonary

embolism [35]. Furthermore, it is known that the prevalence for APC resistance in thrombotic diseases is different in diverse populations [12] and only 10% of Mexican mestizos with the APC resistance phenotype have been found to display FV Leiden mutation [36, 37].

The overall coagulometric determination of PT and APTT, between both COPD groups of patients did not show significant differences. However, COPD/PE patients with double decrease showed a prolongation in clotting time mediated by prothrombin, and 2 of them also showed a prolongation in APTT. In contrast, none of these patients showed evidence of a hemorrhagic event. The prolongation of the clotting time in these patients does not appear to be general due to liver disease or disseminated intravascular coagulation (DIC), since ATIII levels were normal. Mixing experiments to rule out inhibitors and individual factor assays are required to clarify this. Additionally, we can not find any data in the literature about how long after warfarin cessation the clotting levels are normalized. A 20 day interval is reasonable clinically, but we really do not know from actual observational studies that it is enough.

Finally, to address the question whether the abnormalities in Protein C and Protein S are congenital or acquired, it will be suitable to study also a group with PE and not COPD at the same time interval after the event as has been done for the PE/COPD group.

Our results showed a higher frequency of alterations in clotting proteins in patients with COPD and pulmonary embolism. Protein C and protein S were the main deficiencies found, whereas no evidence of APC resistance can be confirmed.

More research is needed to clarify the cause of the decrease of anti-clotting in COPD/PE patients and to establish whether and when prophylaxis against PE should be started, especially during active disease, decreasing the risk of a fatal outcome in COPD patients.

## COMPETING INTERESTS

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally to this work.

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## ABBREVIATIONS

APC resistance	= Activated protein C resistance
APTT	= kaolin-activated partial thromboplastin time
AT-III	= Antithrombin III
COPD	= Chronic obstructive pulmonary disease
DVT	= Deep vein thrombosis
FEV <sub>1</sub> /FVC	= The proportion of the forced vital capacity exhaled in the first second
PC	= Protein C

PE	= Pulmonary embolism
PS	= Protein S
PT	= Prothrombin time
VTE	= Venous thromboembolism.

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