

Inflammatory markers in chronic obstructive pulmonary disease patients with different α 1 antitrypsin genotypes

Danielius Serapinas^{1,2}, Brigita Sitkauskiene¹, Raimundas Sakalauskas¹

¹Department of Pulmonology and Immunology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

²Mykolas Romeris University, Vilnius, Lithuania

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Corresponding author:

Danielius Serapinas PhD
Department of Pulmonology
and Immunology
Medical Academy
Lithuanian University
of Health Sciences
Eiveniu 2
Kaunas LT 50028, Lithuania
Phone: +37037326771
Fax: +37037326953
E-mail:
dserapinas@gmail.com

Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) has been recently defined as a systemic pulmonary inflammatory disease, and congenital α 1 antitrypsin deficiency is one of the well-established genetic risk factors for chronic obstructive pulmonary disease. The aim of our study was to evaluate the possible associations of α 1 antitrypsin with inflammatory markers – CRP, sCD14, TNF- α , sTNFR-1, and sTNFR-2 – in patients with COPD with different α 1 antitrypsin genotypes.

Material and methods: Serum biomarkers from patients ($n = 355$) with COPD, defined according to the GOLD criteria, were analyzed using commercial ELISA kits; α 1 antitrypsin concentrations were determined by nephelometry, and α 1 antitrypsin phenotyping was carried out by means of isoelectric focusing.

Results: No significant differences in CRP, TNF- α , sTNFR-1, sTNFR-2, and sCD14 levels were found comparing COPD patients with different genotypes. In patients without α 1 antitrypsin deficiency (PI*MM), a significant negative correlation between lung function (FEV₁) and serum α 1 antitrypsin ($r = -0.522$, $p = 0.03$) and CRP concentration ($r = -0.590$, $p = 0.011$) was detected. The level of α 1 antitrypsin positively correlated with: a) CRP concentration ($r = 0.671$, $p = 0.005$), b) sCD14 ($r = 0.510$, $p = 0.008$) and c) sTNFR-1 ($r = 0.567$, $p = 0.007$).

Conclusions: In patients without α 1 antitrypsin deficiency, the positive association of α 1 antitrypsin concentration with CRP, sCD14, and sTNFR-1 and the negative association with FEV₁ show the importance of α 1 antitrypsin as a marker of systemic inflammation.

Key words: chronic obstructive pulmonary disease, α 1 antitrypsin, inflammatory markers.

Introduction

Chronic obstructive pulmonary disease (COPD) is a prevalent and costly disease characterized by progressive airflow limitation, related to an abnormal inflammatory response of the lung to long-term tobacco smoke or inhalation of toxic gases [1]. Lung inflammation is further amplified by oxidative stress and proteolytic damage by proteinases [2]. There is increasing evidence of systemic inflammation in patients with COPD [3-8]. Thus, changes of inflammatory mediators can be evaluated not only in the airways and other pulmonary compartments but also in blood [3].

The prevalence of COPD is appreciably higher in current or former heavy smokers aged more than 40 years. However, there is consistent evidence that only 15-30% of smokers develop COPD [1] and some nonsmokers may also develop chronic airflow obstruction, suggesting that the risk of COPD results from a gene-environment interaction. α 1 Antitrypsin (AAT) deficiency is the best-described genetic risk factor for COPD. The primary function of AAT is to inhibit neutrophil elastase. In severe deficiency, anti-elastase protection in the lung interstitium and alveolar space is markedly decreased to about 15-20% of normal levels, similar to the decrease in plasma levels [9-11]. Most of the pathologies related to AAT deficiency are linked to the PI*Z allele, and in clinical practice, 96% of patients with AAT deficiency have a PI*ZZ genotype. The remaining 4% have PI*SZ, PI*MZ, and other rare deficiency genotypes [9].

An important mediator of inflammation in COPD patients is tumor necrosis factor α (TNF- α), produced primarily by activated macrophages [8]. Tumor necrosis factor α exerts its diverse biological effects by binding to two specific cell membrane receptors (mTNFR-1 and mTNFR-2), expressed on a variety of cells. Both receptors can be shed from the cell surface to form soluble TNF- α receptors, sTNFR-1 and sTNFR-2, which can compete with mTNFRs as ligands to TNF and thereby inhibit the effects of TNF- α [8].

Another mechanism leading to a protease-antiprotease imbalance in the lungs during antitrypsin deficiency (PI*ZZ phenotype) is that Z antitrypsin polymerizes abnormally in the lungs and acts as a neutrophil chemo-attractant, leading to the recruitment of neutrophils to the lungs [14]. It has been recently shown that AAT regulates many physiological and pathological processes, which may significantly influence the disease process, including cell-mediated immunity, apoptosis, tumor cell growth, and many others [15]. α 1 Antitrypsin has even been shown to regulate expression of CD14, a receptor for lipopolysaccharides (LPS), in human monocytes *in vitro* [16]. However, the potential role of systemic inflammation in the pathogenesis of COPD with different AAT genotypes has not been well established yet.

The aim of our study was to evaluate the possible associations between AAT and inflammatory markers – C-reactive protein (CRP), soluble CD14 (sCD14), TNF- α , sTNFR-1, and sTNFR-2 – in patients with COPD with different AAT genotypes.

Material and methods

Subjects

The patients for this study were recruited from the regional AAT deficiency and COPD registry of the Department of Pulmonology and Immunol-

ogy, Medical Academy, Lithuanian University of Health Sciences. From the registry, a total of 538 COPD patients without AAT deficiency (PI*MM) were randomly selected, and all 53 patients with AAT deficiency (PI*MZ, PI*SZ, and PI*ZZ genotypes) were selected. From 591 patients invited by post, 392 patients (66%) arrived and agreed to participate in the study. Out of 392 patients, 355 COPD patients (320 and 35 without and with AAT deficiency, respectively) met the following criteria: no use of oral corticosteroids or leukotrienes for at least 6 weeks prior to the study, none of the subjects showed signs of acute respiratory infection at least a month before the investigation, patients were free of systemic steroids for at least 1 month before the study. The patients also met the GOLD [1] spirometric criteria for COPD (ratio of postbronchodilator forced expiratory volume in 1 s (FEV₁) to forced vital capacity (FVC) less than 70% of predicted). Smoking history was calculated in pack-years as the product of tobacco use (in years) and the average number of cigarettes smoked per day/20 (years \times cigarettes per day/20). The study design was approved by the Regional Ethics Committee, and all studied subjects gave their informed consent.

Sample collection and evaluation

Blood samples were drawn in serum tubes, clotted at room temperature (\sim 22°C) for 30-60 min, and centrifuged for 15 min at 4000 rpm. Serum samples were immediately frozen at -70°C for further analysis. Serum concentrations of AAT were determined by nephelometry (Dade Behring Marburg GmbH, Germany) according to the manufacturer's instructions. sCD14, TNF- α , sTNFR-1 and sTNFR-2 levels were determined using Duoset ELISA sets (R&D Systems, MN, USA; detection levels 125 pg/ml, 15.6 pg/ml and 25 pg/ml, respectively). Analysis of CRP in serum was performed using standard assays (Dade Behring, USA, minimum detection level less than 0.15 mg/l). α 1 Antitrypsin phenotyping was carried out by isoelectric focusing (LKB Multiphor II and LKB Macrodrive 5 Constant Power Supply, Amarcham Pharmacia Biotech, Piscataway, NJ, USA) as described previously [17].

Statistical analysis

Statistical analysis was performed using the SPSS 14.0 program. Descriptive statistics were used to tabulate the primary cohort database. Quantitative variables were expressed as means with standard deviations (SD). The differences among means were analyzed for their statistical significance with the one-way ANOVA. The interrelationships among variables were determined using Spearman correlation. A *p* value of less than 0.05 was considered significant.

Results

Demographic data of the 355 studied COPD patients are shown in Table I. The distribution of patients by genotypes was as follows: 320 (90.1%) patients with PI*MM genotype, 25 (7%) with PI*MZ genotype, 7 (2%) with PI*ZZ genotype, and 3 (0.9%) with PI*SZ genotype.

The values of serum biomarkers – AAT, CRP, CD14, TNF- α , sTNFR-1 and sTNFR-2 – in the groups with different AAT genotypes are presented in Table II. Except for expected lower AAT serum concentrations in COPD patients with AAT deficiency, no significant differences in CRP, sCD14, TNF- α , sTNFR-1, and sTNFR-2 levels comparing all genotypic groups studied were seen.

In COPD patients without AAT deficiency (PI*MM genotypic group), a significant negative correlation between lung function, and serum AAT and CRP concentrations was seen as well as a positive correlation between AAT and CRP, sCD14, and sTNFR-1 (Table III).

However, no correlation was documented in COPD patients with AAT deficiency (PI*MM, PI*MZ, and PI*SZ genotypic groups).

Discussion

An important finding of our study is that circulating AAT inversely correlated with FEV₁ in COPD patients without AAT deficiency. Such associations have also been reported before in healthy individuals [18-21]. The SAPALDIA study investigated circulating AAT associations with lung function in the general population and found a negative correlation between AAT level and FEV₁ [19]. The quantity of AAT that diffuses passively from the blood to the lung increases during an inflammatory process, which may take place in COPD [22]. This may indicate increased requirement of AAT to meet the needs of overcoming the release of various enzymes from neutrophilic cells in the lungs, but its protective function may be overrun by the high concentration of proteases [23]. However, other

Table I. General data of study population

Variable	Values
Age [years]	63.4 \pm 11.9
Males/females, n (%)	252 (71)/103 (29)
Smoking status:	
Smokers, n (%)	210 (59.2)
Ex-smokers, n (%)	76 (21.4)
Never smokers, n (%)	69 (19.4)
FVC (mean \pm SD) [% predicted normal]	74.1 \pm 16.2
FEV ₁ (mean \pm SD) [% predicted normal]	47.4 \pm 16.6
FEV ₁ /FVC (mean \pm SD) [%]	54.7 \pm 11.1

Data are presented as mean \pm SD, unless otherwise indicated. FVC – forced vital capacity, FEV₁ – forced expiratory volume in 1 s

studies have not found such associations between serum AAT concentration and FEV₁% predicted value in COPD patients [24]. It appears that many other mechanisms may also be important for lung function and not just for the inflammatory response.

The observed low AAT concentration in PI*ZZ genotype and the FEV₁/AAT association may reflect the dual role of AAT as a biomarker of respiratory disease. The net impact of AAT on lung function seems to be the result of context-dependent (i.e. AAT genotype) and contrasting protective and inflammatory effects in the respiratory tract. On the one hand, elevated levels of serum AAT can reflect a beneficial shift in the protease-antiprotease balance, the cornerstone of the pathophysiological pathway mediating the effect of severe AAT deficiency on COPD. On the other hand, elevated serum AAT can also reflect low-grade inflammatory processes in the lungs [25], which are considered a risk factor for COPD [26]. Significantly higher AAT levels were even reported for AAT deficient (PI*ZZ) patients with COPD as compared to PI*ZZ individuals without COPD, further supporting the hypothesis that AAT levels may also represent an ongoing inflammatory process [27]. Thus, the findings of our

Table II. Serum concentrations of AAT, sCD14, CRP, TNF- α , TNFR-1, and TNFR-2 in COPD patients with different AAT genotypes

Variable	Genotype				Value of <i>p</i> among groups
	PI*MM (n = 320)	PI*MZ (n = 25)	PI*ZZ (n = 7)	PI*SZ (n = 3)	
AAT [g/l]	1.65 \pm 0.48	1.10 \pm 0.30	0.46 \pm 0.40	0.77 \pm 0.19	0.001
sCD14 [μ g/ml]	2.9 \pm 1.3	3.1 \pm 1.5	2.9 \pm 0.4	3.9 \pm 0.2	NS
CRP [mg/l]	9.6 \pm 3.2	10.2 \pm 5.4	8.3 \pm 2.9	7.2 \pm 1.5	NS
TNF- α [pg/ml]	113.7 \pm 25.2	56.1 \pm 11.6	75.4 \pm 22.5	0	NS
sTNFR-1 [pg/ml]	221.9 \pm 77.1	204.4 \pm 62.3	168.1 \pm 49.3	347.4 \pm 109	NS
sTNFR-2 [pg/ml]	231.4 \pm 88.9	219.1 \pm 76.0	219.2 \pm 69.6	223.1 \pm 77.5	NS

All data are presented as mean \pm SD. AAT – α 1 antitrypsin, sCD – soluble leukocyte cluster of differentiation, CRP – C-reactive protein, TNF- α – tumor necrosis factor α , TNFR – tumor necrosis factor α receptor, NS – not significant

Table III. Correlation between inflammatory marker concentrations and lung function (FEV₁, % predicted) in COPD patients without AAT deficiency

Variables	r	Value of p
AAT vs. CRP	0.671	0.005
AAT vs. sCD14	0.510	0.008
AAT vs. sTNFR-1	0.567	0.007
AAT vs. FEV ₁	-0.522	0.03
CRP vs. sTNFR-1	0.507	0.006
CRP vs. FEV ₁	-0.590	0.011
sTNFR-1 vs. sTNFR-2	0.643	0.009

AAT – α 1 antitrypsin, sCD – soluble leukocyte cluster of differentiation, CRP – C-reactive protein, TNF- α – tumor necrosis factor α , TNFR – tumor necrosis factor α receptor, FEV₁ – forced expiratory volume in 1 s, r – Spearman correlation coefficient

study suggest that pulmonary obstruction may be a consequence of the presence of inflammatory stimuli.

Consistent with these results, we could detect a positive relationship between AAT and CRP concentrations. High serum CRP concentrations in severe COPD patients have been reported in previous studies [18, 24, 28]. Gan *et al.* were the first to emphasize the importance of high CRP levels in COPD patients, confirming the systemic inflammation in the stable phase of the disease [26]. Studies of circulating CRP levels in COPD demonstrated that CRP was further elevated during exacerbations, and it was found to predict mortality [29]. Both AAT and CRP are acute-phase proteins. Several studies have found elevated CRP and AAT levels in COPD patients [7, 24], indicating that the inflammatory process is present in disease pathogenesis, and both markers are interrelated.

In addition, we found an inverse correlation between CRP and FEV₁. Even in the healthy population, increases in CRP levels over time were associated with a steeper FEV₁ decline [30, 31]. In these studies, FEV₁ was also inversely associated with CRP concentration. C-reactive protein reflects the total systemic burden of inflammation in several disorders and has been shown to upregulate the production of proinflammatory cytokines [7]. The reasons for the inverse association between systemic inflammation and reduced pulmonary function are not fully understood, but several mechanisms may be involved. Firstly, reduced lung function may be responsible for the observed systemic inflammation. Inflammatory lung or pulmonary epithelial cells have been shown to express CRP and IL-6 [32]. Interleukin-6 may reach the liver via the bloodstream, stimulating the production of CRP and other inflammatory mediators by the liver, sequentially activating pulmonary inflammatory cells during transit through the pulmonary circulation [33, 34]. An alternative mechanism – reverse causation –

cannot be excluded: high levels of cytokines and acute-phase reactants in the peripheral circulation may be a cause rather than a consequence of poor lung function. There is increasing evidence that cytokines play a major role linked to the activation of inflammatory cells and their adhesion to the pulmonary capillary endothelium, leading to changes in endothelial function and increases in pulmonary vascular filtration [18]. Besides, it is known that COPD may even influence venous circulation [35]. Thus, persistence of systemic inflammation may result in damage to the airways, accelerating decline in FEV₁ of COPD patients.

In our study, an important marker, TNF- α , has been analyzed. This inflammatory cytokine is important in COPD pathogenesis [3] and processes where AAT is involved [36, 37]. *In vitro* studies have demonstrated that AAT inhibits TNF- α production [36]. However, no associations between serum TNF- α concentration and other parameters have been found in our study. It is observed that TNF- α levels may be elevated in the sputum, bronchial biopsies, and circulation of COPD patients [3, 26]. Other investigators analyzing TNF- α level in COPD patients did not find any association with the severity of disease [7]. One possible explanation for this could be that these cytokines mainly act in peripheral lung tissues and differences in their levels could be detected in induced sputum but not always in systemic circulation [38]. Thus, in our study, sTNFR-1 was positively correlated with inflammatory markers – AAT and CRP. In addition, a positive correlation between sTNFR-1 and sTNFR-2 was documented. These soluble receptors, which inhibit the inflammatory effect of TNF- α , are expressed and released from many different cells, enabling even elevation of concentration in systemic circulation, where they can be detected [8]. Our results show that elevated sTNFR-1 levels may reflect a systemic inflammatory response.

Recent studies show similar cellular responses in asthma and COPD, with raised levels of neutrophils [39, 40]. Monocytes/macrophages are a significant component of inflammatory infiltrate in COPD [41, 42]. Analyzing sCD14 levels in COPD patients, we did not find any differences in sCD14 levels among different AAT genotypes. Thus, we found a positive correlation between sCD14 and AAT. CD14-dependent mechanisms of inflammation have also been discussed in acute respiratory distress syndrome [43]. The soluble form of CD14 is generated by proteolytic shedding of the membrane-associated form during cellular activation [44]. *In vitro* within 2 h (short time), AAT strongly up-regulates sCD14 secretion and membrane CD14 (mCD14) expression, while after 18 h, it causes a profound decrease in mCD14 expression [16]. This observation provides evidence that a direct rela-

tionship exists between the accumulation of sCD14 and the reciprocal decrease in mCD14 expression. The biological function of sCD14 is not clear so far. An excess of sCD14 is shown to inhibit LPS binding to mCD14 and hence block cellular activation [16]. Recent findings support the hypothesis that modulation of LPS-induced monocyte activation by AAT may be related to the AAT-induced modulation of CD14 levels [36].

These data show that AAT has immunomodulating capacity, and a rapid increase in AAT concentrations during various inflammatory and infectious conditions may enhance the magnitude of inflammatory cell responses to endotoxin and subsequently accelerate resolution of the inflammatory reaction [42, 45]. However, the associations are complex and understanding the interplay of various mediators will require appropriately designed further studies.

In conclusion, the present study indicated that for expected lower AAT serum concentrations in COPD patients with PI*ZZ genotype, there were no significant differences in CRP, sCD14, TNF- α , sTNFR-1, and sTNFR-2 levels comparing patients with different AAT genotypes. In patients without AAT deficiency, the positive association of AAT concentration with CRP, sCD14, and sTNFR-1 and the negative association with FEV1 show the importance of AAT as a marker of systemic inflammation.

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