Clinical and subclinical alveolitis in collagen vascular diseases: contribution of alpha_2-macroglobulin levels in BAL fluid


ABSTRACT: The probability that patients with collagen vascular diseases (CVD) will develop fibrosis is unpredictable. Since changes in bronchoalveolar lavage (BAL) cell data can be observed in CVD patients without evidence of lung involvement, we investigated whether the study of soluble components in BAL could help to distinguish CVD patients with lung involvement (n=15) from those without pulmonary disease (n=37). Our results demonstrate that the alveolitis observed in patients with overt lung involvement is associated with an increase of BAL alpha_2-macroglobulin (alpha_2-MA). In contrast, the BAL alpha_2-MA levels were found to be normal in CVD patients without evidence of pulmonary disease as well as in CVD patients with overt lung involvement treated with steroids. This was observed even in the presence of high neutrophil or lymphocyte counts in BAL. In conclusion, when neutrophils or lymphocytes accumulate in the lungs of CVD patients without evidence of lung damage, in the majority of patients this cell accumulation is not associated with an increase of BAL soluble components.


Chronic interstitial pulmonary disorder is a common complication of collagen vascular diseases (CVD) leading to an interstitial fibrosis similar to that described in idiopathic pulmonary fibrosis [1]. The frequency of pulmonary involvement in each of these disorders varies according to whether physiological, roentgenographic or histological criteria are used to document the lung disease. Clinical observations and bronchoalveolar lavage (BAL) data have suggested that alveolitis is an early manifestation of the disease and may appear prior to fibrosis. Since alveolitis probably mediates at least some of the changes of the alveolar walls that characterize the disease, evaluation of the alveolitis may be useful in staging the activity of the interstitial disease [1, 2]. Furthermore, recent studies have shown that subclinical inflammatory alveolitis might be present in the lower respiratory tract of patients with systemic disorders like CVD without clinical or roentgenological evidence of interstitial lung involvement [3-5]. In addition, the distribution of immune and inflammatory cells in BAL did not significantly differ between patients with CVD with or without pulmonary involvement. Thus, the relevance of the cellular abnormalities found in the bronchoalveolar lavage (BAL) of asymptomatic patients remains obscure [6].

In a previous report, we showed that alpha_2-macroglobulin (alpha_2-MA) is a sensitive parameter for the presence of an alveolitis in interstitial lung disease [7]. In the present study, we evaluate changes of BAL soluble components to discriminate between subclinical alveolitis from symptomatic pulmonary disease associated with CVD.

Materials and methods

Study population

Fifty two consecutive patients with classical criteria for CVD, free of steroid therapy, were prospectively investigated. Each patient in the study was evaluated for evidence of lung involvement. Such diagnosis was assessed through a history of cough or breathlessness or both; presence of bibasilar crackles on physical examination; posteroanterior and lateral chest roentgenograms showing diffuse reticulonodular infiltrates, interpreted without knowledge of BAL findings, pulmonary function tests demonstrating reduced lung volumes and diffusing capacity.

Progressive systemic sclerosis (PSS) (15 cases) was assessed on clinical features and skin histological findings according to the American Rheumatism Association preliminary criteria for the classification of systemic sclerosis [8]. Four patients presented a CREST syndrome (calcinosis, Raynaud's phenomenon, oesophageal dysfunction, sclerodactyly, and telangectasia). Seven patients with classic or definite rheumatoid arthritis (RA) were...
studied. Primary Sjögren's (PSj) syndrome was diagnosed in ten individuals by using the classical signs: xerophthalmia, xerostomia and/or chronic arthritis, a positive Schirmer test and characteristic pathological examination of minor salivary gland [9]. The mixed connective tissue disease (MCTD) group [10] included five patients distinguished from other disease by high levels of serum antibody to nuclear ribonucleoproteins. In four additional patients the diagnosis of dermatomyositis-polymyositis (DPM) was established on clinical data, serum concentrations of creatinine phosphokinase and aldolase and pathological findings on striated muscle biopsy. Finally, a diagnosis of systemic lupus erythematosus (SLE) was proposed for seven patients following the criteria set forth by the American Rheumatism Association [11].

Among the 52 patients, 37 patients (Group I) were free of any history of pulmonary disease, and of clinical, functional and thoracic roentgenographic abnormalities. Medications and smoking habits were obtained in all individuals. Patients with an occupational history relevant to lung disease or failing to exhibit established safety criteria for BAL were excluded from the study. No patient was studied at the time of an acute respiratory infection. Two of the 37 patients presenting a CVD free of lung involvement, were currently receiving various non-steroidal anti-inflammatory agents at comparable doses. In this group there were six patients who had received, or were currently receiving, gold salts therapy (one), or Plaquenil (five). The number of patients treated with these drugs was small and did not appear to influence the results.

Group II included 15 patients with overt lung involvement related to a CVD. Overt lung involvement was defined by the presence of chest X-ray abnormality and/or impairment of lung function tests.

The control group included 16 healthy, nonsmoking individuals with a normal chest X-ray and a normal pulmonary function. The mean age was 38 yrs; all were free of symptoms and signs of pulmonary disease.

Bronchoalveolar lavage

Informed consent was obtained from all patients. BAL was performed through a fibreoptic bronchoscope introduced transnasally into the lower respiratory tract after local anaesthesia with lidocaine. A total of 250 ml of sterile saline solution in aliquots of 5 x 50 ml was instilled in a subsegment of the right middle lobe and gently aspirated. The fluid was collected into sterile siliconized jugs and filtered through gauze to remove gross mucus. The first aliquot representative of the bronchial level was discarded and studies were performed on the following aliquots. The cells were separated from the lavage fluid by low speed centrifugation (800 g for 10 min). The volume of recovered fluid was noted and total cell count expressed as the number of cells per ml of recovered fluid using a Coulter cell counter. Cell differential analysis was performed from cytccentrifuge preparations (Cytospin).

Protein assays

An immunoradiometric assay (IRMA) was used for measurement of the proteins in BAL. This assay previously described in detail, provides a sensitivity in the range of ng·ml<sup>-1</sup> and was performed on non-concentrated BAL fluid [12]. BAL samples were diluted in 20% goat serum in phosphate buffered saline, pH 7.4. Serum levels of the different proteins were determined by immunonephelometry. Results are expressed in coefficient of excretion relative to albumin (RCE).

\[
\text{RCE} = \frac{(\text{protein}) \text{ BAL}}{(\text{protein}) \text{ serum}} \times \frac{(\text{albumin}) \text{ BAL}}{(\text{albumin}) \text{ serum}}
\]

Pulmonary function tests

Forced vital capacity (FVC) was determined using a Jaeger spirometer. Functional residual capacity (FRC) was measured with the helium dilution method. Total lung capacity (TLC) was calculated from residual volume plus inspiratory vital capacity (RV)+(IVC). The carbon monoxide diffusing capacity (DL co) was obtained by a single-breath method and corrected for alveolar volume and haemoglobin. Data were expressed as percentage of the predicted values. Lung function was considered as abnormal when lung volumes were lower than 80% of predicted values and/or when diffusing capacity was lower than 75% of that predicted.

Statistical analysis

Comparisons between patient groups were made using an unpaired t-test; values of p<0.05 were considered significant.

Results

Clinical data and results of pulmonary function tests are given in table 1. BAL total cell counts and differentials in each group are shown in table 2. The mean volumes recovered from the different patient groups were not significantly different (data not shown). Total cell recovery (mean±sd: 29.4±18.9 x 10<sup>3</sup>·ml<sup>-1</sup>) from the BAL of Group II was significantly higher than from the normal individuals (mean±sd: 18.2±5.3 x 10<sup>3</sup>·ml<sup>-1</sup>) and Group I (mean±sd: 14.6±12.7 x 10<sup>3</sup>·ml<sup>-1</sup>) (p<0.01) for the two comparisons. Smoking habits could not explain this difference because the number of smokers was small and was evenly distributed between the groups. An abnormal differential cell count (>mean±2sd: lymphocytes >2.6 x 10<sup>3</sup>·ml<sup>-1</sup>) and/or neutrophils >0.48 x 10<sup>3</sup>·ml<sup>-1</sup>) was noted in 20 of the 37 patients (54%) in group I. Seven showed a lymphocyte alveolitis (2 PSj; 4 PSS; 1 RA), 13 exhibited a neutrophil alveolitis (4 PSj; 6 PSS; 2 RA; 1 MCTD) and 4 cases had a mixed (neutrophil-lymphocyte) alveolitis (2 PSS; 1 PSj; 1 RA) (fig. 1). In group II, all patients except four, presented an increased neutrophil count in BAL. In this latter group, ten patients had a BAL lymphocytosis associated with the neutrophil alveolitis.
Table 1. — Clinical parameters of the patients with collagen vascular disease studied by bronchoalveolar lavage

<table>
<thead>
<tr>
<th>Connective tissue diseases free of pulmonary involvement</th>
<th>Number</th>
<th>Mean age yrs</th>
<th>Sex ratio F/M</th>
<th>Smokers</th>
<th>Abnormal chest X-ray</th>
<th>TLC %</th>
<th>FVC %</th>
<th>TLCO %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Sjögren</td>
<td>9</td>
<td>42</td>
<td>9/0</td>
<td>0</td>
<td>0</td>
<td>104</td>
<td>103</td>
<td>100</td>
</tr>
<tr>
<td>Progressive systemic sclerosis</td>
<td>10</td>
<td>47</td>
<td>7/3</td>
<td>2</td>
<td>0</td>
<td>104</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td>CREST syndrome</td>
<td>1</td>
<td>55</td>
<td>0/1</td>
<td>0</td>
<td>0</td>
<td>101</td>
<td>99</td>
<td>83</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>6</td>
<td>49</td>
<td>5/1</td>
<td>1</td>
<td>0</td>
<td>108</td>
<td>95</td>
<td>121</td>
</tr>
<tr>
<td>Dermatomyositis</td>
<td>3</td>
<td>57</td>
<td>2/1</td>
<td>0</td>
<td>0</td>
<td>109</td>
<td>108</td>
<td>131</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>4</td>
<td>34</td>
<td>4/0</td>
<td>0</td>
<td>0</td>
<td>101</td>
<td>97</td>
<td>93</td>
</tr>
<tr>
<td>Mixed connective tissue disease</td>
<td>4</td>
<td>48</td>
<td>4/0</td>
<td>0</td>
<td>0</td>
<td>112</td>
<td>106</td>
<td>122</td>
</tr>
<tr>
<td>Pulmonary fibrosis untreated in association with a connective tissue disease</td>
<td>15</td>
<td>51</td>
<td>11/4</td>
<td>0</td>
<td>12</td>
<td>86</td>
<td>80</td>
<td>58</td>
</tr>
</tbody>
</table>

Values quoted for TLC, FVC and TLCO are mean and (SD). TLC: total lung capacity; FVC: forced vital capacity; TLCO: carbon monoxide diffusing capacity; CREST: calcinosis, Raynaud's phenomenon, oesophageal dysfunction, sclerodactyl and telangiectasia.

Table 2. — BAL data of the patients with collagen vascular disease and the normal group

<table>
<thead>
<tr>
<th>Connective tissue diseases free of pulmonary involvement</th>
<th>BAL total cell count $10^6$ml$^{-1}$</th>
<th>Macrophages %</th>
<th>Lymphocytes %</th>
<th>Neutrophils %</th>
<th>Eosinophils %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Sjögren</td>
<td>12.5</td>
<td>71.7</td>
<td>24</td>
<td>3.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Progressive systemic sclerosis</td>
<td>22.9</td>
<td>84.1</td>
<td>12</td>
<td>3.2</td>
<td>0.7</td>
</tr>
<tr>
<td>CREST syndrome</td>
<td>10</td>
<td>68</td>
<td>24</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>9.6</td>
<td>83.1</td>
<td>13.6</td>
<td>3.3</td>
<td>0</td>
</tr>
<tr>
<td>Dermatomyositis</td>
<td>7.3</td>
<td>91.7</td>
<td>7.6</td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>6.4</td>
<td>80.8</td>
<td>16.2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Mixed connective tissue disease</td>
<td>12.1</td>
<td>81.3</td>
<td>15.2</td>
<td>3.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Values quoted are mean and (SD). BAL: bronchoalveolar lavage; CREST: see legend to table 1.
Lymphocyte alveolar count

10^4 \cdot m^{-1}\cdot l^{-1}

\begin{align*}
&39.4 \\
&16.1 \\
&14.6
\end{align*}

Neutrophil alveolar count

10^4 \cdot m^{-1}\cdot l^{-1}

\begin{align*}
&34
\end{align*}

Fig. 1. - BAL neutrophil and lymphocyte counts (in number of cells x 10^4·ml^{-1} of recovered BAL) of patients without lung disease (column I) and with lung fibrosis (column II). ●: non-smokers; ○: smokers; dotted areas represent mean+2 SD of normal values; BAL: bronchoalveolar lavage.

Antiproteases in BAL

The results of BAL alpha_2-macroglobulin content, expressed as RCE are shown in figures 2. Group II had a significant increase of alpha_2-MA (mean value 0.48) compared to normals and group I (mean value 0.05 and 0.13, respectively) (p<0.01). However, six patients in group I showed an abnormal value (>mean+2 SD) or alpha_2-MA RCE although no clinical, radiological or functional evidence of lung damage was present. Among these six patients, two (1 PSj and 1 PSS) exhibited a high neutrophil count (0.8 x 10^4·ml^{-1} and 0.5 x 10^4·ml^{-1}, respectively), one PSj had an increased alveolar lymphocyte count (7.1 x 10^4·ml^{-1}) and three patients (1 DPM; 1 MCTD, 1 RA) showed normal cellular data; the patient with RA was under gold therapy when the lavage was performed.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2}
\caption{BAL RCE for, from left to right, alpha_2-MA, alpha_1-PI, IgG, IgM and IgA in CVD patients without lung disease (I) and with lung fibrosis (II). Values of the healthy group are shown in column III. BAL: bronchoalveolar lavage; RCE: coefficient of excretion relative to albumin; CVD: collagen vascular disease.}
\end{figure}
The RCE mean value of alpha-protease inhibitor (alpha-PI) in BAL from the normal group was 1.23. The mean RCE value was not significantly different in the Group I (0.94) but was significantly higher in group II (2.2; p<0.05). However, there was a broad overlap of values between the groups I, II and controls (fig. 2.).

Levels of IgG in lavage from controls (mean 0.74) were not significantly different from that in group I (mean 0.94) (fig. 2). The BAL IgG RCE (mean 1.92) in group II was significantly increased when compared to group I and controls (p<0.05) and was not dependent upon the smoking status of the patients. Group II also had a significantly greater mean BAL IgM level compared to normals and group I (mean 0.66, 0.08 and 0.12, respectively). No significant difference existed between the IgM values in group I and controls (p=0.05). IgM values in group I overlapped with values in group II more than values of alpha,-MA. It must be emphasized that with the immunoradiometric assay, IgM was detected in all samples. IgA in group II (mean 1.60) largely overlapped with the other groups and no significant difference was observed. We found no significant correlation between the RCE of different proteins or between RCE and the numbers of inflammatory cells in BAL.

**The effect of steroid therapy**

In the CVD group exhibiting interstitial lung fibrosis, six patients were evaluated under steroid treatment (mean dose of methylprednisolone 32 mg daily) (four patients at three months and two patients at six months of treatment) (fig. 3).

All patients showed initial high BAL neutrophilia and lowered their BAL neutrophil count under steroid. A concomitant decrease of BAL lymphocytosis was noted when pretreatment values were abnormal (three patients). The large size proteins, alpha,-MA and IgM decreased in BAL, as did the BAL neutrophils, but did not all return to the normal range. IgG values expressed only a trend to lowering. Dramatic drop and normalization of the alpha,-PI values was observed under steroid. By contrast functional data are poorly influenced under treatment since only one out of six patients significantly improved FVC% and TLco%.

**Discussion**

In the present study, we investigated the alveolitis in both established interstitial lung diseases and in patients presenting with CVD but free of any clinical evidence of pulmonary involvement, through the analysis of the soluble components of the BAL compared to cellular data.

Antiproteases and immunoglobulins, potentially reflecting the processes regulating the alveolitis were measured with a sensitive assay. Alpha,-MA represents a unique group of antiproteases of central importance in processing proteases in blood and probably in tissues, through the binding to all classes of proteases [13]. We observed that the local inflammatory process is well reflected in patients with overt lung disease by a striking increase of BAL alpha,-MA when compared to normal values. It is unlikely that the elevated alpha,-MA is secondary only to transudation resulting from the inflammatory process since our data are expressed in coefficient of excretion relative to albumin (RCE) correcting for both variable dilution of BAL and serum concentration of proteins. This finding is consistent with a local pulmonary production of alpha,-MA, perhaps related to a synthesis by alveolar macrophages [14]. The other proteins IgG, IgA and alpha,-PI are also elevated in the BAL from patients with overt lung involvement but a large overlap of the values is observed between the different groups. This further supports the advantage provided by the sensitive changes of BAL alpha,-MA since this large molecule is present in minimal amounts in alveolar fluid collected from normal individuals. The lavage fluid recovered from patients with overt lung involvement also contained increased amounts of IgM as previously reported in BAL from hypersensitivty pneumonitis patients. Although the
molecular weight of IgM is similar to alpha-MA, the overlap between controls and patients with lung fibrosis was greater than for alpha-MA. The lower values of IgM were observed in two cases of CREST syndrome where the alveolitis was reported to be less intense [15, 16]. Previous studies have demonstrated that in normal subjects cigarette smoking can significantly influence the RCE value of IgG in BAL but not RCE values of the other biochemical parameters. Therefore, the results observed for alpha-MA and IgM in the present study do not appear to be related to the smoking status of the patient [7, 17].

Follow-up studies demonstrated that alpha-MA levels in the alveolar fluid reflect changes in the intensity of the inflammatory process. Therefore, analysis of the BAL protein content, expressed as RCE, provides an additional parameter to evaluate the lung inflammatory and immune events during the course of interstitial disorders. Whether the immunoglobulin and antiprotease levels in BAL can be related to the fibrotic process requires a longitudinal study. However, the present data suggest that, when patients with involved lung are treated by steroid, the alveolitis as evaluated by soluble components tends to diminish. Whether these changes are also associated with a steroid-induced clinical responsiveness remains less clear although recent data suggest that monitoring serial lavage cell counts can reflect clinical progress in fibrosing alveolitis [18–21]. This contrasts with a previous study showing that steroid treatment induces no change in the alveolitis of interstitial lung disease associated with CVD as assessed by Gallium-scan and by the percentage of BAL neutrophils and eosinophils [22].

Lymphocytes and/ or neutrophils can accumulate in the lower respiratory tract of patients without evidence of lung damage, a feature found in half of our patients. In contrast, we did not find a significant increase of the soluble components of the BAL in the majority of our patients. In general, alpha-MA levels were no different from the controls and were lower than values measured in patients with obvious lung involvement, supporting the theory that BAL alpha-MA correlates better with the presence of overt lung disease than the BAL neutrophil or lymphocyte subclinical alveolitis. These data may suggest that accumulation of immune and inflammatory cells in the lung of CVD patients could precede an active alveolar inflammatory process associated with the increased local production of antiproteases and immunoglobulins [3, 4, 23, 24].

In conclusion, patients with overt lung involvement are likely to have increased levels of soluble components, in particular a high molecular weight protein such as alpha-MA. The levels of BAL proteins are reversible with steroids although corresponding changes in pulmonary function tests do not occur. Finally, some CVD patients (six in our series) without overt disease may present with an increase of BAL proteins. Longitudinal studies will be required to determine whether the presence of these abnormalities will predict subsequent deterioration and evolution to a fibrotic disorder.

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References


**Alvéolites cliniques et subcliniques dans les collagenoses. La contribution des taux d'alpha-2-macroglobuline dans le lavage broncho-alvéolaire.** J.B. Martinot, B. Wallaert, P.Y. Hatron, C. Francis, C. Voisin, Y. Sibille.

RÉSUMÉ: Il est impossible de prédire si les patients atteints de collagenosé (CVD) vont développer ou non une fibrose. Puisque des modifications cellulaires du lavage broncho-alvéolaire peuvent être observées chez les patients atteints de CVD sans aucune atteinte pulmonaire, nous avons examiné si l'étude des composants solubles du lavage broncho-alvéolaire pourrait aider à discriminer les patients avec atteinte pulmonaire (n=15) de ceux sans atteinte pulmonaire (n=37). Nos résultats démontrent que l'alvéolite observée chez les patients qui ont une atteinte pulmonaire manifeste est associée à une augmentation de l'alpha-2-macroglobuline du lavage broncho-alvéolaire. Par contre, les taux d'alpha-2-macroglobuline du lavage broncho-alvéolaire sont normaux chez les patients souffrant de CVD sans atteinte pulmonaire évidente, aussi bien que chez les patients souffrant de CVD avec atteinte pulmonaire manifeste mais traités par les stéroïdes. Ces faits ont été observés, même en présence de décomptes très augmentés des neutrophiles ou des lymphocytes dans le lavage. En conclusion, lorsque les neutrophiles ou les lymphocytes s'accumulent dans les poumons de patients atteints de CVD sans signe évident d'atteinte pulmonaire, chez la majorité des patients cette augmentation de cellularité n'est pas associée à une augmentation des composants solubles du lavage. *Eur Respir J.*, 1989, 2, 437-443.