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Original Research Article

Increased innate and adaptive immune responses in induced sputum of young smokers

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ABSTRACT

Background and objectives: It is known that chronic obstructive pulmonary disease (COPD) development process is imperceptible and can be asymptomatic for 20 or more years. It is of great importance to diagnose early inflammatory changes that can lead to COPD in young asymptomatic cigarette smokers. The aim of our study was to analyze the cell spectrum of induced sputum (IS) of young cigarette smokers, with emphasis on T-regulatory cells.

Materials and methods: A total of 20 healthy nonallergic smokers, 20 nonsmokers and 20 COPD patients were enrolled in the study. After lung function measurements were taken, we performed sputum induction and analyzed sputum cells. We evaluated the cell count of FOXP3-positive, $CD4^+$ and $CD8^+$ T lymphocytes by immunocytochemistry staining, and the cell count of macrophages and neutrophils by May-Grünwald Giemsa staining.

Results: Induced sputum of smokers contained a higher absolute amount of macrophages and neutrophils when compared to nonsmokers. FOXP3-positive cells in the sputum of young smokers showed a statistically significant increase when compared to nonsmokers. Induced sputum of COPD patients contained an increased absolute amount of neutrophils and FOXP3-positive Treg cells when compared to nonsmokers. Regression analysis showed that the amount of FOXP-3 positive cells, neutrophils and macrophages in the induced sputum was increasing with the number of pack years.

Conclusions: This study demonstrates that young smokers have early inflammatory changes in their airways that not only initiate nonspecific mechanisms recruiting neutrophils, but also involve specific immune mechanisms with recruitment of T regulatory lymphocytes. The lymphocyte response is probably adaptive.

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1. Introduction

Cigarette smoking is a major risk factor for the development of COPD [1]. In addition, the clinical course of COPD can be asymptomatic for 20 or more years. The clinical symptoms due to smoking usually present themselves after approximately 40 years. The ''golden standard'' for diagnosis of COPD remains spirometry. The early diagnosis of COPD is of particular importance especially among young smokers [2].

There is an urgent need for identification of biological markers for the early stage, as well as new molecular targeting therapy for COPD. New potentially relevant biomarkers for an early noninvasive diagnosis of COPD are of particular importance and could be used as a screening tool for the populations at risk. Patients with COPD typically have at least a 10 pack-year history of smoking, but only few of the heavy smokers develop severe airflow limitation. This suggests COPD is not dependent upon the smoke exposure alone.

It is believed that the presence of inflammation in COPD together with the accumulation of components of innate immune system, such as activated macrophages and neutrophil leukocytes, is an important signifier in the disease development [1,3]. More recent studies have postulated that adaptive immune response also contributes to the pathophysiology of COPD [4]. An increased amount of neutrophils in induced sputum is a characteristic feature in patients with COPD [5].

Chronic cigarette smoke exposure increases numbers of alveolar macrophages in the airways lumen of smokers. Smokers' macrophages have an ability to inhibit effects on proliferation of lymphocytes and activities of natural killer (NK) cells [6].

An increased total number of circulating T-lymphocytes has been observed in smokers [6]. It was observed that lymphocytes and macrophages are the predominant cellular elements of the inflammatory infiltrates within the airway walls of patients with COPD. Other studies extended these observations by showing that the numbers of $CD8^+$ lymphocytes in COPD lung were directly related to the degree of airflow limitation [7]. Similarly, in the induced sputum of COPD patients there are higher levels of $CD8^+$ T-lymphocytes [8]. T-lymphocytes can cause tissue injury either by direct cytolytic activities or through the secretion of pro-inflammatory mediators that activate other immune cells. In addition to the generally potent pro-inflammatory effect of $CD4^+$ lymphocytes, a subset of these cells also may impact the progression of COPD by up-regulating the intensity of inflammatory cascades [9].

It is now clearly established that a forkhead box protein 3 (FOXP3) expressed by subset of $CD4^+$ $CD25^+$ T cells, also called regulatory T cells, is essential for the maintenance of self-tolerance and immune homeostasis [10].

$CD4^+$ $CD25^+$ T regulatory (Treg) cells are important in realizing peripheral immunological tolerance, down-regulation of persistent inflammation and prevention of autoimmune reactions by inhibition of other T cell responses [11]. Dysfunction of Treg cells can lead to autoimmune disease, allergy and chronic inflammatory diseases.

We hypothesized that T regulatory cells are involved in the pathogenesis of COPD and that assessment of the numbers of T regulatory cells in the induced sputum could serve as a biomarker for early diagnosis, prognosis and treatment of COPD.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Ethics Committee of the Institute of Experimental and Clinical Medicine, University of Latvia.

2.2. Subjects

A total of 20 healthy nonallergic current smokers (mean age, 21.5 ± 2.6 years; smoking history, 3.03 ± 3.0 pack-years), asked to refrain from smoking at least 2 h before the sample collection, in order to exclude the acute effect, 20 nonsmokers (mean age, 22.4 ± 2.6 years) and 20 COPD patients (mean age, 62.3 ± 2.6 years; smoking history, 39.18 ± 4.8 pack-years) gave informed consent to participate in the study. None of the volunteers had experienced any airway infection at least within one month before the session.

2.3. Study design

All subjects had only one session. We performed lung function measurements, sputum induction, sputum immunocytochemistry, May-Grünwald Giemsa staining and sputum cell analyses. All subjects filled in a questionnaire about their smoking habits. Healthy volunteers were questioned about any complaints or symptoms that could be related with COPD.

Before inclusion in the trial all volunteers were informed about the study design and possible side effects, and signed an informed consent.

2.4. Lung function

Before sputum induction, all subjects underwent spirometry and bronchodilation test with a 200-μg salbutamol (Ventolin™, GlaxoSmithKline) inhalation. We used bronchodilators to make sputum induction easier. We repeated spirometry 15 min after inhalation of salbutamol, using MIR Spirobank II spirometer and following the American Thoracic Society (ATS) and European Respiratory Society (ERS) Spirometry standardization recommendations [12,13]. Repeated spirometry allowed us to ensure that none of the patients has asthma.

2.5. Sputum induction

Sputum induction was performed according to modified protocol validated by E. Pizzichini [14]. For inhalation we used constant concentration of 4% NaCl using an ultrasonic nebulizer (OMRON NE-U17, OMRON Matsusaka Co., Ltd., Japan) with a flow rate of 1 mL/min. Induction was performed for three times, each 5 min long. After each inhalation period volunteers were asked to rinse their mouth with water, to minimize contamination with saliva. Then they were asked to expectorate into a sterile container. Before each inhalation

period FEV_1 levels were evaluated with spirometer. According to the protocol, if FEV_1 drops for more than 20% of the post-bronchodilation value, the procedure should be stopped. We had no such cases.

2.6. Sputum processing

First sputum processing was performed according to modified protocol validated by Efthimiadis et al. [15]. Sputum samples were processed within 2 h after induction. The volume of induced sputum was measured and mixed with equal volume of 0.1% dithiothreitol (DTT, DL-dithiothreitol, minimum 99% titration, Sigma–Aldrich, Inc., St. Louis, USA), which was previously dissolved in Dulbecco's phosphate-buffered saline. Solution was aspirated and dispensed several times with disposable pipette, and then the sample incubated for 15 min in a shaking water bath at 4°C for complete homogenization. Next, incubation sample was filtered on a 48 μg sterile nylon mesh into a preweight tube. A small amount (20 μg) was used to assess total cell count and viability, using tryptan blue dye method and a standard haemocytometer. Remaining sample was centrifuged at 790*g* for 10 min to separate sputum cells from the fluid phase. Supernatants were collected and stored at −80°C. Cell suspension was diluted with phosphate-buffered saline to obtain concentration 1×10^6 cells/mL and doses of 100 μL were used for cytospin slides. Cytospin slides were dried out in the air, wrapped in foil and stored at −80°C before staining.

2.7. Immunocytochemistry

Lymphocytes were stained by immunocytochemical staining, using EnVision polymer kit (DAKO, Denmark, Glostrup). Sections were incubated in 0.5% H_2O_2/PBS to quench endogenous peroxidase activity. The slides were then incubated for 1 h with three different primary monoclonal mouse anti-human antibodies against FOXP3: monoclonal mouse antibody (dilution 1:100, clone 236A/E7; ab 20034, AbCam, UK), mouse monoclonal CD4 (dilution 1:100; clone IR649, M7310, DAKO) and mouse monoclonal CD8 (dilution 1:50; clone IR623, M7310, DAKO). EnVision kit (DAKO) was used for visualization of bounding with the primary antibody. Slides were incubated in a humidity chamber for 30 min with EnVision reagent and then preceding, intervening and subsequent rinses in isotonic buffer (pH 7.6) were applied, three times for 5 min each. 3,3′-diaminobenzidine-tetrahydrochloride (DAB) was applied as chromogen for 7 min.

Slides were analyzed using a light microscope with a video recorder linked to a computerized image system (Motic Image Advanced 3.2, Xiamen, China). FOXP3-positive expression was identified as nuclear immunolocalisation.

The cases were coded and measurements made in a blinded fashion, without knowledge of clinical data for the given patient specimen. The results were expressed as a number of positive cells to a total number of 600 cells per slide.

2.8. May–Grünwald Giemsa staining

Leukocytes (macrophages and neutrophils) were stained with the May–Grünwald–Giemsa staining. Cytospin slides after taking out of cytospin were dried approximately for 24 h. After that slides were fixed in methanol for 10 min and then left to dry again for approximately 24 h or more, if needed. After drying they were ready to stain. Slides were stained with May–Grünwald stain for 9 min, after that washed in distilled buffered water. Then the slides were stained with Giemsa stain for 13 min and washed again in distilled buffered water. Finally they were left to dry for approximately another 3 h.

The cases were coded and measurements made in a blinded fashion, without knowledge of clinical data for the given patient specimen. Differential cell count was performed from the smears on glass slides. We counted 400 cells with nuclei: leukocytes, bronchial epithelium cells and flat epithelium cells.

2.9. Statistical analysis

Statistical analyses were done using Statistica 7.0 software (StatSoft Inc., USA). We verified data consistency to a normal (Gauss) distribution with the distribution analysis sub-module (χ^2 and Kolmogorov–Smirnov tests). Where distribution was not normal, we applied log transformation [16]. Afterwards we ran one factor linear regression analysis between cellular indicators and smoking history. In order to have the data in figures in a non-logarithmic format, they were retransformed after analysis. Since we only transformed the dependent factor, the result was an exponential regression [17].

We considered a regression significant, if $P < 0.05$.

Then we compared the amount of cells in smokers and nonsmokers. For analysis of variance tests, we used similar transformation and normalization as for regression analysis. We also transformed the mean and 95% confidence interval.

3. Results

Table 1 demonstrates clinical patients' characteristics (Table 1). Smokers were on average taller and heavier, but we assumed that this would not influence the goal of the procedure, i.e., evaluation of the cell spectrum in the induced sputum. Both nonsmokers and smokers had normal lung function (FEV_1, FVC, FEV_1/FVC%), but there were statistically significant differences when compared to COPD patients. COPD patients had lower lung function (FEV_1, FVC, FEV_1/FVC%) measurements ($P < 0.01$). The difference in the mean age of each group (non-allergic healthy smokers, healthy nonsmokers and COPD patients) was also statistically significant ($P < 0.01$).

None of healthy volunteers had any complaints about health changes that could be associated with COPD symptoms, such as cough or increased sputum secretion.

3.1. Cell spectrum changes in induced sputum

Induced sputum of smokers had a higher absolute amount of macrophages (147.63 [107.11–203.48] cells $\times 10^4$/mL) compared to nonsmokers (93.48 [67.82–128.85] cells $\times 10^4$/mL) ($P = 0.049$) (Fig. 1). Induced sputum of COPD patients showed a lower absolute amount of macrophages (57.04 [41.38–78.61] cells $\times 10^4$/mL) compared to smokers ($P < 0.001$) and nonsmokers ($P = 0.033$) (Fig. 1). Induced sputum of smokers as well

Table 1 – Subject characteristics.

Parameter	Nonallergic smokers ($n = 20$)	Healthy nonsmokers ($n = 20$)	COPD patients ($n = 20$)
Age, years, mean ± SD	21.5 ± 2.6*	22.4 ± 2.6*	62.3 ± 2.6
Gender, M/F, n	15/5	5/15	18/2
Height, cm, mean ± SD	180.6 ± 4.2*	172.7 ± 4.2**	172.4 ± 4.2
Weight, kg, mean ± SD	72.2 ± 7.0	62.4 ± 7.0*	80.4 ± 7.0
Smoking history, pack-years, mean ± SD	3.03 ± 3.0	–	39.18 ± 4.8
(range)	(0.15–8)		(9–67.5)
FEV_1, % of pred (PRE-), mean ± SD	112.9 ± 7.6*	106.3 ± 7.6*	55.8 ± 7.6
FVC, % of pred, mean ± SD	109.0 ± 7.8*	107.8 ± 7.8*	70.9 ± 7.8
FEV_1/FVC%, mean ± SD	91.2 ± 4.8*	85.8 ± 4.8*	59.3 ± 4.8

* $P < 0.01$ nonallergic smokers compared to COPD patients; healthy nonsmokers compared to COPD patients.
** $P < 0.01$ healthy nonsmokers compared to nonallergic smokers.

contained a higher ($P = 0.038$) absolute amount of neutrophils (78.48 [55.06–111.86], cells $\times 10^4$/mL), compared to non-smokers (46.12 [32.36–65.75], cells $\times 10^4$/mL) (Fig. 2). COPD patients had an increased absolute amount of neutrophils (121.12 [84.97–172.64] cells $\times 10^4$/mL), compared to nonsmokers ($P < 0.001$), and a trend towards statistically significant differences with smokers ($P = 0.088$) (Fig. 2). The amount of FOXP3-positive Treg cells in sputum of young smokers was significantly higher (2.78 [1.77–4.17] cells $\times 10^4$/mL), when compared with nonsmokers (0.39 [0.02–0.90] cells $\times 10^4$/mL, $P < 0.001$) (Fig. 3). COPD patients had a greater count of FOXP3-positive Treg cells (1.85 [1.09–2.89] cells $\times 10^4$/mL, $P = 0.002$), when compared with nonsmokers (Fig. 3).

We did not find any significant statistical differences in the amount of $CD4^+$ and $CD8^+$ in the induced sputum between young smokers and non-smokers. However, there were statistically significant differences in the amount of $CD4^+$ and $CD8^+$, when compared with the COPD patients. The amount of $CD4^+$ T lymphocytes in COPD patients' induced sputum was significantly higher (4.13 [2.69–6.11] cells $\times 10^4$/mL) compared to smokers (1.40 [0.72–2.37] cells $\times 10^4$/mL, $P = 0.002$) and nonsmokers (0.87 [0.33–1.61] cells $\times 10^4$/mL, $P < 0.001$) (Fig. 4). Induced sputum of COPD patients had a higher absolute amount of $CD8^+$ T lymphocytes (2.93 [1.95–4.24] cells $\times 10^4$/mL) compared with smokers (0.74 [0.29–1.33] cells $\times 10^4$/mL, $P < 0.001$) and nonsmokers (0.47 [0.097–0.98] cells $\times 10^4$/mL, $P < 0.001$) (Fig. 5).

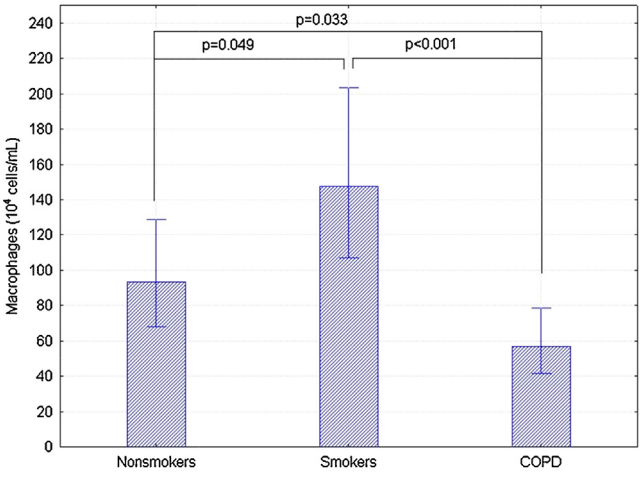

Fig. 1 – Absolute amount of macrophages in induced sputum of nonsmokers ($n = 20$), smokers ($n = 20$) and COPD patients ($n = 20$).

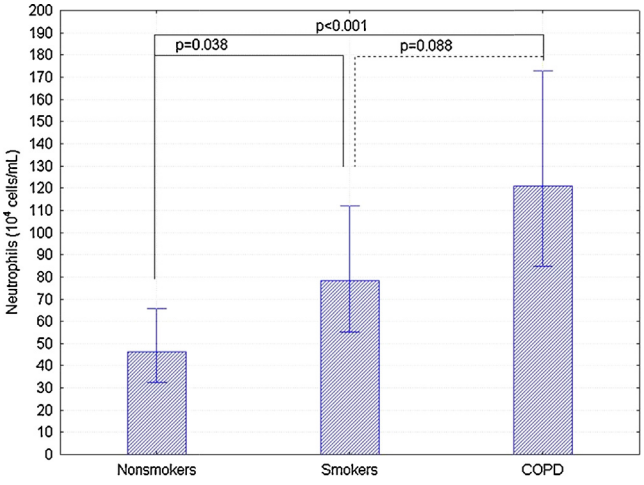

Fig. 2 – Absolute amount of neutrophils in induced sputum of nonsmokers ($n = 20$), smokers ($n = 20$) and COPD patients ($n = 20$).

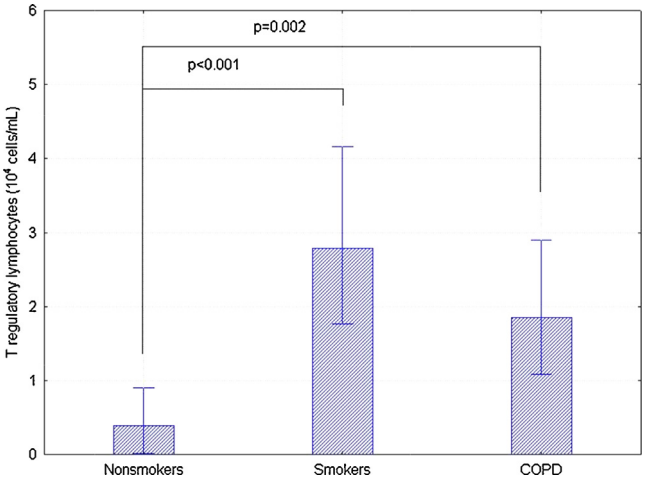

Fig. 3 – Absolute amount of FOXP3-positive T regulatory cells in induced sputum of nonsmokers ($n = 20$), smokers ($n = 20$) and COPD patients ($n = 20$).

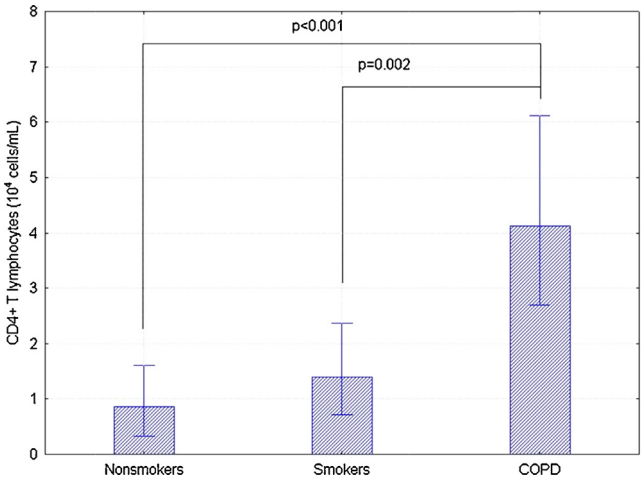

Fig. 4 – Absolute amount of CD4⁺ T lymphocytes in induced sputum of nonsmokers (*n* = 20), smokers (*n* = 20) and COPD patients (*n* = 20).

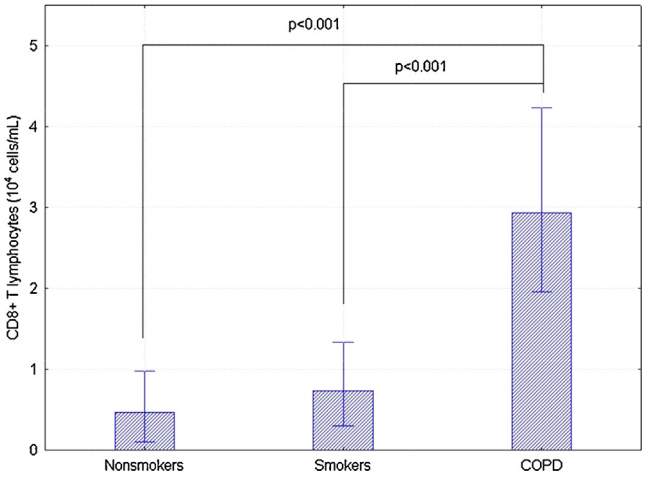

Fig. 5 – Absolute amount of CD8⁺ T lymphocytes in induced sputum of nonsmokers (*n* = 20), smokers (*n* = 20) and COPD patients (*n* = 20).

3.2. *Cell spectrum changes in induced sputum depending on the duration of smoking*

Our study revealed a significant correlation between the numbers of FOXP3-positive T regulatory cells ($r^2 = 0.393$, $P < 0.001$) (Fig. 6), neutrophils ($r^2 = 0.107$, $P = 0.040$) (Fig. 7), and macrophages ($r^2 = 0.108$, $P = 0.038$) (Fig. 8) in induced sputum and smoking history in pack-years. In COPD patients, there was no significant correlation.

4. Discussion

Our study was first to address the changes in induced sputum cells among young smokers with a short (approximately 3 pack-years) smoking history and no symptoms of COPD development. It has shown that despite normal lung function

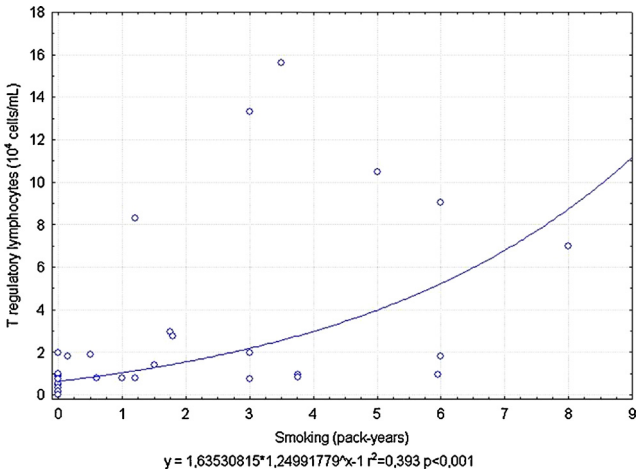

Fig. 6 – The correlation between the numbers of T regulatory cells in induced sputum and smoking history in young smokers and nonsmokers (*n* = 40) (*P* < 0.001).

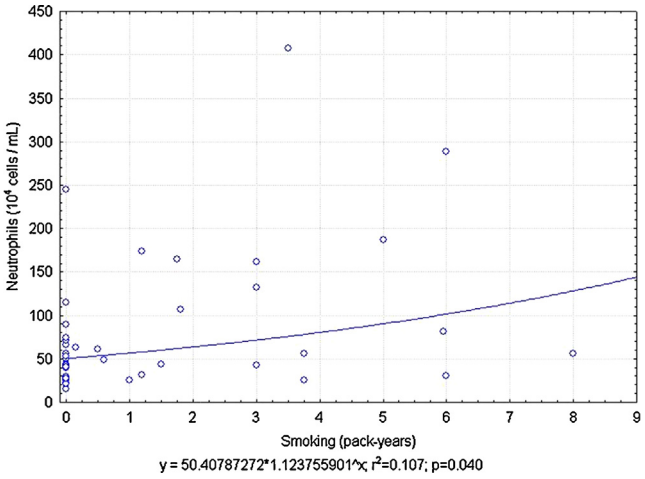

Fig. 7 – The correlation between the numbers of neutrophils in induced sputum and smoking history in young smokers and nonsmokers (*n* = 40) (*P* = 0.040).

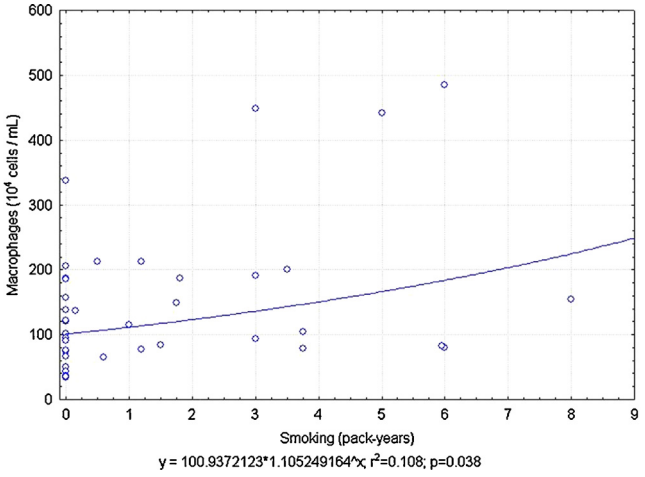

Fig. 8 – The correlation between the numbers of macrophages in induced sputum and smoking history in young smokers and nonsmokers (*n* = 40) (*P* = 0.038).

indices and lack of symptoms, young smokers have an elevated amount of neutrophils in their induced sputum. We also observed a significantly elevated presence of the FOXP3-positive Treg lymphocytes. We found a positive correlation between longer smoking history (amount of cigarettes smoked) and absolute levels of macrophages, neutrophils and FOXP3-positive Treg cells.

However, the determination coefficient for the above correlations was weak. This might be due to difficulties in obtaining a precise cell count, since sputum samples contain also dead cells, which can be in various stages of decomposition.

Previous studies, which match with our results, have found an elevated level of neutrophils in the induced sputum of smokers with COPD [18,19]. Elevated levels of neutrophils are conventionally explained with developments characteristic of a smoking induced chronic inflammation. It is thought that nicotine possesses direct activating and chemoattractant effect upon inflammatory cells. It has been shown that smoking has a direct stimulatory effect on granulocyte production and release from the bone marrow. Induced sputum in COPD contains mainly neutrophils at a percentage that is almost reciprocal to that of macrophages in healthy volunteers. These observations confirm previous studies demonstrating an increased number of neutrophils [20]. It is also established that smoking may increase the retention of neutrophils in the lung [21]. Inflammatory cells are also attracted by the free radicals – reactive oxygen species (ROS) – present in cigarette smoke [22,23]. Both nicotine and free radicals can activate the transcription factor NFkappa-β [24,25], which in turn activates genes for TNF and chemokines production [26]. Chemokines are released by alveolar macrophages [27], T-cells, epithelial cells, as well as the neutrophils themselves [28]. Most important chemokines are the CXC chemokines, for instance, IL-8 (CXCL8), that are able to attract and activate neutrophils, which in turn degranulate, releasing several proteases, for example, elastase, cathepsin G, proteinase 3 and metalloproteases [29]. Proteases consecutively cause tissue damage and mucus hypersecretion. It has been established that smoking diminishes antiprotease effect by causing functional deficit of α1-antitrypsin. The amount of neutrophils in bronchial biopsy materials and induced sputum correlates with severity of COPD and reduced lung function indices [30]. As mentioned before, increased neutrophil amount in IS has been observed among COPD patients as well as healthy smokers with a comparatively long smoking history, such as 25.5 pack years [31]. Our study shows that changes in cell spectrum can be observed already after a few pack years of smoking and that there is a positive correlation between pack years and levels of neutrophils even among those smokers whose lung function indices are still within a normal range. When comparing the induced sputum cell spectrum of COPD patients with the sputum of young smokers and non-smokers, results of our study are consistent with results elsewhere in the literature, stating that the absolute and relative amount of macrophages in COPD patients is lowered [32].

Presence of intrapulmonary inflammation in COPD cases has been a focus of research for many years, and accumulation of active macrophages and polymorphonuclear leukocytes (components of inherited immune system) are recognized as an important part of the COPD development. Recent work has concentrated on the role of adaptive immune system in COPD pathophysiology [1]. Some studies confirm importance of T-cells in triggering and sustaining of inflammation, by secreting mediators and directly interacting with other involved cells [4]. Early studies by Finkelstein et al. showed an increased amount of lymphocytes in COPD patients' airways [33,34]. Data confirms $CD8^+$ T lymphocytes as dominating cells of the immune system on the walls of large airways [35], small airways [7], and lung parenchyma of COPD patients [36,37]. Other authors later pointed out that the overall amount of $CD8^+$ lymphocytes in induced sputum of COPD patients is significantly higher when compared to smokers without COPD and non-smokers [8,32]. Rufino discovered elevated levels of $CD8^+$ and $CD4^+$ lymphocytes in both IS and patients' peripheral blood, which confirms hypothesis that COPD is a systematic inflammation [37]. Tzanakis et al. found no significant difference in levels of $CD8^+$ in IS between the smoking and non-smoking individuals with or without COPD. However, the relative amount of $CD4^+$ lymphocytes was significantly lower among the COPD patients when compared to smokers and nonsmokers without COPD (there was no difference between the two latter groups), but absolute amount of $CD4^+$ T lymphocytes have a tendency to increase [8]. These results are consistent with our results. Arnson et al. reported similar results among the smokers with a smoking history of over 50 pack-years without COPD, observing a lesser relative amount of $CD4^+$ but a higher relative amount of $CD8^+$ lymphocytes [6]. It is believed that $CD8^+$ lymphocytes exhibit various functions in the COPD patients' airways. One of them is serving as defence cells in case of a viral infection, yet it is not clear why the amount of $CD8^+$ does not go down when the infection has passed [37]. $CD8^+$ secrete various cytokines that activate other inflammation cells, for example, macrophages, or else, cause cell death by secreting mediators such as granzymes and perforins [38]. Activated T lymphocytes can activate $CD4^+$ T-cell apoptosis, reducing the CD4/CD8 ratio [39]. Along with extensive research on the role of $CD8^+$ lymphocytes in COPD pathogenesis, the role of $CD4^+$ in development of COPD has also been studied. It is known that $CD4^+$ lymphocytes are responsible for cytokine secretion, thereby regulating the responsive reaction to inflammation and attracting other cells of immune system [40]. Previous studies show that COPD patients have a significantly reduced $CD4^+/CD8^+$ ratio, yet when comparing groups of smokers without COPD and non-smokers the ratio was same [32], which is consistent with our data. In our study, we found no significant changes in the $CD4^+$ and $CD8^+$ lymphocyte levels when comparing young smokers with a short smoking history to non-smokers. COPD patients, in comparison, had elevated amounts of these cells. In the study by Tzanakis et al., the main differences between COPD patients and smokers without COPD and nonsmokers were in the amount of $CD8^+$, and the $CD4^+/CD8^+$ ratio [8]. Similar results were obtained by Rufino et al., who observed an elevation of $CD4^+$ and $CD8^+$ lymphocytes in COPD patients [37]. The authors suggested that the disbalance between $CD4^+$ and $CD8^+$ T lymphocytes might cause abnormal inflammation in COPD. Our study shows that the $CD4^+/CD8^+$ ratio is not changed in young smokers.

As mentioned previously, we observed a significantly higher amount of FOXP3-positive T lymphocytes in smokers when compared to non-smokers, as well as a significant positive correlation between sputum FOXP3-positive T lymphocytes and the smoking history. The $CD4^+$ $CD25^+$ T lymphocytes express transcription factor FOXP3-positive and are called T regulatory lymphocytes. These cells play a major role in the maintenance of self-tolerance and immune homeostasis [10]. FOXP3-positive Treg cells are naturally occurring regulatory T cells that are developmentally programmed under the control of the transcription factor FOXP3-positive [10]. FOXP3-positive expressing Treg cells are capable of suppressing the activation, the proliferation and the effector functions like the production of cytokines of a wide variety of immune effector cells in vivo and in vitro including $CD4^+$ and $CD8^+$ T cells, NK and NK T cells, B cells, and antigen presenting cells, i.e., dendritic cells or macrophages [10]. In our previous studies, our research group found that for both smokers with and without COPD, the level of FOXP3-positive is elevated in walls of large airways when compared to non-smokers. We also found a positive correlation between the duration of smoking history and the amount of FOXP3-positive in large airways. In the small airways walls of COPD patients a lesser amount of FOXP3-positive was found when compared to smokers with normal lung function indices, and with non-smokers. We found no correlation between the amount of FOXP3-positive Treg lymphocytes and smoking pack years [11]. Research by other scholars also shows that the bronchoalveolar lavage fluid (BALF) of smokers with normal lung ventilation indices contains a higher amount of Treg lymphocytes in comparison to the BALF of non-smokers, and the lungs of patients with COPD and emphysema have a lesser amount of FOXP3-positive Treg lymphocytes [41]. Smyth et al. pointed out that long-term cigarette smoke exposure without development of airflow obstruction increased airway T regulatory cell numbers [42]. Our data confirms this observation. It is possible that T regulatory lymphocytes regulate smoking induced changes in the airways of even recent smokers, by reducing activity of the immune system and damages caused by it. However, a study by Roos-Engstrand et al. reported that there were no differences in FOXP3-positive expressions in helper T cells. The percentage of FOXP3-positive expressions in CD25 helper T cells was decreased in smokers when compared to nonsmokers [43]. This suggests that the role for FOXP3-positive T cells in regulating the immune defence in smoking and COPD needs to be further elucidated [43].

It is still an open question, for how long one has to be a smoker for the above described transformations to begin, and in which smokers will it cause COPD. Not all smokers develop COPD. It is likely that COPD development is determined by a host–environmental interaction, combined with a genetic predisposition. Several genes had been suggested as candidates for COPD development, for instance, genes that regulate secretion of inflammation mediators, or production of proteases and antiproteases, etc. Since COPD is a complex disease, discovery of separate pathogenetic transformations does not mean a full understanding of the issue. We plan to continue our research by increasing the amount of young smokers and non-smokers and evaluating the suggested COPD candidate genes in smoking and non-smoking youths, as well as correlating the obtained indices with transformation in the induced sputum cells.

So far we have found that as predicted by our hypothesis, the amount of FOXP-3 positive regulatory T cells is significantly higher among young smokers. We intend to expand our research and introduce larger samples of both smokers and nonsmokers.

5. Conclusions

Our study demonstrated that the airways of young smokers presented the signs of persistent inflammation that not only involved neutrophils, but also recruited Treg lymphocytes, which most probably could be considered as an adaptive response.

Conflict of interest

The authors state no conflict of interest.

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