# Determinants of bronchoalveolar lavage cellularity in idiopathic pulmonary fibrosis

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SCHWARTZ, DAVID A., RICHARD A. HELMERS, CHARLES S. DAYTON, ROBERT K. MERCHANT, AND GARY W. HUNNINGHAKE. *Determinants of bronchoalveolar lavage cellularity in idiopathic pulmonary fibrosis.* J. Appl. Physiol. 71(5): 1688–1693, 1991.—To investigate factors that determine bronchoalveolar lavage (BAL) cellularity in patients with idiopathic pulmonary fibrosis (IPF), we compared BAL cells in patients with IPF ($n$ = 83) to both nonsmoking ($n$ = 111) and smoking ($n$ = 19) normal volunteers. Patients with IPF had higher concentrations of BAL total cells and alveolar macrophages than nonsmoking volunteers and more BAL neutrophils and eosinophils than normal volunteers regardless of smoking status. Among patients with IPF, the numbers of alveolar macrophages, neutrophils, or eosinophils were strongly associated with either smoking status or pack-years of cigarette smoking. In fact, after accounting for cigarette smoking, using multivariate analysis, the only additional factors that were found to be associated with BAL cellularity were age (macrophages and eosinophils) and the percent predicted forced expired volume in 1 s (neutrophils). Additional multivariate models failed to identify a significant relationship between BAL cellularity and either the type of immunosuppressive therapy or other physiological measures of lung function. We conclude that cigarette smoking strongly influences BAL cellularity in patients with IPF. These findings suggest that cigarette smoking may have a role in the pathogenesis of IPF or may adversely affect the prognosis in patients with IPF.

cigarette smoking; immunosuppressive agents

EVALUATION of bronchoalveolar lavage (BAL) has clearly contributed to our understanding of the clinical features and pathogenic mechanisms in interstitial lung diseases (8, 9). In patients with idiopathic pulmonary fibrosis (IPF), the numbers of total BAL cells are increased and specific increases have been observed in the absolute numbers of macrophages, lymphocytes, neutrophils, and eosinophils (8, 9). Among patients with IPF, excess neutrophils (26, 30) and eosinophils (15, 23) have been associated with abnormal lung function and a higher likelihood of disease progression. In contrast, BAL lymphocytes appear to be directly related to an improved response to immunosuppression (15, 26, 30). Thus, for patients with IPF, the cell content of the BAL fluid is thought to be useful in staging the activity of the disease (8, 9), assessing the potential for response to immunosuppressive agents (15, 23, 26, 30), and determining the overall prognosis (8, 9).

However, although the etiology of IPF is unknown, the cell content of BAL fluid may be influenced by factors that are unrelated to the underlying etiology of the interstitial lung disease. For example, among "nondiseased" study subjects, cigarette smoking can significantly influence the numbers, specific types, and function of cells retrieved by BAL (3, 14). Similarly, treatment with immunosuppressive agents may have a role in determining the numbers and types of cells present in BAL fluid. Further understanding of the factors that influence the cellularity of BAL fluid is particularly important as we evaluate the use of BAL to direct our clinical approach to patients with IPF and to understand the pathogenesis of this disorder.

The purpose of this investigation was to identify determinants of BAL cellularity among individuals with IPF. A priori, we hypothesized that cigarette smoking and immunosuppressive therapy would substantially contribute to the amount and type of cells present in the BAL fluid. Our findings indicate that cigarette smoking strongly influences the cellular content of the BAL fluid. However, therapy with corticosteroids or corticosteroids plus cytoxan appears to have very little effect on BAL cellularity.

## METHODS

*Patient population.* In total, 83 patients with idiopathic pulmonary fibrosis (IPF) are included in this study. The patients were identified as part of an ongoing research program in interstitial lung disease at our institution. The diagnosis of IPF was based on standard criteria (6) that included either evidence of interstitial lung disease on chest X-ray or restrictive lung function with an open-lung biopsy demonstrating varying degrees of interstitial fibrosis and intra-alveolar inflammatory cells. Strict exclusionary criteria were established and included no clinically relevant environmental or occupational exposure history; no findings of hypersensitivity pneumonitis, left ventricular failure or systemic disease; and no granulomata or vasculitis on the biopsy specimen. Furthermore, each biopsy specimen was cultured, and patients were included only if the cultures were negative for bacteria, mycobacteria, and fungi. Of 83 patients with IPF, 50 (60%) had an open-lung biopsy and the remaining 33 had a transbronchial biopsy and fulfilled all of the other criteria noted above. On average, these patients were 63 yr of

TABLE 1. *Demographic and clinical characteristics of study subjects with IPF*

| Characteristic | Smoking History | | |
|---|---|---|---|
| | Never ($n$ = 23) | Former ($n$ = 50) | Current ($n$ = 8) |
| Age, yr | 65.2±11.2 | 64.7±13.5 | 42.8±10.0 |
| Race | | | |
| White | 23 (100%) | 49 (98%) | 8 (100%) |
| Black | | 1 (2%) | |
| Sex | | | |
| Male | 9 (39%) | 38 (76%) | 4 (50%) |
| Female | 14 (61%) | 12 (24%) | 4 (50%) |
| Pack-years | 0 | 39.8±21.4 | 37.4±13.7 |

Values are means ± SD for continuous variables and as no. of subjects ($n$); % for categorical variables.

age, only one was black, there was a predominance of men (61%), and also a relatively low proportion of never smokers (27%) (Table 1). These demographic and clinical characteristics are similar to other reported series of patients with IPF (6, 8, 9). However, within our population of patients with IPF, current cigarette smokers tended to be younger than never or former smokers and cigarette smokers (former and current) had a higher prevalence of male subjects than never cigarette smokers (Table 1). Patients were treated with immunosuppressive agents based on the severity and progression of their lung disease. Because this investigation examines the influence of cigarette smoking on the initial bronchoalveolar lavage parameters, many of the study subjects were not being treated at the time of the initial lavage. During the initial evaluation, 50 (60%) patients were on no form of immunosuppression, 22 (27%) were on corticosteroids, and 11 (13%) were on corticosteroids and cytoxan.

One hundred eleven normal nonsmoking volunteers underwent BAL and, in some comparisons, were used as a control population. The mean age of the volunteers was 30 yr (range 20–48 yr). Sixty-five percent were male and 35% were female. For the purposes of this study, nonsmokers were considered to be lifetime nonsmokers (<20 packs of cigarettes in their lifetime). In addition, 19 otherwise healthy smoking volunteers underwent BAL. Although this sample of smokers is relatively small compared with the other study groups, we have included them to evaluate the independent effect of smoking on BAL cell content. None of the normal volunteers (nonsmokers or smokers) had any evidence on history or physical examination of respiratory disease, including asthma, allergic rhinitis, interstitial lung disease, exposure to pneumoconiotic dust, or recent viral illness. These nonsmoking and smoking volunteers have been used as control subjects in other studies (unpublished observations).

*Characterization of smoking history.* Participants were classified as "never smokers" (<20 packs of lifetime cigarettes and no cigarettes in the month before their evaluation), "former smokers" (>20 lifetime packs of cigarettes but stopped at least 1 mo before their evaluation), and "current smokers" (smoked within 1 mo of their evaluation). In the classification of pack-years, never smokers were classified as having 0 pack-yr of cigarette smoking.

*Pulmonary function testing.* The pulmonary function tests consisted of standard spirometry using a Medical Graphics 1070 system (St. Paul, MN) and lung volumes via body plethysmography Medical Graphics 1085 system. A single-breath diffusing capacity was measured using the Medical Graphics 1070 system. The measurements of lung function were performed using standard protocols and the American Thoracic Society guidelines (1) were used to determine acceptability. The predicted normal values used were those of Morris et al. (22) for spirometry, Goldman and Becklake (12) for lung volumes, and Van Ganse et al. (27) for the diffusing capacity. Arterial blood gases were performed on all patients regardless of the use of supplemental oxygen. Alveolar-arterial (A-a) oxygen differences were determined using standard equations, accounting for the use of supplemental oxygen.

*Bronchoalveolar lavage and cell analysis.* Bronchoscopic examination and lavage were performed on all study subjects using our standard method (31). Premedications included atropine sulfate (0.8 mg im), meperidine hydrochloride (75 mg im), and two inhalations of metaproterenol (total 1.3 mg) from a hand-held pressurized canister. The upper airway was anesthetized with Dyclone gargle and aerosolized 4% lidocaine. Lidocaine was also applied topically to the pyriform sinuses and vocal cords. The bronchoscope (Olympus model BF 4B2; 4.9 mm diam at the tip) was advanced into the airways, and the tip was maintained in the wedged position in a subsegmental bronchus throughout the lavage procedure. In all cases, two lavages were performed, and, in most instances, the two lavages were performed in subsegments of the right middle lobe and lingula. Each lavage consisted of 100 ml of saline (5 × 20-ml aliquots).

Immediately after the lavage, the lavage fluid was strained through two layers of surgical 4 × 4 gauze into 50-ml conical tubes. The tubes were centrifuged for 5 min at 200 $g$ and the residual pellet of cells was resuspended and washed twice in Hanks' balanced salt solution (without $Ca^{2+}$ or $Mg^{2+}$). After the second wash, a small aliquot of the sample was removed for a cell count using a hemocytometer. The cells were then washed once more and resuspended in RPMI 1640 medium so that the final concentration was $1 \times 10^7$ cells/ml. The cells present in 10–12 $\mu$l of the $1 \times 10^7$-ml cell suspension were spun onto a glass slide using a filter card and a cytocentrifuge (Cytospin-2; Shandon Southern, Sewickley, PA). After the cells were dried for 2 min, they were stained by using a Diff Quick Stain set (Harleco, Gibbstown, NJ). The cells were counted and classified only after the cytocentrifuge preparation was felt to be satisfactory by the following criteria: negligible staining artifact, uniform dispersal of cells without clumping, essentially no disruption of cells, and <3% airway epithelial cells.

*Statistical analysis.* Univariate comparisons were made to determine whether demographic or clinical variables influenced the amount or type of cells in the BAL fluid. Because we have recently demonstrated that BAL cellularity is not normally distributed (unpublished observations), we used nonparametric statistics to evaluate all of our comparisons. The Mann-Whitney $U$ test and Krusal-Wallis one-way analysis of variance were used to eval-

TABLE 2. *Comparison of BAL cellularity between patients with IPF and both nonsmoking and smoking volunteers*

| | Nonsmoking Volunteers ($n$ = 111) | Smoking Volunteers ($n$ = 19) | IPF Study Subjects ($n$ = 83) |
|---|---|---|---|
| Cells/ml, $\times 10^4$ | 12.7±9.1‡ | 48.9±40.2* | 28.0±24.4 |
| Macrophages/ml, $\times 10^4$ | 12.1±10.0‡ | 47.4±39.6† | 22.4±21.8 |
| Lymphocytes/ml, $\times 10^4$ | 0.8±1.0 | 0.7±0.7 | 1.6±3.0 |
| Neutrophils/ml, $\times 10^4$ | 0.1±0.2‡ | 0.6±0.9* | 2.8±7.3 |
| Eosinophils/ml, $\times 10^4$ | 0.0±0.1‡ | 0.1±0.2‡ | 1.2±2.8 |
| Percent lavage return | 75.9±15.9 | 70.7±17.7 | 73.7±14.7 |

Values are means ± SD; $n$, no. of subjects. $P$ values were computed by comparing nonsmoking volunteers with patients with IPF and by comparing smoking volunteers with patients with IPF. * $P < 0.01$; † $P < 0.001$; ‡ $P < 0.0001$.

uate the relationship between categorical variables and BAL cellularity, and Spearman's correlation coefficient was used to evaluate the relationship between continuous variables and BAL cellularity (7).

We used a multivariate linear regression model (17) to identify the independent determinants of BAL cellularity in patients with IPF. A linear model was generated that incorporated all potential confounders and determined the relative strength of the relationship between BAL cellularity and both cigarette smoking and immunosuppressive therapy. We used a logarithmic transformation (base 10) of the BAL cell counts to account for the underlying distribution of these measures. After a linear model was established, all possible interactions were tested in a stepwise manner to determine if significant improvements could be achieved by inclusion of any of the interactive terms.

## RESULTS

Pulmonary function tests performed immediately before the bronchoscopy demonstrated a mild reduction in lung volumes [total lung capacity = 73.5% predicted, forced vital capacity (FVC) = 62.6% predicted, and residual volume (RV) = 85.2% predicted], a moderate to severe reduction in diffusing capacity (44.1% predicted), and a moderate increase in the A-a oxygen difference (42.2 Torr) in subjects with IPF.

Our study subjects with IPF had an overall increase in the total cells per milliliter of BAL fluid compared with normal nonsmoking volunteers (Table 2). However, smoking volunteers had even higher concentrations of cells than patients with IPF. Patients with IPF were found to have a higher concentration of alveolar macrophages than nonsmoking volunteers and higher concentrations of both neutrophils and eosinophils than normal volunteers regardless of smoking status. The concentration of lymphocytes was slightly higher among patients with IPF; however, it was not significantly different than that observed in either group of volunteers. These findings are consistent with previous reports (8, 9) and do not represent novel findings. However, these findings serve to focus our attention on the factors that are associated with the absolute numbers of different types of BAL cells in individuals with IPF.

To determine the factors that are associated with BAL cellularity in individuals with IPF, we limited the remaining analyses to study subjects with IPF and have focused our analyses on the clinical factors that are associated with the presence of macrophages, lymphocytes, neutrophils, and eosinophils in the BAL fluid. The numbers of BAL macrophages were strongly associated with both age ($r = -0.43$; $P = 0.005$) and smoking status ($P = 0.0005$), with current smokers having a marked increase in the macrophage content of lavage fluid (Tables 3 and 4). A marginal relationship was observed between the concentration of BAL macrophages and both pack-years of smoking ($r = 0.18$; $P = 0.11$) and two measures of lung function: ratio of forced expired volume in 1 s ($FEV_1$) to FVC ($r = -0.15$; $P = 0.20$) and the percent predicted RV ($r = 0.22$; $P = 0.09$) (Table 4). No clear association was observed between the BAL macrophages and either the type of immunosuppressive therapy or other specific measures of lung function. The numbers of lymphocytes

TABLE 3. *Relationship between BAL cellularity and both demographic and clinical categorical factors for patients with IPF*

| | Bronchoalveolar Lavage Cellularity, (cells/ml) × $10^4$ | | | |
|---|---|---|---|---|
| | Macrophages | Lymphocytes | Neutrophils | Eosinophils |
| Sex | | | | |
| Male | 19.5±18.6 | 1.6±2.5 | 2.1±2.1 | 1.3±2.8 |
| Female | 27.0±25.7 | 1.7±3.7 | 3.8±11.5 | 1.1±2.8 |
| Smoking history | | | | |
| Never | 16.6±13.9 } † | 2.0±3.8 | 4.6±13.5 | 0.4±1.3 } * |
| Former | 18.8±15.2 } † | 1.7±2.8 | 2.1±1.9 | 1.5±3.4 } * |
| Current | 63.4±34.1 } † | 0.6±0.6 | 2.3±3.0 | 2.0±1.9 } * |
| Immunosuppressive therapy | | | | |
| None | 22.8±21.9 | 1.8±3.2 | 3.2±9.3 | 1.5±3.5 |
| Steroids | 22.5±24.5 | 1.5±3.2 | 1.9±2.3 | 0.7±1.3 |
| Cytoxan | 20.1±16.6 | 0.9±0.9 | 2.6±1.9 | 1.0±1.5 |
| Borg dyspnea scale | | | | |
| 2 | 24.3±24.3 | 0.9±0.9 | 1.2±1.3 | 1.3±2.4 |
| 3 | 30.7±29.9 | 0.1±0.2 | 3.3±4.4 | 7.8±10.8 |
| 4 | 15.6±9.1 | 0.5±0.4 | 1.0±0.8 | 0.8±1.1 |
| 5 | 24.7±17.1 | 0.5±0.4 | 2.5±2.4 | 2.6±4.8 |

Values are means ± SD. * $P = 0.005$; † $P = 0.0005$.

TABLE 4. *Correlation coefficients for relationship between BAL cellularity and both demographic and clinical continuous factors for patients with IPF*

| | Bronchoalveolar Lavage Cellularity, (cells/ml) ×$10^4$ | | | |
|---|---|---|---|---|
| | Macrophages | Lymphocytes | Neutrophils | Eosinophils |
| Age | −0.43† | 0.01 | 0.10 | −0.33† |
| Pack-years | 0.18 | 0.11 | 0.23* | 0.32† |
| Pulmonary function | | | | |
| $FEV_1$ | −0.14 | −0.13 | −0.25* | −0.20 |
| FVC | −0.01 | −0.10 | −0.17 | −0.11 |
| $FEV_1$/FVC | −0.15 | −0.06 | −0.11 | −0.15 |
| TLC | 0.14 | −0.16 | 0.01 | −0.06 |
| RV | 0.22 | −0.04 | 0.04 | −0.07 |
| $DL_{CO}$ | 0.12 | −0.02 | −0.15 | 0.03 |
| A-a $O_2$ difference | −0.10 | 0.14 | 0.23 | 0.07 |

Percent predicted values are used for forced expired volume in 1 s ($FEV_1$), forced vital capacity (FVC), total lung capacity (TLC), residual volume (RV), and pulmonary diffusing capacity for CO ($DL_{CO}$). Absolute values are used for $FEV_1$/FVC and alveolar-arterial (A-a) $O_2$ difference. * $P \leq 0.05$; † $P \leq 0.005$.

TABLE 5. *Multivariate linear regression for relationship between BAL cellularity and both demographic and clinical variables in patients with IPF*

| | Macrophages | | Neutrophils | | Eosinophils | |
|---|---|---|---|---|---|---|
| | Coefficient ± SE | *P* value | Coefficient ± SE | *P* value | Coefficient ± SE | *P* value |
| Age, yr | 0.007±0.003 | 0.02 | | | 0.01±0.006 | 0.02 |
| Pack-years of smoking | | | 0.02±0.006 | 0.02 | 0.008±0.003 | 0.02 |
| Current smoker | 0.43±0.14 | 0.003 | | | | |
| $FEV_1$ | | | 0.02±0.007 | 0.001 | | |
| Model $r^2$ | 27.1 | | 19.7 | | 19.0 | |

Values are means ± SD.

in BAL fluid were found not to be related to any of the demographic or clinical variables, including smoking status, immunosuppressive therapy, and measures of pulmonary function. The neutrophil content of BAL fluid was directly related to total pack-years of smoking ($r = 0.23$; $P = 0.05$) and inversely related to the percent predicted $FEV_1$ ($r = -0.25$; $P = 0.03$). The eosinophil content was found to be strongly associated with smoking history ($P = 0.005$), pack-years of cigarette smoking($r = 0.32$; $P = 0.006$), and age ($r = -0.33$; $P = 0.002$). Again, no clear association was observed between either BAL neutrophil or eosinophil content and the concurrent use of immunosuppressive agents.

Next, we utilized linear multivariate regression to assess the independence of the observed relationship between cigarette smoking (either smoking history or pack-years) and the BAL cellularity among individuals with IPF. For each of our measures of BAL cellularity (macrophages, neutrophils, and eosinophils), we performed a logarithmic transformation to account for the nonnormal distribution of these measures. The lymphocyte content of the BAL fluid was not analyzed further because no clear relationship was identified with either cigarette smoking or any of the other clinical variables. These multivariate analyses (Table 5) indicate that current cigarette smokers are likely to have higher numbers of alveolar macrophages in BAL fluid. In addition, pack-years of smoking were found to be directly related to the absolute numbers of neutrophils and eosinophils that were observed in the BAL fluid (Table 5). In fact, after accounting for cigarette smoking, the only factors that were found to be associated with BAL cellularity were age (macrophages and eosinophils) and the percent predicted $FEV_1$ (neutrophils). Additional multivariate models failed to identify a significant relationship between BAL cellularity and the type of therapy. No interactive terms were found to significantly contribute to any of the multivariate models.

## DISCUSSION

Our findings indicate that patients with IPF have elevated concentrations of macrophages, neutrophils, and eosinophils in their BAL fluid and that among patients with IPF, these cells are strongly influenced by cigarette smoking behavior. In contrast, BAL cell content does not appear to be related to immunosuppressive therapy. We conclude that cigarette smoking strongly influences BAL cellularity in patients with IPF and appears to account for at least a portion of the changes in BAL cells that have been previously ascribed to the underlying lung disease.

The results of our study extend the observations of other investigators and identify cigarette smoking as an influential determinant of BAL cellularity among patients with IPF. Although previous studies have not directly examined the effect of cigarette smoking on BAL cell content, higher total BAL cell counts and a high percentage of alveolar macrophages have been reported among patients with IPF who smoke cigarettes (15, 29). However, previous studies have not examined the effect of cigarette smoking on the concentration of cells and cell types in the lavage fluid, nor have they investigated the relationship between pack-years of smoking and BAL cell content. Our results demonstrated a strong consistent relationship between cigarette smoking and the concentration of BAL macrophages, neutrophils, and eosinophils in patients with IPF. Importantly, among patients with IPF, BAL neutrophils (26, 30) and eosinophils (15, 23) appear to be directly associated with abnormal lung function and a higher likelihood of disease progression. The most striking association with cigarette smoking was an increase in BAL eosinophils. Increased numbers of eosinophils in BAL have been strongly related to prognosis in patients with IPF and are thought to have an important role in the pathogenesis of this disease (15, 23). Thus the association of these poor prognosticators with cigarette smoking implies that cigarette smoking may either adversely affect the prognosis in IPF or potentiate the chronic inflammatory process in this disease.

Our findings suggest that cigarette smoking may influence the pathogenesis of IPF. Epidemiological studies have demonstrated that IPF appears to be a male-dominated disease with a mean age of onset of 50–60 yr (6). Moreover, cigarette smoking is reported in 60–70% of the patients with IPF (6), which is higher than one would expect in the general population. In fact, our study population confirms these findings: mean age of 63 yr, 61% males, and 70% either former or current smokers. Cigarette smoke has been shown to profoundly alter the constituents and function of inflammatory cells in the alveoli. In addition to excess macrophages and neutrophils (3), BAL cells and fluid from cigarette smokers have clear defects in their phagocytic ability (14), the metabolism of arachidonic acid (18), the balance of protease-antiprotease enzymes (11), the regulation of immunoglobulin production (21), and the ability to regulate

the production fibronectin (28). Similarly, among patients with IPF, alveolar macrophages have been shown to spontaneously produce growth factors (4), fibronectin (24), chemotactic factors (16), and oxygen radicals (25). Although the inflammatory changes in the alveoli are clearly different in patients with IPF and individuals who smoke cigarettes, there exists enough overlap to suggest that exposure to cigarette smoke may have a role in initiating or "fueling" the chronic inflammatory process of IPF.

Previous studies indicate that exposure to cigarette smoke can cause subradiographic interstitial fibrosis and may markedly alter the effect of other fibrogenic agents. Auerbach et al. (2) have demonstrated a strong relationship between histological evidence of interstitial fibrosis and both increasing age and cigarette smoking. However, in the absence of fibrogenic dust inhalation, the development of radiographic evidence of interstitial fibrosis associated with cigarette smoking remains controversial. Cigarette smoke does, however, appear to enhance the development of interstitial fibrosis in workers exposed to asbestos (5). Similarly, patients with rheumatoid arthritis who smoke cigarettes appear to be at higher risk of developing rheumatoid lung disease (10). Moreover, in addition to the high prevalence of smoking in IPF (6), the prevalence of cigarette smoking is higher than expected in patients with histiocytosis X (13). Potential mechanisms accounting for the development of interstitial fibrosis among cigarette smokers include altered regulation and abnormal function of inflammatory cells in the lung parenchyma (11, 14, 18, 28), reduced clearance of inhaled agents (20), and enhanced permeability of airway epithelia (19). Because clinically significant pulmonary fibrosis is not seen in all individuals who smoke cigarettes, we postulate that this exposure potentially acts as a cofactor in either the development or progression of this disease.

The alternate hypothesis also needs to be considered. The inflammatory changes that we have found related to cigarette smoking may not have a role in the pathogenesis of IPF. Smokers with IPF may have two inflammatory processes that are not interactive and do not effect either the response to immunosuppression or the overall prognosis. Moreover, the location of these inflammatory lesions may be quite distinct with cigarette smoke primarily affecting the airways and IPF localized in the alveoli and interstitium. Walters et al. (29) have recently demonstrated that smokers with IPF who are treated with corticosteroids have an increase in their BAL neutrophils while nonsmokers with IPF decrease the percentage of BAL neutrophils after corticosteroid therapy. Although these changes would suggest that smokers are at higher risk of disease progression, these investigators found that the interval changes in BAL neutrophils were unrelated to either the severity or progression of the lung disease (29). Thus the inflammatory changes associated with cigarette smoking clearly influence the BAL cell content but may have very little to do with the pathogenesis of IPF.

Our findings indicate that cigarette smoking influences the inflammatory changes in the alveoli and possibly the airways of patients with IPF. These findings suggest, but do not prove, that smoking may contribute to the development and/or progression of this disease. However, well-designed prospective studies are needed to pursue this hypothesis. In the most limited context, our results clearly identify cigarette smoking as a potential confounding factor that needs to be considered when clinical features and pathogenic mechanisms are evaluated in patients with IPF.

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