Study of anti nuclear and anti smooth muscle antibodies in patients with chronic obstructive pulmonary disease

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Abstract  Background/aim: Autoimmunity is a disease in which the immune system mistakenly attacks the body's own cells and tissues. Sometimes the whole body is attacked, and sometimes only one organ.

The aim of the work: The aim of this study was to evaluate antinuclear and anti smooth muscle antibodies, two common markers of autoimmunity, in COPD and their relation with different components of the disease and disease severity.

Methods: The study included 50 clinically stable COPD patients classified into two groups mild to moderate (group A) and severe to very severe (group B) according to GOLD (2009) [13] criteria plus 30 healthy control subjects (15 smokers and 15 non smokers). Blood levels of ANA and ASMA (measured by ELISA) were recorded.

Results: Levels of both ANA and ASMA were significantly higher in patients than in controls as a whole group but smoker controls showed significantly higher levels of both antibodies than mild to moderate COPD group (group A) indicating that not only smoking is responsible for COPD but other factors also play a role. Also high levels of these antibodies in smoker controls than in non smokers indicate a role of smoking in their development which is augmented by the direct relation with smoking index both in patients and controls.

Conclusion: Both ANA and ASMA levels are elevated in COPD patients compared to controls (smokers and non smokers) and levels elevated in healthy smokers compared to group A COPD patients. Autoimmunity plays a role in the pathogenesis of COPD.

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Introduction

An enhanced and persistent inflammatory response to the inhalation of particles and gases, mostly tobacco smoking, is considered a key pathogenic mechanism of chronic obstructive pulmonary disease (COPD) [7]. Recent reports claim that autoimmunity might play a role in the pathogen-
esis of the disease [21]. Many auto antibodies were found to be elevated in COPD including anti elastin antibody and auto antibodies against pulmonary epithelial and endothelial cells [10].

Antinuclear (ANA) and anti-smooth muscle antibodies are two important markers of autoimmunity which can be easily and accurately assessed [12].

**Subjects and methods**

**Study design**

Prospective case control study.

**Subjects**

This study was performed on 80 subjects, 50 patients were clinically stable COPD patients from those attending the Chest Department at Banha University Hospitals in the period between August 2011 and April 2012 plus 30 age matched apparently healthy control subjects, 15 smokers and 15 non smokers, selected from volunteers and patients relatives.

**Inclusion criteria**

Patients with COPD diagnosed according to [13] criteria.

**Exclusion criteria (due to possible effects on lung function and/or antibody levels)**

- Patients with pneumonectomy, lung cancers [11].
- Patients with residual extensive tuberculous lesion or active disease [24].
- Patients with diabetes mellitus, collagen vascular disorders [25].
- Patients with renal and hepatic disorders and those on systemic anti inflammatory therapy [18].

**Patients and controls were classified into four groups**

- Group (A): Twenty-five, COPD patients grade I and II (FEV1 > 50%).
- Group (B): Twenty-five, COPD patients grade III and IV (FEV1 < 49%).
- Group (C): Fifteen healthy current cigeratte smoker subjects (positive controls).
- Group (D): Fifteen healthy never smoker subjects (negative control).

All subjects were submitted to the following:

1. History taking with the stress on:
   - History of smoking (types and smoking index) [9].
   - History of chest symptoms (cough, expectoration, dyspnea and wheeze).
   - History of any other co-morbidities that may raise the anti nuclear and anti smooth muscle antibodies as diabetes mellitus, tuberculosis, hepatic disease, renal disease, and collagen vascular diseases [25].
   - Clinical examination both general and local.
   - Body mass index (BMI) was calculated as the weight in kg divided by height$^2$.
   - Complete blood count, liver function tests, kidney function tests and erythrocyte sedimentation rate to confirm inclusion and exclusion criteria.
   - Radiological examination: Plain X-ray chest P-A view.
   - Pulmonary function tests (spirometry) before and after broncho dilatation.
2. Anti nuclear antibody measurement (Von Muhlen and Tan [29])

- **Samples**

  This procedure was performed on a serum specimen. Addition of azide or other preservatives to the test samples was avoided as it may adversely affect the results. Grossly hemolyzed or lipemic serum or specimens were also avoided.

  Samples were preserved at $-20\, ^\circ C$ till all samples were collected and then analyzed. Specimens were also mixed well after thawing and prior to testing.

- **Principles of the Procedure**

  Highly purified individual antigens plus extracts from HEp-2 nuclei and nucleoli are bound to the surface of a microwell plate. The antigens include chromatin (dsDNA and histones), Sm/RNP, SS-A, SS-B, Scl-70, centromere, PCNA, Jo-1, mitochondria (M-2) and ribosomal-P protein, as well as the extracts. Anti-chromatin reactivity has recently been shown to be both a sensitive and early marker for SLE. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any ANA antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

- **Calculation of Results**

  The average Optical Density (OD) for each set of duplicates is first determined. The reactivity for each sample can then be calculated by dividing the average OD of the sample by the average OD of the ANA ELISA Low Positive. The result is multiplied by the number of units assigned to the ANA ELISA Low Positive found on the label.

  $\text{Sample Value} = \frac{\text{Sample OD}}{\text{Positive ANA ELISA Low}} \times \text{ANA ELISA Low Positive OD (units)}$

  Reactivity is related to the quantity of antibody present in a non-linear fashion. While increases and decreases in patient antibody concentrations will be reflected in a corresponding rise or fall in reactivity, the change is not proportional (i.e. a doubling of the antibody concentration will not double the...
reactivity). For more accurate quantitation of patient antibody, serial dilutions of the patient sample were run and the last dilution to measure positive in the assay should be reported as the patient’s antibody titer.

- Interpretation of Results

The ELISA assay is very sensitive to technique and is capable of detecting even small differences in patient populations. The sample can then be classified as negative, moderate positive or strong positive according to the manufacturer’s reference shown below.

<table>
<thead>
<tr>
<th>Units/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Moderate positive</td>
</tr>
<tr>
<td>Strong positive</td>
</tr>
</tbody>
</table>

1. A positive result indicates the presence of ANA antibodies and suggests the possibility of autoimmunity.
2. A negative result indicates none of the antibodies tested for are present or are below the levels of the negative cut-off for the assay.

8. Anti smooth muscle antibody (Actin IgG) measurement

- Sampling

The same sample as ANA.

- Principles of the Procedure

Purified F-actin antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any actin antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

- Calculation of Results

The average OD for each set of duplicates is first determined. The reactivity for each sample was then calculated by dividing the average OD of the sample by the average OD of the Actin ELISA low positive.

The result is multiplied by the number of units assigned to the Actin ELISA Low Positive found on the label.

\[
\text{Sample Value} = \frac{\text{Sample OD}}{\text{Positive}} \times \text{Actin ELISA Low Positive OD (units)}
\]

1. A positive result indicates the presence of Actin antibodies and suggests the possibility of an autoimmune disease.
2. A negative result indicates no Actin antibody or levels below the negative cut-off of the assay.

Results

Table 1 shows that 28% of group A were in clinical stage I, 72% in clinical stage II, 72% of group B in clinical stage III and 28% in clinical stage IV and that was statistically significant. The table also shows that 56% of group A were < 60 years old while 44% were > 60 years. In group B 68% were < 60 years old while 32% were > 60 years old. 86.7% of group C and 100% of group D were < 60 years old only 13.3% of group C were > 60 years old and that was statistically significant. Females were not found except in group D (66.7%) and the main presenting symptoms in patients is dyspnea (64%) and cough (36%) and that was statistically non significant. SI and FEV1%, FEF25–75% between 4 groups were statistically significant while BMI was not statistically significant.

Table 2 shows that group B patients have a smoking index significantly higher than both group A and healthy smokers. Healthy smokers have a smoking index significantly higher than group A patients.

Table 3 shows that both ANA and ASMA levels were significantly higher in group B than in group A patients.

Table 4 shows a significant negative correlation between both ANA and ASMA levels and post bronchodilator FEV1% in the whole COPD groups.

Table 5 shows that COPD patients have significantly higher level of ANA and ASMA than in the control group.

Table 6 shows that group B patients have significantly higher levels of ANA and ASMA than both group A and healthy smokers (group C) and non smokers (group D). Healthy smokers (group C) have both ANA and ASMA significantly higher than group A patients and non smokers (group D).

Table 7 shows that there is a non significant relation of patient’s main symptoms to the level of ANA.

Table 8 shows that there is a non significant relation of patient’s main symptoms to the level of ASMA.
Discussion

In the present study, although our patients were randomly selected according to the inclusion and exclusion criteria, all patients were males (100%) (Table 1) indicating a higher prevalence of the disease among males and this is concordant with the COPD literature in which higher prevalence of the disease among males is well known [8]. The higher prevalence of the disease in males may be largely related to the higher rate of smoking and occupational exposure to pollution among men [20].

In a large multicenter study created by Caillaud et al. [6] that included 591 COPD patients it was found that 80.7% were males who were smokers or ex smokers exposed to different vapors and fumes at work place. The authors concluded that, occupational exposure is an independent risk factor for COPD whatever the smoking status of the patients.

Although females are more susceptible to the effect of tobacco smoke than males [26], yet the higher prevalence was seen among males as indicated by absence of females in the patients. Again this is mostly due to factors mentioned above plus a lower incidence of smoking among females in our country due to social, economic and religious factors [23].

In this work levels of ANA and ASMA were found to be higher in COPD patients compared to controls (smokers and non smokers) and the difference between them was statistically significant ($p = 0.001$) (Table 2).

This result agrees with Bonarius et al. [5], who measured serum levels of autoantibodies, anti nuclear antibody (ANA) and anti tissues antibody (ATA) including ASMA in a total 232 subjects, 124 patients with COPD (94 smokers and 30 non smokers) plus 108 control subjects (71 smokers and 37 non smokers) in whom age and gender were matched. In these four subject groups they found seropositivity for ANA to be higher among COPD patients (44%) than controls (22%) and the difference was statistically significant ($p = 0.001$). ASMA were measured in only 12 of the studied patients and all samples were negative. The negative ASMA in these patients disagrees with our results and this discrepancy could be explained by the low number of ASMA assessed patients or a different degree of smoking severity.

The result also agrees with [10] who reported that 32 of 47 patients with COPD (68%) were serum positive for ANA. Also, Hodson and Turner-Warwick [16] found 28% of 50 patients with severe chronic bronchitis to be serum positive for ANA and were significantly higher than that found in age and sex matched non bronchitic controls (4%) which are in support of this study findings.

In a study done by Nunez et al., [22] who measured serum titers of ANA and anti tissue antibodies by immunofluorescence in 328 patients with clinically stable COPD and in 67 healthy controls (31 smokers and 36 non smokers), it was found that 34% of patients had high ANA titer ($>1:160$), eleven times higher than that seen in the control group (3%). They found that 11% of COPD patients had ANA titer ($>1:320$) which was too high.

Also Nunez et al., [22] found that 26% of studied COPD patients showed high ATA titers ($>1:160$) with 4.5 times higher prevalence than controls (6%), more over 21% of their patients showed very high ATA titers ($>1:320$) with ASMA sero-positivity found in most of ATA positive patients ($n = 80$). Results of Nunez et al. [22] support our findings.
Barcelo et al. [3] found that patients with chronic obstructive pulmonary disease showed increased percentages of CD8+ T-lymphocytes, which is consistent with a final maturation activation state of these cells with a correspondingly higher potential for tissue injury, further supporting a potential involvement of the acquired immune response in the pathogenesis of chronic obstructive pulmonary disease.

In this study it was found that the levels of ANA and ASMA were related to the stage of the disease as there was a significant increase in ANA and ASMA levels with increasing the severity of COPD, as severe and very severe COPD cases (group B) showed higher levels of ANA and ASMA than mild and moderate cases (group A), and the difference between them was statistically significant ($P = 0.001$) (Table 3).

Hogg [17] showed that the progression of COPD (from GOLD stage 0 to GOLD stage 4) was associated with increased production of mucus in the lumen of peripheral airways, thickening of the airway wall and amplification of the inflammatory response, mainly due to the accumulation of CD8+ T-cells and, interestingly, B-cells which are responsible for the secretion of antibodies. Moreover they found that, in patients with the most severe stages of COPD, inflammatory cells in the airway wall organize into lymphoid follicles to facilitate antigen presentation to the cells of the adaptive immune response. The authors suggested that these follicles represent an adaptive immune response which may develop in relation to microbial colonization and infection occurring in the later stages of COPD.

The data regarding involvement of B-cells have been recently supported by the observation that these cells are also increased in biopsies of bronchi from patients with increasing severity of COPD [14]. Another observation of increased numbers of plasma cells (i.e. end-stage differentiated B-cells) in and around the mucus secreting glands of patients with chronic bronchitis had been reported by Jeffery and Zhu [19]. Furthermore, in a recent study in human COPD lung and smoking mouse lung models, B-cell follicles were found to be present also in the parenchyma (Van der Strate et al. [28]). The authors had also demonstrated that parenchymal follicular B-cells in human COPD patients to be of oligoclonal origin, supporting B-cells in COPD to accumulate as a result of specific antigenic stimulation. Apart from microbial antigens, cigarette smoke particles or degradation products of lung extracellular matrix may also be the antigenic drive to such B-cell development [1]. Activation of CD8+ T lymphocytes and expansion of B-cells suggest a role in autoimmunity in COPD [1].

Contrary to this study results, Bonarius et al. [5] found that within the COPD studied group there was no association between ANA level and COPD stages as defined by GOLD (2009) [13] criteria. Also this result is not in agreement with [10] who suggested that an autoimmune response is associated with having developed COPD as such and not with the severity of the disease.

Nunez et al. [22] found that 21% of COPD patients with positive ASMA have levels inversely related to lung function impairment ($p = 0.05$). They explained this by airway remodeling in COPD patients as airway smooth muscle cells can synthesize extracellular matrix protein and downregulate matrix metalloproteinases. It is possible that attacking smooth muscle with autoantibodies will disturb their physiology and can enhance production of harmful substances by these cells.

As regards the relation between both ANA and ASMA levels and pulmonary function tests, this study showed a significant negative correlation between both antibodies and post bronchodilator FEV1% pred in the whole COPD group indicating that both ANA and ASMA in some way have a role in the pathology underlying airway dysfunction in these patients (Table 4).

This result agrees with Nunez et al. [22] for ASMA but not for ANA. These authors demonstrated a negative correlation between airway function and ASMA but could not find this relation with ANA. Also Greene et al. [15] found serum

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups Mean ±SD Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA (units/ml)</td>
<td>Group A 33.5 ±21.5 6.61 0.001 S</td>
</tr>
<tr>
<td></td>
<td>Group B 73.36 ±21.65 6.61 0.001 S</td>
</tr>
<tr>
<td>ASMA (units/ml)</td>
<td>Group A 25.32 ±8.41 5.144 0.001 S</td>
</tr>
<tr>
<td></td>
<td>Group B 40.65 ±12.31 5.144 0.001 S</td>
</tr>
</tbody>
</table>

* S = significant.
concentrations of anti tissue antibodies (especially antismooth muscle antigen antibodies), to be present in a fifth of patients with COPD, and correlated with the severity of airflow limitation. On the other hand Bonarius et al. [5] found no relation between both antibodies and lung function impairment. Although ANA is found to be prevalent in COPD patients, Wood et al. [30] could not find a relation between them and smoking status or FEV1.

In this work levels of ANA and ASMA were found to be higher in COPD patients compared to controls (smokers and non smokers) and the difference between them was statistically significant \( (p = 0.001) \) (Table 5).

This result agrees with Bonarius et al. [5], who measured serum levels of autoantibodies, anti nuclear antibody (ANA) and anti tissue antibody (ATA) including ASMA in a total 232 subjects, 124 patients with COPD (94 smokers and 30 non smokers) plus 108 control subjects (71 smokers and 37 non smokers) in whom age and gender were matched. In these four subject groups they found seropositivity for ANA to be higher among COPD patients (44%) than controls (22%) and the difference was statistically significant \( (p = 0.001) \). ASMA were measured in only 12 of studied patients and all samples were negative. The negative ASMA in these patients disagrees with our results and this discrepancy could be explained by the low number of ASMA assessed patients or a different degree of smoking severity.

The result also agrees with [10] who reported that 32 of 47 patients with COPD (68%) were serum positive for ANA. Also, Hodson and Turner-Warwick [16] found 28% of 50 patients with severe chronic bronchitis to be serum positive for ANA and were significantly higher than that found in age and sex matched non bronchitic controls (4%) which are in support of this study findings.

In a study done by Nunez et al., [22] who measured serum titers of ANA and anti tissue antibodies by immunofluorescence in 328 patients with clinically stable COPD and in 67 healthy controls (31 smokers and 36 non smokers), it was found that the levels of ANA were significantly higher in COPD patients (53.68 ± 29.52 Units/ml) than in healthy controls (30.46 ± 30.81 Units/ml) \( (p = 0.001) \) and the levels of ASMA were also significantly higher in COPD patients (32.98 ± 12.99 Units/ml) than in healthy controls (18.86 ± 15.72 Units/ml) \( (p = 0.001) \) (Table 5).

### Table 5: Comparison of ANA and ASMA levels (Units/ml) in COPD as a whole (50 patients) and controls (30 patients).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>Mean ±SD</th>
<th>Student t test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA (units/ml)</td>
<td>COPD</td>
<td>53.68</td>
<td>29.52</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30.46</td>
<td>30.81</td>
<td></td>
</tr>
<tr>
<td>ASMA (units/ml)</td>
<td>COPD</td>
<td>32.98</td>
<td>12.99</td>
<td>*3.86</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>18.86</td>
<td>15.72</td>
<td></td>
</tr>
</tbody>
</table>
* S = significant.

### Table 6: Comparison of ANA and ASMA levels (Units/ml) between both patient groups and healthy smoker controls and non smokers.

<table>
<thead>
<tr>
<th>Variable ((n = 65))</th>
<th>Mean ±SD</th>
<th>F test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>33.5</td>
<td>21.5</td>
<td>30.47</td>
</tr>
<tr>
<td>Group B</td>
<td>73.86</td>
<td>21.65</td>
<td></td>
</tr>
<tr>
<td>Healthy smokers (gp C)</td>
<td>51.59</td>
<td>31.66</td>
<td></td>
</tr>
<tr>
<td>Healthy non smokers (gp D)</td>
<td>9.33</td>
<td>2.54</td>
<td></td>
</tr>
<tr>
<td>ASMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>25.32</td>
<td>8.41</td>
<td>30.9</td>
</tr>
<tr>
<td>Group B</td>
<td>40.65</td>
<td>12.31</td>
<td></td>
</tr>
<tr>
<td>Healthy smokers</td>
<td>30.19</td>
<td>14.85</td>
<td></td>
</tr>
<tr>
<td>Healthy non smokers</td>
<td>7.52</td>
<td>3.96</td>
<td></td>
</tr>
</tbody>
</table>
* S = significant.

### Table 7: Relation between level of ANA (Units/ml) and clinical symptoms in COPD patients.

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>ANA Negative(&lt;20)</th>
<th>Moderate positive(20-60)</th>
<th>Strong positive(&gt;60)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>No 4 %36.4</td>
<td>6 %35.3</td>
<td>8 %36.4</td>
<td>18 %36.0</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>7 %63.6</td>
<td>11 %64.7</td>
<td>14 %63.6</td>
<td>32 %64.0</td>
</tr>
<tr>
<td>Total</td>
<td>11 %100</td>
<td>17 %100</td>
<td>22 %100</td>
<td>50 %100</td>
</tr>
</tbody>
</table>
* NS = non significant.

### Table 8: Relation between level of ASMA (Units/ml) and clinical symptoms in COPD patients.

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>ASMA Negative(&lt;20)</th>
<th>Week positive(20-30)</th>
<th>Moderate to strong positive(&gt;30)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>3 %100</td>
<td>7 %31.8</td>
<td>8 %32.0</td>
<td>18 %36.0</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>0 %0.0</td>
<td>15 %68.2</td>
<td>17 %68.0</td>
<td>32 %64.0</td>
</tr>
<tr>
<td>Total</td>
<td>3 %100</td>
<td>22 %100</td>
<td>25 %100</td>
<td>50 %100</td>
</tr>
</tbody>
</table>
* NS = non significant.
found that 34% of patients had high ANA titer (> 1:160), eleven times higher than that seen in control group (3%). They found that 11% of COPD patients had ANA titer (> 1:320) which was too high.

Also Nunez et al., [22] found that 26% of studied COPD patients showed high ATA titers (> 1:160) with 4.5 times higher prevalence than controls (6%), more over 21% of their patients showed very high ATA titers (> 1:320) with ASMA sero-positivity found in most of ATA positive patients (n = 80). Results of Nunez et al. [22] support our findings.

Barcelo et al. [3] found that patients with chronic obstructive pulmonary disease showed increased percentages of CD8 + T-lymphocytes, which is consistent with a final maturation activation state of these cells with a correspondingly higher potential for tissue injury, further supporting a potential involvement of the acquired immune response in the pathogenesis of chronic obstructive pulmonary disease.

Concerning ANA and ASMA levels, the mean values of ANA and ASMA in group B patients were significantly higher than in group A patients and healthy smokers and healthy non smokers. Healthy smokers have ANA and ASMA mean values significantly higher than group A patients and healthy non smokers (group D) and the difference between them was statistically significant (P = 0.013) (Table 6).

These findings are similar to the those of Bonarius et al. [5], who found seropositivity for ANA to be higher among smoker COPD (40 of 94) than control smokers (17 of 71) and the difference was statistically significant (p = 0.013).

In this work, levels of ANA and ASMA were found to be significantly higher in patients with dyspnea as the main symptom than in those with cough (Table 7 and 8). This indicates that these antibodies are either associated with disease severity which is mainly judged from patient disability due to dyspnea [4], or related to parenchymal injury manifested clinically and radiologically by emphysema and/or vascular injury (pulmonary hypertension). This issue needs a separate work to evaluate.

This result agrees with Bonarius et al. [5] who found that emphysematous phenotype COPD patients (with low attenuation areas on CT scan) have higher levels of ANA. Also Lee et al., [21] demonstrated the presence of antielastin antibodies and Th1 responses in patients with COPD, which were correlated with severity of emphysema.

Also this result is supported by the work of van der Strate et al. [28] who found lymphoid follicles consisting of B-cells and follicular dendritic cells (DCs) with adjacent T-cells to be present both in parenchyma and bronchial walls of patients with emphysema plus an oligoclonal antigen-specific reaction of the B-cells a finding commonly seen in autoimmune disorders. Also Sullivan et al. [27] have shown the presence of CD4+ T-lymphocyte in oligoclonal expansion in the lungs of patients with severe emphysema.

**Conflict of interest**

None declared.

**References**


