Original Article

Latent Pulmonary Inflammation in Patients With Systemic Sclerosis

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A B S T R A C T

Background: Induced sputum is a non-invasive method for studying pulmonary inflammation.
Objectives: To assess pulmonary inflammation by analysis of induced sputum specimens in patients with systemic sclerosis and lung involvement, and to determine whether there is a correlation with the pulmonary function alterations in these patients.
Methods: Twenty-five patients with systemic sclerosis were included (20 women). Patients were divided into 3 groups according to the type of lung involvement: group 1, diffuse interstitial lung disease (n=10); group 2, those with pulmonary arterial hypertension (n=7), and group 3, patients with systemic sclerosis without lung involvement (n=8). All patients underwent a complete lung function study. Induced sputum samples were obtained and differential cell count was performed by optic microscopy.
Results: The mean percentage of sputum neutrophils was 85%, 71%, and 75% for groups 1, 2, and 3, respectively. A significant negative correlation between sputum total cell count and DLCO was seen in group 1 and group 3 (r=−0.733, P=0.016; and r=−0.893, P=0.007, respectively). This negative correlation was not observed in group 2.
Conclusions: Pulmonary inflammation was present in all patients with systemic sclerosis included in the study, regardless of the presence of documented signs of pulmonary involvement. This finding suggests that induced sputum could be helpful for detecting early abnormalities indicative of subclinical pulmonary involvement in patients with systemic sclerosis.

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Inflamación pulmonar latente en pacientes con esclerosis sistémica

R E S U M E N

Antecedentes: El esputo inducido es un método no invasivo para estudiar la inflamación pulmonar.
Objetivos: Estudiar la inflamación pulmonar mediante el análisis de muestras de esputo inducido en pacientes con esclerosis sistémica y afección pulmonar, y determinar si existe correlación con las alteraciones de la función pulmonar observadas en estos pacientes.
Métodos: Se incluyeron 25 pacientes con esclerosis sistémica (20 mujeres). Los pacientes fueron clasificados en 3 grupos, considerando el tipo de afectación pulmonar: grupo 1, enfermedad pulmonar intersticial difusa (n=10); grupo 2, hipertensión arterial pulmonar (n=7); y grupo 3, pacientes con esclerosis sistémica sin afectación pulmonar (n=8). A todos los pacientes se les realizó un estudio completo de función pulmonar y se obtuvieron muestras de esputo inducido. El recuento celular diferencial en las muestras de esputo se realizó mediante microscopy óptica.
Resultados: El porcentaje medio de neutrófilos en esputo inducido fue del 85, del 71 y del 75% para los grupos 1, 2 y 3, respectivamente. Se observó una correlación negativa significativa entre el recuento celular total en esputo inducido y la DLCO en los grupos 1 y 3 (r=−0.733, P=0.016; y r=−0.893, P=0.007, respectivamente). Esta correlación negativa no se observó en el grupo 2.

Palabras clave: Esputo inducido
Neutrófilos
Enfermedad pulmonar intersticial difusa
Hipertensión arterial pulmonar

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Introducción

La prevalencia de fibrosis pulmonar en esclerosis sistémica (SSc) varía entre 25% y 90%, dependiendo del método utilizado para detectar fibrosis intersticial pulmonar (DILD).1 La fibrosis pulmonar se asocia con mayor mortalidad en pacientes con SSc-2,4 y es actualmente la causa principal de muerte en esta población.2,7 Por lo tanto, el diagnóstico temprano de la afectación pulmonar es esencial para evitar la progresión de la enfermedad.

El primer hallazgo de la función pulmonar que se mantiene en SSc es alveolitis. Esta fibrosis alveolitis puede ser detectada mediante pruebas de función pulmonar y tomografía computarizada (HRCT).5,8-10 La función pulmonar es el único examen que se puede utilizar para determinar si la enfermedad es grave lo suficiente para justificar el tratamiento inmediato. La HRCT es el examen más sensible y específico para identificar la presencia de fibrosis pulmonar en SSc, aunque las maniobras ventilatorias pueden ser insuficientes para confirmar el hallazgo en el HRCT.10,11

El lavado broncoalveolar (BAL) de pacientes con SSc y DILD muestra altos conteos de neutrófilos, eosinófilos e histiocitos.12,13 Sin embargo, el BAL no es suficiente evidencia para recomendar el BAL en el tratamiento del paciente. El lavado broncoalveolar es un método invasivo, que se utiliza en el manejo de la inflación de la enfermedad.

El estudio de la función pulmonar durante el tratamiento es una válida técnica para determinar la evolución de la enfermedad.14 Sin embargo, el lavado broncoalveolar es un método invasivo y no se utiliza para determinar la evolución de la enfermedad.

La metodología de este estudio fue el estudio de la función pulmonar en pacientes con SSc. El estudio de la función pulmonar fue realizado en el Hospital Regional de Mérida, España.

Materiales y Métodos

Estudio de la función pulmonar.

El estudio de la función pulmonar se realizó en el Hospital Regional de Mérida, España. El estudio incluyó a 25 pacientes con SSc, quienes fueron consecutivamente evaluados en el Hospital Regional de Mérida, España. El estudio fue aprobado por el Comité de Ética del Hospital Regional de Mérida, España.

El estudio se realizó con el equipo MasterLab (Jaeger, Germany) y se siguieron las recomendaciones de la European Pulmonary Society and American Thoracic Society (ERS y ATS).24,25 El volumen pulmonar se midió mediante plethysmografía y el DLCO (difusión de monóxido de carbono) se midió mediante una técnica de difusión de monóxido de carbono.

El patrón restrictivo ventilatorio fue definido como una capacidad total de los pulmones (TLC) <80% del valor esperado.28 El patrón obstructivo ventilatorio fue definido como un volumen expiratorio forzado en 1 s (FEV1) > FVC ratio >70%, con FEV1 <80% del valor esperado.28 El patrón combinado de la función ventilatoria fue definido como un patrón restrictivo obstructivo.

El DLCO se midió en todos los pacientes. El DLCO se midió en todos los pacientes. El DLCO se midió en todos los pacientes.

Gases sanguíneos.

El estudio de gases sanguíneos fue realizado en el Hospital Regional de Mérida, España. El estudio de gases sanguíneos fue realizado en el Hospital Regional de Mérida, España. El estudio de gases sanguíneos fue realizado en el Hospital Regional de Mérida, España.
Obtaining and Processing the Induced Sputum Samples

The induction and the processing of the sputum were done following the method described by Pizzichini et al., making the patient inhale increasing concentrations of hypertonic saline solution (3%, 4%, and 5%). The sputum samples were analyzed within 2 h after having been obtained.

For the processing of the sputum samples, we selected mucus plugs, which were deposited in an Eppendorf tube and weighed. The sample was treated with a volume of solution of DTT (dithiothreitol) equivalent to 4 times for every mg of sputum obtained; it was agitated during 10 min for homogenization. Later, PBS was added at a volume equivalent to 4 times each mg of sputum, and the sample was agitated for 5 min more. A small portion (15 µl) was used for the total leukocytes count (in a Neubauer chamber) and to determine the cell viability with the trypan blue exclusion method. The rest of the sample was centrifuged and we opted to separate the supernatant from the cellular sediment. With the sediment, we obtained preparations that were stained with May–Grünwald–Giemsa for the differential count, counting 500 non-squamous cells and expressing the result as a percentage of the non-squamous cell total present in the samples. In all the sputum samples, we determined the quantitative cellular content of total cells expressed as cells/ml.

Statistical Analysis

The differences between the groups studied were analyzed using the Kruskal–Wallis test. A P value <.05 was considered significant. In order to evaluate correlations between the cell subtypes in IS and the lung function parameters, Spearman's correlation coefficient was used. In order to carry out the different statistical analyses, the SPSS program (version 13.0) for Windows (SPSS Inc., Chicago, IL) was used.

Results

Demographic Data and Baseline Characteristics

Table 1 shows the demographic data and the baseline characteristics of the study population.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic Variables and Baseline Characteristics of the Patients Included in the Study.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>Total</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>Sex, F:M</td>
<td>20:5</td>
</tr>
<tr>
<td>Age, yearsb</td>
<td>58.52 (14.80)</td>
</tr>
<tr>
<td>[24–82]</td>
<td>[24–82]</td>
</tr>
<tr>
<td>Smoker/non-smoker</td>
<td>2/23</td>
</tr>
<tr>
<td>Disease onset, SSc; SScb</td>
<td>21:4</td>
</tr>
<tr>
<td>[0.7–57]</td>
<td>[0.7–57]</td>
</tr>
<tr>
<td>Treatmenta</td>
<td>7 (28)</td>
</tr>
<tr>
<td>ANAb</td>
<td>25 (100)</td>
</tr>
<tr>
<td>ACAb</td>
<td>14 (56)</td>
</tr>
<tr>
<td>Scl-70a</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Ground glassa</td>
<td>9 (36)</td>
</tr>
<tr>
<td>Reticular patterna</td>
<td>6 (24)</td>
</tr>
</tbody>
</table>

ACA, anti-centromere antibodies; ANA, antinuclear antibodies; DILD, diffuse interstitial lung disease; SSc, diffuse systemic sclerosis; SScb, limited systemic sclerosis; PAH, pulmonary arterial hypertension; NPA, no pulmonary affection.

a Data expressed as n (%).
b Data expressed as mean (SD) [range].

Table 2

<table>
<thead>
<tr>
<th>Values of the Lung Function Parameters Studied.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung Function</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>FVC</td>
</tr>
<tr>
<td>FEV1 (% FEV1/FVC)</td>
</tr>
<tr>
<td>TCL</td>
</tr>
<tr>
<td>RV</td>
</tr>
<tr>
<td>DLCO</td>
</tr>
<tr>
<td>KCO</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>pO2, mm Hg</td>
</tr>
<tr>
<td>pCO2, mm Hg</td>
</tr>
<tr>
<td>SaO2, %</td>
</tr>
<tr>
<td>6MWT, m meters</td>
</tr>
</tbody>
</table>

DLC0, carbon monoxide diffusing capacity; DILD, diffuse interstitial lung disease; FEV1, forced expiratory volume in 1 s; FEV1/FVC ratio expressed as percentage; FVC, forced vital capacity; PAH, pulmonary arterial hypertension; KCO, DLCO/alveolar volume; NS: not significant; 6MWT: 6-min walk test; RV: residual volume; NPA, no pulmonary affection; TCL: total lung capacity.

a Data expressed as means (range).
b Percentage of predicted value.
3 patients of group 1, 2 patients from group 2 and 3 patients from group 3. In these last three patients, the lower values found in the 6MWT were due to osteomuscular affection. PO₂ was <80 mm Hg in 1 patient from group 2 and in one patient from group 3.

**Induced Sputum**

In all patients, the induction of sputum was well-tolerated and no adverse effects were observed. The samples analyzed presented an average percentage of viability (range) of 77% (60–96), 81% (73–87), and 80% (64–94) for groups 1, 2 and 3, respectively. The total and differential counts (%) in the IS of the patients studied are shown in Figs. 1 and 2, respectively. Differences were found in the total cell count between the three groups studied, although they did not reach statistical significance (Fig. 1). A negative correlation was found between the total number of cells in IS and DLCO in the patients with DILD and NPA (r=−0.733, P=.016; and r=−0.893, P=.007, respectively). This negative correlation was not observed in the group of patients with PAH. The mean percentages of neutrophils in IS were 85%, 71%, and 75% for groups 1, 2, and 3, respectively (Fig. 2). No significant differences were found in the differential count among the three groups studied.

**Discussion**

The present study evaluates the cellular profile and the clinical utility of the analysis of IS in patients with SSc, with and without lung affection. Likewise, it researches whether the findings obtained in the analysis of IS correlate with the pulmonary alterations found in this pathology. The most relevant observation of the study is the high percentage of neutrophils found in the sputum of patients with SSc, regardless of whether they have pulmonary affection or not, and the negative correlation between the total number of cells in IS and DLCO in patients with DILD and NPA.

BAL has been used for the early diagnosis of lung affection in SSc. However, it is an invasive technique that is not adequate for follow-up studies over the course of the pathology. One alternative is the cytologic analyses of IS, as it is a non-invasive method that

**Lung Function Studies**

Table 2 summarizes the lung function data of the patients studied. Significant differences were found in the FVC values among the three study groups. The FEV₁ values were also significantly different between group 1 and groups 2 and 3 (P=.032 and P=.009, respectively). In addition, significant differences were obtained in the TLC values between group 1 and groups 2 and 3 (P=.015 and P=.010, respectively).

Eight patients from group 1 showed ventilatory alterations: 7 patients presented restrictive ventilatory disorder and 1 patient showed an obstructive disorder. In the remaining 17 patients (2 from group 1 and all the patients from groups 2 and 3), spirometry and the plethysmography results were normal. The DLCO values were less than 80% of the predicted value in all the patients except in 3 (1 from group 2, and 2 from group 3), who presented normal values. The total distance walked during the 6MWT was <300 m in
can detect pulmonary inflammation. Nevertheless, in spite of the fact that different criteria have been used to define the BAL pattern in SSc, including the presence of eosinophilia, neutrophilia and/or lymphocytosis, these criteria have not been defined for IS samples.

In the present study, a high percentage of neutrophils has been found in patients with SSc compared with healthy individuals. These results are similar to those obtained in two recent studies by Damjanov et al. and Vatrella et al., who observed a significant increase of neutrophils and a low percentage of macrophages in the IS of patients with SSc and with lung affection. In the study by Vilmaz et al., however, the authors find a high percentage of macrophages and lymphocytes in the IS and a reduction in the percentage of neutrophils in patients with SSc. Likewise, these authors find that there is a correlation between the cell profile in IS and BAL in the patients studied. In this sense, there is a disparity between the data obtained in different studies, and Damjanov et al. and Fireman et al. observe that the cell profile in BAL does not correlate with that obtained in IS in the patients with SSc, concluding that it is not possible to make comparisons between both types of samples. This disparity between the data obtained in different studies could be due to the fact that BAL provides information about the distal airway and the alveolar spaces, which are rich in macrophages, while the IS is indicative of the upper and middle airways, presenting abundant neutrophils.

In the present study, the high percentage of neutrophils found in the group of patients that did not present evidence of lung affection could suggest the hypothesis that perhaps there is a sub-clinical pulmonary inflammatory process in SSc. In fact, there is evidence that, indeed, in patients with SSc there is subclinical inflammation. Along these lines, Ferri et al. found characteristic of DILD in autopsies of SSc patients. Nevertheless, clinically significant DILD occurs in less than 50% of patients with SSc. In addition, several authors have observed that studies in BAL generally present anomalies in patients with SSc without lung affection. The clinical significance of this latent subclinical inflammation, both in BAL as well as in IS, is not known. With regards to the evolution of the cellularity in BAL, recent studies have demonstrated that it does not predict the subsequent evolution of the disease, nor the response to the immunosuppressant treatment. Without a doubt, the pulmonary inflammation is what initiates and maintains the fibrotic process; therefore, the treatment with immunosuppressants is indicated in patients with DILD. However, both the decision to initiate treatment as well as the evaluation of the response to it is not done depending on the inflammation detected in the BAL, but instead on the respiratory function repercussions and/or the extension of DILD detected in HRCT. Thus, some authors have suggested that, in patients with latent inflammation, periodical spirometries and radiological exams should be done and immunosuppressant therapy should be considered when alterations are observed in these parameters.

In addition to the latent inflammation found in the IS samples of these patients, the majority of them (88%) presented an early alteration in DLCO. Despite the fact that the patients with DILD and PAH present a decline in DLCO, this should be less frequent in patients with SSc without pulmonary affection. According to several authors, it is possible to see decreases in DLCO in 2–40% of patients without lung alteration, although the significance of this decrease is also debated regarding its use for predicting the evolution of the disease toward DILD or PAH. In the largest series published to date, which includes 152 patients with diffusion alterations and without lung affection, 27% of the cases develop mild DILD during follow-up, with FVC values between 70% and 80% predicted, and 7% develop PAH. In this present series, a lower diffusion was observed in all the patients with DILD, in all the patients with PAH except in one, and in 6 of the 8 patients in the NPA group. In this latter group of patients, there are several factors that could explain this decrease in DLCO. Tobacco is a potential cause, but only one of the patients with SSc and NPA was a smoker. Anemia may also contribute to the decline in DLCO, but none of the patients in this group showed abnormal hemoglobin values. Although it is much less probable, we cannot exclude a possible increase in endogenous CO, which has been observed in conditions that implicate a higher metabolism, such as in SSc, or in pulmonary microcirculation alterations, also present in this pathology. Considering the latent lung inflammation observed in the group of patients without lung affection, it is possible that the decline in DLCO is related with this factor. The inverse correlation found between the number of total cells in the IS samples and the DLCO values support this idea. This hypothesis is reinforced by the fact that this correlation was also found in the group of patients with DILD, but not in the patients with PAH, who also showed less (although not significantly) total cells. Some authors have suggested that a decline in diffusion below 50% predicted or an FVC/DLCO ratio greater than 1.4 could indicate progression toward PAH and a poor prognosis.

In the present study, none of the patients in the NPA group had a decrease in diffusion below 50% predicted. This fact, together with the cell levels in IS, could suggest that the patients with low DILD and an increase in neutrophils in IS present a greater risk for progression toward DILD rather than toward vascular affection.

The study presents some limitations, such as the limited number of patients, which make future studies necessary with larger sample sizes in order to confirm these hypotheses. In addition, there is no control group. However, several studies have researched the normal cell levels in IS, therefore these results can be used to make comparisons, as has been done in this present study. Another limitation is not having a follow-up of the patients with SSc without pulmonary affection, which makes the relationship between the increase of neutrophils in IS and the decrease in DLCO questionable.

In conclusion, in the present study we have observed lung inflammation in all the patients with SSc, regardless of whether there was a presence of pulmonary affection or not. This finding suggests that IS could be useful for the early detection of a sub-clinical pulmonary affection in patients with SSc. In addition, the analysis of IS could be useful as a tool for differentiating between SSc patients with low levels of DLCO and neutrophilic inflammation in IS whose progress could be toward DILD, and those with a normal differential cell count in IS whose progression could be toward PAH. Future follow-up studies are necessary with a greater number of patients in order to confirm the findings of the present study and to more precisely define the role of IS in this pathology.

Conflict of Interest

The authors declare no conflict of interest.

References