Published in final edited form as:

Ann Thorac Surg. 2011 August ; 92(2): 470–477. doi:10.1016/j.athoracsur.2011.04.065.

Circulating Fibrocytes Correlate with Bronchiolitis Obliterans Syndrome Development Following Lung Transplantation: A Novel Clinical Biomarker

Damien J. LaPar, MD¹, Marie D. Burdick², Abbas Emaminia, MD¹, David A. Harris, BA¹, Brett A. Strieter², Ling Liu², Mark Robbins, MD², Irving L. Kron, MD¹, Robert M. Strieter, MD^{2,*}, and Christine L. Lau, MD^{1,*}

¹Division of Thoracic and Cardiovascular Surgery, Department of Surgery, University of Virginia School of Medicine, Charlottesville, Virginia 22908, USA

²Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22908, USA

Abstract

Background—Development of bronchiolitis obliterans syndrome (BOS) following lung transplantation confers increased patient morbidity and mortality. Fibrocytes are circulating, bone marrow derived mesenchymal cell progenitors that influence tissue repair and fibrosis. Accumulated evidence has implicated fibrocytes in chronic pulmonary inflammatory processes. We investigated the correlation of circulating fibrocyte number with BOS development in lung transplant patients.

Methods—We prospectively quantified circulating fibrocyte levels among human lung transplant patients. Patients were stratified according to the development of BOS as indicated by predicted forced expiratory volume in one second (FEV₁). Fibrocyte activity was analyzed by flow cytometry (CD45⁺, collagen 1⁺) in a blinded manner related to clinical presentation.

Results—A total of 39 patients previously underwent double (33.3%), left (25.6%), or right (41.0%) lung transplantation. Average patient age was similar between BOS and non-BOS patients (58.3±3.9 vs. 60.3±2.0 years, $p=0.67$). Males accounted for 61.5% of all patients. Chronic obstructive lung disease was the most common indication for lung transplantation (41.0%). Median FEV₁ was lower among BOS patients compared to non-BOS patients (1.08 vs. 2.18 L/sec, $p=0.001$). Importantly, circulating fibrocyte number was increased in BOS patients compared to non-BOS patients (8.91×10^5 vs. 2.96×10^5 cells/ml, $p=0.03$) by flow cytometry and incrementally increased with advancing BOS stage ($P=0.02$).

Conclusions—Increased circulating fibrocyte levels correlate with the development of bronchiolitis obliterans syndrome following lung transplantation. Increasing fibrocyte levels positively correlates with advancing BOS stage. Quantification of circulating fibrocytes could

© 2011 The Society of Thoracic Surgeons. Published by Elsevier Inc. All rights reserved.

Correspondence to: Christine L. Lau, MD, Associate Professor, Department of Surgery, University of Virginia School of Medicine, PO Box 800679, Charlottesville, VA 22908, Phone: (434) 924-8016, Fax: (434) 924-8603, cl2y@virginia.edu.

*Contributed equally to this study as senior authors

Presented at 47th Annual Meeting of the Society of Thoracic Surgeons, San Diego, CA, January 31, 2011.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

serve as a novel biomarker and possible therapeutic target for BOS development in lung transplant patients.

Keywords

Fibrocyte; Bronchiolitis Obliterans; Biomarker; Lung Transplantation

INTRODUCTION

Bronchiolitis obliterans (BO) and its associated clinical manifestation bronchiolitis obliterans syndrome (BOS) is a form of chronic rejection following lung transplantation characterized by irreversible airway obstruction that affects approximately 45–50% of recipients surviving more than 5 years [1, 2]. BOS remains the leading cause of morbidity and mortality following lung transplantation, and is responsible for compromised long-term survival rates of approximately 20–40% [1, 3–6]. Resulting in a progressive loss of airway structure, the pathogenesis of BO is initiated by a peribronchiolar leukocyte infiltration (lymphocytic bronchiolitis) that disrupts the underlying submucosa and epithelial layers of the transplanted airway [7–10]. Violation of airway integrity is subsequently followed by fibroproliferation and accumulation of extracellular matrix (ECM), culminating in the progressive fibroobliteration of the airway [7–10]. While the step-wise progression of BO has been well characterized, the exact mechanisms underlying the fibroproliferative process remain relatively unknown. Moreover, the ability to predict the development of BOS is difficult following lung transplantation due to a lack of universally accepted, objective diagnostic standards or available biomarkers to detect BO/BOS development or progression.

Circulating fibrocytes are a unique form of bone marrow derived mesenchymal cell progenitors that influence tissue repair and fibrosis. Originally described in 1994, circulating fibrocytes are spindle-shaped progenitor cells that are capable of elaborating various components of the ECM [11]. In response to local injury, these cells selectively migrate to injured areas and differentiate in to fibroblast-like cells. The classic cell surface markers for circulating fibrocytes are the stromal marker collagen-1 (Col-1), and the leukocyte marker CD45 (Col-1⁺CD45⁺) [11]. In addition, a variety of other markers have been described including, the hematopoietic stem cell marker CD34 and vimentin [11]. Recently, accumulating evidence has suggested the important role of fibrocytes in chronic pulmonary inflammatory processes [12–15]. However, the presence and/or role of circulating fibrocytes in the development of BOS remain unreported.

The purpose of this study was to test for the presence of circulating fibrocytes, and if present quantify their levels, among lung transplant recipients with and without BOS. We postulated that increased fibrocyte levels among patients with BOS may help to identify a potential biomarker to assist in early BOS diagnosis as well as monitoring for disease progression.

PATIENTS AND METHODS

Patient Recruitment and Enrollment

Patients were recruited and enrolled in this study from the University of Virginia (UVA) lung transplantation clinic (Charlottesville, VA, USA). All study procedures for patient consent, data collection, and privacy protection were approved by the UVA Institutional Review Board (IRB# 14973). The subject enrollment period was from April 25, 2010–June 15, 2010. Eligible patients for enrollment included those with lung transplants at our institution prior to their clinic visit, as well as patients ≥ 18 years of age, males and females, and patients of all insurance types. A total of 39 patients consented for enrollment in the study during the study period. Patient data was stratified with respect to the development of

post-lung transplantation BOS. The diagnosis of BOS was based upon sequential pulmonary function test performance, demonstrating declining forced expiratory volume in one second (FEV₁) in the absence of other identifiable causes [16]. Patients with BOS included those with stages I, II or III, while non-BOS patients included those without a diagnosis of BOS as well as those with “early BOS” (stage 0-p).

Patient Demographics, Transplantation Characteristics, and Operative Features

A retrospective collection of available patient data was performed through review of enrolled patient medical records. During this process, patient demographics, lung transplantation related details, results of pulmonary function tests, and BOS diagnosis and staging were collected. These data were then merged with laboratory sample results to obtain a master study dataset for subsequent data analysis.

Serum Sample Collection, Preparation, and Processing

Among enrolled subjects, a single 10 milliliters (ml) sample of heparinized venous blood was collected via peripheral, upper extremity venipuncture. Patient samples were collected in sodium heparin vacutainer tubes. Samples were immediately refrigerated for < 24 hours until processing for flow cytometry (FCM). Buffy coat leukocyte populations were prepared from fresh blood by centrifugation as previously described [17, 18]. The samples were subsequently incubated twice in ACK lysis buffer to remove contaminating red blood cells. The cells were then washed and prepared to a concentration of 1.0×10^7 /ml in PBS containing 0.1% FBS.

Flow Cytometric Analysis

Human serum samples were further prepared for flow cytometric analysis to quantify circulating fibrocytes, (Figure 1), among lung transplant recipients with and without BOS development as well as differentiated subpopulations in a blinded manner using previously published methods [13, 14]. Briefly, leukocytes were stained with PerCP anti-CD45 (BD Biosciences, San Jose, CA), PE anti-CD34 (BD Biosciences, San Jose, CA), APC anti-CXCR4 (eBioscience, San Diego, CA), and PE-Cy7 anti-CCR7 (BD Biosciences, San Jose, CA) or isotype controls. Cell permeability was then performed using a commercially available kit (Cytofix/Cytoperm, BD Biosciences) prior to intracellular staining of collagen-1 and α SMA. Intracellular collagen-1 and α SMA was stained with FITC conjugated anti-human Col-1 (Rockland Inc., Gilbertsville, PA) and PE anti-human α SMA (R&D Systems) or isotype controls. The rabbit anti-human collagen-1 and its isotype control antibody was conjugated using Dylight 488 conjugation kit (Thermal Scientific). Stained samples were subsequently analyzed using a fluorescence-activated cell sorting (FACS) Canto II flow cytometer with Diva software (BD Biosciences).

Statistical Analysis

All experimental methodology was designed to test the null hypothesis that circulating fibrocyte levels are not significantly different among lung transplant recipients regardless of the development of BOS. The alternative hypothesis was that circulating fibrocyte counts would significantly differ between lung transplant recipients who developed BOS compared to those who did not develop BOS. Statistical significance was set to an $\alpha < 0.05$. Patient demographics, preoperative factors, operative features, and experimental results were compared by univariate analyses. Categorical data are expressed as within group percentages and were compared by either Pearson's χ^2 or Fisher's exact test. Continuous data are presented as either means $\pm$ standard error of the mean (SEM) for normally distributed data or median [interquartile range] for non-normally distributed data. Normally distributed continuous data were compared using analysis of variance (ANOVA). Non-

normally distributed continuous data were compared between two groups using the Mann-Whitney *U* test for non-parametric data, and comparisons between multiple groups were performed using the Kruskal-Wallis test for K independent samples. All group comparisons were unpaired, and reported *p*-values are two tailed. All statistical analyses were performed using Predictive Analytics SoftWare (PASW) Statistics, version 18.0.0 (IBM Corporation, Somers, NY).

RESULTS

Patient Demographics, Transplantation Characteristics, Operative Features, and BOS Development

The frequency of patient demographics, transplantation characteristics and operative features for all enrolled subjects with and without BOS development are listed in Table 1. A total of 39 patients voluntarily consented for enrollment in this study, and serum samples were collected, processed and analyzed for 100% of enrollees. Males accounted for 61.5% (n=24) of all patients, while females accounted for 38.5% (n=15). The distribution of lung transplantations for the cohort included: double lung transplantation (n=13, 33.3%), left lung transplantation (n=10, 25.6%), and right lung transplantation (n=16, 41.0%). Chronic obstructive pulmonary disease (COPD)/emphysema (n=16, 41.0%), idiopathic pulmonary fibrosis (IPF, n=8, 20.5%), and sarcoidosis (n=5, 12.8%) were the three most common indications for lung transplantation. The overall prevalence of BOS within our study population was 25.6%. Among study cohorts, average patient age (58.3 ± 3.9 years vs. 60.3 ± 2.0 years, $p=0.67$) and gender distribution ($p>0.99$) was similar between BOS and non-BOS patients. Median time from transplant to serum sample collection was longer for patients with BOS development (55.1 [85.4] months) compared to non-BOS patients (41.7 [55.5] months); however this difference did not achieve statistical significance ($p=0.05$). Indications for transplantation were also similar between BOS and non-BOS patients. Importantly, none of the patients requiring transplantation for IPF (n=8) developed BOS.

Circulating Fibrocyte Levels Among Patients With and Without BOS Development

Cell counts as measured by quantitative FACS analysis for overall and differentiated fibrocyte cell populations are detailed in Table 2. Overall circulating fibrocyte counts, defined by CD45- and collagen-1- expressing cells (CD45⁺Col-1⁺), were significantly elevated within lung transplant recipients with BOS development compared to recipients without BOS development (Figure 2a, $p=0.03$). Similar trends were observed for circulating levels of differentiated fibrocyte subpopulations: CD45-collagen-1-CXCR4-expressing cells (CD45⁺Col-1⁺CXCR4⁺, Figure 2b $p=0.02$), CD45-collagen-1-, and CCR7-expressing cells (CD45⁺Col-1⁺CCR7⁺, Figure 2c, $p=0.03$), and CD45-collagen-1-CD34-expressing cells (CD45⁺Col-1⁺CD34⁺, Figure 2d, $p=0.03$). Further all other measured counts of differentiated fibrocyte populations were higher among recipients who developed BOS compared to non-BOS recipients; however, these differences failed to reach statistical significance. Interestingly, fibrocyte counts were not significantly different between those undergoing bilateral (564,000 [772,000]) vs. single (399,500 [917,250], $p=0.98$) lung transplantation. Further a total of 8 patients with IPF underwent, 7 single and 1 double lung transplantation. Of these patients, none developed BOS, and the median fibrocyte level for these patients was 385,000 [425,000].

Comparison of Circulating Fibrocyte Levels by BOS Stage

Further stratification of circulating fibrocyte cell levels as a function of advancing BOS stage is depicted in Figure 3. Overall circulating fibrocyte (CD45⁺Col-1⁺) levels incrementally increased with advancing BOS stage ($p=0.02$), while similar trends were observed for the CD45⁺Col-1⁺CXCR4⁺ expressing subset of fibrocytes ($p=0.04$). Moreover,

circulating levels of CD45⁺Col-1⁺αSMA⁺ fibrocytes and CD45⁺Col-1⁺CCR7⁺ fibrocytes were increased in advanced BOS stages; however, these trends failed to reach statistical significance (all $p > 0.05$).

COMMENT

The results of this study demonstrate important biologic differences among lung transplant recipients and the development of BOS. These data reflect few patient and transplant-related differences among study cohorts. However, this study is the first published report to reveal significant differences in circulating fibrocyte counts among those who developed BOS compared to non-BOS patients. Specifically, these data demonstrate significantly higher levels of circulating fibrocytes among those who developed BOS compared to those without BOS by enumerating differences in collagen-1 expressing CD45⁺ cells. Moreover, similar differences were detected for other circulating fibrocyte subpopulations, including CD45⁺Col-1⁺ cells expressing CCR4⁺, CCR7⁺ and CD34⁺. Differences were also detected among fibrocyte counts upon stratification by BOS stage. Interestingly, circulating fibrocyte count incrementally increased with advancing BOS stage. The present results suggest that circulating fibrocytes may serve as a novel clinical biomarker for the early detection of BOS development among lung transplant recipients, and that routine measurement of fibrocyte levels among BOS patients may help to objectively monitor for disease progression.

Fibrocytes are a unique form of progenitor cells. Initially identified by Bucala and associates, fibrocytes were characterized as a unique fibroblast-like cell population that preferentially extravasated into wounds and contributed to wound repair [11]. By scanning electron microscopy, this group demonstrated that fibrocytes were morphologically distinct from leukocytes. Subsequent investigators have further defined fibrocytes as spindle-shaped cells that are negative for CD14 and nonspecific esterase stain, thus distinguishing them from monocytes or macrophages, as well as cell surface markers for epithelial and endothelial cells [14, 19, 20]. In addition, these cells express several different chemokine receptors and adhesion molecules. Fibrocytes account for approximately 0.1–0.5% of peripherally circulating, non-erythrocyte cells and participate in many biological processes [14, 20, 21]. These cells remain active during tissue remodeling through the elaboration of ECM proteins (collagen I and collagen III) and matrix metalloproteinases as well as secrete various inflammatory mediators (cytokines, chemokines, growth factors) that exert influences through both autocrine and paracrine signaling pathways [21, 22]. Importantly, fibrocytes have been shown to deposit ECM components during various fibroproliferative disorders of the lung [14], and circulating levels have been shown to be elevated in human fibrotic interstitial lung disease [13]. However, the presence of circulating fibrocytes during chronic rejection and BOS development following lung transplantation remains undefined.

The present results are in agreement with previous reports that have documented elevated fibrocyte counts among patients with fibrotic pulmonary disease. In this series, a greater than 3-fold increase [8.9×10^5 cells/ml vs. 2.96×10^5 cells/ml] in overall median CD45⁺Col-1⁺ fibrocyte counts was detected among lung transplant recipients who developed BOS compared to those without BOS development. Previous studies report similar findings among circulating fibrocyte counts in other pulmonary fibrotic processes. Mehrad and colleagues documented increased circulating peripheral blood fibrocytes in human patients with the quintessential fibrotic interstitial lung diseases, usual interstitial pneumonia (UIP) and non-specific interstitial pneumonia (NSIP) compared to healthy lungs [13]. In a similar series of patients with idiopathic pulmonary fibrosis, Moeller and others detected significantly elevated circulating fibrocyte levels among patients with stable IPF and acute disease exacerbation compared to health age-matched volunteers [15]. Furthermore, this group identified increasing fibrocyte counts as an independent predictor of

early mortality and noted significantly decreased mean survival times for patients with elevated fibrocyte counts.

Significant differences in fibrocyte subpopulations among lung transplant recipients with and without BOS further suggest a diverse role of these cells in chronic graft rejection. Among differentiated fibrocyte cell lines, peripheral blood from patients with BOS development demonstrated a 2–3 fold increase in cell counts for CD45⁺Col-1⁺CXCR4⁺ ($p=0.02$), CD45⁺Col-1⁺CCR7⁺ ($p=0.03$), and CD45⁺Col-1⁺CD34⁺ ($p=0.03$) fibrocytes compared to patients without BOS development. Studies in murine models of pulmonary fibrosis have demonstrated variations in cell trafficking for fibrocytes expressing CCR7 and CXCR4 [14]. In these experiments, the intrapulmonary recruitment of CD45⁺Col-1⁺CXCR4⁺ fibrocytes was markedly greater than CD45⁺Col-1⁺CCR7⁺ fibrocytes and correlated with higher collagen deposition within experimental lungs. Thus, in the present study, the significantly higher proportion of CXCR4 expressing fibrocytes suggests a more dominant role compared to fibrocytes expressing CCR7. Furthermore, considering that circulating fibrocytes are capable of differentiating into myofibroblasts *in vitro* and *in vivo* [19, 23], and that prior reports have demonstrated the role of myofibroblasts in pulmonary fibrotic processes [24, 25], the differentiated fibrocyte cell lines expressing the myofibroblast marker, α SMA, were evaluated. Although statistically significant differences among circulating levels of α SMA-expressing fibrocytes or the CXCR4⁺ and CCR7⁺ subsets of the α SMA⁺ fibrocytes was not observed, an important trend of higher cell counts among those who developed BOS compared to those without BOS existed. These observations suggest that circulating fibrocytes may differentiate into a myofibroblast phenotype to contribute to the underlying pathophysiologic process of BOS development and progression.

Increasing fibrocyte counts with advancing BOS stage suggests an increased role of fibrocytes with BOS progression. In order to determine the prognostic capability of quantified fibrocyte levels among lung transplant recipients who develop BOS, we stratified fibrocyte counts by BOS stage (no BOS, stages I, II, and III). As a result, overall fibrocyte counts (CD45⁺Col-1⁺) incrementally increased with advancing BOS stage ($p=0.02$) as well as within the CXCR4⁺ expressing subset of fibrocytes ($p=0.04$). Moreover, although the CCR7⁺ and α SMA⁺ expressing fibrocyte subsets failed to show statistically significant trends, circulating cell counts of each population were, in fact, increased in advanced BOS stages. These results have important clinical significance as the measurement of circulating fibrocytes and differentiated subpopulations may serve as a prognostic biomarker to track BOS progression. On the other hand, the markedly higher fibrocyte counts observed in stage III BOS was less expected as it was initially postulated that overall fibrocyte counts may paradoxically decrease due to a “burnout” of the fibrotic process in end stage BOS.

The current report has several important implications for future investigation, and the presented results remain hypothesis generating. First, considering that the fibroobliterative process of BOS is similar to that of other fibrotic pulmonary diseases, the contribution of the fibroproliferative capabilities of circulating fibrocytes within BOS pathogenesis is of paramount importance. Second, the relative contribution of fibrocytes to the final pathologic features of BOS compared to other resident cells and pre-existing fibroblasts remains unknown. Third, the role of the CXCR4⁺ and α SMA⁺ fibrocyte subsets in the development and progression of BOS requires further characterization. Finally, future experiments to scrutinize the underlying cell trafficking mechanisms of fibrocytes involved in BOS development, including the role of the CXCR4/CXCL12 biologic axis, are warranted within both animal models and human tissue.

Additional factors related to the potential role of circulating fibrocytes and BOS development deserve further discussion. First, the effects of immunosuppression on these bone marrow derived cells should be considered. In this series, all enrolled patients received standard three drug immunosuppressive therapy, consisting of prednisone, tacrolimus (Prograf), and mycophenolate (CellCept), and this treatment strategy remained constant throughout the study period. In addition, none of the patients included in this study were treated with mTOR inhibitors, which have been shown to abrogate fibrocyte cell trafficking in experimental animal models. In addition it is plausible that elevated fibrocyte levels among those undergoing single lung transplantation may, in fact, represent a progression of an underlying disease process in the native lung, which may help to explain a potential protective effect of double lung transplantation on the development of BOS. In this series, median fibrocyte levels were similar among patients undergoing both single and double lung transplants. Thus, the potential confounding influence of such a phenomenon in this series is minimal but should be considered in future investigations.

Despite the significant aforementioned results, this study has noteworthy limitations. Reported findings and data analyses represent the results of patient samples collected at one postoperative point in time. Thus, future studies are required to further characterize changes in fibrocyte levels among transplant recipients with repeated measures of association between fibrocyte counts and the development and progression of BOS. In addition, included analyses are subject to limitations from small sample sizes; however, such an influence would expectedly skew or bias the results in favor of the null hypothesis. To the contrary, despite small sample sizes, statistically significant differences remained upon comparisons of elevated fibrocyte counts among BOS patients compared to non-BOS group. Another potential limitation was the failure to exclude patients receiving lung transplants for IPF. Theoretically, it is conceivable that patients with prior pulmonary fibrosis may represent a group of patients that are more prone to higher circulating fibrocyte levels. Importantly, however, among study cohorts, all patients transplanted for prior IPF failed to develop BOS at the time of study enrollment and were, thus, included in the non-BOS study cohort. The relationship between acute rejection and BOS development was not evaluated in this study, and pathologic confirmation of BO for all patients clinically diagnosed with BOS was not achieved. In addition, negative controls would have been useful to include in order to obtain baseline fibrocyte levels in non-lung transplant patients. Nevertheless, the reported differences in circulating fibrocyte levels among lung transplant recipients with varying BOS stages provides an important clinical contribution through the detection of a potential therapeutic target to combat this dreaded complication.

Conclusion

The present results suggest that increased circulating fibrocyte levels are associated with the development of bronchiolitis obliterans syndrome following lung transplantation. Further, circulating fibrocyte levels appear to incrementally increase with advancing BOS stage. These findings propose that circulating fibrocytes could serve as a novel clinical biomarker for the early detection of BOS development and that measurement of circulating fibrocyte levels could provide transplant pulmonologists/surgeons with an objective method of monitoring for disease progression following lung transplantation. Furthermore, this unique cell population may provide a future therapeutic target to combat chronic graft rejection.

Acknowledgments

This study was supported by Award Numbers 2T32HL007849-11A1 (DJL), 1K08HL094704-01 (CLL), and R01 HL098526 (RMS) from the National Institute of Health and National Heart, Lung, And Blood Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung, And Blood Institute or the National Institute of Health. CLL was also supported by the Thoracic Surgery

Foundation for Research and Education Research Grant and the American Association for Thoracic Surgery John W. Kirklin Research Fellowship.

References

1. Trulock EP, Edwards LB, Taylor DO, Boucek MM, Mohacsi PJ, Keck BM, Hertz MI. The Registry of the International Society for Heart and Lung Transplantation: Twentieth Official adult lung and heart-lung transplant report--2003. *J Heart Lung Transplant*. 2003; 22(6):625–35. [PubMed: 12821160]
2. Vanaudenaerde BM, Meyts I, Vos R, Geudens N, De Wever W, Verbeken EK, Van Raemdonck DE, Dupont LJ, Verleden GM. A dichotomy in bronchiolitis obliterans syndrome after lung transplantation revealed by azithromycin therapy. *Eur Respir J*. 2008; 32(4):832–43. [PubMed: 18827151]
3. Keller CA, Cagle PT, Brown RW, Noon G, Frost AE. Bronchiolitis obliterans in recipients of single, double, and heart-lung transplantation. *Chest*. 1995; 107(4):973–80. [PubMed: 7705164]
4. Reichenspurner H, Girgis RE, Robbins RC, Yun KL, Nitschke M, Berry GJ, Morris RE, Theodore J, Reitz BA. Stanford experience with obliterative bronchiolitis after lung and heart-lung transplantation. *Ann Thorac Surg*. 1996; 62(5):1467–72. discussion 72–3. [PubMed: 8893585]
5. Scott JP, Higenbottam TW, Sharples L, Clelland CA, Smyth RL, Stewart S, Wallwork J. Risk factors for obliterative bronchiolitis in heart-lung transplant recipients. *Transplantation*. 1991; 51(4):813–7. [PubMed: 2014535]
6. Yousem SA, Dauber JA, Keenan R, Paradis IL, Zeevi A, Griffith BP. Does histologic acute rejection in lung allografts predict the development of bronchiolitis obliterans? *Transplantation*. 1991; 52(2):306–9. [PubMed: 1871804]
7. Kelly K, Hertz MI. Obliterative bronchiolitis. *Clin Chest Med*. 1997; 18(2):319–38. [PubMed: 9187825]
8. Paradis I, Yousem S, Griffith B. Airway obstruction and bronchiolitis obliterans after lung transplantation. *Clin Chest Med*. 1993; 14(4):751–63. [PubMed: 8313678]
9. Tazelaar HD, Yousem SA. The pathology of combined heart-lung transplantation: an autopsy study. *Hum Pathol*. 1988; 19(12):1403–16. [PubMed: 3142814]
10. Yousem SA. Lymphocytic bronchitis/bronchiolitis in lung allograft recipients. *Am J Surg Pathol*. 1993; 17(5):491–6. [PubMed: 8470763]
11. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med*. 1994; 1(1):71–81. [PubMed: 8790603]
12. Mehrad B, Burdick MD, Strieter RM. Fibrocyte CXCR4 regulation as a therapeutic target in pulmonary fibrosis. *Int J Biochem Cell Biol*. 2009; 41(8–9):1708–18. [PubMed: 19433312]
13. Mehrad B, Burdick MD, Zisman DA, Keane MP, Belperio JA, Strieter RM. Circulating peripheral blood fibrocytes in human fibrotic interstitial lung disease. *Biochem Biophys Res Commun*. 2007; 353(1):104–8. [PubMed: 17174272]
14. Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY, Belperio JA, Keane MP, Strieter RM. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest*. 2004; 114(3):438–46. [PubMed: 15286810]
15. Moeller A, Gilpin SE, Ask K, Cox G, Cook D, Gauldie J, Margetts PJ, Farkas L, Dobranowski J, Boylan C, O'Byrne PM, Strieter RM, Kolb M. Circulating fibrocytes are an indicator of poor prognosis in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2009; 179(7):588–94. [PubMed: 19151190]
16. Estenne M, Maurer JR, Boehler A, Egan JJ, Frost A, Hertz M, Mallory GB, Snell GI, Yousem S. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. *J Heart Lung Transplant*. 2002; 21(3):297–310. [PubMed: 11897517]
17. Ham JM, Kunkel SL, Dibb CR, Standiford TJ, Rolfe MW, Strieter RM. Chemotactic cytokine (IL-8 and MCP-1) gene expression by human whole blood. *Immunol Invest*. 1991; 20(4):387–94. [PubMed: 1937585]

18. Strieter RM, Remick DG, Ham JM, Colletti LM, Lynch JP 3rd, Kunkel SL. Tumor necrosis factor- α gene expression in human whole blood. *J Leukoc Biol.* 1990; 47(4):366–70. [PubMed: 2319209]
19. Abe R, Donnelly SC, Peng T, Bucala R, Metz CN. Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. *J Immunol.* 2001; 166(12):7556–62. [PubMed: 11390511]
20. Metz CN. Fibrocytes: a unique cell population implicated in wound healing. *Cell Mol Life Sci.* 2003; 60(7):1342–50. [PubMed: 12943223]
21. Quan TE, Cowper S, Wu SP, Bockenstedt LK, Bucala R. Circulating fibrocytes: collagen-secreting cells of the peripheral blood. *Int J Biochem Cell Biol.* 2004; 36(4):598–606. [PubMed: 15010326]
22. Chesney J, Metz C, Stavitsky AB, Bacher M, Bucala R. Regulated production of type I collagen and inflammatory cytokines by peripheral blood fibrocytes. *J Immunol.* 1998; 160(1):419–25. [PubMed: 9551999]
23. Schmidt M, Sun G, Stacey MA, Mori L, Mattoli S. Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. *J Immunol.* 2003; 171(1):380–9. [PubMed: 12817021]
24. Phan SH. The myofibroblast in pulmonary fibrosis. *Chest.* 2002; 122(6 Suppl):286S–89S. [PubMed: 12475801]
25. Xing Z, Tremblay GM, Sime PJ, Gauldie J. Overexpression of granulocyte-macrophage colony-stimulating factor induces pulmonary granulation tissue formation and fibrosis by induction of transforming growth factor- β 1 and myofibroblast accumulation. *Am J Pathol.* 1997; 150(1): 59–66. [PubMed: 9006322]

Figure 1.

Representative flow cytometric analysis of circulating fibrocytes for a lung transplant patient without bronchiolitis obliterans (A-C) compared to a patient with bronchiolitis obliterans development (D-F). (A) Positive population of CD45 cells in patient without BOS. (B) Isotype control for collagen-1 set on CD45-gated cell population in patient without BOS. (C) Positive population for CD45 and collagen-1 cells in patient without BOS. (D) Positive population of CD45 cells in patient with BOS. (E) Isotype control for collagen-1 set on CD45-gated cell population in patient with BOS. (F) Increased positive population for CD45 and collagen-1 cells in patient with BOS. FITC=fluorescein isothiocyanate; SSC-A=side scatter.

Figure 2.

Circulating fibrocytes among lung transplant patients with and without bronchiolitis obliterans development. (A) Represents number of CD45⁺collagen-1⁺ cells. (B) Represents number of CD45⁺collagen-1⁺CXCR4⁺ cells. (C) Represents number of CD45⁺collagen-1⁺CCR7⁺ cells. (D) Represents number of CD45⁺collagen-1⁺CD34⁺ cells. Box and whiskers represent 25–75th and 10–90th percentiles, respectively; transverse lines represent median. BOS=bronchiolitis obliterans; * $p < 0.05$ for independent samples comparison to no BOS development.

Figure 3. Circulating fibrocytes among lung transplant patients by bronchiolitis obliterans stage. No BOS (n=29), Stage I (n=3), Stage II (n=3), Stage III (n=4). Results represent mean $\pm$ standard error. BOS=bronchiolitis obliterans; * p <0.05 vs. all other groups.

Table 1

Patient demographics, transplantation characteristics, and operative features for lung transplant recipients with and without BOS development.

Variable	Overall Frequency	BOS Development (n=10)	No BOS Development (n=29)	p
Patient age (years)*	59.6±1.7	58.3±3.9	60.3±2.0	0.67
Gender[†]				>0.99
Male	24 (61.5%)	6 (60.0%)	18 (62.1%)	
Female	15 (38.5%)	4 (40.0%)	11 (37.9%)	
Indication for Transplant[‡]				0.22
COPD/Emphysema	16 (41.0%)	6 (60.0%)	10 (34.5%)	
Idiopathic Pulmonary Fibrosis	8 (20.5%)	-	8 (27.6%)	
Sarcoidosis	5 (12.8%)	2 (20.0%)	3 (10.3%)	
Cystic Fibrosis	3 (7.7%)	-	3 (10.3%)	
α-1-antitrypsin deficiency	1 (2.6%)	-	1 (3.4%)	
Idiopathic Fibrosing Alveolitis	1 (2.6%)	1 (10.0%)	-	
Interstitial Lung Disease	1 (2.6%)	-	1 (3.4%)	
LAM	1 (2.6%)	-	1 (3.4%)	
Repeat Lung Transplant (BOS)	1 (2.6%)	-	1 (3.4%)	
Occupational Lung Disease	1 (2.6%)	-	1 (3.4%)	
Primary Pulmonary HTN	1 (2.6%)	1 (10.0%)	-	
Lung Transplantation Type[‡]				0.71
Double Lung Transplant	13 (33.3%)	4 (40.0%)	9 (31.0%)	
Left Lung Transplant	10 (25.6%)	3 (30.0%)	7 (24.1%)	
Right Lung Transplant	16 (41.0%)	3 (30.0%)	14 (44.8%)	
FEV₁ (L/sec)[‡]	1.73 [1.29]	1.08 [0.56]	2.18 [1.28]	

* Results reported as mean ± standard error.

[†] Results reported as median [interquartile range].

[‡] Results reported as number (within group %).

BOS=bronchiolitis obliterans syndrome. COPD=chronic obstructive pulmonary disease. FEV₁=forced expiratory volume in 1 second.

Table 2

Circulating fibrocyte counts among lung transplant recipients with and without BOS development.

Circulating Fibrocyte Population	BOS Development (n=10)	No BOS Development (n=29)	<i>p</i>
CD45⁺Col1⁺	891,000 [1,996,750]	296,000 [534,500]	0.03
CD45⁺Col1⁺CXCR4⁺	823,500 [1,502,500]	247,000 [448,700]	0.02
CD45⁺Col1⁺CCR7⁺	116,500 [186,000]	58,700 [146,050]	0.03
CD45⁺Col1⁺SMA⁺	547,500 [842,250]	215,000 [366,500]	0.09
CD45⁺Col1⁺SMA⁺CXCR4⁺	354,000 [395,000]	205,000 [313,100]	0.09
CD45⁺Col1⁺SMA⁺CCR7⁺	126,500 [137,825]	72,000 [108,000]	0.06
CD45⁺Col1⁺SMA⁺CXCR4⁺CCR7⁺	114,500 [111,750]	56,000 [85,650]	0.06
CD45⁺Col1⁺CD34⁺	253,500 [581,000]	124,000 [229,550]	0.03

All results reported as Median [interquartile range].