ORIGINAL ARTICLE

Role of autoimmunity in the pathogenesis of chronic obstructive pulmonary disease

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Abstract *Background:* Chronic obstructive pulmonary disease (COPD) is a major factor for disease related loss of quality of life, health expenditure and loss of productivity. An enhanced and persistent inflammatory response to the inhalation of particles and gases, mostly tobacco smoking, is considered a key pathogenic mechanism of COPD. Recent evidence indicates that autoimmunity plays a significant role in this response.

Aim of the work: This study investigated the role of autoimmunity in the pathogenesis of COPD by determining the prevalence of circulating autoantibodies in these patients and in healthy controls and evaluating their relationship with several disease components.

Patient and methods: This study included 31 COPD patients and 12 healthy non-smoker controls. All individuals were subjected to detailed history taking, full clinical examination, BMI calculation, arterial blood gas analysis, spirometry, 6MWD and assessment of serum level of circulating autoantibodies.

Results: There was a statistically significant difference between COPD patients and healthy controls in the spirometric data, PaO_2, SO_2 and 6MWD; however there was no statistically significant difference between both groups in the prevalence of autoantibodies. Also there was no statistically significant correlation between the prevalence of autoantibodies and other variables such as age, BMI, 6MWD, spirometric and ABG parameters.

Conclusion: The prevalence of positive circulating autoantibodies in COPD patients was found not significant when compared with healthy non smoker control group. This finding does not support a role for autoimmunity in the pathogenesis of COPD. So the hypothesis that autoimmunity plays a role in the pathogenesis in COPD needs further exploration.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common preventable and treatable disease, characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients [1].

It is now increasingly recognized that (COPD)/emphysema presents clinically as a syndrome with pulmonary and extrapulmonary manifestations [2]. Interestingly, only 15–20% of smokers develop COPD, suggesting that genetic predisposition and environmental factors play a role in the pathogenesis of the disease [3].

It has been hypothesized that there may be an autoimmune component to the chronic progressive lung tissue destruction which can persist after smoking cessation [4,5].

There is some evidence to support the contention that there is increased acquired immunity in COPD: (1) both helper (CD4+) and cytotoxic T lymphocytes (CD8+) accumulate in the lung parenchyma of patients with COPD [6]; (2) B lymphocytes form the core of the so-called bronchus-associated lymphoid tissue (BALT) which has been shown to be significantly increased in smokers and in patients with COPD [7,8]; (3) smoking is associated with an expansion of the population of antigen presenting cells on the epithelial surface of the lower respiratory tract [9].

The aim of the present study was to explore the role of autoimmunity in the pathogenesis of COPD by determining the prevalence of circulating autoantibodies in patients with COPD and healthy controls and evaluating their relationship with age, body mass index, spirometric parameters and arterial blood gases.

Subjects

The present study included thirty one male patients who fulfilled the criteria for COPD according to the American Thoracic Society/European Respiratory Society recommendations [10]. All patients were ≥50 years of age and ≥10 pack-year history of cigarette smoking. They were stable at the time of the examination with no other comorbid conditions or autoimmune diseases. Also patients younger than 45 years of age and patients with other pulmonary lesions e.g.; cancer, residual extensive tuberculous lesion or pulmonary fibrosis were excluded.

The control group included twelve healthy male controls with no smoking history.

Methods

All included individuals were subjected to: detailed history taking, full clinical examination, plain chest X-ray (P–A view) to exclude other chest problems. Body mass index, flow/volume loop using body plethysmography with highly transparent box; Sensor-medics V max series, 2130 Spirometer, V6200Autobox, 6200DL, arterial blood gases to estimate $PaCO_2$, PaO_2 and SO_2 using a blood gas analyzer (PHOX PLUS C), six min walk test and serum level of circulating autoantibodies.

Body mass index (BMI), or quetelet index

It is defined as the individual's body weight divided by the square of their height with the value universally being given in units of kg/m^2 [11].

Pulmonary function test

(Flow/volume loop): spirometry indices are reported comparing the individual's value along with the predicted values. The forced vital capacity (FVC), the forced expiratory volume in the first second (FEV1), the ratio of FEV1 to FVC and the forced expiratory flow at 25–75% of FVC (FEF25–75%) were measured. The presence of a post bronchodilator FEV1/FVC ratio < 0.70 confirms the presence of airflow limitation. Bronchodilator challenge test was performed to confirm that the air flow limitation is not fully reversible. Reversible airway obstruction is considered significant when there is an increase in the pre-bronchodilator FEV1 by both greater than 200 ml (*absolute change*) and 12% (*% change*) [12].

Arterial blood gases

One ml of arterial blood was obtained from the radial artery in a heparinized syringe, once the sample is obtained, care is taken to eliminate visible gas bubbles, as these bubbles can dissolve into the sample and cause inaccurate results. The sealed syringe is taken immediately to a blood gas analyzer (PHOX PLUS C) which aspirates this blood from the syringe and measures the partial pressures of arterial oxygen, carbon dioxide and oxygen saturation [13].

Six minute walk test

It was conducted in a 30 m long, flat corridor. Standardized instructions and encouragement were given, according to ATS guidelines [14]. Patients were instructed to walk as far and as fast as possible during the time that is. They were told that they could slow down or even stop if necessary. Standard phrases of encouragement at regular intervals (every 60 s) were used aiming to increase the distance walked. The test was discontinued if the patient had chest pain, intolerable dyspnea, cramps, dizziness, staggering, diaphoresis, pallor or SO_2 < 90% [14].

Assessment of serum level of circulating autoantibodies

Venous blood sample (10 ml) was obtained by peripheral venipuncture in the early morning after fasting overnight. Active smokers were asked to refrain from smoking before 8 h. Blood was centrifuged at 2000 rpm for 10 min immediately after sampling and serum was stored frozen at 80 °C until analysis. Circulating autoantibodies: the serum titers of antinuclear antibodies (ANA) were quantified by indirect immunofluorescence on Hep2 lines (INOVA, San Diego, USA). In ANA positive samples with a homogeneous pattern, we tested the presence of anti-double stranded DNA (dsDNA) antibodies by IFI on *Crithidia luciliae* slides. Anti-tissue autoantibodies (AT) including mitochondrial (AMA), smooth muscle (SMA) and parietal gastric cell (PGC) autoantibodies were determined by immunofluorescence on composite block of rodent liver, kidney and stomach sections (Inmunofluor ANA-AMA-SMA-APCA, MT, Promedt Consulting GmbH, St. Ingbert, Germany). All tests were blindly performed by a technician and reviewed by an immunologist. Both for ANA and AT, titers < 1:80 were considered negative whereas those 1:160, 1:320 and > 1:320 were considered increasingly positive [15].

Statistical analysis

Data were statistically described in terms of mean ± standard deviation (± SD), median and range or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student's *t* test for independent samples. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlation between various variables was done using Spearman rank correlation equation. *p*-Values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

In the present study, there was no statistically significant difference between COPD cases and healthy control group in the mean age and mean value of BMI as shown in Fig. 1, however there was a statistically significant difference between both groups in the spirometric data (Fig. 2), arterial blood gases parameters (Table 1) and 6MWD (Fig. 3).

By comparing COPD patients and healthy control groups in the level of serum circulating autoantibodies, it was found
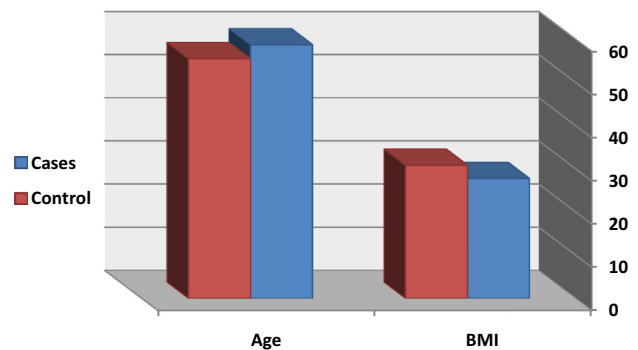

Figure 1 Statistical comparison between COPD cases and control group in mean value of age and BMI.
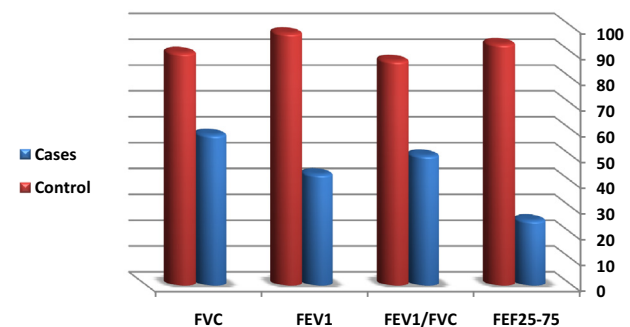

Figure 2 Statistical comparison between COPD cases and control group in the spirometric data.

that there was no statistically significant difference in the level of ANA, ASMA, AMA and APA between both groups (Table 2 and Fig. 4). The presence of Anti-double stranded DNA antibodies was tested only in the two COPD patients with positive homogenous ANA to exclude SLE and it was negative in these patients. Also the comparison between COPD cases and healthy control group was done according to the grade of positivity of ASMA because it may occur in normal healthy individuals but there was no statistically significant difference between both groups even in the + + and + + + categories (Table 3).

As regards the correlation between the level of circulating serum autoantibodies and other disease variables in the study COPD patients such as age, BMI, spirometric data, arterial blood gases parameters and 6MWD, it was found that there was no statistically significant correlation (Fig. 5).

Discussion

COPD is a major public health problem because: (1) it causes significant morbidity and mortality which is expected to increase worldwide in the near future; (2) it imposes an enormous global healthcare cost. However, because the pathogenesis of COPD is poorly understood, treatment is mostly symptomatic and new therapeutic strategies are limited [16,17].

The present study tried to investigate the unexplored role of the immune system in the pathogenesis of COPD and its relationship with age, body mass index, spirometric and blood

Table 1 Statistical comparison between COPD cases and control group in the arterial blood gas parameters.

ABG parameters	Cases (n = 31) Mean ± S.D.	Control group (n = 12) Mean ± S.D.	P-value	Statistical significance
PaO$_2$ (mmHg)	72.42 ± 14.62	86.92 ± 4.21	0.002*	Sig.
PaCO$_2$ (mmHg)	42.23 ± 7.56	39.67 ± 0.78	0.252	N.S
SO$_2$ %	93.23 ± 3.63	97.00 ± 1.21	0.001*	Sig.

* P-Value < 0.05 means statistically significant.
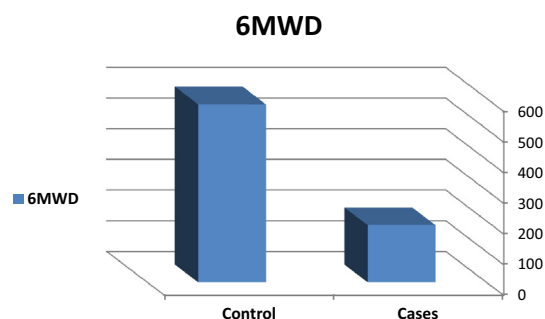
Figure 3 Statistical comparison between COPD cases and control group in 6 min walk distance (6MWD).
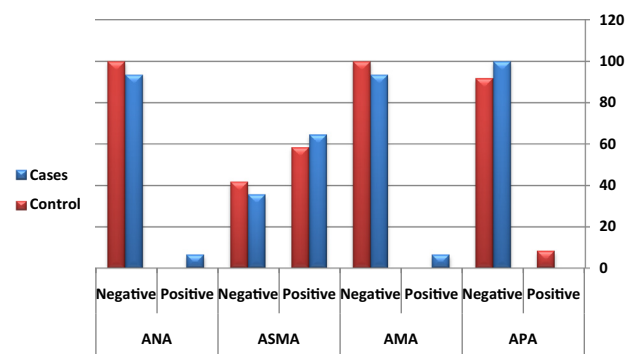
Figure 4 Statistical comparison between COPD cases and control group according to the level of serum circulating auto-antibodies.

Table 2 Statistical comparison between COPD cases and control group according to the level of serum circulating auto-antibodies.

Autoantibodies		Cases (n = 31) No.	% within group	Control group (n = 12) No.	% within group	Total (n = 43) No.	% within group	P-Value	Statistical significance
ANA	−ve	29	93.5	12	100	41	95.3	1.000	N.S
	+ve	2	6.5	0	0	2	4.7		
ASMA	−ve	11	35.5	5	41.7	16	37.2	0.737	N.S
	+ve	20	64.5	7	58.3	27	68.2		
AMA	−ve	29	93.5	12	100	41	95.3	1.000	N.S
	+ve	2	6.5	0	0	2	4.7		
APA	−ve	31	100	11	91.7	42	97.7	0.279	N.S
	+ve	0	0	1	8.3	1	2.3		

Table 3 Statistical comparison between COPD cases and control group according to the grade of positivity of ASMA.

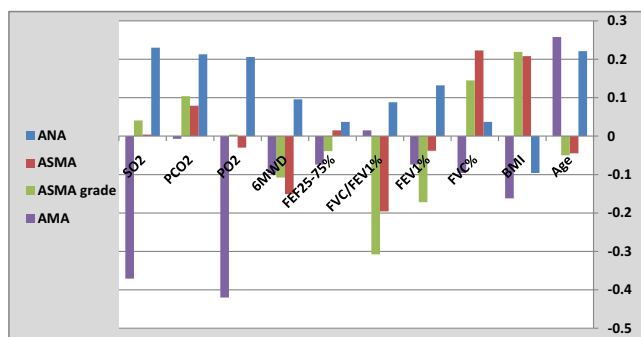| ASMA grade		Cases	Control group	Total
−ve	No.	11	5	16
	% within group	35.5%	41.7%	37.2%
+	No.	15	5	20
	% within group	48.4%	41.7%	46.5%
++	No.	5	1	6
	% within group	16.1%	8.3%	14%
+++	No.	0	1	1
	% within group	0%	8.3%	2.3%
Total	No.	31	12	43
	% within group	100%	100%	100%
		P-value = 0.372		
Statistical significance	N.S.			

Figure 5 Correlation between autoantibodies and Age, BMI, Spirometric data, ABG parameters and 6MWD.

gases parameters. If true, this hypothesis will generate new therapeutic opportunities in COPD.

In The present study the prevalence of positive circulating autoantibodies in clinically stable COPD patients was found not significant when compared with healthy non smoker control group. Also there was no statistically significant correlation between the presence of autoantibodies and the different variables measured in the group of COPD patients such as age, BMI, 6MWD, spirometric and arterial blood gases parameters.

The present study agreed with Cottin et al. [18], who found no evidence for anti-elastin autoantibody response in patients with combined pulmonary fibrosis and emphysema.

Additionally this study agreed with Greene et al. [19], who investigated the presence of autoantibodies against elastin and collagen derived proline–glycine–proline in patients with COPD and α1-antitrypsin deficiency and did not observe increased autoantibody levels.

Also Brandsma et al. [20], did not find an increased presence of autoantibodies against elastin, collagen and decorin in COPD when compared two separate groups of COPD patients and healthy control subjects. However they observed a significant higher level of anti-decorin IgG autoantibodies in COPD ex-smoker than COPD current smokers in one of the two groups.

On the other hand the present study disagreed with Agusti et al. [21] who found that COPD shares many clinical and pathophysiological characteristics of several autoimmune diseases such as rheumatoid arthritis (RA). They explained this by: (1) Smoking is a risk factor for both COPD [22] and RA [23]; (2) Once initiated, the inflammatory response of RA is self-perpetuating [24]. To some extent, this may be analogous to the continued airway inflammation seen after cessation of smoking [25]; (3) exacerbations occur both in RA and COPD. Yet, while in COPD exacerbations are precipitated by external factors such as airway infection and air pollution [26], in RA they are considered an integral part of the disease process, characterized by T cells sensitized to either cartilage epitopes [27] and/or degradation products of intestinal bacteria that mimic them [24] homing to the synovium and contributing to the maintenance or amplification of the inflammatory process; (4) Finally, similarities between the inflammatory cells (neutrophils, macrophages, T lymphocytes) and cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8) involved in the pathogenesis of RA [28] and COPD [6] are remarkable.

Hemminki et al. [29], reported an increased prevalence of COPD in patients with autoimmune disease and suggested that the presence of autoimmunity is a risk factor for the development of COPD. Also Birring et al. [30], previously reported an increased prevalence of autoimmune diseases in patients with COPD who have never smoked and they found that these patients tended to be elderly females and many had a blood lymphopenia, positive autoantibodies and airway inflammation.

The present study also disagreed with Birring et al. [31] and Raj et al. [32] who found a four-fold increase in the prevalence of COPD in patients with autoimmune bowel disease.

Also Tzouvelekis et al. [33] investigated the immunologic profile of a relatively large cohort of patients with CPFE and compared it to that of observed in patients with IPF without emphysema, using a complete panel of clinical, serum and histopathologic markers. They found the following: (1) Increased number of CPFE patients with positive ANA profile compared to patients with IPF without emphysema; (2) Increased number of CPFE patients with elevated serum p-ANCA titers compared to none with IPF without emphysema; (3) Massive infiltration of clusters of CD20+ cells forming lymphoid follicles immediately adjacent to areas of fibroblastic foci in lung biopsy samples from CPFE patients with positive serum immunologic profile (ANCA and/or ANA) compared to patients with negative profile, suggesting the presence of antibody producing B cells within the injured lung.

Núñez and colleagues [34] suggested that an autoimmune component might play a significant role in the pathogenic mechanism of COPD. Also Núñez and colleagues [34] were targeted to find out if ANA and AT can either be nonspecific markers of an ongoing autoimmune response [35] or alternatively, they may be directly involved in the pathogenesis of the disease. They found that ANA titers were not related to lung function, current smoking or comorbidity and that the most frequent antigen specificities of ANA in other autoimmune diseases (ENA, dsDNA) were negative, supporting the possibility that autoantibodies are nonspecific markers of ongoing autoimmune response [36]. By contrast, they found that more than 90% of AT positive patients were SMA positive and AT were inversely related to airflow limitation and DLCO impairment supporting the possibility that autoantibodies may be directly involved in the pathogenesis of the disease.

The present study concluded that autoimmunity itself does not play a significant role in the pathogenic mechanisms of COPD and that the presence of autoantibodies is not related to the pulmonary components of the disease. Further are studies needed to be carried out on a larger number of COPD patients to find out the incidence of autoimmune diseases in COPD, although systemic inflammation associated with COPD could result in several systemic manifestations, such as cardiovascular diseases and metabolic abnormalities Further studies are needed to find out other factors than smoking for the development of COPD since most smokers do not develop COPD aiming for the development of novel diagnostic, prognostic, and/or treatment approaches for this otherwise medically refractory disease.

There may be some limitations and explanations for the findings in the present study: (1) Sex distribution in the study population; the present study included only males, while previous studies which suggested a possible association between autoimmunity and COPD such as this performed by Lee

et al. [37] included male and females with higher percentage of females. (2) Smoking history in the study population; the COPD patients included in this study were only current smokers while some previous studies such as this performed by Brandsma et al. [20] included current, ex-smokers and non smokers and they found a higher level of anti-decorin IgG autoantibodies in COPD ex-smoker compared to COPD current smokers. This was also consistent with the study of Lee et al. [37] in which the majority of COPD patients were ex-smokers. (3) Finally, it is likely that the autoantibody response in COPD can be directed against many different antigens, which may vary between individuals. Such variability will make it more difficult to detect the presence of autoantibodies against a specific antigen in any given group. So investigation of the autoantibody response in COPD should be focused on the presence of specific antibody responses directed against lung tissue components, as Feghali-Bostwick et al. [38] did, when they showed the presence of autoantibodies against lung epithelial cells and also they found the deposition of IgG complexes in the lung.

Conflict of interest

There is no conflict of interest.

References

[1] Global Initiative for Chronic Obstructive Lung Disease (GOLD), Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease, 2011.
[2] E. Wouters, Local and systemic inflammation in chronic obstructive pulmonary disease, Proc. Am. Thorac. Soc. 2 (2005) 26–33.
[3] W. MacNee, Update in chronic obstructive pulmonary disease, AJRCCM 177 (8) (2008) 820–829.
[4] M. Cosio, J. Majo, M. Cosio, Inflammation of the airways and lung parenchyma in COPD: role of T cells, Chest 121 (2002) 160S–165S.
[5] M. Cosio, M. Saetta, A. Agusti, Immunologic aspects of chronic obstructive pulmonary disease, N. Engl. J. Med. 360 (2009) 2445–2454.
[6] M. Saetta, G. Turato, P. Maestrelli, et al, Cellular and structural bases of chronic obstructive pulmonary disease, AJRCCM 163 (2001) 1304–1309.
[7] C. Bosken, J. Hards, K. Gatter, et al, Characterization of the inflammatory reaction in the peripheral airways of cigarette smokers using immune-cytochemistry, Am. Rev. Respir. Dis. 145 (4 Pt. 1) (1992) 911–917.
[8] I. Richmond, G. Pritchard, T. Ashcroft, et al, Bronchus associated lymphoid tissue (BALT) in human lung: its distribution in smokers and non-smokers, Thorax 48 (1993) 1130–1134.
[9] M. Casolaro, J. Bernaudin, C. Saltini, et al, Accumulation of Langerhans' cells on the epithelial surface of the lower respiratory tract in normal subjects in association with cigarette smoking, Am. Rev. Respir. Dis. 137 (1998) 406–411.
[10] B. Celli, W. MacNee, Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper, Eur. Respir. J. 23 (2004) 932–946.
[11] G. Eknoyan, Adolphe quetelet (1796–1874) the average man and indices of obesity, Nephrol. Dial. 23 (1) (2007) 47–51.
[12] Global Initiative for Chronic Obstructive Lung Disease (GOLD), Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease, 2008.
[13] American Association for Respiratory Care (AARC), Clinical practice guideline sampling for arterial blood gas analysis, Respir. Care 37 (8) (1992) 913–917.
[14] American Thoracic Society ATS statement, Guidelines for the six-minute walk test, AJRCCM 166 (1) (2002) 111–117.
[15] R. Tozzoli, N. Bizzaro, E. Tonutti, D. Villalta, D. Bassetti, F. Manoni, A. Piazza, M. Pradella, P. Rizzotti, Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases, Am. J. Clin. Pathol. 117 (2002) 316–324.
[16] C. Murray, A. Lopez, Mortality by cause for eight regions of the world: global burden of disease study, Lancet (1997) 1269–1276.
[17] P. Barnes, Chronic obstructive pulmonary disease, N. Engl. J. Med. 343 (2000) 269–280.
[18] V. Cottin, N. Fabien, C. Khouatra, et al, Anti-elastin autoantibodies are not present in combined pulmonary fibrosis and emphysema, Eur. Respir. J. 33 (2009) 219–221.
[19] C. Greene, T. Low, S. O'Neill, et al, Anti-proline-glycineproline or anti-elastin autoantibodies are not evident in chronic inflammatory lung disease, Am. J. Respir. Crit. Care Med. 181 (2010) 31–35.
[20] C. Brandsma, H. Kerstjens, M. Geerlings, M. Kerkhof, M. Hylkema, D. Postma, W. Timens, The search for autoantibodies against elastin, collagen and decorin in COPD, ERJ 37 (35) (2011) 1289–1292.
[21] A. Agusti, W. MacNee, K. Donaldson, M. Casio, Hypothesis: does COPD have an autoimmune component?, Thorax 58 (2003) 832–834
[22] C. Fletcher, R. Peto, The natural history of chronic airflow obstruction, BMJ 1 (1977) 1645–1648.
[23] D. Hutchinson, L. Shepstone, R. Moots, et al, Heavy cigarette smoking is strongly associated with rheumatoid arthritis, particularly in patients without a family history of RA, Ann. Rheum. Dis. 60 (2001) 223–227.
[24] P. Toivanen, From reactive arthritis to rheumatoid arthritis, J. Autoimmun. 16 (2001) 369–371.
[25] I. Retamales, M. Elliot, B. Meshi, et al, Amplification of inflammation in emphysema and its association with latent adenoviral infection, AJRCCM2 164 (2001) 469–473.
[26] A. Ekberg-Jansson, S. Larsson, C. Lofdahl, Preventing exacerbations of chronic bronchitis and COPD, BMJ 322 (2001) 1259–1261.
[27] A. Guerassimov, Y. Zhang, S. Banerjee, et al, Autoimmunity to cartilage link protein in patients with rheumatoid arthritis and ankylosing spondylitis, J. Rheumatol. 25 (1998) 1480–1484.
[28] W. Arend, Physiology of cytokine pathways in rheumatoid arthritis, Arthritis Rheum. 45 (2001) 101–106.
[29] K. Hemminki, X. Liu, J. Ji, et al, Subsequent COPD and lung cancer in patients with autoimmune disease, ERJ 37 (2011) 463–465.
[30] S. Birring, C. Brightling, P. Bradding, et al, Clinical, radiologic, and induced sputum features of chronic obstructive pulmonary disease in non-smokers: a descriptive study, AJRCC 166 (2002) 1078–1083.
[31] S. Birring, A. Morgan, B. Prudon, et al, Respiratory symptoms in patients with treated hypothyroidism and inflammatory bowel disease, Thorax 58 (2003) 533–536.
[32] A. Raj, S. Birring, R. Green, et al, Prevalence of inflammatory bowel disease in patients with airways disease, Respir. Med. 102 (2008) 780–785.
[33] A. Tzouvelekis, G. Zacharis, A. Oikonomou, et al, Increased incidence of autoimmune markers in patients with combined pulmonary fibrosis and emphysema, BMC Pulm. Med. 13 (2013) 31, http://dx.doi.org/10.1186/1471-2466-13-31.
[34] B. Núñez, J. Sauleda, J.M. Antó, M. Julià, M. Orozco, E. Monsó, A. Noguera, F. Gomez, J. Garcia-Aymerich, A. Agustí, Anti-tissue antibodies are related to lung function in chronic obstructive pulmonary disease, AJRCCM 183 (2011) 1025–1031.

[35] N. Rose, C. Bona, Defining criteria for autoimmune diseases (Witebsky's postulates revisited), Immunol. Today 14 (1993) 426–430.

[36] M. Freemer, T. King, L. Criswell, Association of smoking with dsDNA autoantibody production in systemic lupus erythematosus, Ann. Rheum. Dis. 65 (2006) 581–584.

[37] S. Lee, S. Goswami, A. Grudo, et al, Anti-elastin autoimmunity in tobacco smoking induced emphysema, Nat. Med. 13 (2007) 567–569.

[38] C. Feghali-Bostwick, A. Gadgil, L. Otterbein, et al, Autoantibodies in patients with chronic obstructive pulmonary disease, Am. J. Respir. Crit. Care Med. 177 (2008) 156–163.