CORRELATION OF LOWER RESPIRATORY TRACT INFLAMMATION WITH CHANGES IN LUNG FUNCTION AND CHEST ROENTGENOGRAMS IN PATIENTS WITH UNTREATED TROPICAL PULMONARY EOSINOPHILIA

V K Vijayan, K Sankaran, P Venkatesan, K V Kuppurao

ABSTRACT

Forty-one patients with untreated tropical pulmonary eosinophilia (TPE) were studied to determine whether there was any relationship between lower respiratory tract inflammation and either changes in lung function or abnormalities in chest roentgenograms. Total number of inflammatory cells in bronchoalveolar lavage (BAL) fluid, consisting of alveolar macrophages, lymphocytes, eosinophils and neutrophils had significant negative correlations with transfer factor (TLCO) ($r=-0.519$, $p<0.001$), transfer coefficient (KCO) ($r=-0.312$, $p<0.05$) and total lung capacity (TLC) ($r=-0.352$, $p<0.05$). The absolute count of eosinophils in BAL fluid had a significant negative correlation with TLCO ($r=-0.430$, $p<0.01$) and KCO ($r=-0.300$, $p=0.05$), but not with forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1) or TLC. However, the absolute count of alveolar macrophages had a significant negative correlation with FVC ($r=-0.343$, $p<0.05$), FEV_1 ($r=-0.341$, $p<0.05$) and TLC ($r=-0.305$, $p<0.05$), but not with TLCO or KCO. The total number of lymphocytes had a negative correlation with TLC ($r=-0.315$, $p<0.05$). There was no correlation between the types of cells recovered in BAL fluid and changes in chest radiographs as assessed by the ILO classification for occupational lung diseases. These data suggest that there may be a dissociation of pulmonary pathophysiological changes produced by different inflammatory cells in the lower respiratory tract. Macrophages and lymphocytes may produce more harm to the lung, as evidenced by significant negative correlations of these cells with lung volumes.

Keywords : Tropical pulmonary eosinophilia, lower respiratory tract inflammation, lung function, bronchoalveolar lavage, chest roentgenograms.

SINGAPORE MED J 1991; Vol 32: 122-125

INTRODUCTION

Studies of bronchoalveolar lavage (BAL) in patients with acute untreated tropical pulmonary eosinophilia (TPE) have shown that there is an intense eosinophilic alveolitis[1] and this is associated mainly with a reduction in single-breath transfer factor (TLCO) in most patients[2]. A proportion of patients have obstructive, restrictive and combined ventilatory defects in addition[3,4]. Although there were studies correlating the lower respiratory tract inflammation, as assessed by BAL, to changes in lung function in patients with sarcoidosis and idiopathic pulmonary fibrosis[5,6], such a study has not been reported in patients with tropical pulmonary eosinophilia. As eosinophils have been shown to produce toxic mediators which are injurious to lung parenchyma[7], leading to chronic progressive tissue damage with interstitial fibrosis in bronchopulmonary aspergillosis (a condition similar to tropical pulmonary eosinophilia)[8,9], the impairment in lung function, reflecting underlying lung injury, may be a direct result of the inflammatory reaction in the lower respiratory tract of patients with acute TPE. A study was therefore undertaken to define precisely the respective roles of distinct cell components in alveolar sites (macrophages, lymphocytes, eosinophils and neutrophils) implicated in the pathophysiological changes observed in TPE.

Cardio-Pulmonary Medicine Unit
Tuberculosis Research Centre
Indian Council of Medical Research
Madras 600 031
India

V K Vijayan, MD
Assistant Director

K Sankaran, BSc
Research Assistant

P Venkatesan, M Phil
Research Assistant

K V Kuppurao, PhD
Assistant Research Officer

SUBJECTS AND METHODS

Forty-one consecutive patients (38 males and 3 females, age 15-52 years) with symptoms of one week to six months' duration, and fulfilling the diagnostic criteria of respiratory symptoms such as cough, dyspnoea and nocturnal wheezing, pulmonary infiltrates and peripheral blood eosinophilia ≥ 2000 cells/mm^3 were included in the study. Each individual was evaluated by a detailed history, physical examination, a full plate PA chest radiograph, total and differential leucocyte counts and absolute eosinophil counts in peripheral blood. Stool examinations were done to exclude any infestation with intestinal helminths. Informed consent was obtained from all study subjects. A chest physician (VKV), experienced in reading chest radiographs for nearly 15 years, read all chest radiographs randomly at the end of the study, using the modified ILO U/C classification[10].

Lung Function Tests:

Lung function tests were carried out using transfer test Model C (PK Morgan Ltd, Chatham, UK). Forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) were determined from the best 2 of 3 acceptable forced vital capacity manoeuvres varying by not more than $\pm 5\%$[11]. The largest FVC and FEV_1 values at BTPS were recorded, even if the two values did not come from the same curve. Total lung capacity (TLC) was measured by closed circuit helium dilution method[12] by obtaining, in each patient, two functional residual capacity (FRC) measurements which did not vary by more than $\pm 10\%$. The mean value of these two measurements was used to determine FRC at BTPS. The single-breath diffusing

measurements[12] were carried out in each individual, in duplicate, with an interval of at least 4 minutes, adjusting the inspired volume to 85-90% of previously determined FVC. The values were accepted, if the difference between the two measurements was less than 5% and the highest value was used for analysis. The diffusion capacity per litre of alveolar volume (KCO) was calculated from the ratio of TLCO to effective alveolar volume (V_A). Pulmonary function results were expressed as percent of predicted values obtained from regression equations established in our laboratory[4]. The coefficients of variation for FVC, TLC, TLCO and KCO in our laboratory was less than 2.5%.

Bronchoalveolar lavage:
BAL was performed with a flexible fibreoptic brochoscope as previously described[13] and all bronchoscopies were done under local anaesthesia. Briefly, five 20 ml aliquots of normal saline at room temperature were instilled into each lobe and recovered immediately using suction with 50-100 mm water negative pressure, the bronchoscope being wedged in a sub-segmental bronchus of the right middle lobe, lingula and left lower lobe. The fluid was filtered through gauze to remove mucus and was pooled. An aliquot was used for filter preparations. The total number of cells was estimated on a haemocytometer and expressed as cells/dl. Filter preparations were made on pooled lavage fluid (uncentrifuged cells) as reported by Saltini et al[14] and were stained using haematoxylin-eosin stain. A minimum of 400 cells were counted on each slide by two experienced observers (VKV,KS) and recorded independently. Both observers agreed to within 5% of all lavage analysis and the mean value was used for analysis. BAL was also performed on 17 normal non-smoking individuals as controls. None of these subjects had respiratory symptoms or abnormal physical findings and all had normal chest radiographs and normal pulmonary functions.

All data were expressed as mean ± SEM. Results between groups were compared using two tailed students' 't' test. For correlations, we used Pearson's product moment correlation.

RESULTS

The mean age of the study subjects was 23.9 ± 1.1 years (range 15-52 years). The mean total number of inflammatory and immune effector cells (Table I) in the lower respiratory tract in TPE patients was 160.3 ± 17.1 cells x 10^6/dl (range 24-575 cells x 10^6/dl); this was significantly higher compared to normal subjects ($p<0.001$). Except for two patients, all others had total number of cells greater than 40x10^6/dl. The mean eosinophil percentage (50.0 ± 4.5, range 1-99%) was also significantly higher compared to normals ($p<0.001$) and only two patients had eosinophils of less than 10% in the lower respiratory tract. The mean proportion of alveolar macrophages fell significantly ($p<0.001$) to 43.9 ± 4.2% (range 1 to 91%). One patient with symptoms for only one week had a normal percentage of eosinophils (1%) in the lower respiratory tract; other BAL findings and pulmonary functions were also within normal limits. The absolute (total) numbers of different cell types (total number of cells x differential cell count (%)) are shown in Table I. The mean total number of alveolar macrophages (60.3 ± 6.4) and total number of eosinophils (91.6 ± 16.3) were significantly higher compared to normal subjects ($p<0.001$ for each comparison).

The chest radiographs had a predominance of rounded alveolar opacities in a dense profusion throughout both lung fields. The ILO profusion opacities ranged from 1/1 to 3/3. There was no relationship between radiographic changes and BAL findings or functional changes. Pulmonary function results expressed as percent of predicted values are shown in Fig 1.

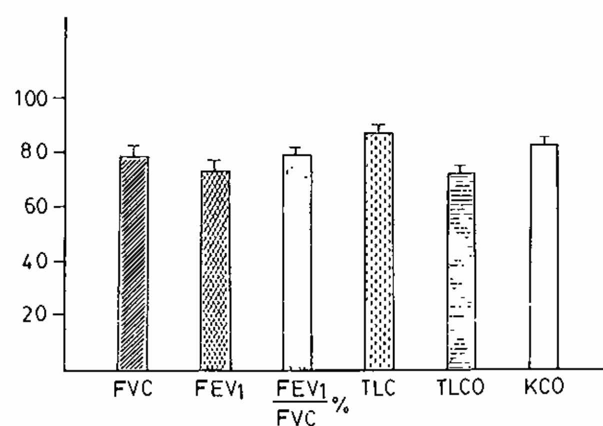
Among the 41 patients, who had TLCO measured, 36 (88%) had results of less than 85% of the predicted. A reduction in both TLC and FVC of less than 85% of predicted was seen in 18 (44%) patients. FEV_1/FVC% was less than 75% in 14 (34%) patients. TLCO had a positive correlation with FVC ($r=0.551$, $p<0.001$), FEV_1($r=0.557$, $p<0.001$) and TLC ($r=0.533$, $p<0.001$).

Table I
BAL findings in patients with tropical pulmonary eosinophilia and healthy control subjects (Mean ± SEM)

	Tropical Pulmonary Eosinophilia (n:41)	Healthy controls (n:17)	P value
Total cells, x10^6/dl	160.3	16.1	<0.001
	±17.1	±1.7	
Macrophages, %	43.9	83.5	<0.001
	±4.2	±1.5	
x10^6/dl	60.3	13.4	<0.001
	±6.4	±1.4	
Lymphocytes, %	3.4	14.9	<0.001
	±0.6	±1.6	
x10^6/dl	4.0	2.5	NS
	±0.9	±0.4	
Eosinophils, %	50.0	0.9	<0.001
	±4.5	±0.3	
x10^6/dl	91.6	0.13	<0.001
	±16.3	±0.04	
Neutrophils, %	2.6	0.7	NS
	±1.4	±0.2	
x10^6/dl	4.3	0.10	NS
	±2.5	±0.03	

NS: Not Significant

Fig. 1
Pulmonary function in patients with tropical pulmonary eosinophilia
(Mean ± SEM)



The correlations of BAL cellular constitutents to various pulmonary function measurements are given in Table II. We found that total number of cells in BAL fluid had a negative correlation with TLC ($r=-0.352$, $p<0.05$), TLCO ($r=-0.519$, $p<0.001$) and KCO ($r=-0.312$, $p<0.05$). Similarly, total number of eosinophils in BAL fluid had a negative correlation with TLCO ($r=-0.430$, $p<0.01$, Fig 2) and KCO ($r=-0.300$, $p=0.05$). However, the total number of alveolar macrophages in BAL fluid had a significant negative correlation with FVC ($r=-0.343$,

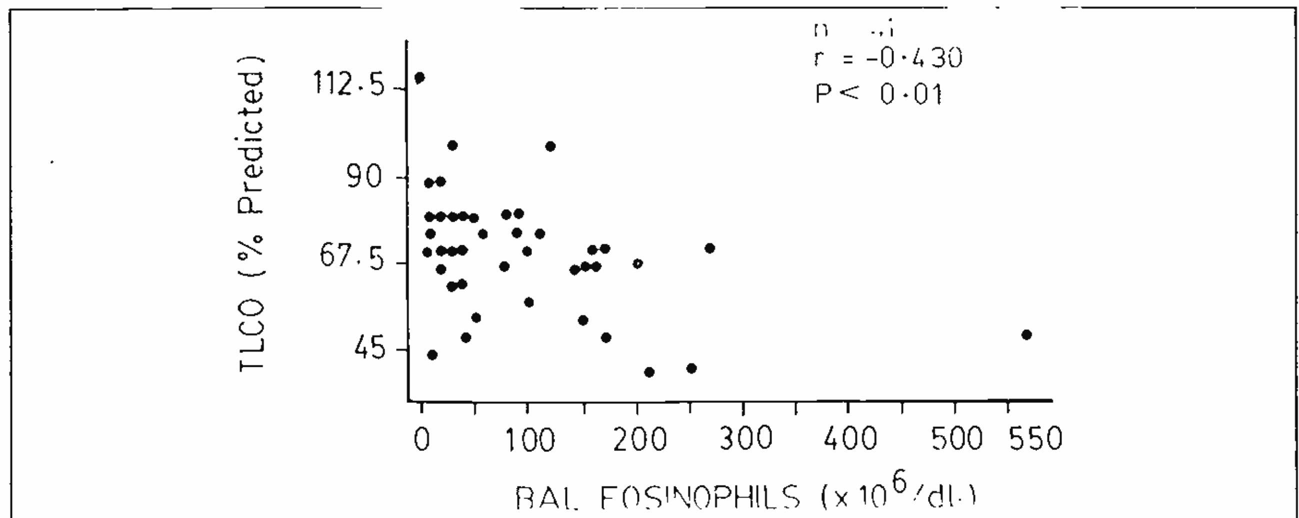
Table II
Correlations of BAL cells with Pulmonary function in TPE

	Total cells x10⁶/dl		Macrophages x10⁶/dl		Lymphocytes x10⁶/dl		Eosinophils x10⁶/dl		Neutrophils x10⁶/dl	
	r	P	r	P	r	P	r	P	r	P
FVC (% Pred.)	-0.287	NS	-0.343	<0.05	-0.171	NS	-0.159	NS	0.059	NS
FEV_1 (% Pred.)	-0.251	NS	-0.341	<0.05	-0.110	NS	-0.131	NS	0.061	NS
TLC (% Pred.)	-0.352	<0.05	-0.305	<0.05	-0.315	<0.05	-0.236	NS	0.025	NS
TLCO (% Pred.)	-0.519	<0.001	-0.287	NS	-0.108	NS	-0.430	<0.01	0.022	NS
KCO (% Pred.)	-0.312	<0.05	-0.097	NS	0.129	NS	-0.300	=0.05	0.010	NS

NS: Not Significant

Fig 2

Relationship of TLCO with BAL Eosinophils



p<0.05), FEV_1 (r=-0.341, p<0.05) and TLC (r=-0.305, p<0.05). TLC also had a significant negative correlation with total number of lymphocytes (r=-0.315, p<0.05) and a total number of lymphocytes had a positive correlation with total number of macrophages (r=0.340, p<0.05).

DISCUSSION

A significant increase in the proportion and total number of eosinophils along with a significant rise in total number of inflammatory and immune effector cells in the lower respiratory tract of patients with untreated tropical pulmonary eosinophilia corroborates our earlier observation of eosinophilic alveolitis in 8 acute tropical pulmonary eosinophilia patients[1]. Although there was a reciprocal significant reduction in proportion of alveolar macrophages, the total number of alveolar macrophages was significantly higher as a result of a significant rise in total number of inflammatory cells. Thus, the inflammation in the lower respiratory tract in patients with untreated TPE is due to an abnormal accumulation of both alveolar macrophages and eosinophils, resulting in macrophage-eosinophilic alveolitis. Similar two cell interactions leading to alveolitis have been described in other interstitial lung diseases as well[15]. Activated macrophages and eosinophils are capable of producing spontaneously various toxic mediators resulting in injury and fibrosis to lung parenchyma[7,16]. The electron microscopic demonstration of severe degranulation with loss of both the core and peripheral portion of granules in eosinophils recovered from the lower respiratory tract of TPE patients[1], suggests that these cells are possibly activated and may be responsible for pathological changes seen in these patients. One of our study subjects, who had a one-week history of symptoms, showed no increase in total number of inflammatory cells or proportion of different cell types in lavage fluid. He had normal pulmonary function including measurements of diffusing capacity. This suggests that in the very early stages of the illness there may not be any influx of eosinophils into the lower respiratory tract.

The finding of a reduced single-breath diffusing capacity in 88% of patients suggests that the alveolitis resulting from the abnormally accumulated inflammatory cells can cause injury to lung parenchyma. The significant correlation of TLCO to lung volumes such as TLC, FVC and FEV_1 further suggests that inflammatory changes in the lower respiratory tract are severe enough to produce reductions in lung volumes as well. Similar observation of a significant correlation of TLCO with FVC has been reported in sarcoidosis[5]. In this study, we could not

find any correlation between absolute number of inflammatory cells recovered by BAL fluid and chest roentgenogram findings or lung function abnormalities. Similar findings have been described in other interstitial lung diseases as well[17,18]. There is no correlation between BAL findings and chest radiographic changes because radiographic abnormalities reflect both inflammatory and fibrotic changes in the lung, while BAL detects only pulmonary inflammation[17]. Hence the lack of correlation between BAL findings and chest radiographic changes in our study subjects with less than 6 months of symptoms suggest the possibility that early fibrosis, in addition to pulmonary inflammation, has occurred in TPE.

The findings of a significant negative correlation of total number of inflammatory cells and absolute eosinophil count in lavage fluid to both TLCO and KCO suggest that the alveolitis, caused by the abnormal accumulation of inflammatory cells and eosinophils in particular, may be responsible for the impairment of diffusing capacity. Similarly, a significant correlation of BAL fluid eosinophilia with a reduced diffusing capacity of the lung, but not with vital capacity or forced expiratory volume has been reported in idiopathic pulmonary fibrosis[19]. However, Watters et al[6] reported that BAL fluid eosinophils had a negative correlation with FVC in idiopathic pulmonary fibrosis, while a negative correlation of BAL fluid lymphocytes with diffusing capacity had been reported in sarcoidosis[5].

Interestingly, the total number of alveolar macrophages had a significant negative correlation with lung volumes such as TLC, FVC and FEV_1, and the absolute lymphocyte count had a significant negative correlation with TLC. Since the recovery of macrophages and lymphocytes was related, as evidenced by a positive correlation between these two types of cells, the reduction in lung volumes in untreated TPE may be due to cytotoxic products liberated by these cells[16]. The findings of a significant negative correlation of the eosinophil count to TLCO and KCO, and also the absolute counts of macrophages and lymphocytes to lung volumes indicate that there may be a dissociation of pathophysiological changes produced by these cells in untreated TPE. The changes may be due to a variety of toxic mediators produced by activated inflammatory cells and the modes of action of these mediators may vary at different sites. Further immunological studies with serial lavage are required to answer these questions.

References

1. Pinkston P, Vijayan VK, Nutman TB et al: Acute tropical pulmonary eosinophilia: Characterisation of the lower respiratory tract inflammation and its response to treatment. J Clin Invest 1987; 80: 216-25.
2. Vijayan VK, Kuppurao KV, Sankaran K, Venkatesan P, Prabhakar R: Diffusing capacity in acute untreated tropical eosinophilia. Indian J Chest Dis All Sci 1988; 30: 71-7.
3. Udwadia FE: Tropical Eosinophilia. In: Pulmonary Eosinophilia. Progress in Respiration Research. S Karger: Basel, 1975: 35-155.
4. Vijayan VK: Tropical eosinophilia: Bronchoalveolar lavage and pulmonary patho-physiology in relation to treatment. PhD Thesis, University of Madras, 1990.
5. Sharma SK, Pande JN, Verma K, Guleria JS: Interrelationship between bronchoalveolar lavage cellular constituents and pulmonary functions in sarcoidosis. Indian J Chest Dis All Sci 1989; 31: 77-84.
6. Watters LC, Schwarz MI, Cherniak RM et al: Idiopathic pulmonary fibrosis: Pretreatment bronchoalveolar lavage cellular constituents and their relationships with lung histopathology and clinical response to therapy. Am Rev Respir Dis 1987; 135: 696-704.
7. Davis WB, Fells GA, Sun X, Gadek JE, Venet A, Crystal RG: Eosinophil-mediated injury to lung parenchymal cells and interstitial matrix. A possible role for eosinophils in chronic inflammatory disorders of the lower respiratory tract. J Clin Invest 1984; 74: 269-78.
8. Safirsteins BH, D'souza MF, Simon G, Tai EH-C, Pepys J: Five year follow-up of allergic bronchopulmonary aspergillosis. Am Rev Respir Dis 1973; 108: 450-9.
9. Capewell S, Chapman BJ, Alexander F, Greening AP, Crompton GK: Corticosteroid treatment and prognosis in pulmonary eosinophilia. Thorax 1989; 44: 925-9.
10. Merchant JA, Reger RB: Classification of the chest radiograph for the pneumoconioses. In: Rom WN. ed. Environmental and occupational medicine. Boston: Little Brown Co, 1983: 113-22.
11. Ferris BG: Editor: Epidemiology standardisation project. Am Rev Respir Dis 1978; 118(Part 2): 62-72.
12. Cotes JE: Lung Function at different stages in life including reference values. In: Lung Function Assessment and Applications in Medicine. Blackwell Scientific Publications: Oxford, 3rd Edition 1975; 112-8; 238-59.
13. Vijayan VK, Pandey VP, Sankaran K, Mehrotra Y, Darbari BS, Misra NP: Bronchoalveolar lavage study in victims of toxic gas leak at Bhopal. Indian J Med Res 1989; 90: 407-14.
14. Saltini C, Hance AJ, Ferrans VJ, Basset F, Bitterman PB, Crystal RG: Accurate quantification of cells recovered by bronchoalveolar lavage. Am Rev Respir Dis 1984; 130: 650-8.
15. Keogh BA, Crystal RG: Alveolitis: The key to interstitial lung disorders. Thorax 1982; 37: 1-10.
16. Crystal RG, Bitterman PB, Rennard SI, Hance AJ, Keogh BA: Interstitial lung disease of unknown aetiology. Disorders characterised by chronic inflammation of the lower respiratory tract. N Engl J Med 1984; 310: 154-66; 235-44.
17. Nugent KM, Peterson MW, Jolles H, Monick MM, Hunninghake GW: Correlation of chest roentgenograms with pulmonary function and bronchoalveolar lavage in interstitial lung diseases. Chest 1989; 96: 1224-7.
18. Turner-Warwick M, Burrows B, Johnson A: Cryptogenic fibrosing alveolitis: Clinical features and their influence on survival. Thorax 1980; 35: 171-80.
19. Hallgren R, Bjermer L, Lundgren R and Venge P: The eosinophil component of the alveolitis in idiopathic pulmonary fibrosis. Signs of eosinophil activation in the lung are related to impaired lung function. Am Rev Respir Dis 1989; 139: 373-7.

4th Congress Of Asian Pacific Association For Laser Medicine And Surgery

(29-31 October 1991)

Further details from :

Academy of Medicine, Singapore
16 College Road #01-01
College of Medicine Building
Singapore 0316
Tel : 2238968
Fax : 2255155
Telex : RS 40173 ACAMED