Detection of Alveolar Fibrocytes in Idiopathic Pulmonary Fibrosis and Systemic Sclerosis

Raphael Borie1,2,9, Christophe Quesnel2,3,9, Sophie Phin4, Marie-Pierre Debray4, Joelle Marchal-Somme2, Kiet Tiev5, Marcel Bonay2,6,7, Aurélie Fabre2,7,8, Paul Soler2, Monique Dehoux2,9, Bruno Crestani1,2,7,9

1 Assistance Publique-Hôpitaux de Paris, Service de Pneumologie A, Centre de Compétences Maladies Rares Pulmonaires, Hôpital Bichat, Paris, France, 2 INSERM, Unité 700, Faculté Bichat, Université Paris 7, Paris, France, 3 Assistance Publique-Hôpitaux de Paris, Service d’Anesthésie et de Réanimation Chirurgicale, Hôpital Tenon, Paris, France, 4 Assistance Publique-Hôpitaux de Paris, Service de Radiologie, Hôpital Bichat, Paris, France, 5 Assistance Publique-Hôpitaux de Paris, Service de Médecine Interne, Hôpital Saint Antoine, Paris, France, 6 Assistance Publique-Hôpitaux de Paris, Service d’Explorations Fonctionnelles, Hôpital Saint Antoine, Paris, France, 7 Université Paris Diderot Paris 7, PRES Sorbonne Paris Cité, Paris, France, 8 Assistance Publique-Hôpitaux de Paris, Service d’Anatomopathologie, Hôpital Bichat, Paris, France, 9 Assistance Publique-Hôpitaux de Paris, Service de Biochimie, Hôpital Bichat, Paris, France

Abstract

Background: Fibrocytes are circulating precursors for fibroblasts. Blood fibrocytes are increased in patients with idiopathic pulmonary fibrosis (IPF). The aim of this study was to determine whether alveolar fibrocytes are detected in bronchoalveolar lavage (BAL), to identify their prognostic value, and their potential association with culture of fibroblasts from BAL.

Methods: We quantified fibrocytes in BAL from 26 patients with IPF, 9 patients with Systemic Sclerosis (SSc)-interstitial lung disease (ILD), and 11 controls. BAL cells were cultured to isolate alveolar fibroblasts.

Results: Fibrocytes were detected in BAL in 14/26 IPF (54%) and 5/9 SSc patients (55%), and never in controls. Fibrocytes were in median 2.5% [0.4–19.7] and 3.0% [2.7–3.7] of BAL cells in IPF and SSc-ILD patients respectively. In IPF patients, the number of alveolar fibrocytes was correlated with the number of alveolar macrophages and was associated with a less severe disease but not with a better outcome. Fibroblasts were cultured from BAL in 12/26 IPF (46%), 5/9 SSc-ILD (65%) and never in controls. The detection of BAL fibrocytes did not predict a positive culture of fibroblasts.

Conclusion: Fibrocytes were detected in BAL fluid in about half of the patients with IPF and SSc-ILD. Their number was associated with less severe disease in IPF patients and did not associate with the capacity to grow fibroblasts from BAL fluid.

Introduction

Lung fibrosis is a common trait of different diseases with different etiologies and different pathophysiological pathways. Lung fibrotic disorders are characterized by accumulation of fibroblasts, myofibroblasts and extra-cellular matrix leading to chronic respiratory failure. The origin of the fibroblasts remains debated and is probably multiple, the respective role of the proliferation of resident fibroblasts or mesenchymal precursors, of the transition of epithelial, endothelial or mesothelial cells toward a mesenchymal phenotype, or the recruitment to the lung of circulating blood mesenchymal precursors is debated [1,2].

In experimental models of acute lung injury (ALI), bone marrow derived cells have been shown to be recruited to the lung and to contribute to normal and pathologic repair [3]. Among these cells, fibrocytes are mesenchymal progenitors derived from hematopoietic precursors, which co-express leukocyte (CD45+) and fibroblast markers (collagen1+) [4]. Fibrocytes are implicated in a wide variety of focal and diffuse remodeling disorders [5], in the skin, lung, liver, kidney, pancreas or in atherosclerosis [6]. The fibrocytes, and their CD14 positive mononuclear precursors [6], are recruited from the blood to organs through multiple chemokine signaling pathways particularly including the CCL2/CCR2 and the CXCL12/CXCR4 pathways [6]. In murine models of ALI, the inhibition of fibrocyte recruitment [7] or the blocking of their differentiation from precursors [8] has been associated with a dramatic limitation of lung fibrosis development. In the blood, fibrocytes could represent almost 1% of the nucleated cells in healthy subjects, 2.7% in stable idiopathic pulmonary fibrosis (IPF) and 14.5% in acute IPF exacerbation (AE) [2]. In patients with IPF, an increased number of circulating fibrocytes has been associated with poor outcome [2]. Fibrocytes have been implicated in the pathophysiology of other fibrotic lung disorders, such as interstitial lung disease associated with systemic...
sclerosis (SSc-ILD), where fibrocytes have been shown to be increased in the peripheral blood and to accumulate in the lung [9]. In patients with acute lung injury (ALI), we described that fibrocytes could be detected in bronchoalveolar lavage (BAL) in almost all patients and that BAL fibrocyte percentage was independently associated with increased mortality [10].

We asked whether fibrocytes could be recruited to the alveolar space in IPF and SSc-ILD and if the detection of fibrocytes in BAL would indicate a worse prognosis in those patients. Furthermore, we determined whether fibrocytes detection was associated with the capacity to grow fibroblasts from BAL.

Patients and Methods

Ethics Statement

The study was approved by the Institutional Review Board of the French learned society for respiratory medicine -Société de Pneumologie de Langue Française. Oral informed consent was obtained from the participants to the study at the time of bronchoscopy and recorded in the clinical charts. Clinical and functional data were collected on a standardized and anonymous collection form.

Methods

A BAL was performed for a diagnostic purpose when a diagnosis of IPF or SSc-ILD was suspected from March 2006 to August 2008, with standard procedures [11]. When enough material was available, BAL was used for research purpose and processed as previously described [11]. Patients fulfilled the 2000 ATS/ERS criteria for diagnosis of IPF (with surgical lung biopsy in 6 patients), and retrospectively fulfilled the 2011 criteria [12,13]. SSc was defined according to international criteria [14]; an ILD was based on the chest CT-scan. The control group included patients without CT-scan evidence of ILD who required BAL. A BAL was performed for a diagnostic purpose when a diagnosis of IPF or SSc-ILD was suspected from March 2006 to August 2008, with standard procedures [11]. Patients fulfilled the 2000 ATS/ERS criteria for diagnosis of IPF (with surgical lung biopsy in 6 patients), and retrospectively fulfilled the 2011 criteria [12,13]. SSc was defined according to international criteria [14]; an ILD was based on the chest CT-scan. The control group included patients without CT-scan evidence of ILD who required BAL.

Pulmonary hypertension was defined by PAPs>40 mm Hg assessed by echocardiography. CT-scan scoring of ground-glass opacities and fibrosis was made according to Kazerooni score [15]. AE of IPF was defined according to the Collard criteria [16]. Progression of fibrosis was defined by a decrease of vital capacity of more than 10% or a decrease of DLCO of more than 15% after exclusion of an AE [17].

BAL processing and flow cytometry analysis.

The BAL was performed and processed as previously described [11]. A differential BAL cell count was performed on a cytospin smear with a Diff quik stain kit (Dade International, Miami, USA). Nine of them were smokers. In all cases bronchoscopy did not display any abnormality.

Chemokines measurements in BAL. CCL2 and CXCL12 concentrations were measured in BAL fluid supernatant by ELISA (Quantikine and DuoSets Kits, R&D systems, Abingdon,UK). The detection threshold of the assays were 15 and 16 pg/ml for CCL2 (Quantikine and Duoset Kits, R&D systems, Abingdon,UK). The detection threshold of the assays were 15 and 16 pg/ml for CCL2 and CXCL12 respectively. When a chemokine concentration was below the detection level of the assay, it was attributed a value of 0.

Statistical Analysis

As the distribution of the % of fibrocytes in the population studied was not normal, and as the number of patients studied in every group was below 30, non parametric tests were used for statistical analysis. Continuous variables were expressed as the median (range) and compared by the Mann-Whitney U test. Categorical variables were expressed as counts and proportions and compared by the Fisher’s exact test. Correlations were assessed with the Spearman correlation test Survival was assessed using the Kaplan–Meier estimator. All tests were two-sided, with

Alveolar Fibrocytes and Fibrosis
p<0.05 indicating statistical significance. All the analyses involved use of the software GraphPad Prism.

Results

Patient Characteristics
Twenty-six patients with IPF, 9 patients with SSc-ILD (cutaneous involvement was diffuse in 8 patients and limited in one) and 11 control patients were included (Table 1 and 2). The HRCT pattern was highly suggestive of nonspecific interstitial pneumonia in 8/9 SSc patients, and suggestive of usual interstitial pneumonia in one SSc patient only. Mean follow-up was 22 months [0–53]. At the end of follow-up, 10 IPF and one SSc patients were deceased, one SSc patient had a lung transplant, 5 patients with IPF and 2 patients with SSc presented a slow progression of fibrosis. The other patients were stable.

Detection of Fibrocytes in BAL
Alveolar fibrocytes, defined as cells expressing CD45 and collagen 1, were detected in 14/26 patients in the IPF group (53%), in 5/9 patients in the SSc-ILD group (55%) and in 0/11 controls (p = 0.006 and p = 0.02 versus controls, respectively). Figure 1 shows a typical image of FACS analysis for one IPF patient and one control. Alveolar fibrocytes were in median 5240/ml [370–29500] and 9700/ml [4815–13650] representing 0.67% [0.4–19.7] and 2.73% [0–3.9] of the BAL cells, in IPF and SSc-ILD patients respectively (Figure 2). In 3 patients, the BAL was performed at the time of an acute exacerbation of IPF. In those patients, fibrocytes were detected in one BAL (8177 fibrocytes/ml) and were absent in two BAL. In view of the limited number of SSc BAL evaluated, further analyses were limited to IPF patients.

In IPF patients, the number of fibrocytes in BAL positively correlated with BAL macrophage counts (p = 0.0006 and p = 0.02, respectively). The number of BAL fibrocytes did not correlate with age, tobacco use, time since diagnosis, quantitative quotation of ground glass opacities, lung volumes or outcome assessed by the risk of progression, AE, transplantation or death. However, an increased number of BAL fibrocytes was associated with higher DLCO (p = 0.01, r = 0.5), higher arterial blood PaO2 at rest (p = 0.08, r = 0.33) and a lower lung CT-scan score of fibrosis (p = 0.04, r = 0.4). The number of alveolar fibrocytes did not correlate with the alveolar concentration of CCL2 and CXCL12 (table 2).

Evolution of BAL fibrocytes counts over time. We had the opportunity to study the evolution of BAL fibrocytes count in three IPF patients who required a second BAL procedure (respectively 8, 95 and 337 days after the first BAL), and in one IPF patient who required two BAL procedures (respectively 260 and 642 days after the first BAL). In all cases, the BAL were performed for a clinical purpose. Alveolar fibrocytes were detectable in the first BAL in 2/3 patients, diminished in the second and were absent in two BAL. In view of the number of BAL fibrocytes did not correlate with the alveolar concentration of CCL2 and CXCL12 (table 2).

Fibroblast culture from BAL. The morphological and immunohistochemical characteristics of cells grown from BAL are shown in Figure 5. The cells were spindle shaped and expressed collagen 1, vimentin and prolly-4-hydroxylase, without expression of desmin, pancytokeratin, CD14, CD31, or CD34. After 28 days of culture, fibroblasts grew from 0/9 of the BAL control cultures (two cultures were stopped because of infection), 12/26 BAL of the IPF patients (P = 0.014 versus controls) and 5/9 of the SSc-ILD patients (P = 0.03 versus controls). Fibroblasts grew from 2/3 BAL performed in patients with AE.

Table 1. Clinical and functional characteristics of the patients.

|                     | IPF       | SSc-ILD   | Controls |
|---------------------|-----------|-----------|----------|
| **Age (years)**     | 74 [56–84]| 62 [46–72]*| 68 [34–79]*|
| **Gender (Male)**   | 19 (73%)  | 4 (44%)   | 9 (81%)  |
| **Smoker (ever)**   | 16 (61%)  | 2 (22%)   | 9 (81%)  |
| **Delay since diagnosis (days)** | 17.5 [0–655] | 146 [5–525] | na |
| **Oral Steroids**   | 6 (23%)   | 1 (11%)   | 0        |
| **Dose (mg)**       | 8.7 [5–40]| 2 (22%)   | 9 (81%)  |
| **Exacerbation**    | 3 (11%)   | 0         | na       |
| **Progression**     | 13 (48%)  | 0         | na       |
| **Lung Function Tests** |            |           |          |
| TLC (% pred)        | 68 [28–105]| 56 [35–94] | 88 [50–118]*|
| VC (% pred)         | 75 [34–108]| 57 [29–134] | 102 [52–109]|
| DLCO (% pred)       | 35 [15–91]| 33 [16–85] | Not available |
| PaO2 (mmHg)         | 76 [45–101]| 90 [39–99] | Not available |
| Pulmonary hypertension (N) | 3 (11%) | 3 (33%) | 0 |

Data are expressed as median [range] or N (%); na: not applicable. *p<0.05. doi:10.1371/journal.pone.0053736.t001

Table 2. Bronchoalveolar lavage results.

|                     | IPF N = 26 | SSc-ILD N = 9 | Controls N = 11 |
|---------------------|------------|---------------|-----------------|
| **Cellularity** (x10^5/ml) | 150 [72–1000] | 310 [48–450] | 180 [30–750] |
| **Macrophages (%)** | 80 [37–95] | 88 [63–92] | 86 [70–94] |
| **Lymphocytes (%)** | 6 [0–20] | 9 [2–26] | 10 [1–22] |
| **Neutrophils (%)** | 10 [2–90] | 6 [0–10] | 4 [1–17] |
| **Eosinophils (%)** | 2 [0–17] | 1 [0–10] | 0 [0–6] |
| **Fibrocytes detection** | 14 (53%) | 5 (55%) | 0 (0%)* |
| **Fibrocytes/ml** | 5240 [370–29500] | 9700 [4815–13650] | 0* |
| **CCL2 (pg/ml)** | 137 [0–1033] | 212 [63–315] | 37 [14–123] |
| **CXCL12 (pg/ml)** | 0 [0–23] | 0 [0–22] | 0 [0–0] |
| **Fibroblast culture (+)** | 12 (46%) | 5 (55%) | 0 (0%)* |

Data are expressed as median [range] or N (%). *p<0.05. doi:10.1371/journal.pone.0053736.t002
P = 0.43). The positivity of fibroblast culture was associated with an increased concentration of CCL2 in BAL fluid at 262 pg/ml [102–1033] vs 64 pg/ml [0–341] in negative cultures, but not with CXCL12.

Discussion

This study demonstrates that fibrocytes are detected in BAL in almost 50% of IPF or SSc-ILD patients whereas these cells are never detected in controls and were in median 5240/ml and 9700/ml in IPF and SSc patients respectively.

Figure 1. Representative flow cytometric analysis of bronchoalveolar lavage cells from a patient with idiopathic pulmonary fibrosis (IPF) and from a control patient. Flow cytometry was used to determine the percentage of fibrocytes. A) Cells were stained for CD45 and collagen 1, and analyzed by flow cytometry. Isotype control staining of cells is shown as red histograms and CD45 or collagen 1 as blue histograms. The negative threshold for all markers was set using the matched IgG isotype control. B) Sample analysis from one patient with IPF. In the left panel, forward scatter/side scatter (FSC/SSC) dot-plot, unstained cells were gated to exclude debris. In the middle panel, CD45+ cells were gated. In the right panel, CD45+ collagen 1+ cells were gated. C) Sample analysis from one control patient. In the left panel, forward scatter/side scatter (FSC/SSC) dot-plot, unstained cells were gated to exclude dead cells. In the middle panel, CD45+ cells were gated. In the right panel, CD45+ collagen 1+ cells were gated. The shape of the gate was chosen to account for autofluorescence. Fluorescein isothiocyanate, FITC; allophycocyanin, APC; forward scatter, FSC-H; side scatter, SSC-H.

doi:10.1371/journal.pone.0053736.g001

Figure 2. Number of fibrocytes in bronchoalveolar lavage in idiopathic pulmonary fibrosis (IPF), Scleroderma patients with interstitial lung disease (SSc) and in controls. Fibrocytes were never detected in controls and were in median 5240/ml and 9700/ml in IPF and SSc patients respectively.

doi:10.1371/journal.pone.0053736.g002

Figure 3. Kaplan-Meier curve of time to death in IPF patients with (solid line) or without (dotted line) alveolar fibrocytes. Survival did not differ between the 2 groups.

doi:10.1371/journal.pone.0053736.g003
this is the first study to identify fibrocytes in the alveolar milieu in patients with fibrotic lung disorders. Fibrocytes are monocyte-derived cells that have features of macrophages and fibroblasts [19]. Increased numbers of circulating fibrocytes have been previously reported in patients with IPF or SSc [2,20] [21] and in the lung of IPF and SSc patients [22] [9]. Andersson-Sjoland and colleagues showed that fibrocytes could be detected in 8/9 IPF lung samples studied and that the number of fibrocytes in lung tissue correlated with the number of fibroblastic foci, suggesting that the number of fibrocytes was an index of the ongoing local fibrotic process [22]. The detection of fibrocytes in BAL fluid could be a minimally invasive way to assess the number of fibrocytes in lung tissue and the severity of fibrogenic process. However, we detected fibrocytes in BAL fluid only in 50% of the patients studied and the presence of fibrocytes in BAL fluid did not associate with the burden of fibrotic tissue as assessed by CT-scan nor with the severity of the disease as assessed by lung function tests results and blood gas abnormalities. In fact we observed the opposite, since higher numbers of fibrocytes were associated with better FVC, better DLCO and lower fibrotic score on HRCT in the patients where fibrocytes could be detected. This observation is surprising as most of the literature available associates circulating fibrocytes numbers with worse prognosis particularly in humans [2,7,8,10,23]. Interestingly, Moeller and colleagues observed that the number of circulating fibrocytes did associate with a reduced survival in a population of IPF patients, whereas they found no correlation between circulating fibrocyte counts and lung function parameters (FVC, TLC, DLCO), 6 minute walking distance or HRCT scores [2]. Our interpretation is that fibrocytes count and differentiation depends on the compartment studied (blood, tissue or BAL for instance) as the differentiation of monocytes to fibrocytes and from fibrocytes to fibroblasts depends greatly on the local microenvironment [24].

The origin of BAL fibrocytes is not unequivocal. Circulating fibrocytes could directly be attracted to the alveolar space because of the local expression of chemokines for fibrocytes (such as CCL2 or CXCL12) already demonstrated in lung fibrosis [22,25]. It is worth noting that Andersson-Sjoland and colleagues measured CXCL12 levels in BAL from 20 IPF patients and 5 controls using the Quantikine assay (R&D systems). They found that CXCL12 was never detected in BAL from controls whereas it was detected in 8 out of 20 (40%) IPF BAL samples. In our hands, CXCL12 was detected only in 1/14 samples analyzed using an assay of similar sensitivity (Quantikine and Dusset Kits, R&D systems). We do not have a clear explanation for this discrepancy. Degradation of the cytokine during the BAL storage is unlikely as we systematically added aprotinin, a broad spectrum serine protease inhibitor, to BAL supernatants.

It is also probable that blood monocytes penetrating the alveolar space in patients with ongoing lung fibrosis could differentiate into fibrocytes, since profibrotic mediators such as IL-4, IL-13, TGF-β and endothelin-1 are present in the fibrotic alveolar milieu and have been shown to induce the differentiation of monocytes to fibrocytes in vitro [19]. According to their origin, it is highly probable that the differentiation markers expressed by fibrocytes could differ. Furthermore, in vitro experiments have shown that fibrocytes may change their differentiation markers with time [26].

We observed that the number of alveolar fibrocytes was positively correlated with the number of alveolar macrophages and negatively correlated with the number of alveolar neutrophils. As most fibrocytes arise from the differentiation of blood monocytes in tissue, the correlation with monocyte/macrophage count was not unexpected, as we observed the same correlation in patients with ALI/ARDS [10]. The negative correlation between the number of alveolar fibrocytes and the number of alveolar

### Table 3. Clinical and functional characteristics of the IPF patients according to the detection of fibrocytes in BAL.

|                | Fibrocytes (+) | Fibrocytes (−) | P value |
|----------------|----------------|----------------|---------|
| Number of patients (%) | 14 (54%)       | 12 (46%)       |         |
| Age                | 73 [56–83]     | 75 [65–84]     | 0.49    |
| Gender (Male)      | 12 (85%)       | 7 (58%)        | 0.12    |
| Smoker (ever)      | 9 (64%)        | 7 (58%)        | 1       |
| Exacerbation       | 1 (7%)         | 2 (28%)        | 0.58    |
| Delay since diagnosis (days) | 13 [0–655] | 17 [0–250] | 0.45 |
| Oral steroids      | 3 (21%)        | 3 (25%)        | 1       |
| Death or lung transplant | 6           | 4              | 0.7     |
| Pulmonary Hypertension (%) | 0           | 3              | 0.08    |
| HRCT fibrosis score | 8 [4–14]      | 8 [6–13]       | 0.98    |
| Fibrocyte count/ml | 5240 [369–29590] | 0 [0–0] |       |
| CCL2 (pg/ml) | 106 [0–350] | 182 [11–1033] | 0.14 |
| CXCL12 (pg/ml) | 0 [0–0] | 0 [0–22.7] | 0.37 |
| Lung function tests |                 |                |         |
| VC (% pred)       | 79 [34–108]    | 70 [40–96]     | 0.7     |
| DLCO (% pred)     | 45 [15–91]     | 45 [58–73]     | 0.06    |
| PaO2 (mm Hg)      | 78 [45–101]    | 76 [59–92]     | 0.48    |

Data are expressed as median [range] or N (%). doi:10.1371/journal.pone.0053736.t003

Figure 4. Evolution of the number of alveolar fibrocytes in 4 patients with IPF where bronchoalveolar lavage fluid was obtained at two or more occasions. Alveolar fibrocytes were no longer detectable in the 2 patients with initial detectable alveolar fibrocytes. In the 2 patients without detectable alveolar fibrocytes at initial evaluation, fibrocytes remained undetectable in subsequent bronchoalveolar lavage fluid analysis. doi:10.1371/journal.pone.0053736.g004
neutrophils could simply reflect the fact that the more macrophages you have, the less neutrophils you have. However, neutrophils could also modulate monocyte to fibrocyte differentiation or fibrocyte recruitment. This has not been specifically studied before but it is scientifically plausible as neutrophil elastase has been shown to cleave both CXCL12 and its receptor CXCR4 [27] and to deactivate endothelial bound CXCL12 and inhibit the subsequent T lymphocytes transendothelial migration [28].

We observed that the number of alveolar fibrocytes inversely correlated with the severity of lung disease as assessed by DLCO, score of fibrosis and PaO2. Most importantly, fibrocytes counts were not increased in patients with AE of IPF, and when BAL were repeated we observed a decrease of the number of alveolar fibrocytes. Altogether, these observations suggest that alveolar fibrocytes expressing CD45 and collagen-1 could be a marker of early fibrotic disease, and that the differentiation of fibrocytes evolve with time.

Fibrocytes differentiate into fibroblast in vitro when appropriately stimulated [19]. There is a debate as to whether fibrocytes can differentiate toward a myofibroblastic phenotype. In this study, we observed that the capacity to grow fibroblasts from BAL did not correlate with the number of alveolar fibrocytes. Other fibroblast precursor are present in BAL, such as local mesenchymal stem cells. Lama and colleagues showed that BAL fibroblast which grew from lung transplant patients were of donor origin, thus demonstrating the role of the local mesenchymal cell precursors in bronchiolitis obliterans due to lung transplant [29]. Other investigators have already shown that fibroblast could be cultured from BAL of IPF and SSc-ILD patients, with negative cultures from control BAL [30]. Surprisingly, although fibroblast could be cultured from almost 50% of the BAL from IPF or SSc-ILD patients and never from control patients, we did not evidence any difference between groups with or without positive cultures.

Our study has some limitations. As we did not collect blood at the time of BAL, we were not able to measure circulating fibrocytes although it would be interesting to compare circulating and alveolar fibrocytes in the same patient. The second limit is the relatively low number of patients included.

In summary, we were able to detect fibrocytes in BAL fluid in about half of the patients with IPF and SSc-ILD. Fibrocytes
Table 4. Clinical and functional characteristics of the IPF patients with positive or negative cultures of fibroblasts.

|                          | Fibroblasts (+) | Fibroblasts (−) | P value |
|--------------------------|-----------------|-----------------|---------|
| Number of patients (%)   | 12 (46%)        | 14 (54%)        |         |
| Age                      | 75 [66–84]      | 72 [56–84]      | 0.4     |
| Gender (Male)            | 7 (58)          | 12 (72)         | 0.06    |
| Smoker (ever)            | 8 (66)          | 8 (57)          | 0.7     |
| Exacerbation             | 2               | 1               | 0.58    |
| Delay since diagnosis (days) | 18 [0–236]  | 7 [0–655]       | 0.74    |
| Death or lung transplant during follow up | 4 (33) | 4 (42) | 1 |
| Oral steroids            | 4 (33)          | 2 (14)          | 0.36    |
| CCL2 (pg/ml)             | 269 [102–1033]  | 64 [0–341]      | 0.02    |
| CXCL12 (pg/ml)           | 0 [0–22.7]      | 0 [0–0]         | 0.54    |
| PaO2 (mm Hg)             | 76 [50–101]     | 77 [45–91]      | 0.8     |
| VC (% pred)              | 79 [40–101]     | 67 [34–108]     | 0.57    |
| DLCO (% pred)            | 35 [15–59]      | 35 [27–91]      | 0.36    |
| Pulmonary Hypertension   | 1               | 2               | 1       |
| HRCT fibrosis score      | 8 [4–13]        | 8 [6–14]        | 0.56    |
| Detection of Fibrocytes in BAL | 5 (41)  | 9 (64)          | 0.43    |

Data are expressed as median [range] or N (%). doi:10.1371/journal.pone.0053736.t004

detection was not associated with the capacity to grow fibroblast from BAL fluid. Further studies are needed to investigate the role of alveolar fibrocytes and their interaction with circulating fibrocytes.

Acknowledgments

We thank Prof. Gabriel Thabut for his helpful advice concerning statistical analysis.

References

1. Lama VN, Phan SH (2006) The extrapulmonary origin of fibroblasts: stem/progenitor cells and beyond. Proc Am Thorac Soc 3: 373–376.
2. Moeller A, Gilpin SE, Ask K, Cox G, Cook D, et al. (2009) Circulating fibrocytes are an indicator of poor prognosis in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 179: 588–594.
3. Hashimot N, Jin H, Liu T, Cherna SW, Phan SH (2004) Bone marrow-derived progenitor cells in pulmonary fibrosis. J Clin Invest 113: 243–252.
4. Bucala R, Spiegel LA, Chenes J, Hogan M, Gerzami A (1994) Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol Med 1: 71–81.
5. Metz CN (2003) Fibrocytes: a unique cell population implicated in wound healing. Cell Mol Life Sci 60: 1342–1350.
6. Mattoli S, Bellini A, Schmidt M (2009) The Role of a Human Hematopoietic Mesenchymal Progenitor in Wound Healing and Fibrotic Diseases and Implications for Therapy. Curr Stem Cell Res Ther: 4: 266–80.
7. Moore BB, Kolodziej J, Thanickial VJ, Cooke K, Moore TA, et al. (2005) CCR2-mediated recruitment of fibrocytes to the alveolar space after fibrotic injury. Am J Pathol 166: 675–684.
8. Pilling D, Roile D, Wang M, Ronkainen SD, Crawford JR, et al. (2007) Reduction of bleomycin-induced pulmonary fibrosis by serum amyloid P. J Immunol 179: 4035–4044.
9. Tourkina E, Bonner M, Oates J, Hofbauer A, Richard M, et al. (2011) Altered monocyte and fibrocyte phenotype and function in scleroderma interstitial lung disease: reversal by caveolin-1 scaffolding domain peptide. Fibrogenesis Tissue Repair 4: 15.
10. Quesnel C, Nardelli L, Piednoir P, Leodnoi P, Leodnoi P, et al. (2012) Alveolar fibrocyte percentage is an independent predictor of poor outcome in patients with acute lung injury. Crit Care Med. 40: 21–8.
11. Quesnel C, Marchand-Adam S, Fabre A, Marchal-Somme J, Philip I, et al. (2008) Regulation of hepatocyte growth factor secretion by fibroblasts in patients with acute lung injury. Am J Physiol Lung Cell Mol Physiol 294: L334–343.
12. (2000) American Thoracic Society. Idiopathic pulmonary fibrosis diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med 161: 646–664.
13. Raghur G, Collard HR, Egan JJ, Martinez FJ, Behr J, et al. (2011) An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 183: 708–824.
14. (1980) Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum 23: 581–590.
15. Kazerooni EA, Martinez FJ, Flint A, Jamadar DA, Gross BH, et al. (1997) Thin-section CT obtained at 10-mm increments versus limited three-level thin-section CT for idiopathic pulmonary fibrosis: correlation with pathologic scoring. AJR Am J Roentgenol 169: 977–983.
16. Collard HR, Moore BB, Fubherty KR, Brown KK, Kaner RJ, et al. (2007) Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 176: 636–643.
17. Ley B, Collard HR, King TE, Jr. (2011) Clinical course and prediction of survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 183: 431–440.
18. Quesnel C, Nardelli L, Piednoir P, Leodnoi P, Leodnoi P, et al. (2010) Alveolar fibroblasts in acute lung injury: biological behaviour and clinical relevance. Eur Respir J 35: 1312–1321.
19. Reikoff RA, Batula R, Herzog EL (2011) Fibrocytes: emerging effector cells in chronic inflammation. Nat Rev Immunol 11: 427–435.
20. Fujisawa A, Kobayashi H, Masuya M, Marnyu M, Nakamura S, et al. (2012) Correlation between circulating fibrocytes and activity and progression of interstitial lung diseases. Respirology. 17: 693–8.
21. Gan Y, Reilkoff R, Peng X, Russell T, Chen Q, et al. (2011) Role of semaphorin 7a signaling in transforming growth factor beta-induced lung fibrosis and scleroderma-related interstitial lung disease. Arthritis Rheum 63: 2484–2494.
22. Anderson-Sjoland A, de Alba CG, Nilberg K, Becerril C, Ramirez R, et al. (2008) Fibrocytes are a potential source of lung fibroblasts in idiopathic pulmonary fibrosis. Int J Biochem Cell Biol 40: 2129–2140.

Author Contributions
Conceived and designed the experiments: RB CQ KT PS MD BC. Performed the experiments: RB CQ SP JMS MB AF. Analyzed the data: RB CQ MPD KT PS MD BC. Contributed reagents/materials/analysis tools: RB CQ MPD KT PS MD BC. Wrote the paper: RB CQ MD BC.
23. Mattoli S, Bellini A, Schmidt M (2009) The role of a human hematopoietic mesenchymal progenitor in wound healing and fibrotic diseases and implications for therapy. Curr Stem Cell Res Ther 4: 266–280.
24. Maharaj SS, Baroke E, Gauldie J, Kolb MR (2012) Fibrocytes in chronic lung disease - Facts and controversies. Palm Pharmacol Ther 25: 263–267.
25. Marchal-Somme J, Uzunhan Y, Marchand-Adam S, Kambouchner M, Valeyre D, et al. (2007) Dendritic cells accumulate in human fibrotic interstitial lung disease. Am J Respir Crit Care Med 176: 1007–1014.
26. Medina A, Ghahary A (2010) Fibrocytes can be reprogrammed to promote tissue remodeling capacity of dermal fibroblasts. Mol Cell Biochem 344: 11–21.
27. Valenzuela-Fernandez A, Plancheaulx T, Baleux F, Staropoli I, Le-Barillec K, et al. (2002) Leukocyte elastase negatively regulates Stromal cell-derived factor-1 (SDF-1)/CXCR4 binding and functions by amino-terminal processing of SDF-1 and CXCR4. J Biol Chem 277: 15677–15689.
28. Rao RM, Betz TV, Lamont DJ, Kim MB, Shaw SK, et al. (2004) Elastase release by transmigrating neutrophils deactivates endothelial-bound SDF-1alpha and attenuates subsequent T lymphocyte transendothelial migration. J Exp Med 200: 713–724.
29. Lama VN, Smith L, Badri L, Flint A, Andrei AC, et al. (2007) Evidence for tissue-resident mesenchymal stem cells in human adult lung from studies of transplanted allografts. J Clin Invest 117: 989–996.
30. Fireman E, Shahar I, Shoval S, Meser G, Dvash S, et al. (2001) Morphological and biochemical properties of alveolar fibroblasts in interstitial lung diseases. Lung 179: 105–117.