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Bronchoalveolar Lavage Pepsin in Acute Exacerbation of Idiopathic Pulmonary Fibrosis

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ABSTRACT

Some patients with idiopathic pulmonary fibrosis experience acute exacerbations in their respiratory status leading to substantial morbidity and mortality. Occult aspiration of gastric contents has been proposed as one possible mechanism leading to these acute exacerbations. We sought to determine if pepsin, a marker of gastric aspiration, is elevated in bronchoalveolar lavage fluid obtained from patients during acute exacerbation of idiopathic pulmonary fibrosis compared to stable disease.

Lavage samples were obtained in a case-control study of well-characterized patients. Acute exacerbation was defined using standard criteria. Levels of lavage pepsin were compared in cases and controls, and were correlated with clinical features and disease course.

Twenty-four cases with acute exacerbations and 30 stable controls were identified. There were no significant differences in baseline demographics between the two groups. Pepsin level was an indicator of acute exacerbation status (p value = 0.04). On average, pepsin appeared higher in patients with acute exacerbations compared to stable controls. This difference was driven by a subgroup of eight patients (33%) with pepsin levels $\geq$ 70 ng/ml. Pepsin level was not an independent predictor of survival time.

These results suggest occult aspiration may play a role in some cases of acute exacerbation of idiopathic pulmonary fibrosis.

Key Words: Acute lung injury, aspiration, gastroesophageal reflux, idiopathic pulmonary fibrosis

Word Count: 200

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is the most common form of idiopathic interstitial pneumonia, with a prevalence of 14 to 42.7 per 100,000 [1]. There is no established therapy and the median survival from the time of diagnosis is approximately 3 years [2, 3]. The natural history of IPF has historically been viewed as one of gradual progression, characterized by a steady decline in lung function over time. More recently it has been recognized that many patients may experience acute deteriorations in their respiratory status after periods of relative stability [4]. When there is no obvious precipitating cause identified (e.g. infection, pulmonary embolism), these acute deteriorations have been termed acute exacerbations of IPF. Acute exacerbations of IPF are characterized by evidence of new ground glass opacities on high-resolution computed tomography (HRCT) scan and diffuse alveolar damage (DAD) on surgical lung biopsy [4-6].

Occult aspiration of gastric contents secondary to gastroesophageal reflux has been proposed as one possible cause of acute exacerbation of IPF [4, 7]. Previous studies suggest that the majority of patients with IPF have gastroesophageal reflux [8-10]. Gastroesophageal reflux is suspected to be a risk factor for pulmonary aspiration [11]. Aspiration of gastric contents can cause acute lung injury, manifest by DAD on lung biopsy [12].

Gastric pepsin is a proteolytic enzyme secreted by gastric chief cells and mucus neck cells as inactive pepsinogen. Bronchoalveolar lavage (BAL) pepsin has been shown to be a useful biomarker for gastric aspiration in the post-lung transplant [13, 14], pediatric [15], asthmatic

[16], and mechanically ventilated populations [17]. In particular, BAL pepsin has been shown to be a highly specific (100%) and sensitive (80%) method for diagnosing gastroesophageal reflux-associated aspiration in children [15]. Gastric pepsin can be detected in the BAL of some normal healthy subjects at very low levels (up to 2.6 ng/ml) [18]. We hypothesized that BAL pepsin levels would be elevated in some patients with acute exacerbation of IPF compared to stable IPF, suggesting that aspiration of gastric contents contributed to a subgroup of acute exacerbations.

MATERIALS AND METHODS

Study Design and Patient Population

This was a case-control study of patients with acute exacerbation of IPF and stable IPF seen at Asan Medical Center from 2000 to 2009. The subjects were enrolled into an Institutional Review Board approved longitudinal cohort study investigating IPF. Informed consent was obtained for all patients.

The diagnosis of IPF was made by multi-disciplinary review according to modified consensus criteria [19]. Stable IPF was defined as a patient with IPF who was not having an exacerbation at the time of BAL. BAL was obtained around the time of the initial IPF diagnosis (median difference in time between IPF diagnosis and BAL was 1 day, interquartile range 9-13 days). These patients were randomly selected from a larger cohort as the control population. The diagnosis of acute exacerbation of IPF was made according to published criteria [4]. Briefly, acute exacerbation was defined by worsening respiratory symptoms within 30 days, new bilateral

ground glass opacities on HRCT, and no evidence of an alternative etiology. All patients with acute exacerbation of IPF and BAL fluid available were included in this study.

Patient demographics, history of tobacco use (categorized as never, former, and current smokers), body mass index (BMI), and pulmonary function values were recorded. Disease co-morbidities were recorded as present or absent based on patient or physician reporting of the disease. Patient characteristics and vital status were obtained using the medical record.

Bronchoscopy and BAL pepsin measurement

BAL was performed as previously reported [20]. For acute exacerbation cases, BAL was performed as part of the diagnostic evaluation of the patients' acute respiratory symptoms. For stable IPF controls, BAL was performed as part of the initial diagnostic evaluation of their interstitial lung disease. For both cases and controls, BAL was performed in a single sub-segment of the right middle lobe or lingula, with at least 100 ml of sterile saline instilled. The BAL fluid was kept on ice and processed within 1 hour of collection then frozen at -80^{o}C. BAL pepsin levels were measured using a commercially available ELISA (USCN Life Science, Inc.).

Radiology review

All HRCT scans were reviewed by a radiologist blinded to the status (case or control) and clinical course of all subjects. Extent of ground-glass opacity, consolidation, honeycombing, reticulation, and overall computed tomography (CT) score were determined [21]. The presence and size (craniocaudal length and diameter) of hiatal hernia, a known risk factor for gastroesophageal reflux [22], was also determined. The craniocaudal dimension was measured

from the superior-most image on which the hiatal hernia was present to the level of the esophageal hiatus. The diameter was measured at the largest transverse dimension of the intra-thoracic component of the hiatal hernia.

Statistical Analysis

Descriptive statistics are presented as mean (standard deviation) or median (inter-quartile range (IQR) 25^{th} percentile, 75^{th} percentile). Inter-group comparisons were performed using *t*-test, Mann-Whitney rank sum test, Chi^2 test or Fischer's Exact test as appropriate. Logistic regression analysis was used to identify predictors of acute exacerbation status. Linear regression analysis was used to analyze the association between independent variables and BAL pepsin level. Cox proportional hazard modeling was performed to identify predictors of survival time in the acute exacerbation cohort. Survival time was calculated from the time of BAL until death or time of censoring. Analysis was also performed using pepsin as a dichotomous variable, using the 95th percentile value from the stable IPF group as the threshold value. All data analysis was performed using STATA version 11. All tests were two-sided and performed at a significance level of 0.05.

RESULTS

Clinical, radiologic, and BAL features of cases and controls

There were a total of 24 acute exacerbation of IPF cases and 30 stable IPF controls identified. There were no significant differences in baseline demographics between these two groups, including age, gender, smoking status, body mass index, and reported co-morbidities (**Table 1**).

There were significant differences in the ground glass opacity score (p value <0.01), the consolidation score (p value 0.01), the reticulation score (p value <0.01), and the overall CT scan score (p value <0.01) between the two groups. (**Table 2**) There was no significant difference in the honeycombing score between the two groups. There was no difference between the two groups in the prevalence or size of radiologically identified hiatal hernia.

The BAL fluid was clear and none of the samples had obvious blood on gross examination. The BAL red blood cell (RBC) count was higher in the acute exacerbation group compared to the stable group (p value <0.01). There was no difference in BAL total cell count between the two groups (**Table 2**). There was a higher percentage of BAL neutrophils (p value = 0.02) and a lower percentage of BAL macrophages (p value <0.01) in the acute exacerbation group compared to the stable group.

Indicators of acute exacerbation status

The median level of BAL pepsin in the acute exacerbation of IPF cases was 46.8 ng/ml, compared to 35.4 ng/ml in the stable IPF controls (p = 0.15, **Figure 1**). On bivariate regression, a one unit increase in BAL pepsin was an indicator of acute exacerbation status (OR 1.02, 95% CI 1.001-1.03, p value 0.04). An increase in BAL pepsin level by one standard deviation (24.8 ng/ml) was associated with an odds ratio of 1.46 (95% CI 1.03, 2.09, p value = 0.04) for acute exacerbation status.

There were no clinical variables associated with acute exacerbation status (**Table 3**). There was no association of prevalence or size of radiologic hiatal hernia with acute exacerbation status. Elevated BAL neutrophil percentage and decreased BAL alveolar macrophage percentage were indicators of acute exacerbation status. After adjusting for BAL neutrophil percentage, BAL pepsin level remained an indicator of acute exacerbation status (OR 1.02, 95% CI 1.002, 1.03, p = 0.03).

BAL pepsin levels and acute exacerbation

Eight (33%) of the acute exacerbation of IPF cases had high BAL pepsin levels (i.e. above the 95th percentile of the stable control population, 70 ng/ml). There were no significant differences in baseline clinical characteristics, including history of gastroesophageal reflux or aspiration, use of prednisone or anti-reflux medications, and survival time, between the high and low pepsin acute exacerbation cases (**Table 4**). Two subjects in the acute exacerbation group were intubated during the time of the BAL. Their BAL pepsin levels were in the low BAL pepsin group (36.8 ng/ml and 31.8 ng/ml). There were also no significant differences in the presence of radiologic hiatal hernia, the percentage of ground glass opacities, consolidation or reticulation between the two groups. The high BAL pepsin group had a higher percentage of BAL lymphocytes compared to the low BAL pepsin group (p value <0.01). There was no significant difference in RBC count between those with low and high BAL pepsin levels (p value 0.24).

Seven subjects in the acute exacerbation cohort also had previously collected BAL fluid at the time of IPF diagnosis (i.e. a corresponding stable sample). In this small subgroup, there was no overall relationship between stable (median 17.3 ng/ml, IQR 0, 92.5 ng/ml) and acute

exacerbation (median 52.5 ng/ml, IQR 8.5, 63.6 ng/ml) BAL pepsin levels (p value 0.61, **Figure 2**). However, only one of the seven subjects had a high BAL pepsin level during their acute exacerbation (220.8 ng/ml); this represented an approximately 10-fold increase in BAL pepsin level compared to the subject's stable value (23.8 ng/ml).

Predictors of BAL pepsin level

In the combined group of cases and controls, clinical variables including age, gender, history of gastroesophageal reflux, history of smoking, BMI, anti-reflux medication use, prednisone use, and intubation status, were not associated with BAL pepsin level (data not shown). Acute exacerbation status and increased reticulation on HRCT were associated with higher BAL pepsin levels (p value = 0.01 and p value = 0.02, respectively). No other radiological variables were significant indicators, including radiologic hiatal hernia. Increased percentage of BAL lymphocytes was associated with increased BAL pepsin level (p value <0.01). There was no association between BAL pepsin level and BAL RBC count (p value 0.79). Within the stable IPF cohort, there was no correlation between BAL pepsin levels and total lung capacity (TLC) percent predicted (**Figure 3**, p value = 0.83) or forced vital capacity (FVC) percent predicted (p value = 0.39).

Survival

The median follow-up time in the acute exacerbation cohort was 74 days (IQR 15, 492). Thirty and 60 day survival were 67% and 58%, respectively. Only 17% of the subjects in the acute exacerbation cohort were alive at the time of final analysis. Analysis of acute exacerbation cases revealed no significant predictors of survival time (**Table 5**). Specifically, BAL pepsin level was

not predictive of survival. Analysis of the stable IPF controls revealed a trend towards higher BAL pepsin levels in those that had died compared to those that were still alive (48.4 vs. 18.3 ng/ml, p value = 0.10).

DISCUSSION

This study addressed the relationship of occult aspiration to acute exacerbation in patients with IPF. Elevated BAL pepsin was predictive of acute exacerbation status on both bivariate and multivariate analysis, driven by the presence of a subgroup of cases (33%) with markedly elevated pepsin levels. Although the relationship is modest, this finding provides evidence that occult aspiration may play a role in acute exacerbation of IPF.

There is a growing interest in the role of gastroesophageal reflux and occult aspiration in patients with IPF [7]. It has been hypothesized that occult aspiration of gastric contents may contribute to the natural history of IPF. One case series described 4 patients with IPF whose clinical course stabilized (based on pulmonary function testing) after receiving medical therapy for gastroesophageal reflux [23]. Another study reviewed 14 patients with IPF awaiting lung transplantation and showed stabilization of oxygen requirements in those patients who had a laparoscopic Nissen fundoplication [24]. These studies support a possible association of gastroesophageal reflux and occult aspiration with disease progression in IPF.

Our study extends this relationship in two ways. First, we found measurable BAL pepsin in most patients with stable IPF, suggesting that occult aspiration is common in IPF. Second, the

association of elevated BAL pepsin with acute exacerbation status provides evidence that occult aspiration may play a role in the natural history of IPF, perhaps through causing some acute exacerbations. An alternative explanation for this association could be that acute respiratory decompensation leads to increased aspiration (and therefore elevated BAL pepsin levels) through increased intrathoracic pressure swings. In our stable IPF cohort, we found no correlation between the severity of disease, as measured by TLC % predicted and FVC % predicted, and BAL pepsin level. However, we do not have pulmonary function test results in the acute exacerbation cohort, and it is possible that microaspiration could occur in the setting of acute respiratory decompensation.

There were no clinical predictors of BAL pepsin level across cases and controls in this study. This finding is consistent with the known discordance between signs and symptoms of gastroesophageal reflux and the presence of pathologic reflux on esophageal pH testing [8, 9]. Importantly, we do not have functional esophageal data (e.g. 24-hour pH monitoring and manometry), so it is unknown if cases with elevated BAL pepsin levels have altered esophageal function. We found no difference in radiologic hiatal hernia between the high and low BAL pepsin groups, which might have helped identify patients at risk for aspiration-related acute exacerbation. There was also no association between BAL pepsin level and survival in cases of acute exacerbation of IPF. This was not unexpected, as acute exacerbation of IPF is characterized by DAD, the natural history of which is likely independent of the underlying etiology.

The BAL differential cell count was associated with acute exacerbation of IPF and BAL pepsin levels. Increased BAL neutrophil percent was an indicator of acute exacerbation and was

suggestive of worse survival. These findings are consistent with data previously published showing BAL neutrophilia is associated with reduced 1-year survival in IPF [25]. Interestingly, the percentage of BAL lymphocytes was higher in acute exacerbation of IPF cases with high BAL pepsin levels. The significance of this finding is unclear, but may suggest that more significant aspiration is associated with lymphocyte recruitment to the lung.

This study was not designed to identify risk factors for the development of acute exacerbation, and therefore does not identify an "at risk" population. Our limited number of acute exacerbation patients with paired samples (n=7) suggest that baseline BAL pepsin levels are not predictive of future acute exacerbation, but this subgroup is underpowered to address this issue. Our data do not support obtaining BAL pepsin levels on stable patients. The relationship of baseline gastroesophageal reflux and aspiration to future acute exacerbation should be the basis for future, longitudinal studies.

We recognize that this study has other limitations. First, BAL pepsin is not a direct measure of aspiration. However, given that pepsin is not normally found in high levels in the lower respiratory tract, its measurement in BAL should be a useful biomarker of aspiration [15, 18, 26]. Second, BAL pepsin level may be affected by factors other than aspiration such as contamination of the bronchoscope with upper airway secretions, variation in the concentration of bronchoalveolar lavagate, patient's position in bed, or recent intubation. These factors are partially controlled for by the inclusion of a stable IPF control group and standardized BAL technique. Third, there is a theoretical concern that we could be detecting pepsinogen from a non-gastric source (i.e. the blood) due to cross-reactivity of the antibody. However, we found no

correlation between the BAL RBC count and the BAL pepsin level. Fourth, the site of the BAL was in either the right middle lobe or lingula, which was not necessarily the most diseased area by HRCT scan. It is possible that the BAL pepsin levels may have been higher had the most diseased area been sampled. Last, given the use of chart review to ascertain medical co-morbidities, we have most likely underestimated the prevalence of gastroesophageal reflux disease and the other co-morbidities in this population.

In summary, this study demonstrates that BAL pepsin is elevated in a subgroup of patients with acute exacerbation of IPF. These findings support the hypothesis that occult aspiration of gastric contents plays a role in acute exacerbation of IPF. Future research should confirm these results in a larger, multi-center study.

REFERENCES

1. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2006; 174: 810-816.

2. Bjoraker JA, Ryu JH, Edwin MK, Myers JL, Tazelaar HD, Schroeder DR, Offord KP. Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1998; 157: 199-203.

3. King TE, Jr., Tooze JA, Schwarz MI, Brown KR, Cherniack RM. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. *Am J Respir Crit Care Med* 2001; 164: 1171-1181.

4. Collard HR, Moore BB, Flaherty KR, Brown KK, Kaner RJ, King TE, Jr., Lasky JA, Loyd JE, Noth I, Olman MA, Raghu G, Roman J, Ryu JH, Zisman DA, Hunninghake GW, Colby TV, Egan JJ, Hansell DM, Johkoh T, Kaminski N, Kim DS, Kondoh Y, Lynch DA, Muller-Quernheim J, Myers JL, Nicholson AG, Selman M, Toews GB, Wells AU, Martinez FJ. Acute exacerbations of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2007; 176: 636-643.

5. 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; 27: 143-150.

6. Travis WD, Colby, T.V., Koss, M.N., Rosado-de-Crhistenson, M.L., Muller, N.L., King Jr., T.E. Lipoid Pneumonia and Chronic Fibrosis. *In:* King DW, ed. Non-Neoplastic Disorders of the Lower Respiratory Tract. American Registry of Pathology and the Armed Forces Institute of Pathology, Washington, DC, 2002.

7. Lee JS, Collard HR, Raghu G, Sweet MP, Hays SR, Campos GM, Golden JA, King TE, Jr. Does chronic microaspiration cause idiopathic pulmonary fibrosis? *Am J Med* 2010; 123: 304-311.

8. Tobin RW, Pope CE, 2nd, Pellegrini CA, Emond MJ, Sillery J, Raghu G. Increased prevalence of gastroesophageal reflux in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1998; 158: 1804-1808.

9. Sweet MP, Patti MG, Leard LE, Golden JA, Hays SR, Hoopes C, Theodore PR. Gastroesophageal reflux in patients with idiopathic pulmonary fibrosis referred for lung transplantation. *J Thorac Cardiovasc Surg* 2007; 133: 1078-1084.

10. Raghu G, Freudenberger TD, Yang S, Curtis JR, Spada C, Hayes J, Sillery JK, Pope CE, 2nd, Pellegrini CA. High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis. *Eur Respir J* 2006; 27: 136-142.

11. Pellegrini CA, DeMeester TR, Johnson LF, Skinner DB. Gastroesophageal reflux and pulmonary aspiration: incidence, functional abnormality, and results of surgical therapy. *Surgery* 1979; 86: 110-119.

12. Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 2000; 342: 1334-1349.

13. Ward C, Forrest IA, Brownlee IA, Johnson GE, Murphy DM, Pearson JP, Dark JH, Corris PA. Pepsin like activity in bronchoalveolar lavage fluid is suggestive of gastric aspiration in lung allografts. *Thorax* 2005; 60: 872-874.

14. Blondeau K, Mertens V, Vanaudenaerde BA, Verleden GM, Van Raemdonck DE, Sifrim D, Dupont LJ. Gastro-oesophageal reflux and gastric aspiration in lung transplant patients with or without chronic rejection. *Eur Respir J* 2008; 31: 707-713.

15. Farrell S, McMaster C, Gibson D, Shields MD, McCallion WA. Pepsin in bronchoalveolar lavage fluid: a specific and sensitive method of diagnosing gastro-oesophageal reflux-related pulmonary aspiration. *J Pediatr Surg* 2006; 41: 289-293.

16. Starosta V, Kitz R, Hartl D, Marcos V, Reinhardt D, Griese M. Bronchoalveolar pepsin, bile acids, oxidation, and inflammation in children with gastroesophageal reflux disease. *Chest* 2007; 132: 1557-1564.

17. Metheny NA, Chang YH, Ye JS, Edwards SJ, Defer J, Dahms TE, Stewart BJ, Stone KS, Clouse RE. Pepsin as a marker for pulmonary aspiration. *Am J Crit Care* 2002; 11: 150-154.

18. Stovold R, Forrest IA, Corris PA, Murphy DM, Smith JA, Decalmer S, Johnson GE, Dark JH, Pearson JP, Ward C. Pepsin, a biomarker of gastric aspiration in lung allografts: a putative association with rejection. *Am J Respir Crit Care Med* 2007; 175: 1298-1303.

19. 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* 2000; 161: 646-664.

20. Kim DS, Jeon YG, Shim TS, Lim CM, Lee SD, Koh Y, Kim WS, Kim WD. The value of interleukin-12 as an activity marker of pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2000; 17: 271-276.

21. Akira M, Hamada H, Sakatani M, Kobayashi C, Nishioka M, Yamamoto S. CT findings during phase of accelerated deterioration in patients with idiopathic pulmonary fibrosis. *AJR Am J Roentgenol* 1997; 168: 79-83.

22. Patti MG, Goldberg HI, Arcerito M, Bortolasi L, Tong J, Way LW. Hiatal hernia size affects lower esophageal sphincter function, esophageal acid exposure, and the degree of mucosal injury. *Am J Surg* 1996; 171: 182-186.

23. Raghu G, Yang ST, Spada C, Hayes J, Pellegrini CA. Sole treatment of acid gastroesophageal reflux in idiopathic pulmonary fibrosis: a case series. *Chest* 2006; 129: 794-800.

24. Linden PA, Gilbert RJ, Yeap BY, Boyle K, Deykin A, Jaklitsch MT, Sugarbaker DJ, Bueno R. Laparoscopic fundoplication in patients with end-stage lung disease awaiting transplantation. *J Thorac Cardiovasc Surg* 2006; 131: 438-446.

25. Kinder BW, Brown KK, Schwarz MI, Ix JH, Kervitsky A, King TE, Jr. Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. *Chest* 2008; 133: 226-232.

26. Ufberg JW, Bushra JS, Patel D, Wong E, Karras DJ, Kueppers F. A new pepsin assay to detect pulmonary aspiration of gastric contents among newly intubated patients. *Am J Emerg Med* 2004; 22: 612-614.

FIGURE LEGENDS

Figure 1: Bronchoalveolar lavage (BAL) pepsin levels in patients with stable idiopathic pulmonary fibrosis (IPF) compared to acute exacerbation of IPF. Median, 25th percentile, and 75th percentile are shown with horizontal lines.

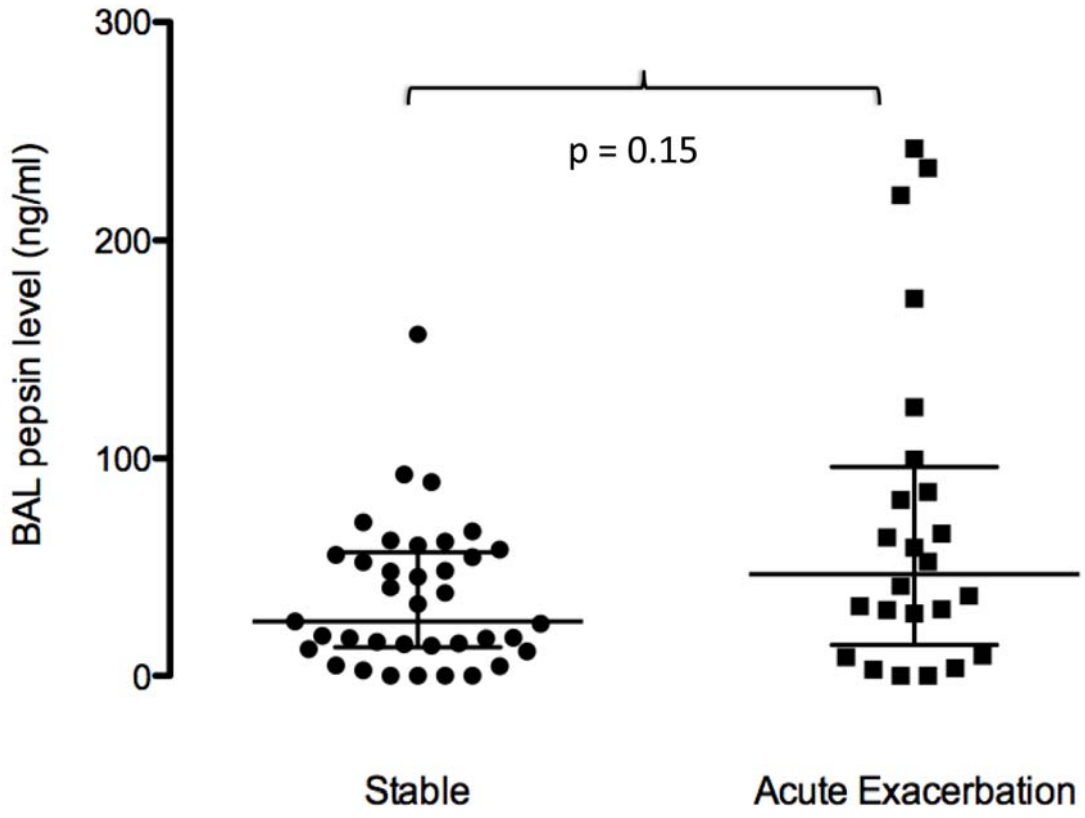

Figure 2: Bronchoalveolar lavage (BAL) pepsin levels in seven patients with matched stable and acute exacerbation of idiopathic pulmonary fibrosis (IPF) BAL samples. Each line represents one study subject. The closed circle and solid line represents the subject who had a high BAL pepsin

level during their acute exacerbation. The open circle and dashed lines represent those subjects who had low BAL pepsin levels during their acute exacerbation.

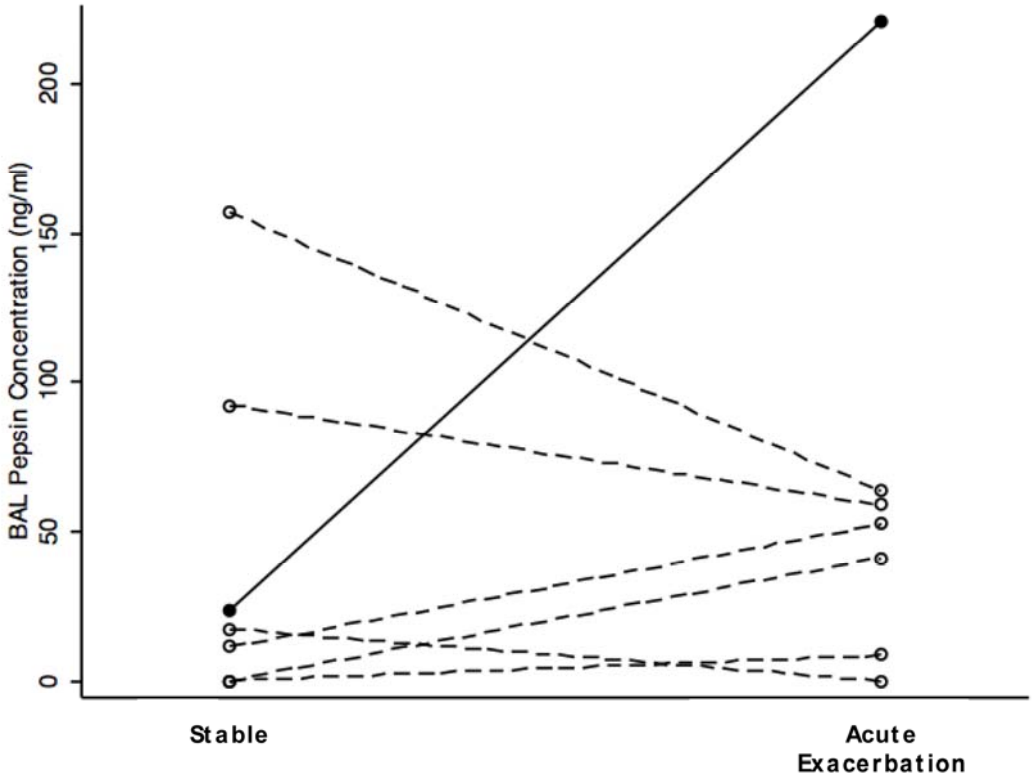

Figure 3: Correlation between bronchoalveolar lavage (BAL) pepsin levels and total lung capacity (TLC) % predicted.

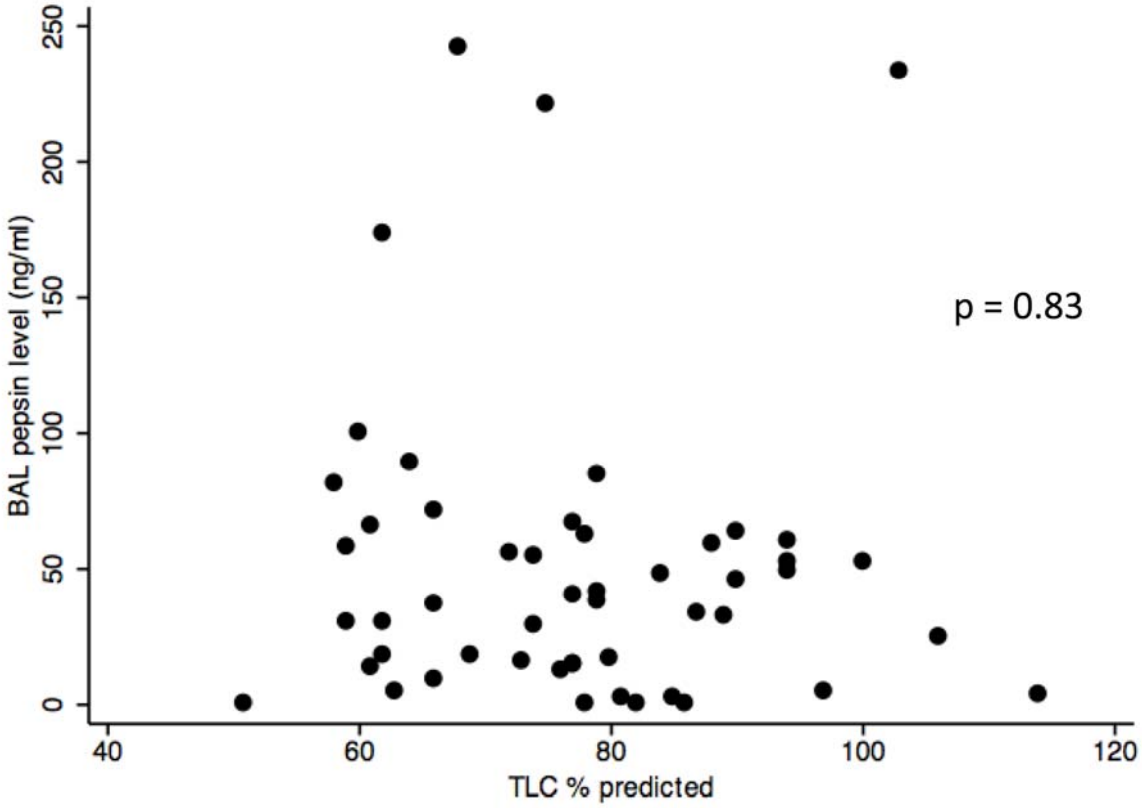

Table 1: Baseline demographics*

	Stable IPF (n=30)	Acute Exacerbation of IPF (n=24)	p value
Age	65 ± 8	64 ± 9	0.91
Male	77% (23)	79% (19)	0.83
Smoking History			
Never smoker	27% (8)	21% (5)	0.62
Former smoker	60% (18)	71% (17)	0.41
Current smoker	13% (4)	8% (2)	0.56
Current Alcohol Use	43% (13)	54% (13)	0.43
Body Mass Index	24.5 ± 3.8	25.2 ± 4.0	0.56
Co-Morbidities			
CAD	3% (1)	13% (3)	0.20
GERD	0% (0)	8% (2)	0.11
OSA	0% (0)	0% (0)	
P-HTN	7% (2)	4% (1)	0.69
Medications			
Prednisone†	17% (10)	63% (15)	<0.01
PPI	0% (0)	4% (1)	0.23
H2 Blockers	20% (6)	38% (9)	0.15
Estrogen	0% (0)	4% (1)	0.26
TLC % predicted	78.5 ± 12.0	n/a	
FVC % predicted	79.6 ± 16.4	n/a	
DLCO % predicted	68.8 ± 17.4	n/a	
Intubated‡	0% (0)	8% (2)	0.11

CAD – coronary artery disease, GERD – gastroesophageal reflux disease, OSA – obstructive sleep apnea, P-HTN – pulmonary hypertension, PPI – proton pump inhibitor, H2 – histamine 2 receptor, TLC – total lung capacity, FVC – forced vital capacity, DLCO – diffusing capacity for carbon monoxide

* - at the time of bronchoalveolar lavage

† - prednisone use at the time of bronchoalveolar lavage

‡- intubated state at the time of bronchoalveolar lavage

Data are presented as % (n), mean ± SD

Table 2: Radiologic and Bronchoalveolar Lavage Fluid Features*

	Stable IPF (n=30)	Acute Exacerbation of IPF (n=24)	p value
Radiologic Features			
Hiatal hernia prevalence	57% (17)	67% (16)	0.45
Hiatal hernia diameter, cm	1.4 (0, 1.5)	1.5 (0, 1.6)	0.39
Hiatal hernia height, cm	1.0 (0, 2.0)	2.0 (0, 2.5)	0.29
Ground glass opacity score, %	2 (0, 5)	18 (6, 27)	<0.01
Consolidation score, %	0 (0, 0)	0 (0, 3)	<0.01
Honeycombing score, %	4 (1, 9)	6 (3, 12)	0.17
Reticulation score, %	6 (3, 8)	9 (5, 27)	<0.01
Overall CT score, %	13 (8, 23)	43 (26, 63)	<0.01
Bronchoalveolar Lavage Fluid Features			
Pepsin concentration, ng/ml	35.4 (14.5, 55.6)	46.8 (18.9, 92.0)	0.15
Total cell count, cells/ml	254 (140, 380)	270 (150, 380)	0.84
Neutrophils, %	6 (2, 10)	13 (6, 28)	0.02
Cells/ml	11 (6, 23)	25 (8, 63)	
Lymphocytes, %	10 (7, 18)	17 (6, 32)	0.25
Cells/ml	23 (11, 70)	26 (8, 70)	
Alveolar macrophages, %	73 (60, 88)	52 (30, 64)	<0.01
Cells/ml	176 (97, 294)	134 (39, 195)	
Eosinophils, %	4 (1, 9)	2 (0, 7)	0.16
Cells/ml	7 (2, 28)	5 (0, 18)	
Red blood cell count, cells/ml	65 (10, 230)	500 (180, 1500)	<0.01

CT – computed tomography

* - at the time of bronchoalveolar lavage

Data are presented as % (n), median (25^{th} percentile, 75^{th} percentile)

Table 3: Variables Associated with Acute Exacerbation Status

Variable	Odds Ratio	95% CI	p value
Clinical			
Age	1.00	0.94, 1.06	0.91
Male	1.16	0.32, 4.24	0.83
Body Mass Index	1.04	0.91, 1.20	0.55
Never smoker	0.72	0.20, 2.59	0.62
Former smoker	1.62	0.52, 5.08	0.41
Current smoker	0.59	0.10, 3.54	0.57
Radiologic			
Hiatal hernia	1.53	0.50, 4.66	0.46
Hiatal hernia height	1.26	0.79, 2.01	0.32
Hiatal hernia diameter	1.32	0.66, 2.67	0.44
Bronchoalveolar Lavage			
BAL pepsin level	1.02	1.001, 1.03	0.04
BAL neutrophil %	1.05	1.00, 1.10	0.03
BAL lymphocyte %	1.03	1.00, 1.07	0.08
BAL alveolar macrophage %	0.95	0.91, 0.98	<0.01

BAL – bronchoalveolar lavage

Table 4: Comparison of Characteristics Between Low and High Bronchoalveolar Lavage Pepsin Levels in Acute Exacerbation of IPF

	Acute Exacerbation Pepsin Low (n=16)	Acute Exacerbation Pepsin High (n=8)	p value
Clinical Characteristics			
Age	65 ± 10	63 ± 8	0.72
Male	69% (11)	100% (8)	0.13
Ever smoker	75% (12)	88% (7)	0.63
Body Mass Index	25.9 ± 4.0	23.6 ± 3.8	0.19
Prednisone use	56% (9)	75% (6)	0.66
Intubated	13% (2)	0% (0)	0.54
Survival time (days)	75 (21, 459)	65 (10, 615)	0.78
Alive at follow-up	12% (2)	25% (2)	0.58
Radiologic Characteristics			
Hiatal hernia prevalence	69% (11)	63% (5)	0.55
Hiatal hernia diameter, cm	1.5 (0, 1.6)	1.2 (0, 1.6)	0.39
Hiatal hernia height, cm	2 (0, 3)	1 (0, 2.3)	0.34
Ground glass opacity score, %	18 (6, 33)	18 (7, 26)	0.82
Consolidation score, %	0 (0, 2)	2 (0, 8)	0.16
Honeycombing score, %	10 (3, 12)	4 (2, 7)	0.23
Reticulation score, %	8 (5, 27)	13 (8, 24)	0.50
Overall CT score, %	45 (28, 63)	43 (24, 58)	0.65
Bronchoalveolar Lavage Fluid Characteristics			
Pepsin concentration, ng/ml	30.3 (6, 46.8)	148.4 (92.0, 227.0)	<0.01
Total cell count, cells/ml	235 (144, 372)	340 (150, 400)	0.53
Neutrophils, %	23 (8, 32)	10 (2, 13)	0.10
Lymphocytes, %	8 (5, 18)	38 (26, 65)	<0.01
Alveolar Macrophages, %	57 (38, 65)	46 (22, 56)	0.11
Eosinophils, %	2 (0, 7)	3 (0, 6)	0.83

Red blood cell count, cells/ml	770 (244, 5875)	390 (130, 750)	0.24

CT – computed tomography

Data are presented as % (n), mean ± SD, median (25^{th} percentile, 75^{th} percentile)

Table 5: Impact of Variables on Survival in Acute Exacerbation of IPF

Variable	Hazards Ratio	95% CI	p value
Clinical			
Age	0.97	0.92, 1.02	0.19
Male	2.19	0.70, 6.82	0.18
Body Mass Index	0.93	0.82, 1.05	0.25
Never smoker	0.56	0.16, 1.94	0.36
Former smoker	1.28	0.46, 3.58	0.64
Current smoker	1.76	0.40, 7.85	0.46
Radiologic			
Hiatal hernia	0.74	0.27 2.01	0.55
Hiatal hernia height	0.91	0.60, 1.38	0.66
Hiatal hernia diameter	0.77	0.42, 1.44	0.42
Ground glass opacity score	1.03	1.00, 1.06	0.08
Consolidation score	1.04	0.94, 1.14	0.43
Reticulation score	0.96	0.91, 1.01	0.15
Overall CT score	1.02	0.99, 1.05	0.21
Bronchoalveolar Lavage			
BAL pepsin level	1.00	0.99, 1.00	0.62
BAL total cell count	1.00	1.00, 1.002	0.35
BAL neutrophil %	1.02	1.00, 1.05	0.05
BAL lymphocyte %	0.99	0.96, 1.02	0.44
BAL alveolar macrophage %	0.99	0.97, 1.01	0.52

CT – computed tomography, BAL – bronchoalveolar lavage