Cyclophosphamide reduces neutrophilic alveolitis in patients with scleroderma lung disease: a retrospective analysis of serial bronchoalveolar lavage investigations

O Kowal-Bielecka, K Kowal, J Rojewska, A Bodzenta-Lukaszyk, Z Siergiejko, M Sierakowska, S Sierakowski

PATIENTS AND METHODS

Patients
We included 21 non-smoking patients in the study, who received intravenous CYC owing to SLD, and evaluated their pulmonary disease before and after 6 months’ treatment. All patients fulfilled the American College of Rheumatology preliminary classification criteria for SSc. Evaluation of the pulmonary disease included estimation of exercise capacity (according to New York Heart Association (NYHA) classification), high resolution computed tomography (HRCT) of the lungs, lung function tests, and BALF analysis. Based on HRCT of the lungs, patients were classified as having predominantly ground-glass opacification, predominantly honeycombing, or mixed pattern. The extent of the disease was evaluated as the percentage of abnormal lungs, as described elsewhere.

The patients had not received immunosuppressive or corticosteroid treatment for more than 5 years before CYC was started. Patients with evidence of respiratory infections were excluded.

In all patients antinuclear and anticentromere antibodies were evaluated using an indirect immunofluorescence test and anti-Scl-70 antibodies by means of an enzyme linked immunosorbent assay (ELISA).

Cyclophosphamide treatment
CYC was given based on the presence of SLD features on HRCT scans of the lungs, plus at least one of the following: significant (>10% of initial value) decrease in forced vital capacity (FVC) within the past 6 months or the presence of neutrophilic alveolitis (NA) as judged by cytological analysis of BALF. CYC was given intravenously, 1.0 g every 30 days for 6 consecutive months. In addition, patients received prednisone (<10 mg/day).

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Full blood cell count, urine analysis, and determination of the erythrocyte sedimentation rate (ESR) were carried out before the first infusion of CYC and repeated monthly during the treatment. C reactive protein (CRP) was measured before the first infusion and 1 month after the last (sixth) infusion of CYC.

Bronchoscopy with bronchoalveolar lavage

Bronchoalveolar lavage was performed with the informed consent of the patient as a part of the routine clinical evaluation of patients with SSc, as previously described.

Scleroderma interstitial lung disease (SLD) is the main cause of death in patients with systemic sclerosis (SSc). Although the pathophysiology of SLD is not clear, histological examination and analysis of bronchoalveolar lavage fluid (BALF) have shown the presence of inflammation in the lower respiratory tract of patients with SLD. Intraovenous cyclophosphamide stabilised or improved the patients’ functional status and lung function tests. The extent of the lungs affected remained unchanged, as assessed with HRCT of the lungs. Patients with SLD and neutrophilic alveolitis (NA) showed greater improvement than patients with normal levels of granulocytes in the bronchoalveolar lavage fluid (BALF). Significant reduction of neutrophils was also seen in the patients with SLD and NA, whereas no significant change was seen in the level of granulocytes in patients with SLD and an initially normal percentage of granulocytes.

Conclusions: Previous reports that patients with SLD with increased levels of granulocytes in BALF are more likely to benefit from treatment with intravenous cyclophosphamide are confirmed. Additionally, clinical improvement in this group of patients is accompanied by a significant decrease in the percentage of granulocytes in BALF.

Objectives: To determine whether cyclophosphamide is beneficial for patients with scleroderma lung disease (SLD).

Methods: The effect of 6 months’ treatment with intravenous cyclophosphamide on the functional capacity of patients, lung function tests, high resolution computed tomography of the lungs, and cytology of bronchoalveolar lavage was evaluated in 21 patients with SLD.

Results: The treatment was well tolerated and all patients completed 6 months’ treatment. Intravenous cyclophosphamide stabilised or improved the patients’ functional status and lung function tests. The extent of the lungs affected remained unchanged, as assessed with HRCT of the lungs. Patients with SLD and neutrophilic alveolitis (NA) showed greater improvement than patients with normal levels of granulocytes in the bronchoalveolar lavage fluid (BALF). Significant reduction of neutrophils was also seen in the patients with SLD and NA, whereas no significant change was seen in the level of granulocytes in patients with SLD and an initially normal percentage of granulocytes.

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Based on the inflammatory hypothesis of the pathogenesis of SLD, cyclophosphamide (CYC) was introduced for treatment of SLD. It has been shown to stabilise or improve lung function and reduce mortality in patients with SLD and alveolitis.

Although CYC is believed to exert its beneficial effects through inhibition of inflammation within the respiratory tract, clear evidence for this effect is still lacking. This study aimed at evaluating changes in BALF in patients treated with CYC owing to SLD.
Differential counts of BALFs were made from the cytospin samples after staining with May-Grunwald-Giemsa stain. Alveolitis was diagnosed when the percentage of neutrophils in the BALF was $>3.0\%$, or the percentage of eosinophils was $>2.5\%$, or both. These cut off values are in agreement with the American Thoracic Society guidelines as well as the reference values obtained in our laboratory.

Statistical analysis
Statistical analysis was performed using the Mann-Whitney U test, Fisher’s exact test, and Wilcoxon’s matched pairs test. Statistical analysis was performed using the Mann–Whitney U test; Fisher’s exact test; Wilcoxon’s matched pairs test. Values of $p<0.05$ were considered significant.

RESULTS
Patient characteristics
Table 1 presents the clinical characteristics of patients with SLD. At baseline, cytological analysis of BALF showed increased percentages of neutrophils in 13/21 (62%) patients. The percentage of eosinophils was increased in two patients only, and in each case this was accompanied by an increased percentage of neutrophils. The remaining 8/21 (38%) patients had normal percentages of granulocytes in their BALFs.

The sex, age, disease duration, disease subset, functional capacity, FVC, and serological status of the patients with NA did not differ significantly from those without NA. HRCT of the lungs disclosed honeycombing as the main pattern in only 1/13 patients with NA, and ground-glass opacification as the main pattern in 1/8 patients without NA only. In the group of patients with NA the mean CRP value was significantly higher than in those without NA, although there were no significant differences in the mean ESR values between the groups.

Results of treatment with cyclophosphamide
All patients completed the 6 months’ course of CYC. Side effects included nausea ($n=10$) and hair loss ($n=1$). In one patient, a woman age 45, introduction of CYC was associated with amenorrhea.

Evaluation of functional capacity according to the NYHA classification showed that after treatment with CYC 13 patients remained stable, whereas the remaining eight improved. Functional capacity did not deteriorate in any of

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<thead>
<tr>
<th>Table 1 Clinical characteristics of the 21 female patients with SLD and the results of treatment with intravenous cyclophosphamide</th>
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</thead>
<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td>NYHA staging, No (%)</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td>FVC (% of predicted)</td>
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<tr>
<td>Percentage of abnormal lung</td>
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<tr>
<td>ESR (mm/1st h)</td>
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<tr>
<td>CRP (mg/l)</td>
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Values are expressed as median (range) unless stated otherwise. *p<0.05 v patients with NA before CYC (Mann-Whitney U test); †p<0.05 v total group of patients before CYC; ‡p<0.05 v patients with NA before CYC; †v0.05 v patients without NA before CYC (Wilcoxon matched pairs test).

Table 2 Influence of treatment with intravenous CYC on cytological analysis of BALF

| Characteristics | All patients (n = 20) | Neutrophilic alveolitis (n = 12) | Without neutrophilic alveolitis (n = 8) |
|---|
| Before CYC | After CYC | Before CYC | After CYC | Before CYC | After CYC |
| BAL recovery (%) | 51.0 (40.0–67.0) | 52.0 (40.0–70.0) | 52.0 (40.0–63.0) | 55.0 (40.0–66.0) | 49.0 (41.0–67.0) | 50.0 (40.0–70.0) |
| Total cell count ($\times 10^4$/ml) | 25.0 (10.0–100.0) | 20.0 (10.0–50.0) | 25.0 (10.0–100.0) | 20.0 (10.0–45.0) | 25.0 (15.0–80.0) | 20.0 (10.0–50.0) |
| Neutrophils (%) | 5.0 (1.0–19.0) | 7.0 (1.0–13.0) | 10.0 (4.0–19.0) | 4.5 (2.0–13.0) | 2.0 (1.0–3.0) | 2.0 (1.0–4.0) |
| Lymphocytes (%) | 19.0 (8.0–53.0) | 20.0 (8.0–50.0) | 16.0 (9.0–38.0) | 19.0 (8.0–53.0) | 26.0 (8.0–53.0) | 23.5 (15.0–39.0) |
| Eosinophils (%) | 0.5 (0.0–5.0) | 0.0 (0.0–1.0) | 1.1 (0.0–5.0) | 0.0 (0.0–1.0) | 0.0 (0.0–1.0) | 0.0 (0.0–1.0) |
| Macrophages (%) | 71.0 (45.0–90.0) | 75.0 (70.0–86.0) | 69.5 (46.0–84.0) | 73.0 (44.0–86.0) | 74.0 (45.0–90.0) | 73.5 (58.0–83.0) |

All values are expressed as median (range). *p<0.05 v patients with NA before CYC (Mann Whitney U test); †p<0.05 v total group of patients before CYC; ‡p<0.05 v patients with NA before CYC (Wilcoxon matched pairs test).

CYC, cyclophosphamide; NA, neutrophilic alveolitis.
been shown that patients with SLD with alveolitis benefit 
lower respiratory tract, but few studies have included serial 
with alveolitis is due to inhibition of inflammation within the 
It is presumed that the beneficial effect of CYC in patients 
improves lung function in patients with SLD.2 4–11 It has also 
Several studies have shown that CYC treatment stabilises or 
A B C

Figure 1 Changes in the total cell numbers (A), the percentages of neutrophils (B), and eosinophils (C) in individual patients with SLD after treatment 
with intravenous CYC. Patients with NA are shown in red, and those without NA, in black.

the patients. The beneficial effect of CYC treatment was seen 
primarily in the group of patients with NA. In this group significantly fewer patients were classified as NYHA class III or IV after the treatment (reduction from nine to 
three patients, Fisher’s exact test; p<0.05).

CYC treatment significantly improved the FVC in the total 
group of patients with SLD. The FVC of patients with NA improved significantly, whereas the FVC in the patients 
without NA remained stable. The percentage of the lung 
affected, as evaluated by HRCT, did not change significantly 
in any of the groups studied.

CYC treatment significantly reduced the ESR and CRP 
values in the whole group of patients with SLD as well as in 
those with and without NA (table 1).

Cytological analysis of BALF before and after 
treatment with CYC

Table 2 and fig 1 show the results of BALF analysis before 
and after CYC treatment.

One patient refused control bronchoscopy, and therefore 
the comparison was done in 20 patients. The mean recovery 
of BALF before and after treatment with CYC was compar-
able. After treatment with intravenous CYC the mean total 
cell number decreased significantly. However, there were no 
significant changes in the mean percentages of particular 
cells before and after treatment with CYC.

However, when the results of CYC were analysed sepa-
rately in patients with NA, significant reduction of the 
percentages of neutrophils and eosinophils were seen. In 
contrast, there were no significant changes in the levels of 
granulocytes after treatment with CYC in the group of 
patients without NA. Figure 1 presents the changes in the 
total cell numbers, the percentages of neutrophils and 
eosinophils in individual patients with SLD after treatment 
with intravenous CYC.

DISCUSSION

Several studies have shown that CYC treatment stabilises or 
improves lung function in patients with SLD.7 8–11 It has also 
been shown that patients with SLD with alveolitis benefit 
from treatment with CYC more than those without alveolitis.9 
It is presumed that the beneficial effect of CYC in patients 
with alveolitis is due to inhibition of inflammation within the 
lower respiratory tract, but few studies have included serial 
analysis of BALF in patients with SLD.2 4 10–13 Moreover, most 
of the studies included a very limited number of patients, and 
their results are inconsistent.

Silver et al and Giacomelli et al did not find any significant 
differences in serial BALF analysis in five and 17 patients 
with SSc, treated with CYC because of alveolitis, respec-
tively.2 10 Varai et al reported a significant decrease in the total 
cell number, but not in the percentages of granulocytes in 
BALF from another five patients treated with CYC owing to 
SLD.6 Schnabel et al found a significant reduction in the 
percentages of granulocytes in six patients receiving CYC 
because of interstitial lung disease related to collagen 
vascular diseases, including two patients with SLD.15 
Recently, we have shown that treatment with intravenous 
CYC significantly decreased the total cell number and the 
percentage of granulocytes in six patients with NA due to 
SLD.13 The significant decrease in the percentage of neutro-
phils was accompanied by a significant decrease in the 
concentration of leukotriene B4, which is a strong chemoki-
netic factor for neutrophils.13

In the present study 6 months’ treatment with intravenous 
CYC stabilised or improved the functional capacity and 
significantly improved the FVC in patients with SLD; these 
results are similar to the results of previous reports.4–11 15 We 
found no significant changes in HRCT scans of the lungs 
after treatment, which may be a result of the relatively high 
high number of patients with fibrotic changes. Cytological 
analysis of BALF showed a significant reduction in the total number 
of cells in the BALF after treatment with CYC, which is in 
agreement with observation of Varai et al.10 Like Giacomelli et 
al16 we found no significant change in the percentages of 
granulocytes in the whole group of 20 patients. However, 
when patients with NA were analysed separately, a sig-
nificant reduction in the percentages of neutrophils and 
eosinophils was shown. In contrast, in a group of patients 
without NA there were no significant changes in the 
percentages of neutrophils.

Together with the significant reduction of granulocytes in 
the BALF, there was a significant improvement in functional 
capacity and FVC in patients with NA, whereas in the group 
of patients without NA functional capacity and lung function 
remained stable. This is in agreement with the results of 
White et al, who showed significant improvement in lung 
function and survival after CYC treatment in patients with 
NA in comparison with those without NA.6

We also found that serum CRP levels were significantly 
higher in patients with NA than in those without, although 
the ESR values did not differ significantly between the two 
groups. Therefore, our results suggest that CRP, as a more 
specific biochemical marker of inflammation, may be helpful 
in identifying patients with alveolitis. Our patients with NA 
approved after CYC treatment, which is in agreement with a 
previous report of Akesson et al, who showed that patients 
with SLD with raised acute phase protein levels are more 
likely to benefit from treatment with CYC.3 Like Akesson et al, 
we observed a significant decrease in CRP and ESR levels 
after treatment with CYC.

In summary, our study suggests that one of the mechan-
isms responsible for the beneficial effect of CYC in SLD is 
reduction of NA in the lungs.
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