Bronchoalveolar Lavage and Response to Cyclophosphamide in Scleroderma Interstitial Lung Disease

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Rationale: The presence of inflammatory cells on bronchoalveolar lavage is often used to predict disease activity and the need for therapy in systemic sclerosis-associated interstitial lung disease.

Objectives: To evaluate whether lavage cellularity identifies distinct subsets of disease and/or predicts cyclophosphamide responsiveness.

Methods: Patients underwent baseline lavage and/or high-resolution computed tomography as part of a randomized placebo-controlled trial of cyclophosphamide versus placebo (Scleroderma Lung Study) to determine the effect of therapy on forced vital capacity. Patients with 3% or greater polymorphonuclear and/or 2% or greater eosinophilic leukocytes on lavage and/or ground-glass opacification on computed tomography were eligible for enrollment.

Measurements and Main Results: Lavage was performed in 201 individuals, including 141 of the 158 randomized patients. Abnormal cellularity was present in 101 of these cases (71.6%) and defined a population with a higher percentage of men ($P = 0.04$), more severe lung function, including a worse forced vital capacity ($P = 0.003$), worse total lung capacity ($P = 0.005$) and diffusing capacity of the lung for carbon monoxide ($P = 0.004$), more extensive ground-glass opacity ($P = 0.005$), and more extensive fibrosis in the right middle lobe ($P = 0.005$). Despite these relationships, the presence or absence of an abnormal cell differential was not an independent predictor of disease progression or response to cyclophosphamide at 1 year ($P =$ not significant).

Conclusions: The presence of an abnormal lavage in the Scleroderma Lung Study defined patients with more advanced interstitial lung disease but added no additional value to physiologic and computed tomography findings as a predictor of progression or treatment response.

Clinical trial registered with www.clinicaltrials.gov (NCT 00004563).

Keywords: systemic sclerosis; Scleroderma Lung Study

Interstitial lung disease (ILD) in systemic sclerosis (SSc) is common and remains the leading cause of death (1–3). Considerable heterogeneity exists in the severity and progression of

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Bronchoalveolar lavage (BAL) has been used to determine disease activity in systemic sclerosis-associated interstitial lung disease.

What This Study Adds to the Field

BAL with increased percentages of polymorphonuclear leukocytes and/or eosinophils is correlated with worse baseline fibrosis. However, there is no difference in the response of FVC to cyclophosphamide between individuals with or without an abnormally cellular BAL.

lung disease. However, a subset of patients with SSc ILD follows a course of progressive decline (4–6). In these patients, inflammatory changes in the lung occur early in the course (7–9) and progress over time (10, 11). Although lung parenchymal abnormalities may be absent on chest computed tomography (CT) at the earliest stage of ILD, most patients develop ground-glass opacities (GGO) and parenchymal lung distortion. Open lung biopsy in these individuals usually demonstrates either cellular or fibrotic nonspecific interstitial pneumonitis (NSIP) (12, 13). These observations have suggested that immunosuppressive therapy for active inflammation with cyclophosphamide (CYC) may limit the development of lung fibrosis (6, 14–22). The randomized, placebo-controlled Scleroderma Lung Study (SLS), which demonstrated a beneficial effect of oral CYC in slowing progression of restrictive lung disease and improving dyspnea and health-related quality-of-life measures, confirmed these observations (23).

Bronchoalveolar lavage (BAL) has been used in SSc to define a population of patients whose lung function will likely decline without therapy (4, 6, 10, 14). The best correlates of subsequent decline in forced vital capacity (FVC) have included the percentage of polymorphonuclear leukocytes (PMNs) and eosinophils on a standardized BAL from either the right middle lobe (RML) or lingula (4, 14, 24). However, these cellular components of BAL have also been seen in other ILDs, such as idiopathic NSIP, idiopathic pulmonary fibrosis (25), and cryptogenic organizing pneumonia (26). In these diseases, BAL cell counts and cellular differentials have been variably associated with disease severity (25, 27), but in most studies these do not predict subsequent disease progression (28, 29).

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High-resolution CT of the chest (chest HRCT) is able to detect and characterize abnormalities associated with scleroderma lung disease (30–34). In the SLS, the baseline chest HRCT fibrosis score was the strongest correlate of disease progression in those assigned to the placebo arm and of the difference between 1-year FVC in the CYC versus placebo groups (23). Fibrosis of the lung produces airway distortion and traction bronchiectasis that is often associated with abnormalities of mucus clearance and excess BAL neutrophils (35). In this context, prior associations between BAL cellularity and outcome may have represented an epiphenomenon that would have been better characterized by the extent of fibrosis on chest CT, particularly since chest HRCT is universally available and less invasive than BAL.

This article reports the BAL characteristics in the SLS cohort and explores the utility of BAL with or without chest HRCT to define a CYC-responsive SSc population. Some of the results of these studies have been previously reported in the form of abstracts (36–38).

METHODS

Patient Selection

The SLS is a multicenter, randomized, double-blind, parallel comparison of CYC versus placebo in dyspneic SSc patients with ILD that enrolled patients between September 2000 and January 2004. All subjects provided written, informed consent. Eligible subjects had onset of their first non-Raynaud's manifestation within 7 years and an FVC between 45 and 85% predicted. All subjects had been nonsmokers for at least 6 months. Complete inclusion and exclusion criteria appear in Table E1 of the online supplement. Patients meeting initial screening criteria were invited to undergo HRCT and BAL, and those with GGO on HRCT and/or a positive BAL ($\geq 3.0\%$ neutrophils and/or $\geq 2.0\%$ eosinophils) were randomized at a 1:1 ratio to receive CYC (≤ 2 mg/kg/d) or matching placebo in a double-blind fashion for 1 year. Pulmonary function was assessed every 3 months during the first year to determine the primary endpoint of %predicted FVC at 12 months, adjusted for baseline. Full methods have been published previously (23).

BAL

A videotape demonstrating the standard BAL procedure and specimen processing was used to standardize the procedure across sites. BAL was performed by serially instilling and manually aspirating four 60-ml aliquots of room temperature saline in the RML. The volume of recovered fluid was recorded. Samples were passed through sterile 100- μ m filters, and pooled. Total cell counts were recorded and cytopspins prepared with 25,000 to 50,000 cells/slide. Slides were stained with a modified Wright's stain (Diff-Quik; Dade International, Aguada, Puerto Rico) with one set read locally and another forwarded to the BAL core where differential counts were determined by two expert readers (R.M.S., M.B.B., or C.S.) and averaged. When the results from two readers differed with respect to eligibility, a consensus reading was performed on a double-headed microscope.

Pulmonary Function Testing

A standardized pulmonary function protocol was used across all sites, performed by study-certified pulmonary function technologists, as described previously (23), in accordance with published standards (39). Dyspnea was assessed at baseline and follow-up visits using the Mahler baseline and transitional dyspnea indices, respectively (40).

Chest HRCT

Chest HRCT was performed in the prone position at full inspiration and again at end expiration using a standard imaging protocol that is detailed in the online supplement.

Statistical Analysis

Summary statistics were generated using Student's *t* or chi-square analysis. Selected correlation coefficients were computed using Ken-

dall's τ . Comparisons in BAL interpretation were assessed using the κ statistic. Stepwise multiple regression analyses were constructed to determine whether the distribution of BAL return was predicted by clinical features of SSc. The primary outcome was the 1-year FVC% predicted for the entire group and BAL subsets. A prespecified analysis of covariance was used to assess the FVC% predicted at 1 year, adjusting for baseline FVC, HRCT fibrosis score, and treatment group, and using a Huber covariance estimation to account for influential extreme values. FVC was not available for all patients at 1 year and a missing-at-random multiple imputation model was used. The Mantel-Haenszel test determined if patient subsets were predictive of CYC response.

RESULTS

Identification of Two BAL Cohorts

A total of 267 subjects were screened for eligibility at 13 clinical centers throughout the United States. Of these, 201 underwent BAL without a serious adverse event (Figure 1). A total of 158 study-eligible subjects were randomized, of whom 141 had undergone both an evaluable BAL and chest HRCT. These 141 individuals make up the population examined in this article.

A positive BAL was observed in 101 (71.6%) of these subjects and an abnormal chest HRCT demonstrating any form of GGO was present in 125 subjects (88.7%). The distribution of cellular findings on BAL is shown in Figure E1. Two cohorts, 101 subjects with a positive BAL and 40 subjects with a negative BAL (no increase in BAL neutrophils or eosinophils), were defined for further analysis (Table 1). The screening HRCT had no GGO or fibrosis in 15.8% of individuals. Twelve subjects also had a BAL lymphocytosis of greater than 10%, but there was no overall difference in the distribution of lymphocytes between the BAL-positive (BAL⁺) and BAL-negative (BAL⁻) groups and no specific correlation of lymphocytosis with HRCT findings or CYC responsiveness.

Comparison of Cell Differentials between Local and Core Readers

In general, cell differential counts performed at the clinical centers correlated well with those performed at the core laboratory with respect to both the percentage of neutrophils (κ coefficient, 0.72; 95% confidence interval [CI], 0.60–0.84) and eosinophils (κ coefficient, 0.60; CI, 0.46–0.74) and with the participant's assignment as either BAL⁺ or BAL⁻ according to the entry criteria (κ coefficient, 0.75; CI, 0.63–0.88).

In the absence of the core reading, none of the 101 BAL⁺ subjects would have been ruled ineligible by these criteria and 5 of the 40 BAL⁻ subjects would have been identified as belonging to the BAL⁺ cohort. However, with the combined use of BAL and/or HRCT as entry criteria, all 141 of the subjects still would have been enrolled into the trial regardless of whether the BAL differential was performed at the clinical center or the BAL core.

Baseline Characteristics of the BAL⁺ and BAL⁻ Cohorts

Baseline demographics for all participants and for the BAL⁺ and BAL⁻ subsets are detailed in Table 2. There were more men in the BAL⁺ group than in the BAL⁻ group ($P = 0.04$), but no other differences were identified with respect to age, the length of time since the onset of non-Raynaud's phenomenon SSc symptoms, the presence of limited versus diffuse SSc manifestations, skin scores, or prior therapy with immunosuppressive agents or prednisone. However, the BAL⁺ and BAL⁻ cohorts were distinctively different with respect to their baseline characteristics on pulmonary function testing and HRCT analysis as detailed in Table 3. As a group, subjects with a positive BAL exhibited significantly worse % predicted values for FVC

Figure 1. Screening and enrollment diagram for the Scleroderma Lung Study (SLS) and the cohort with evaluable bronchoalveolar lavage (BAL) results. HRCT = high-resolution computed tomography.

($P = 0.003$), FEV_1 ($P = 0.015$), total lung capacity (TLC) ($P = 0.005$), and diffusing capacity of the lung for carbon monoxide (DL_{CO}) ($P = 0.004$). There was also a trend ($P < 0.1$) toward a higher percentage of the BAL^+ subjects as compared with BAL^- subjects with pure GGO (54% positive vs. 14% positive) or fibrosis (93% positive vs. 32% positive) on the quantitative visual analysis of their HRCT. In addition, the BAL^+ group exhibited a significantly higher total score with respect to the overall extent of pure GGO on HRCT (sum of the Likert score for each of the different lung regions; $P = 0.005$). When the HRCT analysis was limited to the RML, the specific site of the BAL, the score for fibrosis was also significantly worse in

the BAL^+ group ($P = 0.005$). RML fibrosis remained the only correlate of the BAL^+ group ($P < 0.02$) when added to RML ground-glass and RML honeycombing in a simple logistic regression model.

Univariate correlates of % PMNs and % eosinophils similar to the global BAL^+ correlates are presented in the online supplement. A cough producing sputum was correlated with % PMNs but not % eosinophils.

The volume of BAL recovered from the RML averaged 115.4 ± 41.3 ml (48% of instilled volume) for all subjects, but was significantly greater in the BAL^- subset than the BAL^+ subset (131.6 ± 35.6 vs. 109.1 ± 41.8 ml, $P = 0.003$) (Table 1). The nature of this difference, and the cause for the wide intersubject variability in BAL return (range, 26–229 ml) was investigated by univariate correlations to a variety of patient variables. Significant relationships identified by this approach were then examined by a multiple regression technique (Table 4). Prior cigarette smoking, TLC %predicted, FEV_1/FVC , and the presence of 3% or more PMNs were identified as independent correlates of BAL volume.

TABLE 1. BRONCHOALVEOLAR LAVAGE CHARACTERISTICS AND/OR THE PRESENCE OF GROUND-GLASS OPACITY AT SCREENING

	BAL ⁻ Cohort (n = 40)	BAL ⁺ Cohort (n = 101)	P Value*
BAL characteristics			
Total cells/ μ l†	33.0 ± 31.1	27.3 ± 28.5	0.3065
BAL volume recovered, ml	131.6 ± 35.6	109.1 ± 41.8	0.0032
% PMNs	1.2 ± 0.7	8.4 ± 7.7	<0.0001
% Eosinophils	0.5 ± 0.4	3.9 ± 4.8	<0.0001
% Lymphocytes	4.7 ± 4.1	5.1 ± 6.5	0.7209
Screening HRCT			
Percent with any GGO	100	84.2	0.0060
Percent with any RML GGO	26.3	41.2	0.1175

Definition of abbreviations: BAL = bronchoalveolar lavage; GGO = ground-glass opacity; HRCT = high-resolution computed tomography; PMNs = polymorphonuclear leukocytes; RML = right middle lobe.

BAL⁻ cohort defined as having less than 3% neutrophils and less than 2% eosinophils; BAL⁺ cohort defined as having 3% or more of neutrophils and/or 2% or more of eosinophils.

* Two-tailed Student's *t* test or Fisher's exact test.

† n = 38 for BAL⁻ and 94 for BAL⁺ cohorts due to missing data for total cell counts.

Relationship between BAL Findings and SLS Outcomes

BAL cellular differentials were examined to identify relationships between the presence and extent of abnormality and the change in baseline-adjusted FVC over the first 12 months of treatment. Only 126 of the 158 randomized subjects had both baseline BAL results and met the primary analysis criteria of completing at least 3 months of therapy and having follow-up FVC measurements available at 6, 9, and/or 12 months. Within this defined cohort, assignment to the placebo arm was associated with a significant decline in %predicted FVC over time (Figure 2B; $P = 0.0001$, linear mixed model). When examined with respect to the findings on BAL, there was not a significant difference in the course of subjects who were BAL^+ versus those who were BAL^- ($P = 0.73$, linear mixed model). Even

TABLE 2. DEMOGRAPHICS OF BRONCHOALVEOLAR LAVAGE-POSITIVE AND BRONCHOALVEOLAR LAVAGE-NEGATIVE SUBJECTS

	All Subjects	BAL ⁺	BAL ⁻	P Value*
No. of subjects	141	101	40	
Age, yr	48.6 ± 12.0	49.0 ± 12.9	47.6 ± 9.7	0.56
Sex, % female/male	72/28	67/33	85/15	0.04
Diffuse SSc, %	57.9	54.5	66.7	0.25
SSc disease duration				
RP duration	5.2 ± 5.8	4.9 ± 5.3	6.1 ± 7.0	0.27
Duration of first non-RP manifestation	3.1 ± 2.1	3.0 ± 2.1	3.4 ± 2.0	0.32
Smoking %				
Never	61.8	60.8	64.1	0.85
Past	38.2	39.2	35.9	
Using prednisone at any time during the study, % of subjects	32.6	33.7	30.0	0.84
Mean daily dose, mg	9.3 ± 5.9	9.7 ± 6.7	8.2 ± 2.5	0.48
Modified Rodnan skin score at entry	14.4 ± 10.8	14.1 ± 10.6	15.0 ± 11.3	0.67
Musculoskeletal				
Creatine phosphokinase (ratio of observed/upper limit normal)	130.7 ± 143.0	141.5 ± 151.2	100.1 ± 113.7	0.19
Joint tenderness count (0–8)	1.05 ± 2.07	0.94 ± 1.97	1.34 ± 2.30	0.31
Joint swelling count (0–8)	0.63 ± 1.52	0.55 ± 1.42	0.82 ± 1.75	0.36
Proximal muscle weakness, % of subjects	10.5	9.4	13.2	0.54
Tendon friction rubs, % of subjects	16.3	15.8	17.5	0.81
Renal				
Serum creatinine, mg/dl	0.75 ± 0.22	0.76 ± 0.22	0.73 ± 0.24	0.51
Prior immunosuppressive therapy, %†	40.7	37.6	48.7	0.25

Definition of abbreviations: BAL = bronchoalveolar lavage; RP = Raynaud's phenomenon; SSc = systemic sclerosis.

Data are presented as mean ± SD. BAL⁺ cohort defined as having 3% or more of neutrophils and/or 2% or more of eosinophils; BAL⁻ cohort defined as having less than 3% neutrophils and less than 2% eosinophils.

* P value of comparison between patient subgroups: using two-sample *t* test/Fisher's exact test.

† Prior history of treatment with azathioprine, cyclophosphamide, cyclosporin, potaba, methotrexate, D-penicillamine, or photopheresis.

when corrected for both the baseline FVC and the severity of fibrosis (worst fibrosis score) on HRCT (23), which were worse in those with a positive BAL, there were no detectable differences in the change over time of the %predicted FVC ($P = 0.12$) between the BAL⁺ and BAL⁻ groups. Consistent with this result, a multiple regression analysis failed to identify any specific BAL finding (including total cellularity, total lavage volume returned, or the percentage of PMNs, lymphocytes, or

eosinophils) as an independent predictor of the primary outcome.

Although those assigned to CYC had a significantly better baseline adjusted %predicted FVC at 12 months than those on placebo in the primary analysis for the SLS (23), this difference between treatment groups no longer reached a statistically significant level when the analysis was limited to the 126 patients with evaluable BAL cell differentials ($P = 0.075$). We therefore

TABLE 3. BASELINE PULMONARY AND COMPUTED TOMOGRAPHIC FEATURES

	All Subjects	BAL ⁺	BAL ⁻	P Value*
Pulmonary Features				
No. of subjects	141	101	40	
Baseline dyspnea index (0–12)	5.7 ± 1.7	5.7 ± 1.7	5.6 ± 1.7	0.7564
Cough index (0–4)†	1.6 ± 0.6	1.7 ± 0.6	1.5 ± 0.5	0.1465
FVC, %predicted	68.5 ± 11.9	66.7 ± 11.5	73.2 ± 11.6	0.0027
FEV ₁ , %predicted	69.3 ± 12.5	67.7 ± 12.7	73.4 ± 11.3	0.0149
TLC, %predicted	69.7 ± 13.2	67.7 ± 13.2	74.6 ± 11.9	0.0053
D _{LCO} , %predicted	47.6 ± 14.3	45.5 ± 14.0	53.0 ± 13.8	0.0044
CT Findings				
Frequency of findings, n (%)				
Pure GGO	68 (48)	54 (53)	14 (35)	0.062
Fibrosis	125 (89)	93 (92)	32 (80)	0.073
Honeycombing	53 (38)	41 (41)	12 (30)	0.260
Total score				
Pure GGO	7.16 ± 4.04	7.90 ± 3.94	5.26 ± 3.70	0.005
Fibrosis	2.49 ± 3.04	2.70 ± 3.04	1.92 ± 3.01	0.180
Honeycombing	1.43 ± 2.33	1.56 ± 2.40	1.08 ± 2.15	0.280
RML score				
Pure GGO	0.42 ± 0.59	0.46 ± 0.60	0.32 ± 0.51	0.190
Fibrosis	0.89 ± 0.65	0.99 ± 0.66	0.64 ± 0.58	0.0053
Honeycombing	0.15 ± 0.32	0.17 ± 0.34	0.092 ± 0.26	0.210

Definition of abbreviations: BAL = bronchoalveolar lavage; CT = computed tomography; GGO = ground-glass opacity; RML = right middle lobe.

Data are presented as mean ± SD. BAL⁺ cohort defined as having 3% or more of neutrophils and/or 2% or more of eosinophils; BAL⁻ cohort defined as having less than 3% neutrophils and less than 2% eosinophils.

* P value of comparison between patient subgroups using two-sample *t* test/Fisher's exact test.

† Cough index graded on a 4-point Likert scale.

TABLE 4. BRONCHOALVEOLAR LAVAGE RECOVERY VOLUME WAS INFLUENCED BY PREVIOUS SMOKING, TOTAL LUNG CAPACITY, FEV₁/FVC, AND THE PERCENTAGE OF POLYMORPHONUCLEAR LEUKOCYTES IN LAVAGE

Systemic Sclerosis Features Found Significant on Univariate Testing	Estimate*	P Value
BAL PMN, %	-1.24	0.010
FEV ₁ /FVC	1.52	0.021
Cigarette smoking, pack-years	17.19	0.031
Total lung capacity, %predicted	0.75	0.047
Serum WBC, per mm ³	2.26	0.111
Gastroesophageal reflux	-14.50	0.188
DL _{CO} , %predicted	0.32	0.337
CPK units	-0.02	0.395
Creatinine units	-7.67	0.677
FEV ₁ , % predicted	-0.15	0.710

Definition of abbreviations: BAL = bronchoalveolar lavage; CPK = creatine phosphokinase; PMN = polymorphonuclear leukocytes; WBC = white blood cells.

* Effect estimates for results of stepwise multiple regression analysis. Statistically significant features are in bold type.

examined the impact of BAL-defined cohorts on the proportion of subjects who had either a stable or improving %predicted FVC at 12 months versus the proportion of subjects with a worsening %predicted FVC (Figure 3). A significantly higher proportion of those assigned to receive CYC had a stable or improving FVC compared with placebo ($P = 0.015$, Fisher's exact test). When divided into the BAL⁺ and BAL⁻ cohorts, a significant treatment effect for CYC was only observed in those who were in the BAL⁺ ($P = 0.034$) and not in the BAL⁻ cohort ($P = 0.42$).

A small but unique subpopulation included 14 individuals who enrolled with a positive BAL but normal HRCT for whom 1-year FVC data were available (see Figure E2). There was no difference in the percentage of individuals who improved with CYC (3 of 6; 50%) compared with the individuals who received placebo (4 of 8; 50%), although the very small size of this subpopulation could lead to misleading results. However, there also was no difference compared with the larger population with a positive BAL and any GGO on HRCT who were treated with CYC (21 of 40; 52.5%) ($P =$ not significant).

DISCUSSION

The poor prognosis associated with SSc ILD, its variable response to therapy, and the toxicities associated with treatment emphasize the importance of identifying patients who are more likely to have progressive disease and/or to respond to treatment. Historically, progressive shortness of breath, declining lung function tests, and/or a changing radiograph have triggered clinicians to initiate therapy. In 1993, Silver and colleagues reported that FVC stabilized or improved when a group of 14 dyspneic patients with SSc and with a declining FVC and alveolitis on their BAL were treated with oral CYC (16). In a retrospective case study, White and coworkers also reported a strong correlation between inflammatory changes on BAL ($\geq 3\%$ neutrophils and/or $\geq 2\%$ eosinophils) and the course of FVC and DL_{CO} over time depending on whether or not the patients were treated with CYC (14). Patients with SSc and ILD and a negative BAL had, on average, stable values for FVC and DL_{CO} over the mean observation period of 16 months. In contrast, those with a positive BAL who were not treated experienced a significant decline in lung function, whereas those with a positive BAL who were treated with CYC experienced an improvement in their FVC and DL_{CO} over time.

Figure 2. Course of %predicted FVC over 1 year in Scleroderma Lung Study subjects assigned to the placebo arm. (A) The %predicted FVC at each time point after starting therapy was compared with the baseline value to calculate the change from baseline for all subjects who were assigned to the placebo group, had an evaluable baseline bronchoalveolar lavage (BAL), and completed a follow-up measurement of FVC at the 6, 9, and/or 12-month time point ($n = 66$ subjects). (B) The change from baseline for the %predicted FVC at each time point stratified by the subject's assignment as either BAL positive (BAL⁺) (44 subjects) or BAL negative (BAL⁻) (22 subjects). There was a significant decline in FVC at 1 year in patients assigned to placebo ($P = 0.0001$, linear mixed model), but no significant difference between the course of decline comparing the BAL⁺ and BAL⁻ groups ($P = 0.74$, linear mixed model).

With the assumption that inflammation causes progressive fibrosis and that CYC mediates its effects by reducing lung inflammatory cell numbers (41), the measurement of inflammatory cells on BAL has become a common practice for identifying patients with SSc and ILD who warrant immunosuppression. However, controversy has remained about the reproducibility of BAL cell differentials in general and the utility of a single RML lavage as a measure of lung inflammatory cells (43), and the relationship between BAL cellularity and disease activity (11). With respect to this issue, the SLS provided a unique opportunity to prospectively study the relationship between BAL cell differentials and the progression of SSc ILD in a randomized controlled trial in which patients were treated with either oral CYC or placebo and carefully monitored over the course of 1 year.

There were several features of the SLS that were designed to reduce bias related to the interpretation of the BAL cell differentials. First, a combination of chest HRCT and BAL entry criteria was used in a redundant manner to define patients with active ILD. As such, the study population contained a mixture of BAL⁺ and BAL⁻ patients, with the BAL⁻ cohort representing 28% of those enrolled. In addition, the cell differentials were read independently by two out of three experienced readers and the results presented as the average interpretation, thereby reducing individual selection bias. All discordant interpretations, which would have resulted in a different eligibility assignment, were resolved by consensus. Finally, BAL cell differentials were read at both the clinical center and at the core to evaluate the feasibility and reproducibility of BAL readings in the clinical setting. Our results demonstrate a high degree of correlation between reading sites and that, by using both HRCT and BAL as entry criteria, there was little chance for differences between readers to have influenced the outcome. As such, the SLS demonstrated that a multicenter study using BAL can be performed with minimal variance between centers, if appropriate efforts to standardize the procedure are used.

The first goal of our analysis was to determine whether the presence of a positive BAL identified a unique cohort of patients with SSc and ILD with respect to their baseline features or the rate of progression of their disease. Indeed, the BAL⁺ cohort had significantly more severe lung disease by pulmonary

Figure 3. Frequency distribution of the change in %predicted FVC over 1 year stratified according to treatment group. The change in %predicted FVC, comparing the value at 1 year with the value at baseline, was calculated for each participant in the Scleroderma Lung Study who had an evaluable baseline bronchoalveolar lavage (BAL) and had completed a follow-up measurement of FVC at the 6, 9, and/or 12-month time point (126 subjects). Frequency distribution curves were calculated based on the magnitude of the change in %predicted FVC, stratified by their assignment to either the cyclophosphamide (CYC) or placebo groups, and displayed for all subjects (A), those with a positive BAL (B), and those with a negative BAL (C). There were significantly more subjects who had a stable or improving FVC when treated with CYC than with placebo when all subjects were analyzed ($P = 0.015$) and for the BAL-positive cohort ($P = 0.034$), but not for the BAL-negative cohort ($P = 0.42$, Fisher's exact test). CYC, solid bars; placebo, hatched bars.

function criteria, more pure GGO on HRCT, and a trend toward more extensive fibrosis. In fact, when findings on the BAL were correlated to a regional analysis of the RML on HRCT, the most significant difference between the BAL⁺ and BAL⁻ groups was the severity of fibrosis, which was worse in those with a positive BAL. A similar relationship between fibrosis and a positive BAL was recently reported in a smaller study of SSc ILD by Clements and colleagues (43).

For some years, idiopathic pulmonary fibrosis was believed to be an inflammatory disease on the basis of BAL studies, until it was later recognized that usual interstitial pneumonia on open lung biopsy does not have as many inflammatory cells as were seen on BAL. One explanation is that lung fibrosis causes traction of small airways that leads to mucus retention and cellularity in the small airways. Whether NSIP associated with SSc shares this quality remains unknown. The results of this study would suggest that BAL cellularity may have little to do with pathogenesis and is simply an epiphenomenon. Therefore, the "alveolitis" that has historically been characterized as the lesion of SSc ILD may not be of alveolar origin at all. Alternatively, GGO likely represents a mixture of microscopic fibrosis, interstitial cellularity, and excesses of alveolar cells that characterize NSIP.

The difference in baseline lung disease and fibrosis between the BAL⁺ and BAL⁻ subsets also raises concern about the uncontrolled comparison of these two groups in prior studies—which assumed that they were equal in all other aspects. A multivariate analysis from the main SLS identified two independent factors that were related to the severity of lung function at the conclusion of the study: baseline FVC and baseline fibrosis as measured by HRCT (23). There was a significant and negative correlation between the baseline fibrosis score on HRCT and the 12-month value for %predicted FVC, suggesting that the presence of fibrosis is a predictor of progressive disease. As such, prior studies using BAL findings as a predictor of disease activity may have inadvertently selected for more extensive disease at baseline. In our current analysis, we evaluated the changes in %predicted FVC over time using both the measured values and after adjusting outcomes for the observed differences in baseline FVC and fibrosis, neither of which identified a difference in the course of untreated disease between those subjects who were BAL⁺ and those who were BAL⁻. In addition, past cigarette smoking that could impact BAL recovery or cellularity was not found to impact changes in %predicted FVC in either the CYC or the placebo group.

The second goal of this analysis was to determine whether the presence of a positive BAL predicts a subset of patients more likely to respond to CYC treatment. The only baseline variables correlated with a differential treatment response as

measured by the difference in baseline-adjusted %predicted FVC at 12 months were the baseline FVC and the baseline HRCT fibrosis score. Neither BAL cellularity nor identification as BAL⁺ or BAL⁻ by entry criteria predicted outcome when subjected to a multivariate analysis. Unfortunately, the SLS was not sufficiently powered to allow subgroup stratification with respect to its primary analysis comparing the CYC and placebo arms for any variables. Therefore, the analysis we performed in this study was an effort to define the degree of colinearity with baseline FVC and the baseline HRCT fibrosis score and determine if any statistical measure of BAL cellularity could be correlated with FVC change. Any correlation found would necessarily be less robust than the baseline FVC and the baseline HRCT fibrosis score for clinical care.

We examined the impact of stratification by BAL subgroup on the proportion of subjects in whom the %predicted FVC was either stable or improving versus deteriorating. A significant treatment effect from CYC was observed in the BAL⁺ group, but not in the BAL⁻ group. However, given the considerably smaller sample size of the BAL⁻ group, it remains difficult to conclude that there is a true association between BAL cellularity on BAL and response to CYC.

A potential limitation in assessing the utility of BAL in this study was that lavage was performed exclusively in the RML, regardless of the site of disease activity by chest HRCT. Some studies have suggested that interlobar variation of BAL cellularity is small in the majority of individuals (42); however, higher percentages of eosinophils and PMNs are found when the BAL is performed in an area of chest HRCT abnormality in idiopathic pulmonary fibrosis (35) and in SSc (43). As such, our approach may have underestimated the number of BAL⁺ subjects and reduced our ability to correlate BAL findings with outcomes. Future studies could consider multilobar BAL as an alternative sampling strategy, similar to the targeting of open lung biopsies from multiple areas of variably involved lung in ILD.

The clinical importance of the current analysis is significant. If chest HRCT and pulmonary function testing are better predictors of a beneficial CYC response than BAL, then BAL should rarely be performed for clinical care in SSc unless infection is suspected (43, 44). If fibrosis is the important feature that drives ILD progression, a strong argument can be advanced that therapy for SSc ILD should be instituted when the chest HRCT shows fibrosis, regardless of BAL findings. Because fibrosis will thereafter be present on all subsequent CT scans, further clinical trials will be needed to determine optimal duration of treatment.

In summary, despite the correlation of a positive BAL to measures of lung fibrosis and the severity of baseline lung function abnormality, BAL was not helpful in predicting either

the course of disease in the placebo group or the response to CYC as measured by FVC %predicted. When subjected to the rigorous analysis of a randomized controlled trial, the clinical utility of BAL cellularity as a routine test to assess the activity of SSc ILD appears limited in the setting in which pulmonary function and HRCT can more readily be obtained. The utility of BAL for other biomarkers of disease activity or clinical response to therapy is not precluded by this study. Instead, BAL may remain a useful tool to exclude lung infections when clinically indicated, and to obtain cells and epithelial lining fluid for studies of SSc ILD pathogenesis.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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