

Systemic inflammation in COPD in relation to smoking status

Infiammazione sistemica nella BPCO in rapporto alla condizione tabagica

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ABSTRACT

Background and aims: Smoking is the main risk factor for the development of chronic obstructive pulmonary disease (COPD) that has been recently defined as a systemic pulmonary inflammatory disease. However, the impact of smoking itself on systemic inflammation in COPD patients has not yet been well established. The aim of our study was to investigate the association between inflammatory markers and smoking status.

Material and methods: We compared 202 current smokers, 61 ex-smokers and 57 never-smokers, all COPD patients. Assessments included medical history, spirometry, alpha-1 antitrypsin (AAT) genotyping, serum AAT, C-reactive protein (CRP), tumor necrosis factor (TNF)- α , and soluble tumor necrosis factor receptor (sTNFR)-1 and sTNFR-2 concentrations.

Results: AAT and CRP concentrations in smokers (1.75 ± 0.51 g/L and 14.4 [9.5–20.5] mg/L) and ex-smokers (1.69 ± 0.43 g/L and 12.3 [8.7–16.3] mg/L) were higher than in never-smokers (1.49 ± 0.38 g/L and 5.1 [2.5–8.7] mg/L; $p < 0.05$). sTNFR-1 level was higher in smokers than ex-smokers or never-smokers (241.2 pg/mL [145.3–349.4] vs. 213.7 pg/mL [147.1–280.3] and 205.2 pg/mL [125–275]; $p < 0.05$).

Conclusions: Our data confirm that smoking is associated with increased levels of AAT, CRP, and sTNFR-1 in COPD patients, an array of systemic inflammation markers that continue to be active even after smoking cessation.

Keywords: Alpha-1 antitrypsin, COPD, inflammatory markers.

RIASSUNTO

Introduzione e obiettivi: Il fumo è la principale causa della broncopneumopatia cronica ostruttiva (BPCO), patologia che è stata recentemente definita come una malattia infiammatoria sistemica. Tuttavia, i dati relativi all'influenza del fumo

sull'infiammazione sistemica per i malati di BPCO non sono sufficienti. Scopo della ricerca è quello di stabilire i collegamenti tra i marcatori dell'infiammazione e l'abitudine al fumo.

Materiali e metodi: Sono stati presi in esame 320 malati di BPCO: 202 fumatori, 61 ex fumatori, 57 non fumatori. Sono stati valutati: anamnesi, spirometria, genotipo di alfa-1 antitripsina (AAT), concentrazioni sieriche di AAT, proteina C reattiva (CRP), fattore di necrosi tumorale alfa (TNF- α) e delle frazioni solubili dei recettori per il fattore di necrosi tumorale (sTNFR-1, sTNFR-2).

Risultati: I livelli di AAT e di CRP dei fumatori ($1,75 \pm 0,51$ g/l e $14,4$ mg/l [9,5–20,5]) e negli ex-fumatori ($1,69 \pm 0,43$ g/l e $12,3$ mg/l [8,7–16,3]) sono risultati più alti dei non fumatori ($1,49 \pm 0,38$ g/l e $5,1$ mg/l [2,5–8,7]; $p < 0,05$). I livelli di TNFR-1 sono risultati più alti nei fumatori rispetto agli ex-fumatori e ai non fumatori ($241,2$ pg/ml [145,3–349,4] versus $213,7$ pg/ml [147,1–280,3] e $205,2$ pg/ml [125–275]; $p < 0,05$).

Conclusioni: L'aumento delle concentrazioni di AAT e CRP nel siero dei pazienti fumatori ed ex-fumatori con BPCO, rispetto ai non fumatori, indica che l'infiammazione sistemica si prolunga anche dopo la cessazione del fumo, anche se le concentrazioni di sTNFR risultano inferiori dopo la cessazione.

Parole chiave: Alfa-1 antitripsina, BPCO, marcatori dell'infiammazione.

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 lungs to long-term tobacco smoke or inhalation of toxic gases [1]. The

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prevalence of COPD is appreciably higher in current or former heavy smokers aged > 40 years. Smoking may lead to COPD in 15-30% of those who smoke [2]. There is a growing body of evidence supporting the presence of systemic inflammation in patients with COPD [3-8]. Tumor necrosis factor α (TNF- α), a powerful proinflammatory cytokine primarily produced by activated macrophages, is thought to play a critical role in the pathogenesis of COPD by promoting and maintaining the expression and release of various proinflammatory mediators, which lead to tissue damage and remodeling [8-9]. Lung inflammation is further amplified by oxidative stress and proteolytic damage by proteinases. Smoking itself may also cause a protease-antiprotease imbalance in the lungs by reducing the functional activity of the major antiprotease – alpha-1 antitrypsin (AAT) – in the lung interstitium and alveolar lining fluid, and by increasing the amount of elastolytic proteases released in the lung [10]. Moreover, smoking increases the number of neutrophils, macrophages, and T cells, which release proinflammatory mediators into the lung and airways [11]. A number of studies suggest that AAT can also exhibit biological activities independently of the inhibition of serine proteases: namely, an immunoregulatory effect, inhibition of neutrophil superoxide production, and antimicrobial activity [12].

Little is known about the mechanism responsible for increased levels of inflammatory markers, especially TNF- α and its inhibitory soluble receptors, in the serum of COPD patients, and a direct impact of active smoking on these markers has not been well established. The aim of our study was to evaluate the concentrations of AAT, C-reactive protein (CRP), TNF- α , and soluble TNF receptors 1 and 2 (sTNFR-1 and sTNFR-2) in COPD patients in relation to smoking habit.

MATERIALS AND METHODS

Subjects

A total of 320 stable outpatients with COPD were examined: 202 current smokers, 61 ex-smokers (quit since at least 1 year), and 57 never-smokers followed up at the Department of Pulmonology and Immunology, Medical Academy, Lithuanian University of Health Sciences.

Patients met the following inclusion 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 1 month before the investigation; patients were free of systemic steroids for at least 1 month before the study; none of the patients had congenital AAT deficiency. All patients met the Global Initiative for Obstructive Lung Disease (GOLD) [1] recommended spirometric criteria for COPD (ratio of postbronchodilator forced expiratory volume in one second [FEV₁] to forced vital capacity [FVC] less than 70%). When grouping the patients into smokers, ex-smokers, and never-smokers, we relied only on reported

smoking status. Several studies [13] have reported trends of underestimation when smoking prevalence is based on self-report, and varying sensitivity levels for self-reported estimates depending on the population studied and the medium in which the biological sample is measured [13]. However, there are studies showing that reported smoking could be an acceptable assessment because prevalence estimates of smoking were similar to self-reports and cotinine assessment [14].

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 cig. 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 for 30-60 min, and centrifuged for 15 min at 4000 rpm. Serum samples were immediately frozen at -70°C for further analysis. AAT phenotyping was carried out by means of isoelectric focusing (LKB Multiphor II and LKB Macrodrive 5 Constant Power Supply, Amersham Pharmacia Biotech, Piscataway, NJ, USA). Serum AAT concentration was determined by nephelometry (Dade Behring Marburg GmbH, Germany) according to the manufacturer's instructions. TNF- α , sTNFR-1, and sTNFR-2 levels were determined using Duoset ELISA sets (R&D Systems, MN, USA; detection levels, 125, 15.6, 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).

Statistical analysis

Descriptive statistics were used to tabulate the primary cohort database. Quantitative variables were expressed as means \pm standard deviation (SD) or median (25th-75th percentile). Normally distributed data were compared using Student's *t* test and one-way ANOVA. The Mann-Whitney U and Kruskal-Wallis H tests were used for comparison of non normally distributed data to determine if they differed significantly. A *p* value of less than 0.05 was considered significant. Statistical analysis was performed using the SPSS 14.0 program.

RESULTS

The majority of the patients were male ($n = 250$, 78.1%), mostly with moderate-to-severe COPD (Table I). Mean age was 63.4 ± 11.9 years, and 82% of the patients were current or former smokers with an average smoking history of 22.7 ± 12.9 pack-years. Serum levels of biomarkers AAT, CRP, TNF- α , sTNFR-1, and sTNFR-2 are presented in Table I.

We found positive associations between active and former smoking habit and higher AAT and CRP levels. Figure 1 shows that AAT concentration was higher in smokers (1.75 ± 0.51 g/L) and ex-smokers (1.69 ± 0.43 g/L) than in never-smokers (1.49 ± 0.38

TABLE I: GENERAL PATIENT CHARACTERISTICS AND CONCENTRATIONS OF INFLAMMATORY MARKERS

Variable	Value
Age, years	63.4 ± 12.2
Males/females (%)	250 (78.1)/70 (21.9)
Smoking status (%):	
Smokers	202 (63.1)
Ex-smokers	61 (19.1)
Never-smokers	57 (17.8)
FVC, % predicted	74.5 ± 17.1
FEV ₁ , % predicted	47.0 ± 16.2
FEV ₁ /FVC, %	54.8 ± 10.7
AAT, g/L	1.65 ± 0.48
CRP, mg/L	9.6 (4.2–14.5)
TNF- α , pg/mL	113.7 (30.2–162.1)
sTNFR-1, pg/mL	221.9 (149.2–270.5)
sTNFR-2, pg/mL	231.4 ± 101.9

Definition of abbreviations: AAT, alpha-1 antitrypsin; CRP, C-reactive protein; sTNFR, soluble tumor necrosis factor α receptor; TNF- α , tumor necrosis factor α .

Data are presented as n (%), mean ± SD, or median (25th–75th percentiles).

g/L) ($p < 0.05$). CRP concentration was also higher in smokers and ex-smokers than never-smokers (14.4 [9.5–20.5] mg/L and 12.3 [8.7–16.3] mg/L vs. 5.1 [2.5–8.7] mg/L; $p < 0.05$) (Figure 2).

sTNFR-1 levels were higher in smokers than ex-smokers or never-smokers (241.2 [145.3–349.4] pg/mL vs. 213.7 [147.1–280.3] pg/mL and 205.2 [125–275] pg/mL; $p < 0.05$) (Figure 3).

No differences in TNF- α and sTNFR-2 concentrations were observed in relation to smoking status ($p > 0.05$) (Table II).

DISCUSSION

An important message of this study is that current smokers and ex-smokers had higher circulating AAT and CRP levels than never-smokers. These results suggest that smoking may be associated with higher AAT and CRP production in the liver of COPD patients and mechanisms related to systemic inflammation, which continues even after smoking cessation. Even in healthy individuals, positive associations between active smoking and AAT levels have been reported [15]. The amount of AAT that diffuses

passively from the blood to the lungs increases during an inflammatory process, as occurs in COPD [16]. This may indicate an increased requirement of AAT to meet the need of overcoming the release of various enzymes from neutrophilic cells in the lungs, but its protective function may be overwhelmed by the high concentration of proteases [17]. Elevated AAT levels in smokers and ex-smokers reflect the dual role of AAT as a respiratory disease biomarker. The net impact of AAT on lung function seems to be a result of context-dependent (i.e. AAT genotype) and contrasting protective and inflammatory effects in the respiratory tract. On the one hand, elevated serum AAT can show a beneficial shift in the protease-antiprotease balance, the centrepiece of the pathophysiological pathway mediating the effect of severe congenital AAT deficiency on COPD. On the other hand, elevated serum AAT can also reflect a low-grade chronic inflammatory process in the lungs [18], which is considered a risk factor for COPD [19,20].

CRP reflects the total systemic burden of inflammation in several disorders and has been shown to up-regulate the production of proinflammatory cytokines [5,7]. Gan and co-workers were the first to highlight the importance of high CRP levels in COPD patients, confirming the systemic inflammation in the stable phase of the disease [19]. Studies of circulating CRP in COPD have demonstrated that CRP levels are further elevated during exacerbations, and they were also found to be a mortality predictor [21]. Several previous studies have also shown higher serum CRP levels in COPD patients than in non-smoking controls [22–23]. However, other studies did not find such differences in CRP levels [24]. These controversial data indicate that each patient has his/her own unique and different mechanism of disease development.

There is a consensus about the presence of small airway and lung parenchyma inflammation in smokers and COPD patients [25–27]. Local inflammation is characterized by increased numbers of inflammatory cells, such as neutrophils, lymphocytes, and macrophages, and higher TNF- α and IL-8 concentrations in smokers than healthy controls [25,26,28–30]. However, after smoking cessation, the inflammatory state changes only in asymptomatic ex-smokers, but not in COPD ex-smokers [31–32]. Gamble et al. compared bronchial biopsy specimens from COPD current smokers and COPD ex-smokers and did not find any differences in cell

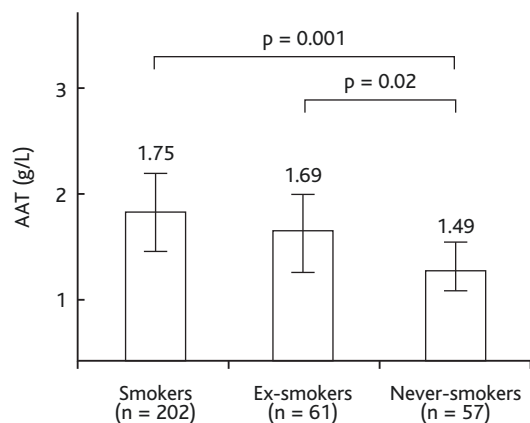
TABLE II: SERUM CONCENTRATIONS OF TNF- α AND sTNFR-2 IN COPD SMOKERS, EX-SMOKERS AND NEVER-SMOKERS

Variable	Smokers (n = 202)	Ex-smokers (n = 61)	Never-smokers (n = 57)	P
TNF- α , pg/ml	114.1 (33.4–154.2)	115.4 (44.2–167.6)	110.0 (37.5–155.3)	0.3
sTNFR-2, pg/ml	233.6 ± 107.1	227.6 ± 82.4	223.4 ± 63.6	0.07

Definition of abbreviations: sTNFR-2, soluble tumor necrosis factor α receptor 2; TNF- α , tumor necrosis factor α .

Data are presented as mean ± SD or median (25th–75th percentiles).

FIGURE 1: SERUM AAT CONCENTRATION IN COPD SMOKERS, EX-SMOKERS AND NEVER-SMOKERS

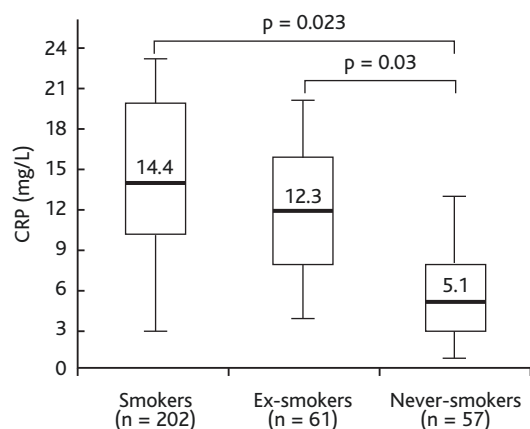


Definition of abbreviation: AAT, alpha-1 antitrypsin. Data are presented as mean ± SD.

counts or inflammatory markers, including TNF- α , between the groups [31]. TNF- α is an inflammatory cytokine important not only for COPD pathogenesis [3,33], but also for the function of the main neutrophil protease inhibitor – AAT [12,34]. We found no associations between TNF- α serum concentration and smoking status in our study. Some studies have reported elevated TNF- α levels in the sputum, bronchial biopsy specimens, and circulation of COPD patients as compared to controls [3,19]. However, other authors did not find such associations [35].

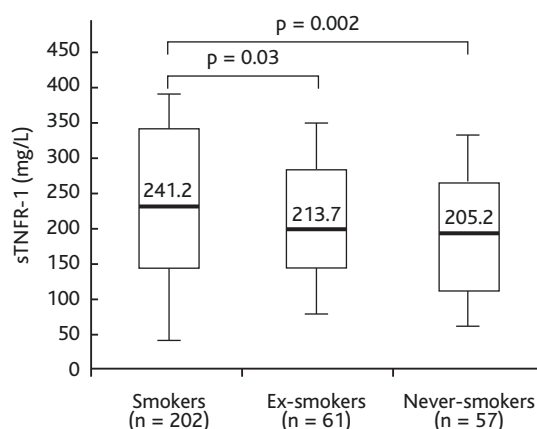
On the contrary, in our study, sTNFR-1 level was higher in smokers than ex-smokers and never-smokers. These soluble receptors, which inhibit the in-

FIGURE 2: SERUM CRP CONCENTRATION IN COPD SMOKERS, EX-SMOKERS AND NEVER-SMOKERS



Definition of abbreviation: CRP, C-reactive protein. Data are presented as median (25th–75th percentile).

FIGURE 3: SERUM sTNFR-1 CONCENTRATION IN COPD SMOKERS, EX-SMOKERS AND NEVER-SMOKERS



Definition of abbreviation: sTNFR-1, soluble tumor necrosis factor α receptor 1.

Data are presented as median (25th–75th percentile).

flammatory effect of TNF- α , are expressed and released from many different cells, causing also the elevation of their concentration in systemic circulation, where they can be detected [8,36]. Our results show that elevated sTNFR-1 levels in smokers may show a systemic inflammatory response. In a study by Vernooij et al., circulating levels of sTNFR-1 and sTNFR-2 did not differ when comparing current smokers with ex-smokers in the COPD and control groups [27]. In another study, a steady decline in serum sTNFR-1 and sTNFR-2 levels was observed after smoking cessation [37].

Several authors suggest that circulating sTNFR levels reflect a systemic inflammatory state better than do individual short-lived cytokine levels and should be better predictors of inflammation than TNF [37]. Smoking cessation is the only clinical intervention associated with reduced FEV₁ decline in COPD patients [38]. However, to date, no reduction in airway inflammation has been demonstrated when COPD ex-smokers are compared to COPD current smokers [32,33]. We speculate that the reduced sTNFR-1 concentrations in ex-smokers and never-smokers compared to current smokers observed in our study may partially explain some of the systemic benefit associated with smoking cessation. However, we did not find any differences in sTNFR-2 levels in relation to smoking status. Despite the fact that TNF receptors are considered to be markers of a proinflammatory state inducible by cytokines such as TNF- α , they present different biological functions [39,40].

We did not analyze local inflammation in our study, and some data suggest that local and systemic inflammation may be regulated differently [28]. Thus, the association among inflammatory markers is complex, and a better understanding of the interplay among various mediators will require appropriately designed studies in the future.

In conclusion, our data confirm that the impact of smoking status on systemic inflammation differs according to the type of inflammatory marker analyzed.

Higher levels of inflammatory markers in current smokers and ex-smokers indicate that in COPD patients inflammation continues for many years after smoking cessation.

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