Subclinical pulmonary involvement assessed by bronchoalveolar lavage in patients with early undifferentiated connective tissue disease

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Abstract

Objective
To assess the presence of neutrophil and lymphocyte fibrosing alveolitis by bronchoalveolar lavage in patients with early undifferentiated connective tissue disease (EUCTD) and systemic sclerosis (SSc).

Method
Bronchoalveolar lavage (BAL) was performed in 13 patients with EUCTD who exhibited signs of lung involvement by non-invasive methods including lung function tests and high resolution computed tomography. The mean age of cases was 48.1 ± 6.6, and the mean disease duration was 1.8 ± 0.8 years. Differential cell counts of BAL were evaluated. Eleven patients with systemic sclerosis and 5 healthy control subjects were also investigated.

Results
Eleven of the 13 EUCTD and 10 of the 11 SSc patients showed an elevated total cell number (above the median cell/ml of control + 2SD) in the BAL fluid. In patients with EUCTD, the lymphocyte count was elevated in 6, and the polymorphonuclear neutrophil count in 2 patients. One of the patients with EUCTD had simultaneously elevated lymphocyte and neutrophil granulocyte counts. In the SSc group, 6 patients had an elevated lymphocyte and 6 an increased neutrophil count. Three of these cases had both increased neutrophil and elevated lymphocyte counts, simultaneously.

Conclusion
Subclinical, predominantly lymphocyte alveolitis can be present in patients with EUCTD. Patients with SSc tend to exhibit neutrophil alveolitis.

Key words
Early undifferentiated connective tissue disease, systemic sclerosis, lung fibrosis, alveolitis, bronchoalveolar lavage.

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Introduction
The term undifferentiated connective tissue disease (UCTD) is used to describe a group of patients with systemic connective tissue disorders that lack definitive characteristics of any particular well-defined disease (1-3). Several patients with UCTD later develop a well-defined connective tissue disease such as systemic sclerosis (SSc) (4, 5) or systemic lupus erythematosus (SLE) (5, 6), although a significant portion of the patients with UCTD tend to remain in a permanently stable clinical condition during the follow up (5-8). The leading clinical symptoms in UCTD are Raynaud’s phenomenon, non-erosive polyarthritis and/or keratoconjunctivitis sicca (1,9-11) accompanied by a series of other clinical-laboratory findings (1,12,13). The presence of antinuclear antibodies also seems to be an important finding, as has recently been proposed (14). Lung fibrosis/alveolitis may also be among the manifestations of UCTD (1,4,6,15). Lung fibrosis plays an important role in the prognosis and outcome of connective tissue diseases, including SSc and inflammatory myopathies; therefore, the early detection of interstitial lung abnormalities are of high priority (16,17). Subclinical alveolitis also seems to be present in well defined connective tissue diseases such as inflammatory myopathies or SLE (18-21); however, little is known about the lung symptoms in patients with early UCTD and cases which are potential candidates for the later development of a connective tissue disease (4,15,22). Bronchoalveolar lavage is regarded as a safe procedure for the investigation of various forms of interstitial lung diseases and their activity. Single site BAL usually correctly detects the presence of alveolitis (18). In the case of alveolitis, there is a characteristic increase in alveolar macrophages and an increase in both the absolute number and the percentage of granulocytes (neutrophils and eosinophils). This type of alveolitis seems to predominate in SSc (23-25). In the other form of alveolitis, the lymphocyte count is increased in the BAL fluid. Lymphocyte alveolitis can be detected in patients with Sjögren’s syndrome, rheumatoid arthritis, and systemic lupus erythematosus. Lymphocytosis in certain cases with SSc can also be detected (20, 26). Our aim in this study was to identify patients with ongoing lymphocyte alveolitis among cases with EUCTD. Furthermore, we characterised the type of alveolitis (neutrophil or lymphocyte) and compared these results to the findings for patients with SSc.

Patients and methods
Disease definitions and clinical protocol
The diagnosis of patients with EUCTD was based on the criteria used by the previous investigators including Calvo-Alén et al. (6) and Alarcón et al. (1) with substantial modifications (27) (Table I). Cases with overlap syndromes (a disease state in which criteria for more than one connective tissue disease are fulfilled) (28), and patients with mixed connective tissue disease (29) were definitely excluded from this study. The onset of the disease was the appearance of any of the following symptoms/signs: Raynaud’s phenomenon, polyarthritis, keratoconjunctivitis sicca or increased ESR. For the diagnosis of patients with SSc, the preliminary ACR criteria were used (30). Cases with overlap syndromes (28), and patients with mixed connective tissue disease (29) were excluded from this study except SSc patients with coexisting myositis.

Chest radiograph, spirometry/diffusion capacity, and ECG were investigated in all cases. A Schirmer’s test was routinely performed in all patients. In the case of symptoms compatible with polynuropathy, an electrophysiologic investigation was performed. Nailfold capillary microscopy was performed in all patients by the same investigator (Z.N.). Signs of scleroderma capillary pattern were investigated (31).

ESR, CRP, rheumatoid factor, and antinuclear antibody (on HEp-2 cells and rat liver sections) were tested in all patients. Anti-dsDNA, anti-Rnp, anti-SS-A, anti-topoisomerase I, anti-cardiolipin IgG and IgM were investigated by conventional ELISA tests (for anti-dsDNA, anti-SS-A antibodies, Cogent

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the ESR value was above 40 mm/hr for also obtained. Non-smokers were incapable to stop smoking for at least 4 months.

Thirty-nine cases with early undifferentiated connective tissue disease and 35 patients with SSc were evaluated in all cases as well. Increased sedimentation rate was recorded, if the ESR value was above 40 mm/hr for a period of at least 6 weeks without any other known cause.

**Study subjects**

Thirty-nine cases with early undifferentiated connective tissue disease and 35 patients with SSc were evaluated in Nephrological Center and 2nd Department of Internal Medicine of the University of Pécs, between August 1995 and May 1999. Informed consent was obtained from all patients. The permission of the local ethical committee was also obtained. Non-smokers were investigated. In smokers, the investigation was also performed if the patient was able to stop smoking for at least 4 months.

The 39 early UCTD cases were investigated. HRCT was performed in 24 cases with at least minimal changes (mild basal reticular or reticulonodular pattern) on chest x-ray, and/or DLCO/VA < 80%. Seven out of the 24 patients who did not stop smoking were not further studied. From the 17 remaining cases, 13 agreed to undergo bronchoalveolar lavage.

All 13 patients with UCTD were females with a mean age 48.1 ± 6.6 years. The mean disease duration was 1.8 ± 0.8 years. Poliarthritis/arthralgia was detected in 9 cases, Raynaud’s phenomenon was shown in 12 cases, and decreased lacrimal secretion in 9 cases. Increased ESR was detected in 3 and oesophageal dysmotility in 1 case. Peripheral neuropathy was verified in one case. Alopecia was present in 2 cases. No kidney symptoms, cardiac involvement, rash, dermatitis, nor sclerodactyly were detected. Antinuclear antibody positivity was detected in 7 cases by indirect immunofluorescence. Homogeneous staining pattern was found in 2, fine speckled in 4, and antinuclear antibody in 1 case. Anti-dsDNA autoantibody was found in 3, anti-SS-B in 2, increased anti-cardiolipin IgG in 3, and increased rheumatoid factor in 4 cases, respectively. We present here patients with early UCTD; therefore they may not fulfill the proposed criteria for established UCTD (14). Similar to other investigators, a significant proportion but not all cases were found to be ANA positive (32). We found an SSc pattern on nailfold capillary microscopy in 2 cases. All cases showed lung involvement with signs of fibrosis on chest X-ray. Ground glass opacity on HRCT was detected in 12 cases, and fibrosis was shown in 13 patients on HRCT. Honeycombing was shown in one case (Table II).

HRCT was performed in 28/35 cases with SSc because of the presence of lung involvement. In 10 cases, BAL was not performed because of either the elderly age of the particular patient (>60 years) or the long disease duration (>10 years). All of these excluded cases belonged to the lcSSc subset. From the remaining 18 cases one continued smoking. Two further cases were also excluded because of the presence of a significant ischemic heart disease. Four cases who fulfilled our selection criteria for performing BAL did not agree to participate in the study. All but 2 patients were females. The mean age of the patients was 48.5 ± 8.4 years. The mean disease duration was 5.1 ± 4.0 years. Seven patients belonged to the limited cutaneous systemic sclerosis (lcSSc), and 4 patients to the diffuse cutaneous systemic sclerosis (dcSSc) subset (33). Four patients had oesophageal dysmotility and 8 of them suffered from sicca syndrome. No cases with renal and only one patient with cardiac involvement were detected. One patient showed myositis. In 6 cases the typical capillary microscopic pattern of systemic sclerosis was found. One antinuclear body positive patient and 7 antitopoiso-merase I antibody positive cases were found in this group (Table III). Anti-SS-A antibodies were found in 2, anti-SS-B in three, anti-cardiolipin in 2 cases, respectively. Ten cases showed lung involvement with signs of fibrosis on chest X-ray. Ground glass opacity on HRCT was detected in 9 cases, and

**Table I. Classification criteria for EUCTD.**

<table>
<thead>
<tr>
<th>Major symptoms</th>
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<tbody>
<tr>
<td>Raynaud’s phenomenon</td>
</tr>
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<td>Non-erosive, symmetric polyarthritis</td>
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<tr>
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<tr>
<td>Arthritis/arthralgia</td>
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<td>Rash/dermatitis or alopecia or oral ulcers or UV light sensitivity</td>
</tr>
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</tr>
<tr>
<td>Lung fibrosis/alveolitis and/or decreased diffusing capacity/decreased vital capacity</td>
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<td>Swallowing problems/pyrosis with oesophageal dysmotility</td>
</tr>
<tr>
<td>Cardiac involvement</td>
</tr>
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<td>Pleuritis/pericarditis</td>
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Peripheral neuropathy/central nervous system symptoms

- Sclerodactyly and/or swollen fingers/oedema of fingers or skin ulcers (otherwise unexplained)
- Elevated ESR/CRP
- Positive antinuclear antibody test with homogeneous, fine speckled, nucleolar, antinuclear antibody staining pattern or specific autoantibody positivity (anti-dsDNA, anti-SS-A, anti-Rnp, anti-centromere, anti-topoisomerase I, repeatedly positive anti-cardiolipin IgG or IgM by ELISA test)
- Abnormal nailfold capillarmicroscopy findings compatible with the presence of scleroderma capillary pattern (31)

Early undifferentiated connective tissue disease was diagnosed if at least one of the major symptoms, and at least 3 further minor and/or major symptoms were fulfilled (27). Cases fulfilling a well established connective tissue disease including systemic lupus erythematosus, systemic sclerosis, rheumatoid arthritis, inflammatory myopathies, primary Sjögren’s syndrome, and mixed connective tissue disease were excluded.

Diagnostics Ltd, UK were used. ELISA tests for anti-Rnp, anti-topoisomerase I, anti-cardiolipin antibodies were purchased from Corgenix Corp., CO, USA. Urine analysis (proteinauria, hematuria, casts), and the presence of leukopenia/thrombocytopenia were investigated in all cases as well. Increased sedimentation rate was recorded, if the ESR value was above 40 mm/hr for a period of at least 6 weeks without any other known cause.

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- Abnormal nailfold capillarmicroscopy findings compatible with the presence of scleroderma capillary pattern (31)
fibrosis was shown in all 11 patients on HRCT. In 2 cases severe, advanced fibrosis was revealed (Table II).

Five non-smoking healthy individuals (3 females and 2 males) without any lung parenchymal abnormality were also investigated as controls. The mean age of the controls was 23.0 ± 2.0 years.

Investigation of the lung involvement
Beside the physical examination by an experienced pulmonologist (HZ), lung manifestation was characterised by the detection of bibasilar or diffuse pulmonary fibrosis on chest x-ray. Pulmonary function tests included spirometry, recording of maximum expiratory flow volume curves and maximum flow at 75, 50, and 25% of vital capacity, determination of residual volume and airway resistance by body plethysmography (System 2800 Whole Body Plethysmograph, SensorMedics, USA). Parameters analysed included inspiratory vital capacity (IVC), total lung capacity (TLC), diffusing capacity for carbon monoxide (DLCO), and carbon monoxide diffusing capacity adjusted for alveolar volume (DLCO/VA). Patients were considered to have a restrictive ventilatory impairment if IVC and TLC were less than 80% of the predicted normals. Diffusing impairment was diagnosed if the DLCO was less than 80% of the predicted value. Quality control procedure and reference values were performed according to the European Community of Coal and Steel (34). Lung involvement was encoded in cases of signs of lung fibrosis on chest x-ray, and/or restrictive ventilatory failure, and/or decreased diffusing capacity. Minor reticulonodular changes on chest X-ray were carefully evaluated. Cases with signs of lung involvement underwent a high-resolution computed tomography (HRCT). HRCT was performed as previously described with minor modifications (16, 20). Lung HRCT scans were performed using a Siemens Somatom AR HP (non-spiral, third generation) computer tomograph with the patient in the supine AP position, under normal breathing, with HiRes Lung scan type, basically in the native mode without contrast material. The scan parameters were as follows: slice thickness 2 mm, detector side collimator setting 2 mm, with 10 mm slice distance, 130 kV and 210 mAs and 3 sec/scan, 20 slices, giving a total scan time of approx. 3 min. with positioning and measurement overhead. The windowing method chosen was the default HiRes Lung setting (c:1000, w:-700) with minor modifications for optimum image evaluation. Ground glass opacity with and without fibrosis, lung fibrosis, subpleural and diffuse honeycombing were blindly evaluated by the same two investigators (EJ, KD). Minor reticulonodular changes on chest X-ray were carefully evaluated. The inter observer variation was less than 5%.

**Bronchoalveolar lavage**
A standard fiberoptic bronchoscopy was used as previously described (20) with minor modifications. The BAL was carried out in one of the subsegmental bronchi of the middle lobe by the injection of 5 aliquots of 40 ml of saline at room temperature, which was re-aspirated by gentle syringe suction. 50 ml plastic syringes were used for this purpose. The recovery was 60 ± 14% in UCTD, 58 ± 21% in SSc and 82 ± 3% in the control group. In the bronchoalveolar lavage fluid of the healthy controls the total cell count was 5.9 x
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The average percentage of lymphocytes and neutrophil granulocytes was 8.3% (1.7-9.9 x 10^4 cell/ml) and 2.7% (0-3.0 x 10^3 cell/ml), respectively. Physiologic saline slightly alkalised by NaHCO₃ was used for the BAL. The BAL fluid was filtered through gauze and immediately centrifuged (400g, 4 °C, 15 min.). The cell pellet was used for further studies. Differential cell counts was determined by using May-Grünwald-Giemsa staining. Viability was measured by trypan blue exclusion dye test, and was always above 95%. Samples with red blood cell contamination were excluded from further tests.

### Flow cytometry

The cells were pooled and washed twice (250g, 5 min, 4°C) in staining buffer (PBS without Ca²⁺ and Mg²⁺, 0.1% (w/v) BSA, 0.1% (w/v) sodium azide, pH = 7.4, stored at 4°C). Cells were stained in plastic tubes (5 x 10⁵ cells in 100 ml staining buffer containing a previously determined optimal concentration of a fluorochrome-conjugated antibody for cell surface antigens or appropriate negative (isotype) control). To identify the T lymphocyte subpopulations the following direct labeled mouse anti-human antibodies against surface antigens were used: CD3 PE-Cy5, CD4 FITC, CD8 PE (all from Immunotech, France). The following direct labeled isotype control antibodies were used to adjust the instrument settings and to determine the levels of specific positive staining: IgG1-FITC, IgG1-PE, IgG1 PE-Cy5 (Immunotech, France). Following incubation at 4°C for 30 min in the dark, two washing steps with staining buffer were performed and the pellet gained by centrifugation was used (250g, 5 min, 4°C). The resuspended cells were fixed in 1 % formaldehyde in PBS. Cells were further analysed by flow cytometry (FACSCalibur, BD; CellQuest software). To create the lymphocyte gate the dot plot of the physical light scatter parameters (forward scatter/side scatter) was used. To select the T cells the CD3 positive lymphocytes were further gated using an FLIII./cell count histogram. 5000 events for T cells were acquired. The selected cells were further analyzed to their CD4 and CD8 staining, in a FLI./FLII. dot plot. The ratio of the CD4+/CD8+ cells were determined by quadrant statistic.

### Table III. Total and differential cell count (cell/ml) in bronchoalveolar lavage fluid of patients with early undifferentiated connective tissue disease and systemic sclerosis.

<table>
<thead>
<tr>
<th>P</th>
<th>Disease²</th>
<th>Ab³</th>
<th>Total cell count x10⁵</th>
<th>Macrophage count x10⁴</th>
<th>Lymphocyte count x10⁴</th>
<th>Neutrophil granulocyte count x10⁴</th>
<th>CD3⁺</th>
<th>CD3⁺ CD4⁺</th>
<th>CD3⁺ CD8⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td></td>
<td>0.96</td>
<td>0.77</td>
<td>1.81</td>
<td>0.13</td>
<td>1.47</td>
<td>1.21</td>
<td>0.18</td>
</tr>
<tr>
<td>2</td>
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<td></td>
<td>3.65</td>
<td>3.58</td>
<td>0.18</td>
<td>0.55</td>
<td>0.04</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>EUCTD</td>
<td></td>
<td>6.34</td>
<td>6.26</td>
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<td>0.40</td>
<td>0.16</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
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<td>1.65</td>
<td>1.24</td>
<td>0.28</td>
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<tr>
<td>5</td>
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<td></td>
<td>1.83</td>
<td>1.66</td>
<td>1.28</td>
<td>0.37</td>
<td>0.35</td>
<td>0.23</td>
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<tr>
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<td>0.62</td>
<td>0.73</td>
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<td>0.47</td>
<td>0.16</td>
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<tr>
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<td>1.33</td>
<td>1.27</td>
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<td>0.00</td>
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<td>0.05</td>
<td>0.02</td>
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<tr>
<td>9</td>
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<td>1.80</td>
<td>1.84</td>
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<td>1.07</td>
<td>0.83</td>
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</tr>
<tr>
<td>10</td>
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<td>0.86</td>
<td>8.80</td>
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<td>7.48</td>
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<tr>
<td>11</td>
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<td>1.93</td>
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<tr>
<td>12</td>
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<td>1.11</td>
<td>1.36</td>
<td>0.05</td>
<td>1.19</td>
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<td>1.85</td>
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<td>-</td>
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<tr>
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<td>ACA</td>
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<td>1.11</td>
<td>1.80</td>
<td>0.00</td>
<td>1.55</td>
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<tr>
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<td>10.90</td>
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<td>ATA</td>
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<td>1.31</td>
<td>1.45</td>
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<td>1.02</td>
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<tr>
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<td>lcSSc</td>
<td>ATA</td>
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<td>1.01</td>
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<td>0.71</td>
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<tr>
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<td>ATA</td>
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<td>0.34</td>
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<td>0.39</td>
<td>0.20</td>
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<td>1.21</td>
<td>1.06</td>
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<td>0.82</td>
<td>0.14</td>
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<td>0.31</td>
<td>0.16</td>
<td>0.22</td>
<td>0.12</td>
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</tr>
</tbody>
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1 Patients, see Table II.
2 EUCTD: early undifferentiated connective tissue disease, lcSSc: limited cutaneous systemic sclerosis, dcSSc: diffuse cutaneous systemic sclerosis
Data analysis
The values in patients were regarded as abnormally increased if they exceeded the median value observed in control subjects by 2 or more standard deviations. Lymphocyte or granulocyte alveolitis was diagnosed if the absolute cell count of either of the cell population was elevated. If both lymphocyte and granulocyte count were elevated it was interpreted as mixed alveolitis. Non-parametric analysis was used for group comparisons of the quantitative data, since different segments of BAL cellular components were not normally distributed and/or the SD-s of the different groups differed significantly. The Mann-Whitney U-test was used for comparisons between two groups.

Results
Both patients with EUCTD and SSc exhibited a significant increase in the total cell count of the BAL compared to controls (p < 0.05, p < 0.01, respectively; Fig. 1). In EUCTD, the average cell count was 1.8 ± 1.6 x 10^5 cell/ml. Patients with SSc had 1.6 ± 4.2 x 10^5 cell/ml in the BAL fluid, whereas the control group exhibited 0.6 ± 0.1 x 10^5 cell/ml. Eleven of the 13 EUCTD and 10 of the 11 SSc patient showed an increased total cell number above the median of controls + 2 SD in the BAL (Table III).

The alveolar macrophage count was also significantly increased both in EUCTD and SSc (p < 0.01; p < 0.05, respectively) compared to controls. The average alveolar macrophage count was in EUCTD 1.3 ± 1.6x10^5 macrophage/ml, and in SSc 1.3 ± 3.6 x 10^5 macrophage/ml, whereas the control group exhibited 0.5 ± 0.1x10^5 macrophage/ml (Table III).

In EUCTD, the total lymphocyte count was 1.3 ± 2.3 x 10^4 lymphocyte/ml. Patients with SSc exhibited 1.3 ± 3.0 x 10^4 lymphocyte/ml in the BAL (versus the control group of 0.4 ± 0.4 x 10^4 lymphocyte/ml). The average polymorphonuclear neutrophil count was in EUCTD 1.2 ± 1.7 x 10^3 neutrophil/ml, in SSc 3.9 ± 48.7 x 10^3 neutrophil/ml (versus the control group of 1.3 ± 1.1 x 10^3 neutrophil granulocyte/ml) (Table III). In cases with EUCTD, the lymphocyte count was above the median of controls + 2 SD in six, the polymorphonuclear neutrophil count in two patients and both cell types simultaneously in one other patient. In the SSc group, 3 patients had an elevated lymphocyte and 3 an increased neutrophil count. Additionally, 3 other cases had both increased neutrophil and elevated lymphocyte count, simultaneously (Fig. 2).

The total T lymphocyte count (8.2 ± 24.3 x 10^3 lymphocyte/ml) was significantly elevated in the group of the SSc patients compared to the controls (p < 0.05) (Fig. 3). The average T lymphocyte count was in EUCTD 8.6 ± 20.6 x 10^3 lymphocyte/ml versus the control group of 1.8 ± 2.0 x 10^3 lymphocyte/ml (Table III). A significantly increased Tc (CD3+ CD4- CD8+) lymphocyte count was detected in patients with SSc (p < 0.05) (Fig. 3). The elevated T lymphocyte count was explained by an increase of the alveolar Tc cell number whilst the Th (CD3+ CD4+ CD8-) count did not differ significantly between the investigated groups. The same tendency was observed in the EUCTD group as well, but the data did not reach the level of statistical significance (Fig. 3). The CD4/CD8 ratio did not differ significantly between the compared groups (2.7 ± 1.7, 2.1 ± 1.5, 2.2 ± 1.3, 1.8 ± 1.5).
During the follow-up, only one patient received methylprednisolone as characterized by BAL is associated with the type of alveolitis (38). In contrast to neutrophil alveolitis, lymphocyte alveolitis seems to respond well to corticosteroid treatment (39).

Pulmonary fibrosis is a frequent and serious complication of scleroderma (4, 16,19,24,40,41) which plays an important role in the prognosis and outcome of SSc (4, 17). Active alveolitis as characterized by BAL is associated with progressive pulmonary disease in SSc patients (42). Our present findings also confirm the well-known fact that both lung fibrosis and active alveolitis are frequently found in SSc (Tables II, III). In accordance with previous investigators (23,25), we also detected an increased amount of absolute cell num-

3.5 ± 1.4; in UCTD, in SSc and control group, respectively) (Table III). Among the cases with SSc, 7 patients had anti-topoisomerase I antibody (Table III). From these cases, 2 exhibited lymphocytosis, 2 neutrophil alveolitis and 3 mixed alveolitis (with simultaneously increased lymphocyte and granulocyte counts). Two of these latter cases showed an eosinophilic alveolitis as well. One single anti-centromere antibody positive case with neutrophil alveolitis was also identified (Table III). From the 4 cases with early scleroderma (disease duration ≥ 2 years), 2 had mixed alveolitis, and one patient exhibited a lymphocyte alveolitis (Table II, III). Decreased diffusion capacity was found in all cases with early scleroderma (Table II).

Apart from one patient with EUCTD and 2 cases with SSc we have some follow-up data on clinical findings, chest X-ray, and lung function parameters. In UCTD during the follow-up of 20.9 ± 9.2 months no significant changes were detected on the chest x-ray and furthermore, lung function parameters remained stable as well (data not shown). During the follow-up, only one patient with EUCTD developed SLE; all the other cases remained clinically stable. This latter case with SLE did not show any significant changes in chest X-ray, and in her lung function parameters.

In EUCTD 6 patients with lymphocyte alveolitis received methylprednisolon therapy (initial dose of 28-32 mg/day, gradually tapered to 6-12 mg for 3-18 months) because of lymphocyte alveolitis. In these cases no other cytostatic/immunosuppressive therapy were administered. The lung function parameters of these patients did not show a progression during the follow up. Two patients required methylprednisolon treatment showed more than a 10 percent decline in diffusion capacity values during the follow up, and one patient ameliorated more than 10%.

No significant difference was detected in the lung function parameters, when these methylprednisolon treated cases were compared with the remaining 6 cases with EUCTD (data not shown). With regard to the patients with SSc, 5 patients received bolus cyclophosphamide therapy (600-1000 mg/month, 6-12 times) because of neutrophil alveolitis or declining lung function parameters during the follow-up (19.7 ± 9.2 months). These cases also received low dose methylprednisolon treatment (6-12 mg/day). Patients with cyclophosphamide therapy did not show any considerable change in their lung function parameters. Furthermore, we couldn’t detect any significant differences between the lung function parameters of these cases with cyclophosphamide therapy compared to the rest of the investigated cases with SSc.

**Discussion**

Subclinical pulmonary involvement, as assessed by non-invasive investigations and bronchoalveolar lavage, may be present in cases with very early connective tissue symptoms because an inflammatory process of the lower respiratory tract may appear prior to fibrosis (22, 36). Although increasing data are accumulating (1-9, 11-13, 37), the natural evolution of early undifferentiated CTD is not well characterized; therefore little is known about the frequency of the early lung involvement in early undifferentiated connective tissue disease (1,6).

Two major groups of alveolitis can be distinguished. Lymphocyte alveolitis is often detected in patients with extra-thoracic granulomatosis or with certain collagen vascular diseases. Neutrophil alveolitis seems to be the major finding in collagen vascular diseases, especially SSc and dermat-polymyositis. Therapeutic and prognostic aspects require specification of the type of alveolitis (lymphocytosis or granulocytosis or mixed forms) in patients with connective tissue disease. For this purpose, only BAL seems to be an appropriate method; the non-invasive diagnostic procedures cannot adequately predict the type of alveolitis (38). In contrast to neutrophil alveolitis, lymphocyte alveolitis seems to respond well to corticosteroid treatment (39).
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ber in SSC (Fig. 1) indicating a net cell influx, and furthermore we found neutrophil and/or lymphocyte alveolitis in the great majority of patients (Table III). DcSSc is characterised by extensive skin involvement and severe internal organ involvement (33). In our study, cases with dcSSc also showed more severe lung symptoms (Table II, III). Anti-topoisomerase antibodies are usually present in dcSSc, and anti-topoisomerase I antibody positive cases usually exhibit an extensive lung disease (43-45) which was also confirmed by our study because 5 of the 7 anti-topoisomerase positive patients exhibited alveolitis with increased granulocyte cell number (Table III).

It is noteworthy that 2 out of the 4 early scleroderma cases showed neutrophil type of alveolitis, and all of these patients had a decreased diffusion capacity. Although the number of investigated cases is low, these findings may suggest that BAL is a valuable method for the selection of those early cases who may need a more aggressive treatment. The appearance of significant lung symptoms is a bad prognostic sign in SSC (17), and furthermore, the combination of decreased DLCO, increased ESR and proteinuria apparently seems to cause the worst prognosis in SSC (46).

The anticientromere autoantibody (ACA) is present in the milder form of SSC, namely in lcSSc. Patients with lcSSc have a skin involvement limited to hands, face, feet, and forearm, subcutaneous calcinosis, telangiectasia and they may have a late incidence of pulmonary hypertension without any significant interstitial lung disease (33). In the lcSSc group, we have identified one case with ACA positivity and neutrophil alveolitis indicating that this is not a general rule, and a significant interstitial lung disease may appear in certain cases (Table III). In our previous HRCT studies, we indicated that even severe lung fibrosis may be occasionally present in the lcSSc subset (16).

Pulmonary involvement in SSC is characterised by an increased number of CD8+ T cells in BAL fluids (47, 48). Our results confirm this finding (Fig. 3). The tendency of an increased CD8+ T cell population was also observed in EUCTD without reaching the level of statistical significance.

Our preliminary follow up data demonstrated that the lung function parameters were stabilized (but not significantly improved) in the cyclophosphamide/corticosteroid treated patients. These findings are similar to a previous report (49). In accordance with other authors (50-52), we think that cyclophosphamide/corticosteroid therapy may be useful in early scleroderma cases with neutrophil alveolitis to stop the decline of lung function. Obviously further controlled studies with a larger number of investigated cases are required to test this hypothesis.

Our findings indicate that a subclinical alveolitis as assessed by BAL cell analysis can be present in patients with EUCTD. A net cell influx as a sign of alveolitis was detected among our cases of EUCTD, indicating that the lung interstitial inflammation may be a very early event in the disease process (Fig. 1). In contrast to SSC, in patients with EUCTD predominantly lymphocyte alveolitis can be observed (Fig. 2).

Follow up studies are required to identify those patients with EUCTD who are candidates for a later development of a clinically significant interstitial lung disease.

With regard to the normal values used in our study, they seem to be similar to those of previous investigators (25, 46, 47), in spite of the low number and younger age of our control group. We demonstrated that patients with early undifferentiated connective tissue disease also exhibit interstitial lung disease, therefore screening tests for the investigation of lung disease (chest X-ray, DLCO, spirometry) are required. Some further investigations (HRCT, BAL) may also be necessary in a subgroup of patients to prove the presence of alveolitis in these cases with EUCTD.

Further studies are required to clarify whether the early identification and treatment of the alveolitis in EUCTD has a significant impact on the outcome. Our preliminary follow up findings indicate that EUCTD patients with lymphocyte alveolitis probably do not tend to develop severe lung fibrosis.

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