Egyptian Journal of Chest Diseases and Tuberculosis (2012) **61**, 129–134

The Egyptian Society of Chest Diseases and Tuberculosis

Egyptian Journal of Chest Diseases and Tuberculosis

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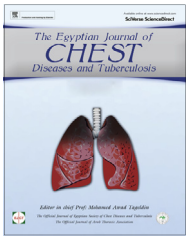

ORIGINAL ARTICLE

Role of BAL cellularity in assessment of severity of idiopathic pulmonary fibrosis

M. Abdel Khalik, M. Zakaria *, M. Khorshed, S. Ahmed, Y. Elhenawy

Cairo University, Egypt

Received 18 November 2012; accepted 13 February 2013
Available online 11 March 2013

KEYWORDS

Bronchoalveolar lavage cellularity (BAL);
Idiopathic pulmonary fibrosis (IPF);
Assessment of severity

Abstract *Background/aim:* Study the role of BAL cellularity in assessment of the degree of severity of IPF.

Methods: Forty IPF patients underwent, history taking, clinical examination, HRCT chest, ABGs, 6MWT, spirometry, echocardiography, FOB and BAL.

Results: Neutrophils were present in 28 patients, mean value 57.25 and SD ± 31.27. The second predominant cell was Alveolar Macrophages with mean value 24.25 and SD ± 27.828.HRCT pattern and BAL cellularity, showed the most predominant cell neutrophils in the ground glass and Honey combing on HRCT. There was no statistical significance between the sex and the BAL cellularity, the most predominant cell was neutrophils in both sexes. Corticosteroids did not have an effect on BAL cellularity and the predominant cell was neutrophils in IPF patients using steroids and those not. BAL cellularity in relation to treatment by azathioprine and/or acetlycysteine showed no statistical significance between patients using those drugs and those not, the most predominant cell was neutrophils in both groups.Correlation between BAL cellularity and age, showed no statistical significance but there was a negative correlation between age to neutrophils and lymphocytes. There was a negative correlation between neutrophils and $PaCO_2$, PaO_2, SO_2, FVC%, FEV1 and 6MWT with no statistical significance but with significance to FEF25–75%.Macrophages showed a positive correlation with age, PaO_2, 6MWT, FVC%, FEV1% with no statistical significance but with significance to SO_2 and FEF25–75% and a negative correlation with $PaCO_2$ and PASP.

Conclusion: There was a positive correlation between neutrophils and severity of the disease and a negative correlation between Macrophages and severity of the disease. Most commonly used drugs did not show any effect on BAL cellularity.

* Corresponding author. Tel.: +20 0122 210 2700.
E-mail address: mw_khalil@hotmail.com (M. Zakaria).

Peer review under responsibility of The Egyptian Society of Chest Diseases and Tuberculosis.

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Introduction

IPF is defined as a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause, occurring primarily in older adults, limited to the lungs, and associated with the histopathologic and/or radiologic pattern of UIP [6].

IPF is a fatal lung disease; the natural history is variable and unpredictable. Most patients with IPF demonstrate a gradual worsening of the lung function over years; a minority of patients remains stable or declines rapidly. Some patients may experience episodes of acute respiratory worsening despite previous stability [6].

Disease progression is manifested by increasing respiratory symptoms, worsening pulmonary function test results, progressive fibrosis on HRCT, acute respiratory decline, or death. Cellular analyses of BAL can be useful in the diagnosis of certain forms of ILD and in the evaluation of patients with suspected IPF [6].

Materials and methods

This study was conducted on 40 patients form Chest Department – Kasr El-Aini Hospital.

(A) Inclusion criteria

All patients were fulfilling major and minor criteria of ATS/ERS consensus classification of the idiopathic interstitial pneumonias [9].

(B) Exclusion criteria

1. Systemic disease such as connective tissue or hepatic disorders, Vasculitis, and Sarcoidosis.
2. Malignancy or drug induced.

All cases were subjected to the following:

1. Full history taking.
2. Full clinical examination.
3. High resolution CT chest.
4. Arterial blood gases.
5. Pulmonary function tests.
6. Echocardiography.
7. Six minute walk test.
8. FOB and lavage for cellularity.

Flexible bronchoscopy and bronchoalveolar lavage cellular analysis.

- The procedure was performed using a flexible fiberoptic bronchoscope PENTAX (FB-18 RX).
- Premedication: includes an intramuscular injection of atropine (0.6 mg) 30 min before bronchoscopy.
- Monitoring: with pulse oximeter.
- Technique:
  - Oxygen is supplied to ensure that SpO_2 is above 90%.
  - Local anesthesia includes: topical xylocaine 1% to anesthetize vocal cords and the airways.
  - Bronchoscope was introduced intranasally and a thorough inspection of all segments was done where it was wedged into one of the segments of the right lower lobe.
  - BAL was performed using 100 mL of sterile saline injected through the bronchoscope working channel and then extracted using the mechanical suction with a negative pressure of about 60–80 mmHg to avoid airway collapse.

Analysis of the bronchoalveolar lavage fluid for differential cell count

After the volume of recovered BAL fluid had been assessed macroscopically for its volume, color and aspect, the fluid was filtered through a layer of sterile gauze, centrifuged for 15–20 min at 500*g*, then re-suspended in sterile saline. Total cell counts were assessed in a Neubauer chamber and viability was determined by the trypan blue exclusion test and proved to be more than 75%. Direct smears and cytospin smears were prepared. The cytospin preparation was centrifuged at 200*g* for 6 min in a cytocentrifuge (Shandon Cytospin 3, Thermo Electron Corporation). The cytospin smear was air-dried. Both the direct and cytospin smears were stained with modified Wright stain (May-Grünwald-Giemsa). A 150- to 200-cell nucleated differential count from 5 or more fields of the stained cytospin preparation was made by using the 100× oil objective [10].

Statistical analysis

Quantitative data were presented as minimum, maximum, means and standard deviation (SD) values. One way ANOVA (Analysis of Variance) was used to compare between means among mild, moderate and severe asthma patients. Duncan's test for pair-wise comparisons was used to determine significant differences between means when ANOVA test is significant.

Qualitative data were presented as frequencies and percentages. The Chi-square (χ^2) test was used for studying the comparisons and associations between different qualitative variables.

The significance level was set at $P \leqslant 0.05$. Statistical analysis was performed with SPSS 16.0® (Statistical Package for Scientific Studies) for Windows® (SPSS, Inc., Chicago, IL, USA).

Results

This study included 32 female IPF patients (80%) and 8 male IPF patients (20%) with a mean age of 47.7 years.

HRCT chest showed that, Honey combing was the predominant pattern in 65% of patients, 20% of patients had ground glass appearance and only 15% of patients had predominantly reticulation on HRCT.

Arterial blood gases showed mean PaO_2 was 62.45 with SD ± 18.62 while the mean value of $PaCO_2$ was 40 with SD ± 9.66. Although the mean PaO_2 was 62.45, 80% of the IPF study patients had Type I respiratory failure.

As regards the pulmonary function test parameters, the mean FVC% was 45.35 with SD ± 17.43 while the mean FEV1% was 42.4 with SD ± 21.95 consistent with the restrictive pattern.

The mean value of 6MWT in the current study was 160.57 m with SD ± 50.74.

Sixty percent of the patients did not have pulmonary hypertension while 40% had pulmonary hypertension with a mean value of 19.55 mmHg and SD ± 26.01 (Table 1).

Correlations.	
1. Age	There is a negative correlation between age and BAL neutrophils and lymphocytes
2. PCO_2	There is a negative correlation to neutrophils and macrophages
3. PO_2	There is a negative correlation to neutrophils and lymphocytes
4. SO_2	There is a negative correlation to neutrophils and lymphocytes There is a positive correlation to macrophages with *P*-value: 0.04 denoting statistical significance
5. 6MWT	There is a negative correlation to neutrophils
6. FVC%	There is a negative correlation to neutrophils and epithelial cells
7. FEV1	There is a negative correlation to neutrophils
8. FEF25–75%	There is a negative correlation to neutrophils with *P*-value: 0.003 denoting statistical significance There is a positive correlation to macrophages with *P*-value: 0.02 denoting statistical significance
9. PASP	There is a positive correlation to lymphocytes with *P*-value: 0.035 denoting statistical significance There is a negative correlation to macrophages

Table 1 Patients on medications during study.

Medications		No.	%
Steroids	No	12	30
	Yes	28	70
Azathioprine	No	20	50
	Yes	20	50
ACC	No	18	45
	Yes	22	55

50% of patients were on steroids, azathioprine and acetyl cysteine.
5% of patients were on steroids and acetyl cysteine.
15% of patients were on steroid only.
30% of patients were without treatment.

Table 2 BAL cellularity in the study.

	Mean ± SD (No. = 40)
Neutrophils (No. = 38)	57.25 ± 31.266
Lymphocytes (No. = 14)	4.75 ± 8.188
Macrophages (No. = 28)	24.25 ± 27.828
Epithelial cells (No. = 24)	13.75 ± 22.353

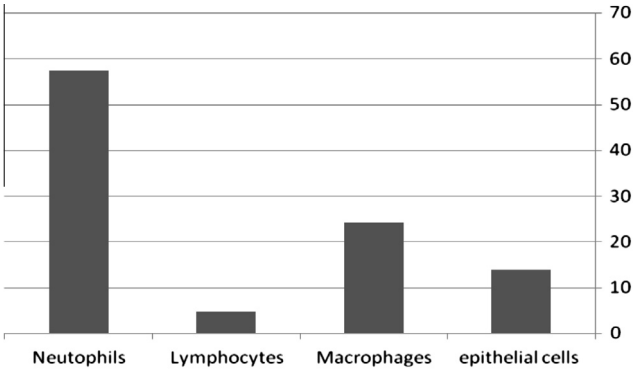

Figure 1 BAL cellularity in the study.

Discussion

Our study indicated a higher incidence of IPF among females compared to males. This is contradicting the ATS/ERS International Multidisciplinary Consensus Classification of the IPF stating that more males have been reported to have IPF than females, our mean age was consistent with ATS/ERS statement.

In this study the differential cellular analysis in the bronchoalveolar lavage showed that the predominant cell is neutrophils followed by alveolar macrophages (Table 2; Fig. 1).

Obayashi et al. [5] stated that although the neutrophil count in BAL could not represent the distribution of neutrophil in the lung, high levels of neutrophil elastase were demonstrated in lung parenchyma and also in both BAL and sera. Therefore, neutrophils might indeed play an important role in the pathogenesis of IPF.

Schwartz et al. [7], showed that patients with IPF had higher concentrations of BAL alveolar macrophages than non-smoking volunteers.

Alternatively activated macrophages (M2 phenotype) are crucial in the pathogenesis of IPF enhancing fibrogenesis of fibroblasts by providing profibrogenic factors, favoring cell growth, collagen formation and tissue repair [2].

By looking at the relation between the HRCT pattern and the BAL cellularity, it was found that the most predominant cell was neutrophils in the ground glass and Honey combing HRCT pattern (Table 3).

Agusti et al. [1] stated that BAL neutrophilia correlated with the total extent of pulmonary abnormalities on the HRCT scan and with the extent of the ground glass pattern in the lavaged lobe. These findings are supported by the fact that the inflammatory process in IPF is characterized by a sustained accumulation of neutrophils in the alveolar spaces.

Also Wells et al. [12] stated that BAL neutrophil levels increase with increasingly extensive disease on CT.

Regarding the statistical comparison between males and females according to BAL cellularity, there is no statistical

Table 3 Relation between HRCT pattern and BAL cellularity in the current study.

HRCT Pattern		Neutrophils	Lymphocytes	Macrophages	Epithelial cells
Ground Glass	No.	8	8	8	8
	Mean ± SD	67.50 ± 12.583	2.50 ± 5.000	21.25 ± 11.815	8.75 ± 8.539
Honey combing	No.	26	26	26	26
	Mean ± SD	56.92 ± 32.502	6.15 ± 9.608	30.00 ± 32.210	6.92 ± 8.301
Reticulations	No.	6	6	6	6
	Mean ± SD	45.00 ± 47.697	1.67 ± 2.887	3.33 ± 5.774	50.0 ± 43.589
Total	No.	40	40	40	40
	Mean ± SD	57.25 ± 31.26	4.75 ± 8.188	24.25 ± 27.828	13.75 ± 22.353
P-Value		0.740	0.756	0.193	0.371
Statistical significance	Not significant				

There is no statistical significance between the HRCT pattern and the BAL cellularity however the most predominant cell was neutrophils in the Ground glass and Honey combing HRCT pattern.

Table 4 Statistical comparison between males and females to BAL cellularity.

Sex		Neutrophils	Lymphocytes	Macrophages	Epithelial cells
Female	No.	32	32	32	32
	Mean ± SD	52.19 ± 32.299	4.69 ± 8.056	26.88 ± 30.434	16.25 ± 24.393
Male	No.	8	8	8	8
	Mean ± SD	77.50 ± 17.078	5.00 ± 10.0	13.75 ± 9.465	3.75 ± 4.787
Total	No.	40	40	40	40
	Mean ± SD	57.25 ± 31.266	4.75 ± 8.188	24.25 ± 27.828	13.75 ± 22.353
P-Value		0.178	0.824	0.554	0.385
Statistical significance	No statistical significance				

There is no statistical significance between the sex of the patient and the BAL cellularity however the most predominant cell was neutrophils in both male and female.

Table 5 BAL cellularity in patients using steroids.

Steroid TTT		Neutrophils	Lymphocytes	Macrophages	Epithelial cells
Yes	No.	28	28	28	28
	M ± SD	58.93 ± 28.701	6.79 ± 9.116	17.50 ± 20.732	16.79 ± 25.915
No	No.	12	12	12	12
	M ± SD	53.33 ± 39.328	0.0 ± 0.00	40.00 ± 37.417	6.67 ± 8.165
Total	No.	40	40	40	40
	M ± SD	57.25 ± 31.266	4.75 ± 8.188	24.25 ± 27.828	13.75 ± 22.353
P-Value		0.771	0.041	0.153	0.548
Statistical significance		N.S	Sig.	N.S	N.S

There was a statistical significance between patients not on steroid and the absence of lymphocytes in BAL.

significance between the sex of the patient and the BAL cellularity however the most predominant cell was neutrophils in both male and female (Table 4).

As regards BAL cellularity in patients using steroids, it was found that the predominant cell was neutrophils in both patients using steroids and in those not using it (Table 5). This may indicate that steroids did not have an effect on BAL cellularity.

The current study disagreed with Smith [8] who found that one group of IPF patients showed an observed decrease in neutrophils during corticosteroid therapy. However the other group failed to confirm the first group findings and showed that an increase in neutrophils alone is associated with a poor prognosis.

BAL cellularity in relation to treatment by azathioprine showed no statistical significance between patients on azathio-

Table 6 BAL cellularity in patients using azathioprine.

Azathioprine		Neutrophils	Lymphocytes	Macrophages	Epithelial cells
Yes	No.	20	20	20	20
	M ± SD	52.5 ± 30.482	6.5 ± 10.554	20.50 ± 23.623	20.50 ± 29.856
No	No.	20	20	20	20
	M ± SD	62.00 ± 32.931	3.00 ± 4.830	28.00 ± 32.335	7.00 ± 7.888
Total	No.	40	40	40	40
	M ± SD	57.25 ± 31.266	4.75 ± 8.188	24.25 ± 27.828	13.75 ± 22.353
P-Value		0.424	0.562	0.590	0.529
Statistical significance		No statistical significance			

There is no statistical significance between whether the patient was on azathioprine or not and the BAL cellularity, however the most predominant cell was neutrophils in both groups.

Table 7 BAL cellularity in patients using acetylcysteine.

ACC TTT		Neutrophils	Lymphocytes	Macrophages	Epithelial cells
Yes	No.	22	22	22	22
	M ± SD	52.27 ± 28.928	6.82 ± 10.068	20.45 ± 22.411	20.45 ± 28.324
No	No.	18	18	18	18
	M ± SD	63.33 ± 34.641	2.22 ± 4.410	28.89 ± 34.167	5.56 ± 6.821
Total	No.	40	40	40	40
	M ± SD	57.25 ± 31.266	4.75 ± 8.188	24.25 ± 27.828	13.75 ± 22.353
P-Value		0.320	0.263	0.643	0.268
Statistical significance		No statistical significance			

There is no statistical significance between whether the patient was on acetylcysteine or not and the BAL cellularity, however the most predominant cell was neutrophils in both groups.

prine and those not using it, however the most predominant cell was neutrophils in both groups (Table 6). Also another study failed to identify a significant relationship between BAL cellularity and either type of immunosuppressive therapy [7].

BAL cellularity in relation to treatment by acetylcysteine showed no statistical significance between patients on acetylcysteine or those not using it, however the most predominant cell was neutrophils in both groups (Table 7).

As regards the correlation between the BAL cellularity and the age, there was no statistical significance but there was a negative correlation between age to neutrophils and lymphocytes. Also there was a negative correlation between neutrophils and $PaCO_2$, PaO_2, SO_2, FVC%, FEV1 and 6MWT with no statistical significance but with significance to FEF25–75% (Table 8).

This may indicate that BAL neutrophilia was associated with worse oxygenation parameters and poor pulmonary function test parameters so BAL neutrophils may be associated with poor prognosis or more severe cases.

Kinder et al. [4] found that BAL fluid neutrophilia may identify a subset of patients with disease that is more ''active'' or at a period of acceleration. They also stated that BAL fluid neutrophilia has identified patients during an accelerated phase of tissue damage that may predispose them to an acute exacerbation.

The prognostic value of BAL fluid differential cell count in patients with idiopathic pulmonary fibrosis (IPF) is unknown. We hypothesized that baseline BAL fluid cell count differential (i.e., elevated levels of neutrophils and eosinophils, or reduced levels of lymphocytes) would predict higher mortality among persons with IPF [4].

There are difficulties in interpreting the prognostic significance of a BAL neutrophilia. It has been argued that a prominent neutrophilia is indicative of a more progressive course in IPF [11].

An increased percentage of neutrophils alone or an increased percentage of neutrophils, along with either increased eosinophils or normal to decreased lymphocytes, is associated with clinical deterioration over the following 6–12 months [8].

In the current study, macrophages also show a positive correlation with age, PaO_2, 6MWT, FVC%, FEV1% with no statistical significance but with significance to SO_2 and to FEF25–75% and negative correlation with $PaCO_2$ and PASP (Table 8). This may indicate that increased macrophages in the BAL cellular analysis is associated with better oxygenation parameters, PFT parameters and 6MWT.

In contradiction to the current study, there was a study that has shown that alveolar macrophages from patients with idiopathic pulmonary fibrosis induce a non-type-1 T-helper-like response and that this altered accessory cell function may contribute to the predominance of a type-2 T-helper-like

Table 8 Correlation between BAL and different parameters.

		Neutrophils	Lymphocytes	Macrophages	Epithelial cells
Age	Correlation coefficient	−0.196	−0.188	0.135	0.388
	P-Value	0.407	0.427	0.572	0.091
	Statistical significance	N.S	N.S	N.S	N.S
PCO_2	Correlation coefficient	−0.077	0.332	−0.091	0.161
	P-Value	0.748	0.152	0.702	0.498
	Statistical significance	N.S	N.S	N.S	N.S
PO_2	Correlation coefficient	−0.374	−0.383	0.417	0.099
	P-Value	0.104	0.095	0.068	0.679
	Statistical significance	N.S	N.S	N.S	N.S
SO_2	Correlation coefficient	−0.410	−0.376	0.458	0.097
	P-Value	0.073	0.102	0.042	0.683
	Statistical significance	N.S	N.S	Sig.	N.S
6MWT	Correlation coefficient	−0.422	0.072	0.434	0.105
	P-Value	0.064	0.762	0.056	0.658
	Statistical significance	N.S	N.S	N.S	N.S
FVC%	Correlation coefficient	−0.262	0.429	0.130	−0.048
	P-Value	0.264	0.059	0.584	0.840
	Statistical significance	N.S	N.S	N.S	N.S
FEV1%	Correlation coefficient	−0.402	0.231	0.140	0.288
	P-Value	0.079	0.328	0.557	0.217
	Statistical significance	N.S	N.S	N.S	N.S
FEF25–75%	Correlation coefficient	−0.625	0.045	0.517	0.193
	P-Value	0.003	0.850	0.020	0.416
	Statistical significance	Sig.	N.S	Sig.	N.S
PASP	Correlation coefficient	0.021	0.473	−0.353	0.244
	P-Value	0.929	0.035	0.127	0.300
	Statistical significance	N.S	Sig.	N.S	N.S

inflammatory response in the pulmonary interstitium of patients with idiopathic pulmonary fibrosis [3].

References

[1] C. Agusti, A. Xaubet, P. Luburich, M.C. Ayuso, J. Roca, R. Rodriguez-Roisin, Computed tomography-guided Bronchoalveolar lavage in idiopathic pulmonary fibrosis, Thorax 51 (1996) 841–845.

[2] E. Bargagli, A. Prasse, C. Olivieri, J. Muller-Quernheim, P. Rottoli, Macrophage-derived biomarkers of idiopathic pulmonary fibrosis, Pulm. Med. (2011), http://dx.doi.org/10.1155/717130.

[3] H. Furuie, H. Yamasaki, M. Suga, M. Ando, Altered accessory cell function of alveolar macrophages: a possible mechanism for induction of Th2 secretory profile in idiopathic pulmonary fibrosis, Eur. Respir. J. 10 (1997) 787–794.

[4] B.W. Kinder, K.K. Brown, M.I. Schwarz, H. Joachim IX, A. Kervitsky, T.E. King Jr., Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis, Chest 133 (2008) 226–232.

[5] Y. Obayashi, I. Yamadori, J. Fujita, T. Yoshinouchi, N. Ueda, J. Takahara, The role of neutrophils in the pathogenesis of idiopathic pulmonary fibrosis, Chest 112 (5) (1997) 1338–1343.

[6] G. Raghu, H.R. Collard, J.J. Egan, F.J. Martinez, J. Behr, K.K. Brown, T.V. Colby, J.F. Cordier, K.R. Flaherty, J.A. Lasky, D.A. Lynch, J.H. Ryu, J.J. Swigris, A.U. Wells, J. Ancochea, D. Bouros, C. Carvalho, U. Costabel, M. Ebina, D.M. Hansell, T. Johkoh, D.S. Kim, TE King Jr., Y. Kondoh, J. Myers, N.L. Müller, A.G. Nicholson, L. Richeldi, M. Selman, R.F. Dudden, B.S. Griss, S.L. Protzko, H.J. Schünemann, ATS/ERS/JRS/ALAT. Idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management, Am. J. Respir. Crit. Care Med. 183 (2011) 788–824, 201.

[7] D.A. Schwartz, R.A. Helmers, C.S. Dayton, R.K. Merchant, G.W. Hunninghake, Determinants of bronchoalveolar lavage cellularity in idiopathic pulmonary fibrosis, J. Appl. Physiol. 71 (5) (1991) 1688–1693.

[8] L.J. Smith, Bronchoalveolar lavage today, Chest 80 (1981) 251–252.

[9] W.D. Travis, W.D. Travis, T.E. King Jr., E.D. Bateman, D.A. Lynch, F. Capron, D. Center, C.G. Brambilla, K.K. Brown, A.L. Cherniaev, U. Costabel, et al, American Thoracic Society/European Respiratory Society International multidisciplinary consensus classification of the idiopathic interstitial pneumonias, Am. J. Respir. Crit. Care Med. 165 (2002) 277–304.

[10] L. Welker, R.A. Jorres, U. Costabel, H. Magnussen, Predictive value of BAL cell differentials in the diagnosis of interstitial lung diseases, Eur. Respir. J. 24 (2004) 1000–1006.

[11] A.U. Wells, The clinical utility of bronchoalveolar lavage in diffuse parenchymal lung disease, Eur. Respir. Rev. 19 (117) (2010) 237–241.

[12] A.U. Wells, D.M. Hansell, P.L. Haslam, M.B. Rubens, J. Cailes, C.M. Black, R.M. du Bois, Bronchoalveolar lavage cellularity lone cryptogenic fibrosing alveolitis compared with the fibrosing alveolitis of systemic sclerosis, Am. J. Respir. Crit. Care Med. 157 (1998) 1474–1482.