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Low-dose pulse cyclophosphamide in interstitial lung disease associated with systemic sclerosis (SSc-ILD): Efficacy of maintenance immunosuppression in responders and non-responders

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ABSTRACT

Objective: To investigate the long-term disease course of patients with recently deteriorated systemic sclerosis (SSC)-interstitial lung disease (ILD) undergoing continuous immunosuppressive treatment with cyclophosphamide (CYC) as induction therapy.
Methods: A total of 45 consecutive SSc patients were treated with weekly pulses of 500 mg of CYC up to 10-g cumulative dose followed by azathioprine (AZA) in those experiencing improvement (> 10% increase) or stabilization of both forced vital capacity and diffusion lung capacity for carbon dioxide and by micophenolic acid (MMF) in those experiencing deterioration (> 10% decrease of either parameter). The follow-up ranged from 6 to 62 months post-CYC regimen (median = 36 months).
Results: Overall, 39 patients completed the CYC regimen. Of them, 24 (61.5%) experienced improvement or stabilization of lung function parameters and received AZA; the remaining 15 received MMF. During follow-up, lung function parameters improved in 3 (12.5%), remained stable in 18 (75%), and worsened in 3 (12.5%) AZA-treated patients, whereas they worsened in 8 (67%) and remained stable in 4 (33%) MMF-treated patients. The incidence of improvement or stabilization was significantly higher in AZA-treated than in MMF-treated patients ($p = 0.001$). The time to the decline of lung function was significantly shorter in CYC non-responders, and CYC unresponsiveness was predictive of lung function worsening over time in a multivariate analysis (HR = 9.14; 95% CI: 2.28–36.64; $p = 0.0018$).
Conclusion: Our study supports the use of low-dose pulse CYC as induction therapy of recently deteriorated SSc-ILD. Moreover, it suggests that AZA should be administered to CYC-responsive patients but does not show any definite effect of MMF in unresponsive patients.

Introduction

Systemic sclerosis (SSc) is an autoimmune systemic disease characterized by skin fibrosis, vascular abnormalities, and internal organ involvement and associated with a shortened survival [1]. Interstitial lung disease (ILD) is one of the leading causes of death in SSc patients and is still a therapeutic challenge [1–3]. Since the pivotal article by Silver et al. [3], a number of studies have investigated the efficacy of cyclophosphamide (CYC) in the treatment of ILD in SSc patients, including 2 randomized controlled studies, namely the Scleroderma Lung Study (SLS) [4] and the Fibrosing Alveolitis in Scleroderma Trial (FAST) [5], and many observational studies [3,6–22].

Based on the results of these studies, the European Scleroderma Trial and Research (EUSTAR) group recommended that patients with SSc-ILD be treated with CYC [23]. Subsequently, however, 2 meta-analyses did not find any clinically significant difference with respect to placebo in the SLS and FAST controlled trials and found just a freezing effect in observational studies [24,25]. Moreover, the SLS trial pointed out that the small differences observed between the treatment group and the placebo gradually vanished from 6 months after CYC interruption, which suggests a role for a maintenance immunosuppressive treatment [26]. In that context, Berezne et al. [15] retrospectively investigated the course of SSc-ILD in 27 SSc patients and reported that 14 of the 19 patients responding to a 6-monthly course of pulse CYC and treated with

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azathioprine (AZA) for the following 18 months had improved or stable respiratory function at 2-year follow-up. We previously reported that the protocol devised by researchers at St. Thomas's Hospital in London [27], namely, CYC at weekly low-dose pulses (500 mg) followed by oral AZA, was effective and safe in patients with early diffuse SSc-ILD [28,29].

We undertook the present 6-year, prospective, observational study to assess the effectiveness of low-dose pulse CYC up to 10 g, followed, according to a treat-to-target strategy, by AZA in responder patients and by micophenolic acid (MMF) in non-responders.

Materials and methods

Patients admitted from November 1, 2007 to October 31, 2012 to the Rheumatology Unit of the Second University of Naples and satisfying the 1980 American College of Rheumatology criteria for the classification of SSc [30] and/or the LeRoy and Medsger [31] criteria for early SSc, who had experienced a recent worsening of lung function, were enrolled in the study after giving their written informed consent.

All patients were assessed with the EUSTAR Minimal Essential Data Set [32] and were divided into 2 subsets (diffuse and limited cutaneous; dc- and lc-SSc) according to the subsetting scheme of LeRoy et al. [33]. Autoantibody profile was investigated as previously described [34]. Disease duration was calculated from the onset of the first non-Raynaud's phenomenon sign/symptom.

To be included in the study, patients had to show a significant deterioration of lung function during the previous 6 months, i.e., a decrease of forced vital capacity (FVC) and/or diffusion lung capacity for carbon dioxide (DLCO) greater than 10% of the respective predicted values with respect to previous values, in absence of a chronic obstructive pulmonary disease. In patients experiencing an isolated reduction of DLCO, a predefined workup designed to rule out pulmonary hypertension and to ascribe the finding to ILD was made. This consisted an evaluation of systolic pulmonary arterial pressure (sPAP) at echocardiography, serum pro-brain natriuretic peptide [35], and high-resolution computed tomography (HRCT) of the lungs. A bronchoalveolar lavage (BAL) analysis was proposed in patients without changes on HRCT consistent with SSc-ILD to confirm the presence of alveolitis and rule out an infection. Pulmonary function tests including FVC, forced expiratory volume in 1 min (FEV1), and DLCO, expressed as percentages of predicted values based on age, sex, and height and corrected for hemoglobin level, were performed according to techniques accepted by the American Thoracic Society (ATS) [36,37]. The percentage predicted DLCO reported value was the average of at least 2 acceptable tests meeting the reproducibility requirements of ATS [36]. At chest HRCT, images were obtained with 1-mm collimation and 10-mm intervals at maximal end-inspiratory phase with the patient in a supine position using a high spatial frequency algorithm. If needed, prone scans were added to distinguish gravity-related changes from structural abnormalities. The presence of bilateral ground-glass and/or fibrotic abnormalities involving at least both bases was considered consistent with SSc-ILD. The extent of the disease was defined according to Goh et al. [38]. A bronchoalveolar lavage analysis was performed according to a standardized procedure. Alveolitis was diagnosed when the percentage of neutrophils in the BAL fluid was $\geq 3\%$ or when the percentage of eosinophils was $\geq 2\%$, or both [39].

Induction CYC therapy

Patients were treated with weekly pulses of 500 mg of CYC up to a cumulative dose of 10 g (20 pulses). All patients received oral corticosteroids (7.5–10 mg/day prednisone equivalent) and standard therapy with proton pump inhibitors, calcium channel blockers, antiplatelet agents, and vitamin D supplementation. No other immunosuppressive drug was allowed.

To prevent CYC-induced cystitis, hydration and Mesna of 100 mg (before each CYC pulse and 4 and 8 h after) were administered. To monitor toxic effects, weekly blood count, liver function tests (alanine aminotransferase and aspartate aminotransferase), and urinalysis were performed. If the white blood cells level was below 3000 per mm^3 or neutrophils below 1500 per mm^3 and/or if liver enzymes level increased above 2.0 times the upper normal range value, the subsequent CYC pulse was delayed until normalization of these parameters.

All patients received oral trimethoprim–sulfamethoxazole (160–800 mg) 3 times per week to prevent *Pneumocystis jirovecii* infection.

Sequential therapy with AZA or MMF

At the end of the low-dose CYC pulses, patients were reevaluated at 6-month intervals by history, clinical examination, and pulmonary function tests. At completion of the 20 planned CYC pulses, patients experiencing a significant functional improvement (i.e., an FVC increase $>10\%$ of predicted value with respect to entry values) were defined "improved," those with an FVC change between -10% and $+10\%$ were defined "stable," and those with an FVC decline $>10\%$ were defined "worsened." In addition, we also considered "worsened" patients with a stable FVC and a DLCO decrease $>10\%$ vs basal values. Patients with improved or stable disease were defined "CYC responders." Those with a stable FVC who developed a decrease of DLCO $>10\%$ and those whose FVC had worsened were defined "CYC non-responders." CYC responders were treated with oral azathioprine (AZA) (2 mg/kg/day), whereas CYC non-responders were treated with oral micophenolic acid (MMF) (2 g/day). To monitor toxic effects of these drugs, blood count and liver function tests (alanine aminotransferase and aspartate aminotransferase) were performed before starting the therapy, after 2 and 4 weeks, and then every month.

Patients were monitored for a median of 48 months (range: 18–72). Those undergoing AZA or MMF treatment and experiencing either an improvement or a stabilization of FVC vs values recorded upon completion of CYC pulses continued the treatment up to October 31, 2013; those showing a decrease of FVC and/or DLCO $>10\%$ were considered as treatment failure and censored at this point for the analysis of the effectiveness and were monitored only for safety and disease status up to October 31, 2013.

Finally, since DLCO can present a significant degree of variability and it is also influenced by vascular pulmonary disease, we performed a post-hoc analysis limited to the 13 patients enrolled for a decline of FVC associated or not with a decline in DLCO, and we used the FVC as response parameter both at the end of CYC induction therapy and during maintenance immunosuppression with AZA or MMF. Moreover, we also analyzed the treatment effectiveness in patients at a higher risk to worsen, i.e., anticentromere (ACA) negative with at least one feature among anti-Scl-70 positivity, a significant FVC worsening, or a diffuse extension of fibrosis at HRCT, using FVC as the measure to define the response.

The study was approved by the Ethics Committee of the Azienda Ospedaliera Seconda Università di Napoli.

Statistical analysis

Continuous variables were analyzed with unpaired Student's *t*-test or with the Mann–Whitney test as appropriate. The chi-square test or Fisher's exact test was applied for categorical

variables. Kaplan–Meier curves and the log-rank test were used to analyze differences in lung function changes in different subgroups of patients. Logistic regression analysis was used to assess the correlations between response to CYC and baseline features of each patient including demographic, clinical and serological factors, pulmonary function, HRCT scores, and smoking status. Univariate and multivariate Cox regression analyses were performed to identify the predictors of lung parameters worsening during the follow-up. $p < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS software, version 12.0.1 (SPSS Inc., Chicago, IL).

Results

Baseline features of the patients

From November 1, 2007 to October 31, 2012 45 patients readmitted to the Rheumatology Unit of the Second University of Naples were found to present a decrease of FVC and/or DLCO > 10% compared with previous values. All of them were enrolled in the study after giving a written informed consent. Most were women (91.1%) with a mean (SD) age and disease duration from first non-Raynaud phenomenon sign/symptom of 49.8 ± 13.3 years and 6.9 ± 5.7 years, respectively. Among the patients, 8 (17.7%) had dcSSc and 27 (60%) were anti-Scl-70-positive. At enrollment, mean FVC was 81.4 ± 15.9% and mean DLCO was 51.4 ± 11.9% in the whole cohort. In detail, the mean percent FVC was 84.8 ± 14.1% in lcSSc and 64.5 ± 15.2% in dcSSc patients ($p = 0.0007$); the mean DLCO was 56.6 ± 11.5% in lcSSc patients and 41.5 ± 13.4% in dcSSc patients ($p = 0.002$). In each patient, the presence of chronic obstructive respiratory disease was ruled out. CYC therapy was administered because of a significant decrease of FVC ± DLCO in 16 patients (35.5%) and of DLCO in 29 (64.4%). In detail, considering all the cohort, the FVC mean (SD) percentage before the significant decline was 85.8 ± 14.6 (last FVC pre-treatment was 81.4 ± 15.9%), the mean (SD) percentage DLCO was 62.3 ± 14.7 (last DLCO pre-treatment was 51.4 ± 11.9%). The median interval between the 2 evaluations was 8 months. As concerns patients enrolled for a decline of FVC ± DLCO, the mean (SD) FVC and DLCO values preceding the significant fall were 76.0 ± 9.5 (last FVC pre-treatment was 64.7 ± 9.4%) and 60.8 ± 11 (last DLCO pre-treatment was 47.8 ± 13.2%), respectively. Among patients enrolled for an isolated fall of DLCO, the previous DLCO value was 65.6 ± 11.4% (last DLCO pre-treatment was 53.0 ± 11.3%). All the latter patients had normal serum NT-proBNP levels, 16/29 presented HRCT findings consistent with ILD; the remaining 13 underwent a BAL analysis, which revealed alveolitis and ruled out infection.

Table 1 lists the main epidemiologic, clinical, and laboratory features of the patients enrolled. Among the 45 patients, 11 had been treated with methotrexate for skin disease and/or musculoskeletal involvement. The 20 low-dose pulse CYC regimen was completed in 39 patients. CYC was discontinued in 4 patients for safety reasons: 1 because of onset of urticarial lesions (after 10 infusions), 1 because of drug-induced fever (after 7 infusions), 1 because of skin induration prevented access to a peripheral venous, and the patient declined implant of a central catheter (after 7 infusions), and 1 because of thrombocytopenia (after 12 infusions). Overall, 2 patients withdrew consent (after 3 and 12 infusions).

Upon completion of CYC pulses, 24/39 (61.5%) patients were defined "CYC responders." In detail, FVC improved in 9/39 (23.0%), and both FVC and DLCO stabilized in 15/39 (38.4%). Among the 9 improved patients, 3 had been enrolled due to a reduction of FVC and DLCO and 6 due to an isolated decline of DLCO ascribed to ILD, as assessed by lung HRCT in 4 and BAL analysis in 2 patients. Among the 15 stable patients, 6 had been enrolled because of a reduction of both FVC and DLCO and 9 because of an isolated DLCO reduction. Of the 20 (25%) patients with a stable FVC, 5 experienced a reduction of DLCO > 10% (2 had been enrolled because of a reduction of both FVC and DLCO and 3 because of an isolated reduction in DLCO). Finally, 10/39 patients (25.6%) were defined "worse" (4 had been enrolled for a reduction of both FVC and DLCO and 6 for an isolated reduction of DLCO). There were no significant difference between CYC responders and non-responders in terms of epidemiologic, clinical, and laboratory features at study entry, except for the extent of HRCT lung involvement (Table 2). Actually, a limited HRCT disease extent according to Goh et al. [38] was found to be associated with CYC response at multiple logistic regression analyses (OR = 5.25; 95% CI: 1.05–25.78; $p = 0.034$).

Table 1
Main epidemiologic, clinical, and laboratory features of patients enrolled in the study

Patients' characteristics	
Age (yrs) mean ± SD	49.86 ± 13.33
Females, *n* (%)	41 (91.1)
Diffuse disease, *n* (%)	8 (17.7)
Disease duration from first non-Raynaud, yrs, mean ± SD	6.9 ± 5.7
Dyspnea, *n* (%)	18 (40.0)
Body mass index (kg/m^2), mean ± SD	25.8 ± 4.7
Modified Rodnan skin score median (range)	3 (0–27)
FVC %, mean ± SD	81.46 ± 15.94
DLCO%, mean ± SD	51.48 ± 11.95
Antinuclear antibodies, *n* (%)	45 (100)
Anticentromere antibodies, *n* (%)	9 (20)
Anti-Scl-70 antibodies, *n* (%)	27 (60)
Nucleolar pattern, *n* (%)	1 (2)
HAQ-DI score median (range)	0.25 (0–2.625)
Previous immunosuppressive treatment, *n* (%)	11 (24.4)
Smoking status	
Current smoker, *n* (%)	12 (26.6)
Ex-smoker, *n* (%)	6 (13.4)
Never-smoker, *n* (%)	27 (60)

Yrs, years; SD, standard deviation; *n*, number; FVC, forced vital capacity; DLCO, diffusion lung capacity for carbon dioxide; HAQ-DI, health assessment questionnaire-disability index.

Effectiveness of AZA or MMF

After completion of CYC pulses, patients were monitored for a median of 36 months (range: 6–62). Duration of follow-up did not differ between CYC responders (median = 39 months; range: 6–62) and CYC non-responders (median = 47 months; range: 12–60) ($p = 0.213$). The 24 CYC responders received AZA, whereas the 12 CYC non-responders, i.e., 7 with a decreased FVC and 5 with a stable FVC associated with DLCO deterioration > 10%, received MMF. The 3 other non-responders were enrolled in a clinical trial with imatinib (NCT00573326) and were not considered in this follow-up analysis.

Among the 24 CYC responders, FVC improved in 3 (12.5%), remained stable in 18 (75%), and worsened in 3 (12.5%). DLCO worsened only in 1 patient who had a parallel decline of FVC. Of the 3 improved patients, 1 had been enrolled because of a reduction of FVC and DLCO and 2 because of an isolated reduction of DLCO. Of the 18 stable patients, 5 were enrolled in the study because of a decline of FVC ± DLCO and 12 because of an isolated reduction of DLCO. Of the 12 patients treated with MMF, FVC worsened in 3 patients (25%); it remained stable in 9 (75%) patients, among whom, however, 5 had a significant decrease of DLCO. Therefore, a further decline of lung function was detected in 8 of the 12 patients treated with MMF.

Table 2
Baseline main epidemiologic, clinical, and laboratory features of CYC responders compared to CYC non-responders

	CYC responders, $n = 24$	CYC non-responders, $n = 15$	p
Age (yrs) mean ± SD	52.5 ± 13.5	46.4 ± 11.4	0.09
Females, *n* (%)	22 (91.6)	13 (86.6)	0.630
Diffuse disease, *n* (%)	2 (8.3)	3 (20.0)	0.354
Disease duration from Raynaud onset (yrs), mean ± SD	12.8 ± 12.1	10.67 ± 9.4	0.457
Dyspnea, *n* %	11 (45.8)	6 (40.0)	0.752
Body mass index (kg/m^2), median (range)	25.6 (19.5–37.5)	26.8 (19.9–38.7)	0.09
Modified Rodnan skin score, median (range)	3 (0–14)	2 (0–27)	0.898
FVC %, mean ± SD	84.0 ± 16.0	77.6 ± 15.3	0.109
DLCO%, mean ± SD	51.8 ± 11.1	51.94 ± 12.7	0.709
sPAP (mmHg), mean ± SD	18.8 (15.3)	20.53 (10.8)	0.722
Antinuclear antibodies, *n* (%)	24 (100)	15 (100)	–
Anticentromere antibodies, *n* (%)	6 (25.0)	1 (6.6)	0.215
Anti-Scl-70 antibodies, *n* (%)	13 (54.1)	11 (73.3)	0.317
Nucleolar pattern, *n* (%)	1 (4.1)	0 (0)	1.00
HAQ-DI score, median (range)	0.125 (0–2.625)	0.375 (0–1.875)	0.851
Smoking status			
Current smoker, *n* (%)	6 (25)	3 (20.0)	1.00
Ex-smoker, *n* (%)	2 (8.3)	3 (20.0)	0.629
Nonsmoker, *n* (%)	16 (66.6)	9 (60.0)	0.739
HRCT abnormalities			
Limited disease by Goh score, *n* (%)	17 (70.8)	6 (40.0)	0.030

Yrs, years; FVC, forced vital capacity; DLCO, diffusion lung for carbon monoxide; HAQ-DI, health assessment questionnaire-disability index; HRCT, high-resolution computed tomography.

Figure 1 provides an overview of the disease course in our series.

The rate of improvement or stabilization was significantly higher in CYC responders treated with AZA than in CYC non-responders treated with MMF (21/24 vs 4/12; $p = 0.001$).

Considering completion of CYC pulses as time 0, Kaplan–Meier curves showed a significantly longer time to FVC and/or DLCO worsening in lcSSc and CYC responders compared to dcSSc patients ($\chi^2 = 4.95$, $p = 0.02$) and CYC non-responders ($\chi^2 = 16.02$; $p = 0.023$), respectively (Fig. 2). CYC non-responsiveness was the only feature predictive of lung function worsening over time in multivariate analysis (HR = 9.14; 95% CI: 2.28–36.64; $p = 0.0018$).

The 13 SSc patients enrolled for a decline of FVC associated or not with a decline in DLCO were mostly females (84.6%),

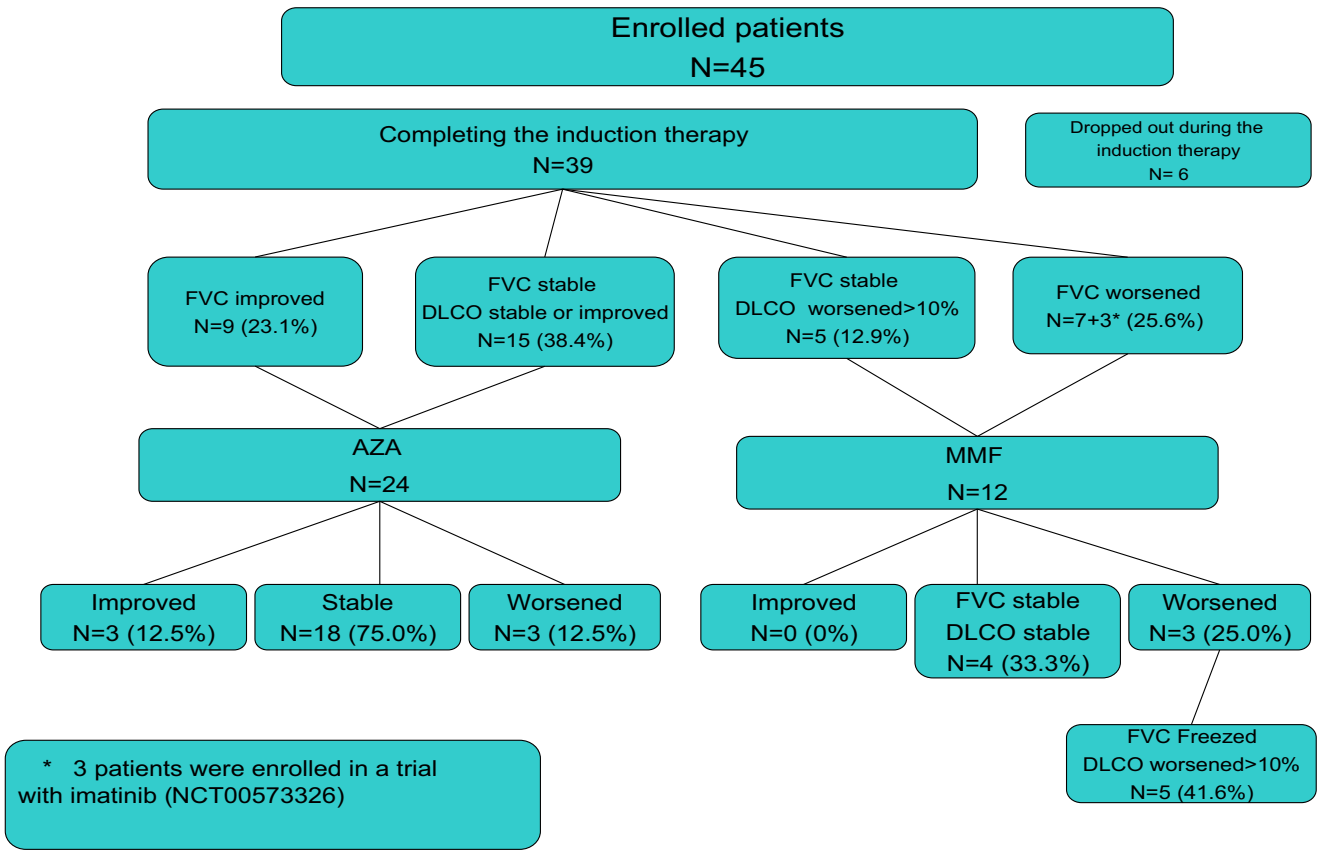

Fig. 1. Summary of the disease course in our series. FVC, forced vital capacity; DLCO, diffusion lung capacity for carbon dioxide; AZA, azathioprine; MMF, micophenolic acid.

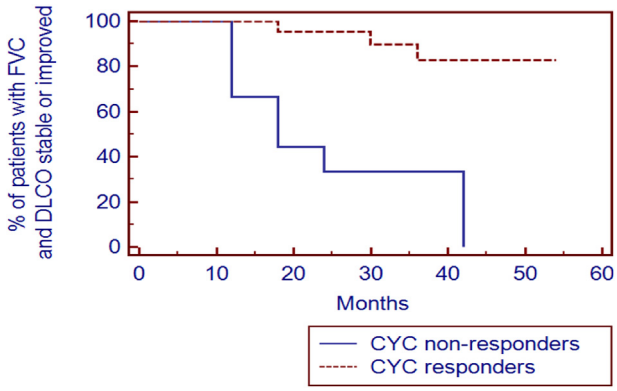

Fig. 2. Time to worsening FVC and/or DLCO in CYC responders and CYC non-responders.

anti-Scl-70 positive (84.6%), a mean age of 47.6 ± 12.6 years, and a mean disease duration from first non-Raynaud onset of 6.9 ± 7.2 years. They presented at baseline FVC (% of the predicted values) values ranging from 49 to 74 (median = 68) and DLCO values ranging from 28 to 72 (median = 48). Of them, 9 (69.2%) resulted to be CYC responders (in 3 FVC improved, i.e., increased > 10% with respect to basal value, and in 6 it remained stable, i.e., declined less than 10% or increased less than 10%) at the end of induction therapy. This figure was not statistically different from that regarded in the 26 patients enrolled for a decline in DLCO ($p = 0.704$).

The 16 SSc patients at higher risk of worsening (i.e., ACA-negative patients with at least one feature among anti-Scl-70 positivity, a significant FVC worsening, or a diffuse extension of fibrosis at HRCT) enrolled were mostly females (81.2%), with a mean age of 47.1 ± 11.3 years and a mean disease duration from first non-Raynaud onset of 6.4 ± 6.9 years. Of them, 13 were anti-Scl-70 positive (6 with a recent significant FVC decline, 2 with extensive fibrosis at HRCT, and 5 with both features). The remaining 3 patients were ANA positive without any autoantibody specificity; 2 had been enrolled for a significant FVC worsening; 1 presented an extensive fibrosis at HRCT. The patients presented baseline FVC (% of the predicted values) values ranging from 49 to 78 (median = 68) and DLCO ranging from 28 to 76 (median = 48). Of them, 11 (68.7%) resulted to be CYC responders (in 3 FVC improved, i.e., increased > 10% with respect to basal value, and in 8 it remained stable, i.e., declined less than 10% or increased less than 10%) at the end of induction therapy. Among the 11 responders, 8 received AZA and 3 received MMF for a significant worsening of DLCO. All the CYC non-responders received MMF. Overall, 3 of 11 CYC responders (all treated with MMF) and 1 of 5 CYC non-responders ($p = 1.00$) worsened during the follow-up. However, a median shorter follow-up was recorded in non-responders (12 months vs 36 months; $p = 0.02$). This could explain the lack of difference in response rate in this subanalysis.

Overall, no patient treated with AZA and 4 of 8 patients treated with MMF ($p = 0.07$) worsened during follow-up. The median follow-up was not significantly different between the 2 groups ($p = 0.08$).

Adverse events

Adverse events caused CYC discontinuation in 3 instances: in 1 because of drug-induced fever, in 1 because of thrombocytopenia, and in 1 because of the onset of urticarial lesions requiring hospital admission. Transient adverse events not requiring CYC discontinuation were nausea in 13 of 45 patients (28.8%), urinary infections in 6 patients (13.3%), upper respiratory tract infections in 4 patients (8.8%), diarrhea in 4 patients (8.8%), myalgias and muscular cramps in 3 patients (6.6%), and hypertransaminasemia in 1 patient (2.2%). Transient microhematuria was recorded in 6 patients, but hemorrhagic cystitis was not detected at cystoscopy. Patients treated with AZA experienced the following adverse events: pneumonitis (1 patient), transient hair loss (2 patients), and transient hypertransaminasemia (2 patients). None of them discontinued the drug. Adverse events resulted in MMF discontinuation in 2 instances. In 1 patient, the drug was discontinued because of the onset of articular and muscular pain and in another because of a low platelet count. The latter patient did not experience lung function deterioration. Transient adverse events recorded in patients taking MMF were dyspepsia (3 patients), herpes zoster infection (1 patient), and pneumonitis (1 patient).

One patient who worsened on AZA after 18 months of treatment and dropped out from the effectiveness study died from lung cancer 2 years after CYC discontinuation. One patient developed breast cancer 4 months after CYC discontinuation due to poor venous access after 7 pulses; thus, a causal relationship between the drug and cancer seems unlikely. Lastly, 1 non-responder to CYC died from respiratory insufficiency 5 years after completing CYC therapy.

Discussion

This is the first, long-term observational study on continuous immunosuppressive treatment in patients with SSc-ILD. We undertook this prospective, observational study to investigate the efficacy and safety profile of low-dose pulse CYC up to 10 g and the long-term disease course of SSc-ILD under continuous immunosuppressive treatment with either AZA or MMF depending on the response to the first CYC course.

First, we would underline a peculiar characteristic of our series, which has been reported by others [40], namely, a high percentage of anti-Scl-70-positive cases. This feature might affect the comparison between our data and those of others but does not affect the conclusions of the study.

In our study, 29 of the 45 original set of patients had been enrolled because of a decline of DLCO only, and 5 of the patients completing the CYC course were defined “non-responders” for the same reason. DLCO is not considered a validated outcome measure of SSc-ILD since it can be influenced by lung vascular disease [41]. However, we excluded such an involvement in our patients and can thus affirm that DLCO decrease depended on ILD in all cases.

The initial CYC course resulted in an improvement/stabilization of lung function in 62% of the 39 patients who completed the regimen. Patients enrolled for an isolated reduction of DLCO had response rates similar to those enrolled for a reduction of FVC ± DLCO in terms of both CYC response after 20 pulses (9/13 vs 15/26; $p = 0.728$) and number of patients in whom maintenance therapy led to improvement or stabilization of lung parameters (5/12 vs 6/24; $p = 0.445$). This response rate is similar to that reported in a small prospective study of 13 SSc patients treated monthly with intravenous CYC and methylprednisolone pulses, i.e., stabilization of lung function at 48 months in 60% of patients [20]. It is also similar to that reported in the following 2 retrospective studies: by White et al. [9], in 39 SSc patients with alveolitis diagnosed by BAL analysis or lung biopsy and treated with oral (35 patients) or pulse (4 patients) CYC and monitored for 16 months, and by Mittoo et al. [22], who followed up 38 SSc patients treated with oral CYC for alveolitis diagnosed by BAL analysis for a median of 4.5 years.

Since DLCO is not considered a primary end point in ILD treatment studies for both pathophysiological and variability reasons [42] and an isolated DLCO was shown by Steen et al. [41] to predict a PAH in 11% of 153 SSc patients, we performed a

post-hoc analysis limited to the 13 patients enrolled for a decline of FVC associated or not with a decline in DLCO. We used the FVC as response parameter both at the end of CYC induction therapy and after maintenance immunosuppression with AZA or MMF. We detected a favorable outcome in a percentage of patients not different from that included in the study for a decline in DLCO both at the end of induction (9/13 vs 15/26; $p = 0.728$) and of maintenance immunosuppression treatment (5/12 vs 6/24; $p = 0.445$). These results support the validity of the conclusions made considering the changes detected in the whole series. In that regard, it is important to underline that 20 of the 73 patients (27%) admitted to Pittsburgh Unit with an isolated reduction of DLCO developed a restrictive pattern during the follow-up and, more importantly, 22% of them already presented pulmonary fibrosis as detected by chest x-ray at baseline and 43% were found to present this feature at the end of a 5.4-year follow-up (range: 2.0–13.2) [41]. Accordingly, we would be inclined to think that our patients are similar to these Pittsburgh patients.

Since the course of SSc-ILD is variable [24,25], it could be hypothesized that the results registered in patients who presented stable values over time refer to a subset of SSc-ILD patients who were not going to decline. In that regard, we would underline that FVC freezing has long been used to identify treatment responders [9,10]. Moreover, obtaining such a result with a less-aggressive treatment seems worthwhile. CYC treatment in ILD-ACA-positive patients enrolled for a decline in DLCO could be regarded as a useless intervention because of the low propensity of such patients to worsen. In order to avoid this bias, we conducted a further post-hoc analysis restricted to the 16 SSc patients a at higher risk to worsen, i.e., ACA-negative patients with at least one feature among anti-Scl-70 positivity, a significant FVC worsening, or a diffuse extension of fibrosis at HRCT. In these patients also, we registered a high percentage of response to treatment by using FVC as an outcome measure. Therefore, whether or not SSc-ILD patients with a recent isolated decline of DLCO should be treated, the present study shows that the low-dose CYC pulse regimen also works in SSC-ILD patients with a high risk of worsening.

Unlike other studies, we enrolled only patients with recent deterioration of lung function and found that a limited extent of lung involvement at HRCT according to Goh et al. [38] was predictive of CYC response. These data mean that the low-dose pulse CYC regimen used by us shows its greatest effectiveness in these conditions.

SLS pointed out the need of continuous immunosuppressive treatment because of the observation that differences between patients treated with CYC and placebo vanished since 18 months after CYC introduction. In this context, Berezne et al. [15] treated 27 patients affected by a worsening ILD with 6-monthly CYC pulses followed by AZA for 18 months. Of the patients who failed to respond to CYC, 5 were treated with MMF. They reported that the percentage of patients with stable or improved FVC or total lung capacity (TLC) after 2 years of follow-up was not significantly different from the one recorded by us after 48 months. Similar to Berezne et al. [15], we administered AZA to CYC responders but used MMF in CYC non-responders according to a predefined strategy. The incidence of improvement during follow-up was 12.5% in AZA patients, whereas no patient on MMF improved. Lung function stabilized in 75.0% of our patients taking AZA compared to only 33% of patients treated with MMF. Therefore, MMF was able to arrest the deterioration of lung function experienced under CYC in 4 out of 12 (33%) of patients who completed the CYC course and were found to be unresponsive to it.

Our data support the use of CYC as induction therapy in SSc-ILD. Nevertheless, CYC cannot be identified as the gold-standard induction therapy to be introduced in any SSc-ILD patient. Further studies are needed to understand which patients should be selected for each drug that is so far shown to be effective in SSc-ILD, i.e., cyclophosphamide, azathioprine, mycophenolate, and rituximab [3–22,43–48]. Moreover, the moderate dosage of MMF used by us in CYC non-responders might have negatively influenced the results obtained with this drug.

Other drugs have been tried as first-line treatment in patients affected by ILD, but the evidence cannot be considered conclusive. Rituximab, as first-line treatment, has been reported to increase both FVC and DLCO in 8 patients treated after 1 [43] and 2 years of follow-up [44]. Imatinib has been found to preserve lung function in 20 patients [45]. MMF has been reported to improve DLCO and stabilize FVC after 4–6 months of treatment in 6 patients [46], to improve FVC but not DLCO after 1 year in 11/13 patients [47], and to stabilize either FVC or DLCO in 17 patients monitored for 1 year and in 8 patients monitored for 2 years [48]. However, a recent case–control study did not find any difference in lung function parameter changes between patients treated with MMF and those treated with CYC and monitored for 2 years [49]. However, a deterioration of lung HRCT findings was observed in patients treated with MMF but not in those treated with CYC [49].

Few studies have evaluated how to manage SSc-ILD patients after CYC failure. Furuya and Kuwana [50] did not find bosentan effective in 9 patients ineligible or unresponsive to CYC. In a case series, 7 patients were treated with MMF as maintenance therapy after CYC, but the authors did not state if patients were CYC responders or not [51]. Haroon et al. [52] report a patient unresponsive to CYC in whom treatment with rituximab led to a clinical improvement.

We found that MMF at 2 g/day had only a “stabilizing” effect in 4/12 patients unresponsive to CYC. Consequently, MMF at that dosage should not be considered the strategy of choice in patients unresponsive to CYC. In an observational trial conducted with 15 SSc patients affected by early diffuse disease treated with MMF up to 3 g/day, Derk et al. [53] observed significant improvements in skin scores, peripheral vascular involvement, and patient-perceived health status and no worsening in pulmonary function parameters. Our study does not exclude that MMF at a higher dosage could be more effective. Nevertheless, the treatment of SSc-ILD unresponsive to CYC still represents a challenge. The lack of serial lung HRCTs could be regarded as a limitation of our study. However, the extent and the type of lung involvement detected by HRCT are considered a possible secondary outcome measure [42], and, more importantly, HRCT changes over time do not necessarily parallel FVC/DLCO changes [49].

Enrolling SSc patients with a recent deterioration of lung physiology parameters has been considered a tool to confine the study to patients with active disease. Nevertheless, because of their long disease duration, our patients could be considered at low risk to develop end-stage lung disease. However, Khanna et al. [54] pointed out FVC deterioration over time that was similar in patients with disease duration from the 1st non-Raynaud's phenomenon >4 years and in those with a disease duration <4 years. Moreover, as pointed out by Medsger et al. [55], even a difference in FVC or DLCO as small as 10% (i.e., between grade 1 and grade 2 or grade 2 and grade 3 in lung severity scale) can affect survival. Therefore, we think that the results of our study are worthwhile, in particular, if the safety of the protocol used by us is considered. Actually, we used the St. Thomas's Hospital scheme because it was associated with a lower burden of side effects compared to long-term oral CYC intake. CYC administered at a dosage as high as 2 mg/kg/day per os for long periods could cause such severe side effects as infections, infertility, and cancer [56]. In point of fact, the burden of adverse events detected by us is somewhat lower that than reported in previous studies with oral CYC. In the study by White et al. [9], of the 39 patients with alveolitis treated with oral CYC, 4 (10.2%) were hospitalized for

infection, 2 (5%) developed hemorrhagic cystitis, and 1 (3%) developed alopecia. Tashkin et al. [4] reported that leukopenia (19 of 79; 24%), neutropenia (7 of 79; 8.8%), and pneumonia (6 of 79 patients; 7.5%) were more frequent in patients treated with CYC than in a placebo group, and moreover, withdrawals were more numerous in the treated patients. Moreover, despite the short follow-up period, 3 malignant cancers were diagnosed. On the contrary, we had to discontinue CYC for adverse events only in 3 cases. Notably, only 1 serious adverse event was recorded, and the potential consequences of oral CYC intake such as alopecia, hemorrhagic cystitis, or cancers did not occur. There was only 1 case of hematologic toxicity (thrombocytopenia). Therefore, our study supports the use of the low-dose pulse protocol.

Conclusion

We found that about 60% of patients who completed the low-pulse dose regimen, which is associated to a lower burden of side effects, experienced an improvement or freezing of lung function, i.e., figures similar to those recorded with higher CYC doses. Moreover, we found that AZA further improved or stabilized lung function in about 90% of them, whereas MMF, at a dosage of 2 g/day, was just able to freeze lung function in 33% of CYC non-responders. Therefore, our results support the use of low-dose pulse CYC, followed by AZA, in CYC responders but do not show any definite effect of MMF, at least at that dosage, in CYC non-responders.

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