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Acute Exacerbations and Pulmonary Hypertension in Advanced Idiopathic Pulmonary Fibrosis

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Short Running Head: Exacerbations and Pulmonary Hypertension in Advanced IPF

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Abstract

Aims: To evaluate the risk factors for and outcomes of acute exacerbations (AE) in patients with advanced idiopathic pulmonary fibrosis (IPF), and to examine the relationship between disease severity and neovascularization in explanted IPF lung tissue.

Methods: IPF patients assessed for lung transplantation (n=55) were grouped into AE (n=27) and non-AE (NAE) groups. Haemodynamic data was collected at baseline, at time of AE and at lung transplantation. Histological analysis and CD31 immunostaining to quantify microvessel density (MVD) was performed on the explanted lung tissue of all transplanted patients (n=13).

Results: AE were associated with increased mortality ($p = 0.0015$). Pulmonary hypertension (PH) at baseline and AE were associated with poor survival ($p < 0.01$). PH at baseline was associated with a significant risk of AE (HR = 2.217, $p = 0.041$). Neovascularization was significantly increased in areas of cellular fibrosis and significantly decreased in honeycomb areas. There was a significant inverse correlation between mPAP and MVD in honeycomb areas.

Conclusion: AE are associated with significantly increased mortality in patients with advanced IPF. PH is associated with the subsequent development of an AE and with poor survival. Both neovascularization and its correlation with mPAP vary significantly according to severity of fibrosis.

Introduction

The natural history of idiopathic pulmonary fibrosis (IPF) has been described as a progressive decline in pulmonary function leading to death from respiratory failure or complicating comorbidity. (1) More recently, abrupt deteriorations of patients' clinical status leading to a more unpredictable clinical course have been acknowledged. These acute, clinically significant deteriorations of unknown cause in patients with underlying IPF have been defined as 'acute exacerbations' (AE). (2) AE are histologically characterized as diffuse alveolar damage (DAD) superimposed on underlying usual interstitial pneumonia (UIP). (3, 4)

The natural history of the impact of AE on IPF patients remains to be further elucidated. Estimates of the incidence and mortality rate of AE vary greatly, depending on the diagnostic criteria and follow-up period used. (3, 5) Although there have been reports of acute respiratory deterioration occurring in patients with IPF after thoracic surgery (6) and bronchoalveolar lavage, (3) there are currently no known risk factors for AE. A recent study evaluated the incidence, risk factors for and outcomes of AE of patients with mildly severe IPF, (7) but the impact of AE on patients with advanced disease has not been evaluated. In addition, the potential association between pulmonary hypertension (PH), a disease process which is increasingly recognized as having important implications in IPF patients, (8) and subsequent development of an AE has not been evaluated.

The relative roles played by neovascularization and vascular regression in the pathogenesis of IPF remain uncertain. Previous histological studies have shown a heterogeneous pattern of vessel turnover, with the extent of both increased capillary density and vascular regression varying according to the extent of the tissue fibrosis present. (9) Consequently, the importance of considering disease severity when analyzing these vascular changes has been acknowledged. (10) The relationship between neovascularization and disease severity (in terms of histological severity of disease and pulmonary function) has been previously studied.

(9, 11) However, the relationship between these pulmonary microvascular changes and pulmonary arterial pressures has not been evaluated.

The purpose of this study was to therefore evaluate the incidence, risk factors for and outcomes of AE in patients with advanced IPF awaiting lung transplantation, and to examine the relationship between PH at baseline and subsequent AE. Secondly, we undertook to examine the relationship between neovascularization and disease severity (in terms of histological tissue type, pulmonary function and PH) in the explanted lung tissue of patients with advanced IPF.

Methods

Subjects

All IPF patients who were assessed for lung transplantation between 2005 and 2010 at the Irish National Lung Transplant Unit in the Mater Misericordiae University Hospital, Dublin, Ireland were included in this study. All patients (n=55) met accepted diagnostic criteria for IPF. The study was approved by the Ethics and Audit Committee and informed consent was obtained from the patients. AE were defined as an acute onset of increased dyspnoea and hypoxia with progressive infiltrates on chest x-ray or high resolution CT (HRCT) of the thorax, within the preceding 30 days, in the absence of infection, pulmonary embolism or cardiac failure (as confirmed by clinical, radiological, echocardiographic and microbiological analysis). (2) Patients who re-presented with a possible AE during the study period were evaluated using a standardized diagnostic methodology which included a chest x-ray or HRCT of the thorax, white cell count, c-reactive protein, d-dimer level (with CT pulmonary angiogram if indicated), sputum microbiological analysis and echocardiogram. Patients were

sub-grouped according to the definition of AE into those with AE (AE group) (n=27) and those without AE (NAE group) (n=28) (Table 1). Patients were also sub-grouped into those who underwent lung transplantation over the follow-up period (n=13) and those who did not.

The Irish National Lung Transplant database and patient charts provided the data set. Baseline patient demographics (age, gender, blood group, cytotoxic antibody status, body mass index) were recorded. Pulmonary function testing (including forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), total lung capacity (TLC) and diffusion capacity of the lung for carbon monoxide (D_{LCO})) is performed on all patients who are assessed for lung transplantation. Transthoracic echocardiographic data (left ventricular ejection fraction, right ventricular systolic pressure) was collected both at the time of initial lung transplant assessment and during an AE. Right heart catheterization (RHC) data (pulmonary arterial pressure, pulmonary capillary wedge pressure (PCWP), cardiac output (CO)) was collected both at the time of initial transplant assessment and also at the time of lung transplantation. PH was defined as a mean pulmonary arterial pressure (mPAP) $\geq$ 25 mm Hg on RHC. The explanted lung tissue from the thirteen lung transplant recipients underwent blinded histological assessment (AF) for evidence of pulmonary vascular changes and DAD.

Histological Analysis

Microvessel density (MVD) (or neovascularization) was quantified for cases using CD31 immunostaining. Paraffin embedded 4μm sections were deparaffinised and processed on an automatic Ventana XT Immunostainer. They were then subjected to antigen retrieval (Ventana Protease for 12 minutes) and incubated with antibody CD31 (Dako, ref M0823, dilution 1:40) for 32 minutes at 37 °C. Antibody detection was done using a Ventana UltraView Detection Kit.

As UIP presents a heterogeneous distribution of fibrosis, 8 different fields at a magnification x20 were assessed in the explanted lung of each transplant recipient, including significant/cellular interstitial fibrosis (4 fields) as previously described, and honeycomb areas (4 fields). (12) Architecturally preserved lung tissue present in the same explanted lung was used as an internal "normal" control.

Digital images of CD31 stained lung fibrosis were captured with a Nikon Digital Sight DS-5M camera, and total tissue area stained with CD31 was quantified using Image J software (NIH, Bethesda, MD). MVD was assessed on inverted and binary images, and the percentage area covered by vessels in areas of fibrosis, honeycomb and control areas was calculated for each case, as previously described. (13) Mean values for MVD were then calculated by averaging the percentage area covered by vessels.

Statistical Analysis

Baseline characteristics were summarized for comparison purposes by calculating the mean and standard deviation. Baseline demographic data, pulmonary function, echocardiographic data and RHC data of the AE and NAE groups were compared using the Mann-Whitney test. Interval changes in echocardiogram and RHC data were assessed using the Wilcoxon test. A Cox regression analysis was used to identify significant variables capable of predicting an AE or acting as prognostic factors. Survival between the AE and NAE groups was evaluated using a Kaplan-Meier survival curve and the log-rank test. Spearman rank correlation was used as a non-parametric measure of correlation between echocardiographic and right heart catheterization data, and between histological and pulmonary arterial pressure data.

All tests were two-tailed, and a p-value < 0.05 was assumed to represent statistical significance. All statistical analysis was performed using SPSS software (SPSS 16.0.2, SPSS Inc., an IBM Company).

Results

Baseline Characteristics

The baseline characteristics of the 55 patients with IPF who were included in this study are presented in Table 1. Baseline characteristics of patients in the AE (n=27) and NAE (n=28) groups are also shown. There was no statistically significant difference in demographic, pulmonary function or cardiovascular data between both groups.

Incidence and effects of AE on patient survival

The incidence of AE was 19.11% per annum. As lung transplantation alters the natural course of IPF, transplantation events (n=13) over the follow-up period were censored when performing survival analysis. AE were associated with a significant increase in mortality in these patients, with 16 patients dying in the AE group and 6 patients dying in the NAE group ($p = 0.0015$) (Figure 1). The majority of patients died during an AE, with 11 (68.75%) patients dying during an AE. The median time interval from initial assessment to AE in patients who did not undergo lung transplantation was 6 months, and the median survival of these patients post-AE was 1 month.

Table 2 shows the prognostic impact of factors on the survival of patients who did not undergo lung transplantation over the study period. Cox univariate analysis revealed significant associations between occurrence of AE over the study period (HR: 4.0374, 95% CI: 1.568-10.399, $p = 0.004$), baseline mPAP (HR: 1.080, 95% CI: 1.013-1.152, $p = 0.033$) and PH at baseline (HR: 5.201, 95% CI: 1.987-13.609, $p = 0.0008$) and poor overall survival. Multivariate Cox analysis showed that occurrence of AE (HR: 2.924, 95% CI: 1.089-7.852, $p = 0.0342$) and PH (HR: 4.749, 95% CI: 1.278-17.641, $p = 0.0206$) were associated with poorer overall survival over the study period.

Pulmonary hypertension

PH at baseline was associated with a significant risk of AE (HR 2.217, 95% confidence interval 1.005 to 4.889, p = 0.041), with 12 of the 17 patients who had PH at baseline subsequently experiencing an AE during follow-up period. There were no significant other associations between baseline variables and development of an AE (Table 3).

Mean right ventricular systolic pressure (RVSP) on echocardiogram increased significantly from 28.69 mm Hg (± 13.14) at baseline to 37.00 mm Hg (± 15.64) at the time of AE (n = 27, p = 0.0025). Mean left ventricular ejection fraction (LVEF) remained unchanged, with a mean LVEF of 59.37% (± 7.42) at assessment and 60.03% (± 8.85) at the time of AE (n = 27, p = 0.529). There was significant correlation between overall baseline RVSP measured by echocardiogram and right heart catheterization (rho = 0.41, p = 0.0069). Correlation between baseline RVSP measured by echocardiogram and right heart catheterization for the AE group was also significant (rho = 0.44, p = 0.039), and approached significance for the NAE group (rho = 0.38, p = 0.096).

Lung Transplantation

Thirteen (23.64%) of the 55 patients in this study underwent lung transplantation over the follow-up period. All lung transplants were single organ transplants. None of the 13 patients who received lung transplants died during the follow-up period. The characteristics of patients at the time of lung transplant are shown in Table 4.

MPAP increased significantly from 21.39 mm Hg at baseline to 36.21 mm Hg at the time of transplant (n = 13, p = 0.027) (Figure 2). MPAP also increased significantly from 20.92 mm Hg at initial assessment to 37.96 mm Hg at the time of transplant in the AE group (n = 8, p = 0.039). The increase in mPAP from 22.33 mm Hg to 33.40 mm Hg in the NAE group was not statistically significant (n = 5, p = 0.887) (Figure 2). PCWP was similar at baseline (7.64 mmHg) and at the time of transplant (8.54 mmHg).

Histological Analysis of Explanted Lung Tissue

There was histological evidence of pulmonary vascular changes in the explanted lung tissue of all 13 patients who underwent lung transplantation (Figure 3B). The histological pattern of UIP was found in 11 cases, and DAD was found in 4 cases (Figure 3A). In 3 of these 4 cases, the patients were being treated for an AE at the time of lung transplantation.

MVD was significantly increased in cellular fibrotic areas compared to both control areas ($p < 0.001$) and honeycomb areas ($p < 0.001$) (Figure 4), while MVD was significantly decreased in honeycomb areas compared to control areas ($p = 0.04$). There was a significant inverse correlation between mPAP and MVD in honeycomb areas (rho = -0.637, $p = 0.019$) (Figure 5) but not in cellular fibrotic areas (rho = -0.258, $p = 0.394$) (Figure 6). PH at the time of lung transplantation did not correlate significantly with MVD in cellular fibrotic areas (rho = -0.312, $p = 0.299$) or MVD in honeycomb areas (rho = -0.445, $p = 0.127$). Pulmonary function (FVC, DLCo) did not correlate significantly with MVD in cellular fibrotic or honeycomb areas. There was no significant difference in MVD in cellular fibrotic or honeycomb areas in explanted lung tissue with histological evidence of DAD compared to tissue without evidence of DAD.

Discussion

In this study, we found that AE are common and are associated with significantly increased mortality in patients with advanced IPF. PH at baseline was associated with the subsequent development of an AE and with poor overall survival, and mPAP was shown to increase significantly over time in patients who had experienced an AE. We have also shown that neovascularization is significantly increased in areas of cellular fibrosis and significantly

decreased in honeycomb areas, and that there is a significant inverse relationship between mPAP and neovascularization in areas of honeycombing

Reports on the incidence and mortality from AE of IPF have varied greatly. (3, 5) This is likely due to differences in study design, the definition of AE used and the severity of the disease. In a recent retrospective review of 461 patients with IPF (limited disease with a mean baseline FVC ≥ 72% predicted and mean baseline DLco ≥ 62% predicted), the 1-year incidence of AE was 11.6%, and the median survival post-onset of AE was 2.2 months. (7) In contrast to that study, patients in our study had advanced IPF, and AE were associated with a slightly higher incidence and a lower median survival post-AE. Our study therefore suggests that AE are more common and have an even poorer outcome in patients with advanced IPF. Previous reports have also indicated that a steady decline in pulmonary function is the major manifestation of disease progression in IPF. (14, 15) The short time interval from initial assessment to AE seen in this study, coupled with the poor outcome following AE suggests that AE may also contribute to and be a manifestation of disease progression. It also emphasizes the importance of urgent lung transplantation for these patients.

In this study, both univariate and multivariate logistic analysis indicates that PH is an independent predictor for development of an AE in patients with advanced IPF. Risk factors for AE are largely unknown. One recent study which looked at risk factors for developing an AE, did not evaluate PH as a potential predictor. (7) In that study, non-smokers with lower lung function were found to be at increased risk of AE. However, patients in that study had only mildly severe disease. This contrasted to our study where all patients had severe IPF and had been referred for lung transplantation. (7) Indeed, previous studies of patients with IPF referred for lung transplantation have shown that lung function is not predictive of survival in this cohort of patients with advanced disease.(16, 17)

PH has been shown to be common in patients with IPF. Previous studies have shown a prevalence of 32 to 46% in patients with advanced disease. (8, 18-20) The incidence of PH and the mPAP have also been shown to increase over time in patients undergoing lung transplantation, (18) and the presence of PH has been associated with an increased risk of mortality in IPF patients with advanced disease. (8, 21, 22) In this study, PH at baseline (in addition to occurrence of AE over the follow-up period) was found to be an independent predictor of increased mortality. Furthermore, both the number of patients with PH and the mPAP increased over time in both the AE and NAE groups at the time of transplant, and the increase in mPAP was statistically significant in the AE group but not in the NAE group. Given the progression of pulmonary arterial pressures over time, and the high incidence of PH and its association with AE, these results support the hypothesis that patients with advanced IPF should be evaluated for occult PH. This data also provides further evidence for considering pulmonary vasodilator therapy in these patients. (23, 24)

The effect of AE of IPF on pulmonary arterial pressures is largely unknown. Previous studies on a small number of patients suggest that mean RVSP is elevated during AE, (4) and that PH (as evidenced by histological findings) is implicated in disease progression or AE. (25) In our study, mean RVSP increased significantly from baseline to the time of AE while mean LVEF remained stable. MPAP also increased significantly over time in the AE group. It is not clear whether PH is a causative factor itself or whether it results from AE and disease progression. Indeed, IPF with pulmonary hypertension may represent a distinct clinical phenotype (IPF-PH). (1) The role of pulmonary vasodilator therapies in the treatment of AE also remains to be established.

It is uncertain as to whether the primary vascular abnormality in IPF results from an increase or a reduction in neovascularization. Establishing if neovascularization plays a key role in abnormal extracellular matrix remodeling may have important therapeutic implications.

Previous studies have shown that both increased capillary density and vascular regression occur in IPF, and indeed vary according to the extent of the disease. (9, 26) In contrast to previous studies where tissue from non-fibrotic/disease-free lungs was used as controls, (9, 12) our study used 'normal' (i.e. non-diseased) lung tissue from within the same lung as controls. The authors believe that this is an important consideration in order to study the well-described heterogeneous pattern of both fibrosis and vascularization throughout the whole lung.

The results of our study support the results of previous studies which show a heterogeneous pattern of neovascularization according to disease severity. (9, 26) Neovascularization was significantly increased in areas of cellular fibrosis (areas of increased extracellular matrix where neovascularization is composed of numerous capillaries and small thin walled vessels), and significantly decreased in honeycomb areas (less cellular areas with dense collagenous tissue). The increased MVD in less fibrotic areas may represent a compensatory response to hypovascularity in areas of dense fibrosis (honeycomb areas). (10) Alternatively, it may be actively involved in the fibrogenic process, (10) or may be involved in tissue regeneration. (9) Further studies are required to answer this question.

Another interesting finding of this study was that mPAP at the time of lung transplant was significantly inversely correlated with MVD in honeycomb areas but not with areas of cellular fibrosis. In a previous study which evaluated the relationship between mPAP and pulmonary venous changes in explanted IPF tissue, there was no significant correlation found. (27) The findings in our study (which evaluates both arterial and venous changes) may be explained by the differences in vascular changes observed in differing fibrotic areas. In honeycomb areas, vascular changes were as those observed in idiopathic pulmonary arterial hypertension, with medial hypertrophy and intimal fibrosis of the muscular arteries and intimal fibrosis of the pulmonary veins. (28) These vessels may contribute most significantly to increased

pulmonary vascular resistance, while the neovascularization seen in cellular fibrotic areas may represent an adaptive proliferative change in response to altered vascular resistance rather than a primary event. The pathogenesis of PH in the setting of pulmonary fibrosis is believed to relate to the effect of alveolar hypoxia with subsequent vascular remodeling, and to the reduction of the vascular bed by fibrosis. (29) Our results support the role of this latter factor in the pathogenesis of PH in IPF. Alternatively, our results may suggest that honeycombing remodeling may play a major role in vascular resistance in IPF. Further studies are therefore required to evaluate these hypotheses. Pulmonary function has been shown to be a poor surrogate of pulmonary vascular disease in IPF, and this may explain the lack of correlation between pulmonary function measures and MVD seen in this study. (19)

In addition to its retrospective design, the main limitation of this study relates to the cohort of IPF patients used, namely patients with advanced disease. Histological analysis was also performed on explanted IPF tissue pre-lung transplantation. Therefore, these results cannot be extended to all patients with IPF. Echocardiography was also used to assess haemodynamic changes during AE. Although RHC is the only definitive method of diagnosing PH, higher right ventricular systolic pressures on echocardiogram are suggestive of pulmonary hypertension, and RHC is recommended in these cases. (30) Given the obvious difficulties in performing RHCs (and other potential surrogate markers such as 6-minute walk tests and D_{LCO}) in acutely unwell patients, echocardiography is currently the most practical method for assessing pulmonary arterial pressure during AE, and significant increases in RVSP as seen in this study are suggestive of increased pulmonary arterial pressure. (31)

Another potential limitation relates to the differences in the methodology of RHC at initial assessment and at the time of transplantation. Baseline RHC was performed in an awake state in the cardiac catheterization laboratory. Transplant RHC was performed under general

anaesthesia in the operating room. The induction agents used in general anaesthesia may reduce pulmonary arterial pressure. (32) Therefore, the mPAP readings obtained under general anaesthesia at the time of transplant may have been underestimated. Furthermore, results from a previous study using similar RHC methodology support this method of comparing pulmonary arterial pressures under these clinical circumstances. (18) Although we did not have a full set of haemodynamic variables at the time of transplant and were therefore unable to assess for a serial change in cardiac output, the close similarity between baseline and transplant PCWP suggests that the anaesthetic induction agents also had little effect on left ventricular function (which could have confounded pulmonary arterial pressure results). In conclusion, this study has shown that AE are common and are associated with significantly increased mortality in patients with advanced IPF. PH at baseline was associated with the subsequent development of an AE and with poor overall survival, and mPAP was shown to increase significantly over time in patients who had experienced an AE. We have also shown that neovascularization and its correlation with mPAP varies significantly according to the severity of fibrosis.

References

1. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011 Mar 15;183(6):788-824.

2. Collard HR, Moore BB, Flaherty KR, Brown KK, Kaner RJ, King TE, Jr., et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2007 Oct 1;176(7):636-43.

3. Kim DS, Park JH, Park BK, Lee JS, Nicholson AG, Colby T. Acute exacerbation of idiopathic pulmonary fibrosis: frequency and clinical features. Eur Respir J 2006 Jan;27(1):143-50.

4. Parambil JG, Myers JL, Ryu JH. Histopathologic features and outcome of patients with acute exacerbation of idiopathic pulmonary fibrosis undergoing surgical lung biopsy. Chest 2005 Nov;128(5):3310-5.

5. Homma S, Sakamoto S, Kawabata M, Kishi K, Tsuboi E, Motoi N, et al. Cyclosporin treatment in steroid-resistant and acutely exacerbated interstitial pneumonia. Intern Med 2005 Nov;44(11):1144-50.

6. Kondoh Y, Taniguchi H, Kitaichi M, Yokoi T, Johkoh T, Oishi T, et al. Acute exacerbation of interstitial pneumonia following surgical lung biopsy. Respir Med 2006 Oct;100(10):1753-9.

7. Song JW, Hong SB, Lim CM, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. Eur Respir J 2011 Feb;37(2):356-63.

8. Lettieri CJ, Nathan SD, Barnett SD, Ahmad S, Shorr AF. Prevalence and outcomes of pulmonary arterial hypertension in advanced idiopathic pulmonary fibrosis. Chest 2006 Mar;129(3):746-52.

9. Ebina M, Shimizukawa M, Shibata N, Kimura Y, Suzuki T, Endo M, et al. Heterogeneous increase in CD34-positive alveolar capillaries in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2004 Jun 1;169(11):1203-8.

10. Renzoni EA. Neovascularization in idiopathic pulmonary fibrosis: too much or too little? Am J Respir Crit Care Med 2004 Jun 1;169(11):1179-80.

11. Parra ER, David YR, da Costa LR, Ab'Saber A, Sousa R, Kairalla RA, et al. Heterogeneous remodeling of lung vessels in idiopathic pulmonary fibrosis. Lung 2005 Jul-Aug;183(4):291-300.

12. Renzoni EA, Walsh DA, Salmon M, Wells AU, Sestini P, Nicholson AG, et al. Interstitial vascularity in fibrosing alveolitis. Am J Respir Crit Care Med 2003 Feb 1;167(3):438-43.

13. Beaudry P, Force J, Naumov GN, Wang A, Baker CH, Ryan A, et al. Differential effects of vascular endothelial growth factor receptor-2 inhibitor ZD6474 on circulating endothelial progenitors and mature circulating endothelial cells: implications for use as a surrogate marker of antiangiogenic activity. Clin Cancer Res 2005 May 1;11(9):3514-22.

14. Jeon K, Chung MP, Lee KS, Chung MJ, Han J, Koh WJ, et al. Prognostic factors and causes of death in Korean patients with idiopathic pulmonary fibrosis. Respir Med 2006 Mar;100(3):451-7.

15. Martinez FJ, Safrin S, Weycker D, Starko KM, Bradford WZ, King TE, Jr., et al. The clinical course of patients with idiopathic pulmonary fibrosis. Ann Intern Med 2005 Jun 21;142(12 Pt 1):963-7.

16. Thabut G, Mal H, Castier Y, Groussard O, Brugiere O, Marrash-Chahla R, et al. Survival benefit of lung transplantation for patients with idiopathic pulmonary fibrosis. J Thorac Cardiovasc Surg 2003 Aug;126(2):469-75.

17. Shitrit D, Gershman Y, Peled N, Medalion B, Saute M, Amital A, et al. Risk factors for death while awaiting lung transplantation in Israeli patients: 1997-2006. Eur J Cardiothorac Surg 2008 Aug;34(2):444-8.

18. Nathan SD, Shlobin OA, Ahmad S, Koch J, Barnett SD, Ad N, et al. Serial development of pulmonary hypertension in patients with idiopathic pulmonary fibrosis. Respiration 2008;76(3):288-94.

19. Nathan SD, Shlobin OA, Ahmad S, Urbanek S, Barnett SD. Pulmonary hypertension and pulmonary function testing in idiopathic pulmonary fibrosis. Chest 2007 Mar;131(3):657-63.

20. Shorr AF, Wainright JL, Cors CS, Lettieri CJ, Nathan SD. Pulmonary hypertension in patients with pulmonary fibrosis awaiting lung transplant. Eur Respir J 2007 Oct;30(4):715-21.

21. Nadrous HF, Pellikka PA, Krowka MJ, Swanson KL, Chaowalit N, Decker PA, et al. The impact of pulmonary hypertension on survival in patients with idiopathic pulmonary fibrosis. Chest 2005 Dec;128(6 Suppl):616S-7S.

22. Mejia M, Carrillo G, Rojas-Serrano J, Estrada A, Suarez T, Alonso D, et al. Idiopathic pulmonary fibrosis and emphysema: decreased survival associated with severe pulmonary arterial hypertension. Chest 2009 Jul;136(1):10-5.

23. Collard HR, Anstrom KJ, Schwarz MI, Zisman DA. Sildenafil improves walk distance in idiopathic pulmonary fibrosis. Chest 2007 Mar;131(3):897-9.

24. Zisman DA, Schwarz M, Anstrom KJ, Collard HR, Flaherty KR, Hunninghake GW. A controlled trial of sildenafil in advanced idiopathic pulmonary fibrosis. N Engl J Med 2010 Aug 12;363(7):620-8.

25. Daniels CE, Yi ES, Ryu JH. Autopsy findings in 42 consecutive patients with idiopathic pulmonary fibrosis. Eur Respir J 2008 Jul;32(1):170-4.

26. Parra ER, Silverio da Costa LR, Ab'Saber A, Ribeiro de Carvalho CR, Kairalla RA, Fernezlian SM, et al. Nonhomogeneous density of CD34 and VCAM-1 alveolar capillaries in major types of idiopathic interstitial pneumonia. Lung 2005 Sep-Oct;183(5):363-73.

27. Colombat M, Mal H, Groussard O, Capron F, Thabut G, Jebrak G, et al. Pulmonary vascular lesions in end-stage idiopathic pulmonary fibrosis: Histopathologic study on lung explant specimens and correlations with pulmonary hemodynamics. Hum Pathol 2007 Jan;38(1):60-5.

28. Pietra GG, Capron F, Stewart S, Leone O, Humbert M, Robbins IM, et al. Pathologic assessment of vasculopathies in pulmonary hypertension. J Am Coll Cardiol 2004 Jun 16;43(12 Suppl S):25S-32S.

29. Weitzenblum E, Chaouat A. Pulmonary hypertension due to chronic hypoxic lung disease. In: Peacock AJ, Rubin LJ, editors. Pulmonary circulation: diseases and their treatment. London: Arnold; 2004. p. 374-86.

30. Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Respir J 2009 Dec;34(6):1219-63.

31. Janda S, Shahidi N, Gin K, Swiston J. Diagnostic accuracy of echocardiography for pulmonary hypertension: a systematic review and meta-analysis. Heart 2011 Apr;97(8):612-22.

32. Gilman AG, Rall TW, Nies AS, Taylor P. The Pharmacological Basis of Therapeutics. 8th ed. New York: Macmillan; 1990.

Table 1. Baseline Characteristics of Patients at Time of Initial Assessment. *

Parameter	AE (n = 27)	NAE (n = 28)	Overall (n = 55)
Age (years)	61.92 (5.27)	58.15 (8.94)	60.04 (7.51)
Gender (no. of male patients)	20	21	41
Body Mass Index	26.87 (2.96)	26.02 (4.29)	26.44 (3.76)
FVC (% of predicted value)	59.32 (14.02)	60.17 (21.62)	59.76 (18.20)

FEV_1 (% of predicted value)	63.46 (14.48)	60.58 (18.80)	61.96 (16.77)
DL_{CO} (% of predicted value)	30.73 (10.18)	29.13 (12.87)	29.91 (11.51)
TLC (% of predicted value)	62.81 (11.59)	65.45 (13.52)	64.13 (12.53)
Cytotoxic Abs (no. of patients)	14	12	26
LVEF (%)	59.37 (7.42)	59.71 (5.04)	59.53 (6.35)
RVSP (Echo) (mm Hg)	28.69 (13.14)	28.08 (8.91)	28.39 (10.88)
RVSP (RHC) (mm Hg)	32.45 (6.62)	30.40 (6.97)	31.43 (6.78)
mPAP (mm Hg)	22.47 (8.17)	19.78 (4.87)	21.13 (6.80)
PCWP (mm Hg)	7.47 (4.21)	8.00 (3.35)	7.73 (3.87)
CO (L/min)	5.29 (1.39)	5.54 (1.47)	5.40 (1.41)

***** Data are presented as mean (SD). AE = acute exacerbation; NAE = non-acute exacerbation; FVC = forced vital capacity; FEV_1 = forced expiratory volume in one second; DL_{CO} = diffusion capacity of the lung for carbon monoxide; TLC = total lung capacity; Cytotoxic Abs = cytotoxic antibodies; LVEF = left ventricular ejection fraction on echocardiogram; RVSP (Echo) = right ventricular systolic pressure measured by echocardiogram; RVSP (Echo) = right ventricular systolic pressure measured during right heart catheterization; mPAP = mean pulmonary arterial pressure during right heart catheterization; PCWP = pulmonary capillary wedge pressure during right heart catheterization; CO = cardiac output measured during right heart catheteriszation; † $p < 0.05$.

Table 2. Prognostic factors for overall survival from initial assessment of non-transplanted patients over the follow-up period (n=42).

Parameter	Hazard Ratio	95% CI	P
Univariate Cox Analysis			
Age	0.991	0.941-1.045	0.749
Male	0.549	0.220-1.373	0.203
Body Mass Index	0.984	0.886-1.092	0.758
FVC (% of predicted value)	0.987	0.966-1.007	0.187

FEV_1 (% of predicted value)	0.995	0.973-1.017	0.646
DL_{CO} (% of predicted value)	1.002	0.962-1.044	0.918
TLC (% of predicted value)	0.981	0.946-1.017	0.299
LVEF (%)	0.989	0.921-1.061	0.752
RVSP (mm Hg)	1.005	0.966-1.045	0.824
mPAP (mm Hg)	1.080	1.013-1.152	0.033
PH	5.201	1.987-13.61	< 0.001
Occurrence of AE #	4.037	1.568-10.40	0.004
Multivariate Cox Analysis			
PH	4.749	1.278-17.64	0.021
Occurrence of AE #	2.924	1.089-7.852	0.055
mPAP	0.989	0.898-1.090	0.827

CI = confidence interval; FVC = forced vital capacity; FEV_1 = forced expiratory volume in one second; DL_{CO} = diffusion capacity of the lung for carbon monoxide; TLC = total lung capacity; LVEF = left ventricular ejection fraction on echocardiogram; RVSP = right ventricular systolic pressure; mPAP = mean pulmonary arterial pressure during right heart catheterization; PH = pulmonary hypertension; AE = acute exacerbation; # Overall occurrence over follow-up period.

Table 3. Risk Factors at the time of initial assessment for Acute Exacerbation.

Parameter	Hazard Ratio	95% CI	P
Univariate Cox Analysis			
Age	1.023	0.967-1.081	0.435
Male	0.599	0.250-1.434	0.253
Body Mass Index	1.043	0.939-1.159	0.437
FVC (% of predicted value)	0.999	0.979-1.019	0.928
FEV_1 (% of predicted value)	1.008	0.987-1.031	0.438
DL_{CO} (% of predicted value)	1.003	0.967-1.041	0.873

TLC (% of predicted value)	0.982	0.946-1.019	0.347
LVEF (%)	0.989	0.922-1.062	0.767
RVSP (mm Hg)	1.010	0.977-1.042	0.597
mPAP (mm Hg)	1.043	0.977-1.114	0.210
PH	2.217	1.005-4.889	0.041
PCWP (mm Hg)	0.938	0.843-1.044	0.241
Multivariate Cox Analysis			
Male	0.587	0.398-1.139	0.182
PH	2.510	1.119-5.628	0.026

CI = confidence interval; FVC = forced vital capacity; FEV_1 = forced expiratory volume in one second; DL_{CO} = diffusion capacity of the lung for carbon monoxide; TLC = total lung capacity; LVEF = left ventricular ejection fraction on echocardiogram; RVSP = right ventricular systolic pressure; mPAP = mean pulmonary arterial pressure during right heart catheterization; PH = pulmonary hypertension; PCWP = pulmonary capillary wedge pressure during right heart catheterization;

Table 4. Characteristics of Patients (n = 13) at Time of Lung Transplantation

Parameter	Value
Age	57.69 (5.42)
Gender (no. of males)	10
Body Mass Index	26.73 (3.02)
Cytotoxic Antibodies (no. of patients)	6
LVEF (%)	60.73 (5.16)
mPAP (mm Hg)	36.21 (16.73)
PCWP (mm Hg)	8.54 (4.68)

* Data are presented as mean (SD). LVEF = left ventricular ejection fraction on echocardiogram; mPAP = mean pulmonary arterial pressure during right heart catheterization; PCWP = pulmonary capillary wedge pressure during right heart catheterization;

Figure Legends

Figure 1. Survival of Patients in the Acute and Non-Acute Exacerbation Groups. As lung transplantation alters the natural course of IPF, transplantation events (n=13) over the follow-up period were censored when performing this Kaplan-Meier survival analysis. AE = acute exacerbation; NAE = non-acute exacerbation; mths = months; * p = 0.0015.

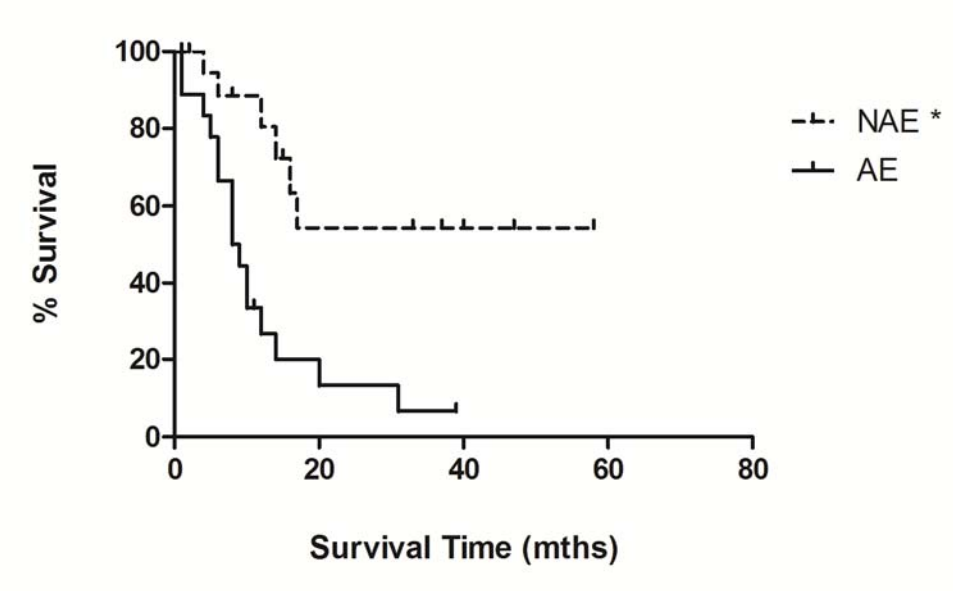

Figure 2. Mean Pulmonary Arterial Pressure in Lung Transplant Recipients (Acute Exacerbation (n = 8), Non-Acute Exacerbation (n = 5) and Overall (n = 13)) at baseline and at the time of transplantation. mPAP = Mean Pulmonary Arterial Pressure; AE = Acute Exacerbation; NAE = Non-Acute Exacerbation; Overall = overall mean;

* p = 0.039, mPAP at baseline compared to mPAP at time of transplantation in transplant recipients who had experienced an AE; ** p = 0.027, mPAP at baseline compared to mPAP at time of transplantation in all transplant recipients; Median (interquartile range) of mPAP at baseline and at time of transplant are as follows – AE: 18.00 (12.75 to 27.50) and 32.17 (26.42 to 52.42), NAE: 25.33 (19.00 to 27.70) and 21.00 (17.84 to 55.17), Overall: 23.00 (15.00 to 27.00) and 30.67 (20.50 to 55.00).

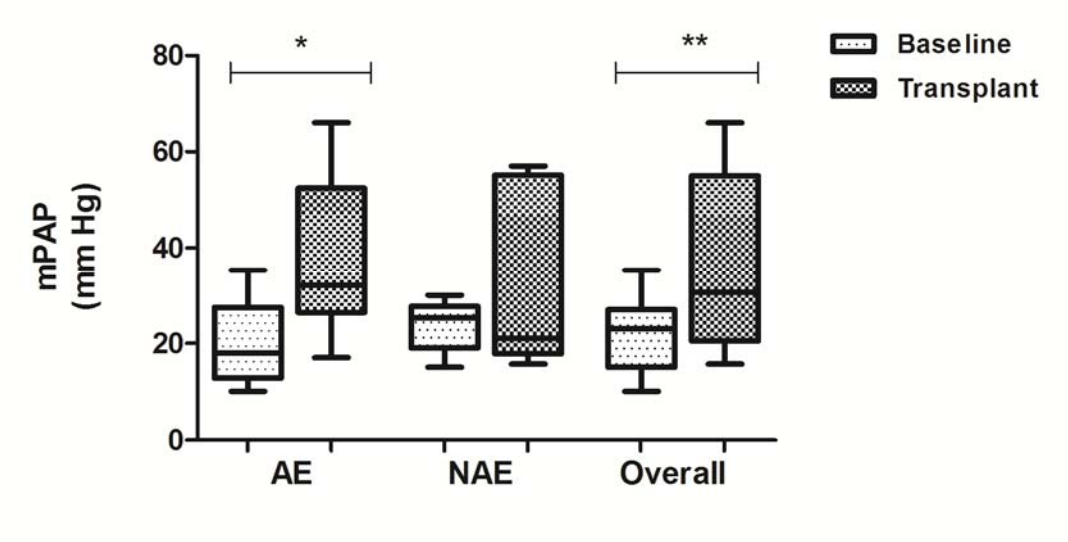

Figure 3. Explanted lung histology: A) IPF lung showing diffuse alveolar damage with hyaline membranes lining the alveolar spaces, as seen in acute exacerbations (Martius, Scarlet and Blue stain, x20); B) branch of pulmonary artery (arrow) adjacent to fibroblastic foci (*) showing asymmetrical intimal thickening as seen in pulmonary hypertension (H&E, x20); C and D) CD31 staining showing increased number of capillaries in IPF lung and honey comb areas (x20) compared to normal control (insert).

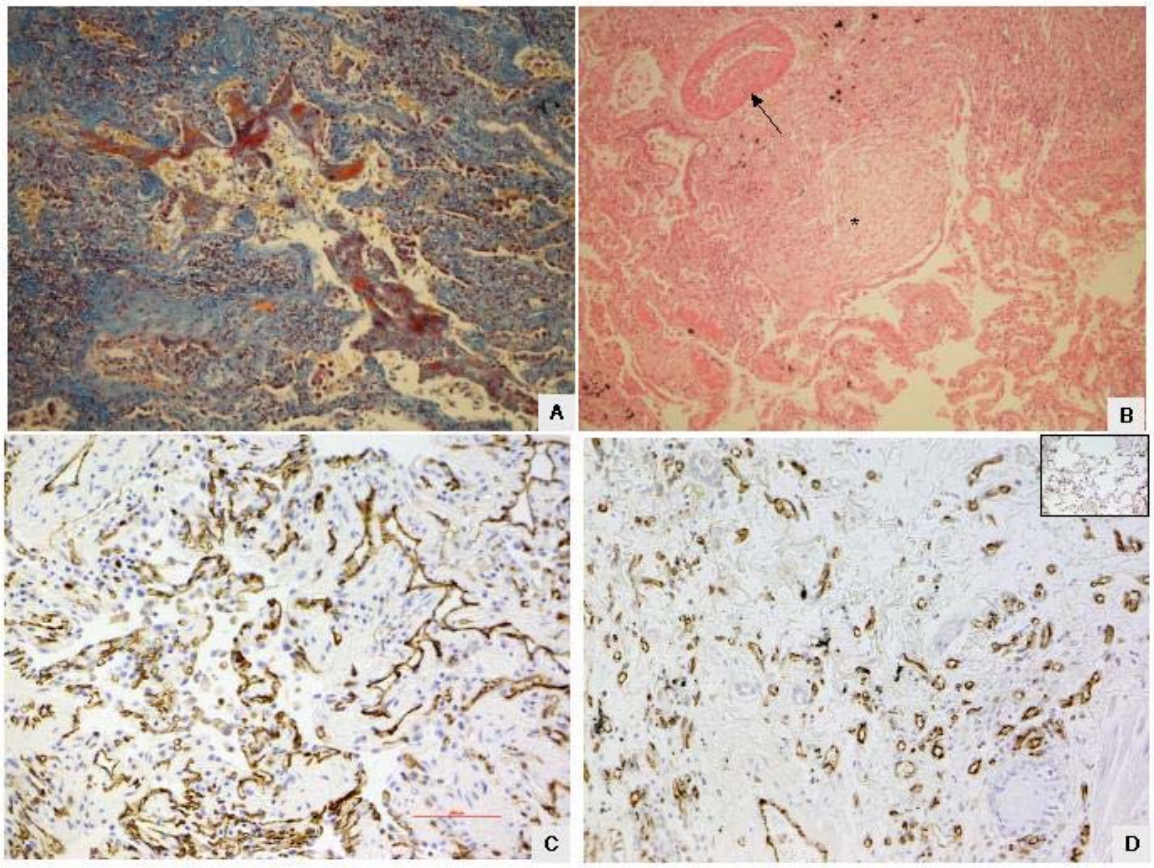

Figure 4. Mean Microvessel Density (%) in Control (non-diseased) areas and in areas of Cellular Fibrosis and Honeycombing. MVD = mean microvessel density; Fibrosis = Cellular Fibrosis; * $p < 0.0001$, MVD in areas of cellular fibrosis compared to control areas and MVD in areas of cellular fibrosis compared to areas of honeycomb; ** $p = 0.04$, MVD in areas of honeycomb compared to control areas; Median (interquartile range) of MVD (%) as follows – Honeycomb: 9.29 (7.54 to 10.47), Cellular Fibrosis: 23.14 (21.68 to 27.57), Control: 11.74 (9.14 to 14.11).

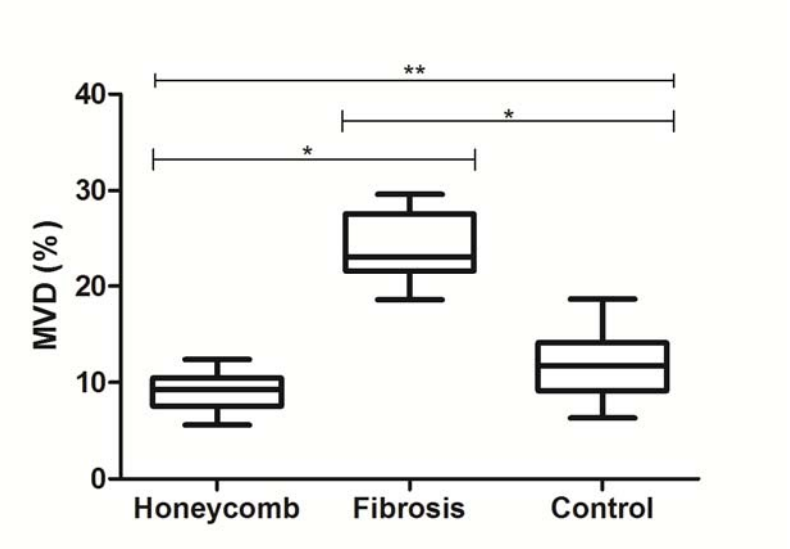

Figure 5. Relationship between Mean Microvessel Density in areas of Honeycombing and Mean Pulmonary Arterial Pressure. MVD = mean microvessel density in areas of honeycombing; mPAP = mean pulmonary arterial pressure; Spearman correlation rho = -0.637, p = 0.019.

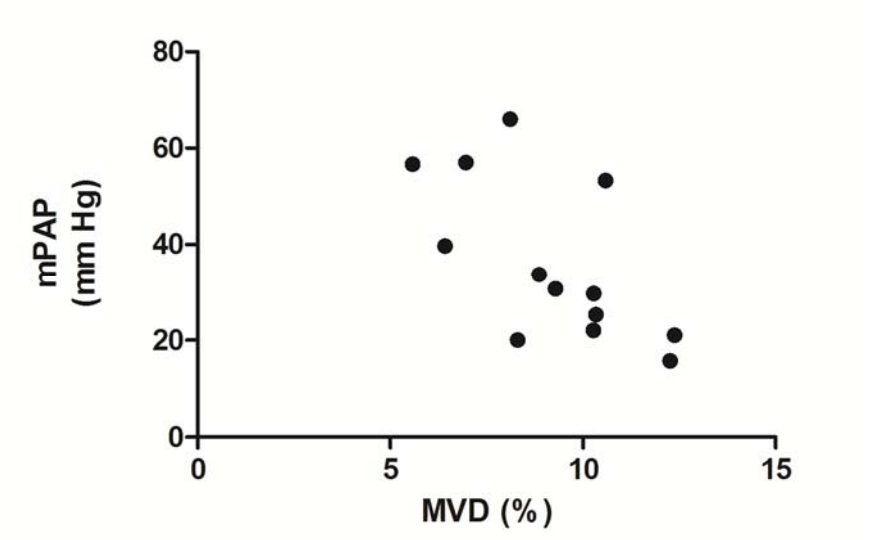

Figure 6. Relationship between Mean Microvessel Density in areas of Cellular Fibrosis and Mean Pulmonary Arterial Pressure. MVD = mean microvessel density in areas of cellular fibrosis; mPAP = mean pulmonary arterial pressure; Spearman correlation rho = -0.258, p = 0.394.