Original Article

Suberosis and Bird Fancier's Disease: a comparative study of radiological, functional and bronchoalveolar lavage profiles

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Summary. Hypersensitivity Pneumonitis (HP) is an immunologically mediated interstitial lung disease that may result from repeated inhalation of many different environmental agents. Heterogeneity of the clinical presentation and bronchoalveolar lavage profiles have been described, possibly related to different occupational exposures. The aim of our study was to compare bronchoalveolar lavage fluid (BALF), clinical, functional and radiological characteristics of the two most frequent forms of HP seen in our practice: Suberosis (an HP related to moldy cork dust exposure) and bird fancier's disease (BFD).

We included 81 patients with Suberosis, with a mean age of 38.8±11.3 years and a mean exposure of 20.0±10.5 years and 32 patients with BFD, with a mean age of 46.3±11.8 years and mean exposure of 10.5 ± 1.0 years.

Patients with BFD had more acute forms, while subacute and chronic presentations predominated in Suberosis. Restrictive defect was the most frequent pattern of lung function impairment, and more severe in BFD. Ground glass opacities were the most frequent pattern in high-resolution computed tomography. A normal chest x-ray was more frequently seen in Suberosis. Both types of HP had lymphocytic alveolitis in BALF: Suberosis - 6.6±5.7 x 10^5 ml-1 cells, 58.8 ± 18.9% lymphocytes; bird fancier's disease - 9.0±6.5 x 105 ml-1 cells, 61.7±22.2% lymphocytes. Although BALF CD8+ lymphocytes predominated in both diseases, the proportion of CD4+ and CD4/CD8 ratios were significantly higher in bird fancier's disease (Suberosis: 0.47±0.33 versus BFD: 1.1±1.5; p <0.005). Moreover, BALF cellularity and mast cell counts were also significantly higher in BFD.

In conclusion, Suberosis and bird fancier's disease are HP with different clinical and laboratory profiles, suggesting that despite their pathophysiological similarities, different antigenic exposures may cause different immune and inflammatory response dynamics in the lung.

Key Words: Bronchoalveolar lavage, HRCT, Suberosis, Bird Fancier's Disease

Introduction

Hypersensitivity Pneumonitis (HP) is an immunologically mediated interstitial lung disease resulting from repeated inhalation of a variety of environmental agents[1]. There are many different etiologies of HP, such as farmer's lung [2] and bird fancier's disease [3] and, according with clinical presentation, acute, subacute and chronic forms have been described. The diagnosis is based on a combination of clinical features, radiographic abnormalities, lung function tests and immunological tests [4,5]. High resolution computed tomography (HCRT) [6] and bronchoalveolar lavage (BAL) [7] have considerably improved diagnostic accuracy in interstitial lung diseases, including HP [8] .

Although all HP share a common immune-mediated pathogenesis, some factors, like the intensity of exposure and diverse nature of the antigens, may lead to subtle clinical differences [3,9]. In fact, a low grade protracted exposure may favor the development of the chronic presentations of the disease, with lung fibrosis being the major outcome in bird fancier's disease [10]. Moreover, BAL lymphocytes subpopulations vary with the type of HP [9] and, concerning HRCT findings, emphysema predominates in farmer's lung [11].

Suberosis is a HP caused by inhalation of *Penicillium*

glabrum that contaminates cork during its industrial processing[12]. It is especially prevalent in Portugal, and together with bird fancier's disease represents the most frequent type of HP in this country.

The aim of our study was to evaluate the clinical presentation, radiological and bronchoalveolar lavage findings of patients with suberosis in comparison with those of bird fancier's disease.

Materials and Methods

Our study included 113 patients with HP: a) 81 with Suberosis, with a mean age of 38.8±11.3 years, (63 male, 18 female) and b) 32 patients with bird fancier's disease, with a mean age of 46.3±11.8 years, (20 male, 12 female).

All patients were referred to an occupational lung disorders clinic for diagnosis and were still symptomatic and not receiving any treatment. The diagnostic work-up included lung function tests, chest x-ray, HCRT and BAL.

Diagnostic criteria:

After confirming a relevant occupational exposure to moldy cork dust or birds, the diagnosis of HP was considered in the presence of: 1) cough and exertional dyspnea, with mialgia, fatigue or weight loss, and 2) a lymphocytic alveolitis in BAL fluid (≥ 22% of recovered cells) [13], with 3) lung function impairment or radiological abnormalities.

The *acute form* was defined by respiratory complaints that occurred within hours of antigenic exposure, with systemic symptoms, and resolving within days after removal from exposure; the *subacute form* was considered when progressive symptoms occurred over days or weeks; the *chronic form* was characterized by an insidious onset of respiratory complaints in patients with a long and persistent exposure to the organic dust [1].

Pulmonary Function Tests

Static lung volumes and pulmonary diffusion capacity (DLCO), by the single-breath method, were measured using a body plethismograph *(6200 Autobox DL, SensorMedics, Yorba Linda, USA)*, and dynamic lung volumes by mass flow sensors *(Vmax229, SensorMedics, Yorba Linda, USA)*, with the patients in the seated position according to standard procedure [14].The predicted values of Quanjer et al [15] were used.

A restrictive pattern was considered when the forced vital capacity (FVC) was less than 80% and/or total lung capacity (TLC) less than 80%, with a normal forced expiratory volume in one second (FEV1). A FEV1/FVC ratio less than 75% or a residual volume (RV) superior to 120% defined an obstructive pattern.

High Resolution Computed Tomography

High Resolution Computed Tomography (HRCT) examination, performed with a model 9800 scanner *(GE Medical Systems, Milwalkee, Wis)*, was done in 51 (63%) patients with Suberosis and 27 (84%) with BFD. For each subject, 1 to 5 mm thick slices were obtained at 10 mm intervals, using 120kw and 40 mA. Each scan was examined for the presence of the following: 1) areas of attenuation with the appearance of *«ground glass»*, 2) *reticulation and/or parenchimal nodules* and 3) fibrosis.

The classification was performed by the authors (AM and JMS) and a thoracic radiologist, all experienced in the evaluation of interstitial lung diseases.

Fiberoptic bronchoscopy with bronchoalveolar lavage (BAL)

BAL was performed according to the recommendations of the European Society of Pneumology Task Group on BAL [16]. Briefly, four aliquots of 50ml sterile saline (37ºC), were instilled in the middle lobe and gently aspirated with a syringe after each instillation. The recovered fluid (BALF) was pooled discarding the first aliquot and total cell numbers (Neubauer chamber) and viability (trypan blue exclusion) were estimated. Cell differentials were obtained by counting 500 cells on glass cover preparations stained with May-Grünwald-Giemsa.

Measurement of BALF lymphocyte subsets was performed by two-color direct immunofluorescence staining and flow cytometry. Briefly, $2.5x10^5$ BALF cells were incubated for 30 min on ice in the dark, with 10 µl of FITC or PE conjugated monoclonal antibodies anti-CD45, CD14, CD2, CD19, CD4 and CD8 *(Beckton Dickinson, Mountain View, CA.)*. After washing and fixation, cells were analyzed on a flow cytometer *(FACScan, Becton Dickinson)* and data of 10,000 events acquired and stored in list mode. The lymphocyte gate was established based on forward and side scatter adjusted with a forward scatter/ CD45 contour plot [17].

Statistical analysis

All data were analyzed with the statistical package SPSS® for Windows® (SPSS Inc., Chicago, IL). Simple descriptive analysis was predominantly used and, unless otherwise stated, data are reported as mean ± SD and frequencies as n (%). Differences between the two patient groups (Suberosis and BFD) were analysed using non-parametric tests. Fisher's exact test and Pearson Chi squared test were used for independent category data.

Comparison of independent continuous data with patient groups as the ordinal level of measurement were analysed with Mann-Whitney's U Test. For multiple independent samples Kruskal-Wallis Test was used. Spearman correlation was used for comparison between continuous data namely BAL and Lung function parameters. Significance was accepted at 5%.

Results

Clinical presentation

The 81 patients with Suberosis had a mean exposure of 20.0 ± 10.5 years in the cork stoppers industry (punching and sorting of bottle corks) and the majority (74.1%) were still working at the time of study. Non-exposed patients (n=21) were away from exposure for 14.8± 16.6 months. Seven (9.1%) were smokers and 10 (13%) ex-smokers. The acute disease form occurred in 9 (12.2%), subacute forms in 33 (44.6%) and the chronic presentation in 32 (43.2%) cases.

The 32 patients with Bird fancier's disease had a mean exposure of 10.5 ± 1.0 year. The majority (84%) were pigeon breeders. Two (6.5%) patients were smokers and 6 (19.4%) were ex-smokers. Twenty-two (88%) were still exposed at the time of the study. Non-exposed patients (n=5) were away from exposure for 6.6±9.8 months. Acute disease form occurred in 10 (40%) patients, subacute in 9 (36%) and chronic in 6 (24%).

Bird fancier's lung had more acute forms while Suberosis had more frequent subacute or chronic presentations (p= 0.006). Moreover, patients with Suberosis had a longer duration of exposure (p<0.001).

Radiology

Chest x-rays were normal in 22.7% of Suberosis cases and only in 3.2% of BFD patients (p= 0.017). In 53.8% of the cases with normal chest radiographs HRCT scans were abnormal. The most frequent HRCT pattern found in both diseases was ground glass opacifications (Fig 1 and 2). However, there was no significant difference between predominant HRCT patterns in both HP and according with clinical presentations.

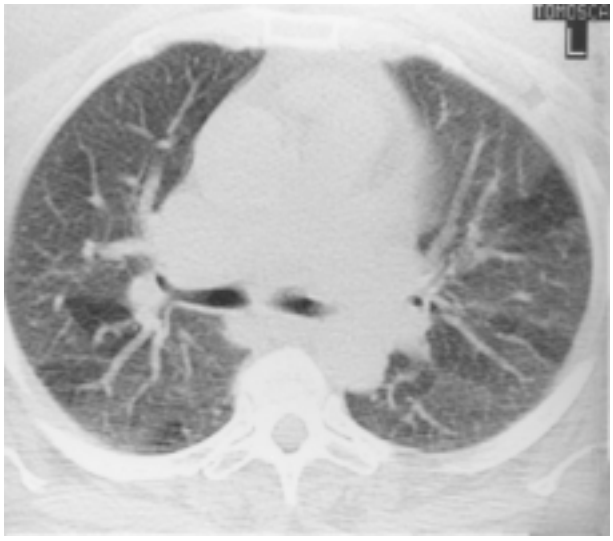

Figure 1. HRCT scan of a pigeon breeder with acute presentation, showing patchy areas of ground glass attenuation in both lungs.

Lung function

In Suberosis there was a restrictive impairment in 47.1%, while an obstructive syndrome occurred in 5.7% and 30% had normal lung function. In Bird fancier's disease, 63.3% patients had a restrictive syndrome, 10 % an obstructive syndrome and 6.7% had normal lung function. There were no significant differences in the distribution of lung function patterns (restrictive/obstructive) between Suberosis and BFD, and between different clinical presentations (acute/subacute/chronic). Moreover, Suberosis had significantly more normal lung function tests (p=0.018)

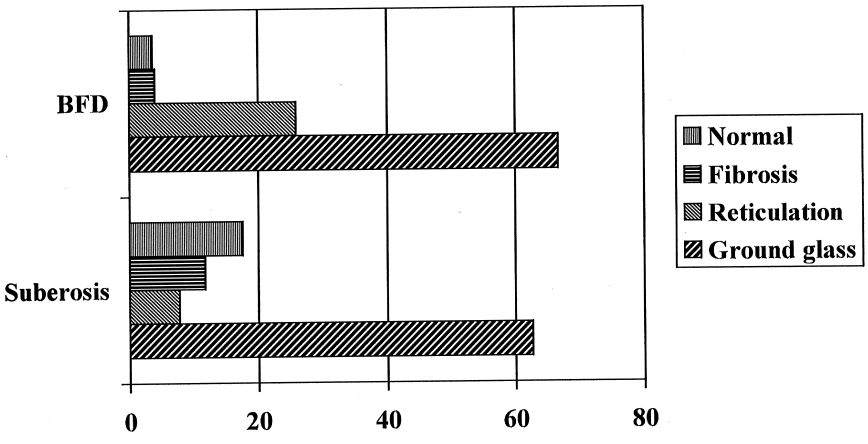

Figure 2. Predominant (in percentage) HRCT patterns in Suberosis and BFD.

DLCO was the most frequently abnormal parameter: 55% of patients had values under 80% of predicted: 48.2% in Suberosis and 70.8% in BFD, p=0.03. We found a significant difference between TLC (p= 0.036), RV/TLC (p=0.020), DLCO (p=0.000) and DLCO/VA (p=0.002), with lower levels in BFD compared with Suberosis (Table 1).

BAL findings (Table 2)

Both types of HP had a lymphocytic alveolitis (Suberosis: 58.8 ± 18.9%, BDF: 61.7 ± 22.2%; p=n.s.) with predominant CD8+ lymphocytes, with Bird fancier's disease having a higher total cell count (6.6 ± 5.7 x 10^5 ml^{-1} cells *versus* 9.0 ± 6.5 x 10^5 ml^{-1} cells; p=0.025)-table 2. Moreover, BFD showed higher total

Table 1. Bronchoalveolar lavage cell counts in BFD and Suberosis (data are presented as mean and standard deviation).

	Suberosis	**BFD**	**p**
FVC % pred	83.8 ± 24.5	76.2 ± 18.6	**n.s**
FEV1 % pred	80.3 ± 26.0	76.3 ± 18.8	**n.s**
FEV1/FVC	87.9 ± 16.4	89.3 ± 11.6	**n.s.**
TLC % pred	92.5 ± 17.9	83.7 ± 16.3	**0.04**
RV/TLC	119.2 ± 38.6	86.9 ± 57.6	**0.02**
DLCO % pred	83.1 ± 24	60.9 ± 24	**<0.001**
DLCO/VA % pred	95.2 ± 23.2	76.8 ± 27.7	**0.002**

n.s. = non significant - FVC = Forced Vital Capacity - FEV1 = forced expiratory volume in 1 second - TLC = total lung capacity - RV = residual volume - DLCO = pulmonary diffusion capacity - VA = alveolar volume - % pred = % of predicted.

Table 2. Pulmonary Function Tests in BFD and Suberosis (data are presented as mean and standard deviation)

	Suberosis	**BFD**	**p**
Total cell counts	6.6 ± 5.7 x 105ml-1	9.0 ± 6.5 x 105ml-1	**0.025**
Macrophages	34.4 ± 17.8 %	30.5 ± 21.4%	**n.s.**
Lymphocytes	58.8 ± 18.9 %	61.7 ± 22.2 %	**n.s.**
Neutrophils	5.7 ± 7.6 %	4.4 ± 5.7 %	**n.s.**
Eosinophils	0.7 ± 1.1 %	1.3 ± 2.3 %	**n.s.**
Mast cells	0.04 ± 0.1 %	0.3 ± 0.6 %	**0.002**
CD4+ lymphocytes	23.5 ± 10.6 % 1.0 ± 1.1 x 103ml-1	35.0 ± 17.3 % 2.1 ± 1.5	**<0.001** **<0.001**
CD8+ lymphocytes	56.3 ± 13 % 2.5 ± 6.6 x 103ml-1	45.8 ± 18.8 % 3.2 ± 3.7	**0.004** **n.s.**
CD4/CD8 ratio Median [interquartile range]	0.5 ± 0.3 0.4 [0.2-0.6]	1.1 ± 1.5 0.6[0.4-1.7]	**0.002**

n.s. = non significant

lymphocyte counts (p= 0.048), CD4/CD8 ratio (p=0.002), total CD4+ lymphocytes, and also a higher proportion of mast cells (p=0.002).

In Suberosis we only found a significantly higher proportion of CD8+ lymphocytes (p=0.004). Concerning patients away from exposure at the time of the study, the only significant finding in BALF data was a significantly lower proportion of CD8+ lymphocytes in Suberosis (exposed: 55.5 ± 15.5% *versus* non-exposed 49.3± 15.1%, p=0.012), with no significant difference in their total numbers (exposed: 2.8±2.9 x 10^3 ml-1 *versus* non-exposed: 2.0±2.0 x 10^3 ml^{-1}, p=0.415).

No significant differences were found in BALF differential counts in smokers (data not shown).

Relationship between clinical presentations, functional, radiological and BALF data

In patients with an acute presentation we found significantly higher BALF total cell counts (9.9±7.8 *versus* 6.5±5.5 x 10^3 ml^{-1}, p=0.04) and mast cell numbers (0.23 ± 0.38% versus 0.08 ±0.26, p=0.04). No significant differences were found concerning neutrophil (7.3 ±12.3 % versus 5.1±5.9 %) and lymphocyte counts (61.6 ±19.2 % *versus* 58.4±20.3 %)

In the BFD group, a significant negative correlation between DLCO and BALF total cell counts was found (rs= -0.4, p=0.03), and acute patients (40% of all patients) had significantly higher BALF CD8+ lymphocytes (54.5 ±15.8 % *versus* 34.1 ± 14.2 % and 5.5±5.2 *versus* 1.4.±1.1 x 10^3 ml^{-1}, p=0.02).

Suberosis patients had significantly different BALF profiles in relation to HRCT patterns: those with a ground glass pattern had significantly more BAL lymphocytes (65.8±16.6% *versus* 51.6±18.2%, p=0.012) and patients with a fibrosis pattern had significantly more mast cells (0.15±0.28% *versus* 0.01±0.04%, p=0.029). Moreover, when we analyzed different exposure intervals in Suberosis (<10 years, 10-20 years and >20 years), we found progressively lower values of FEV1/FVC (p=0.044), total cell counts (p=0.001), total lymphocyte counts (p=0.001), total CD4 (p=0.002), and CD8 lymphocyte counts (p=0.001) (Figure 3).

Discussion

Hypersensitivity Pneumonitis is a complex syndrome, with a varying intensity of response that may depend on the type of antigen exposure: soluble pigeon antigens typically evoke a less intense reaction and response compared with the particulate fungal antigens [18]. The form and course of the disease may be determined by factors such as the intensity and frequency of antigen exposure, the severity of the first attack and host factors governing immunological responses, namely HLA alleles and TNF_ promotor gene polymorphisms [19-22].

In our study, both HP forms belonged to the same age and gender group (35 to 45 year old male adults), in accordance with male predominance in both activities (cork industry and pigeon fanciers). Patients with BFD had more acute forms, according with the usual intermittent high intensity exposure experienced by bird

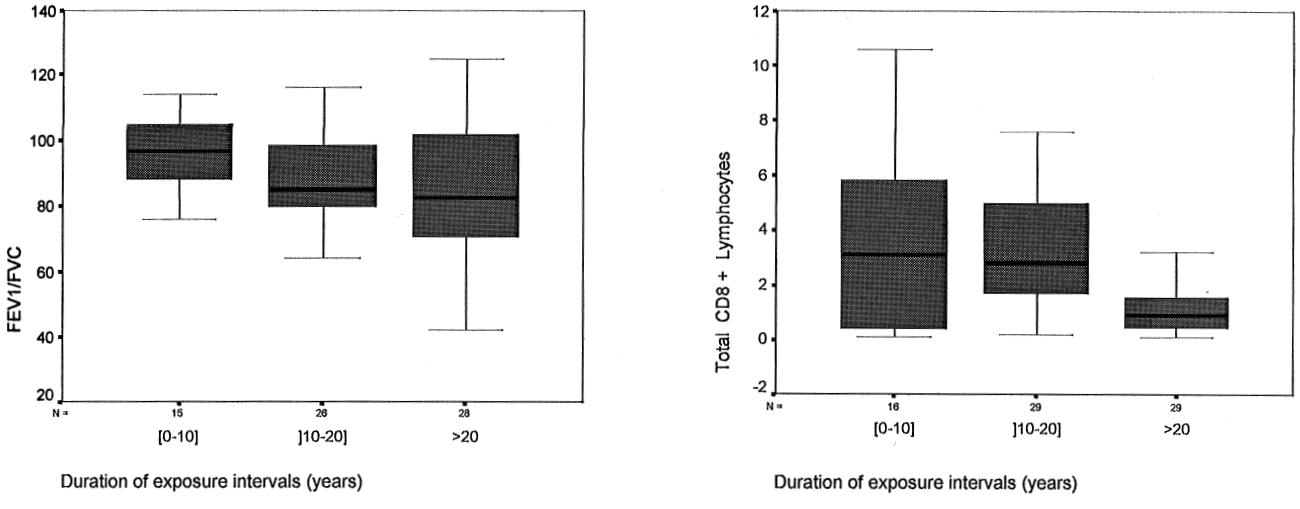

Figure 3. Progressively lower FEV/FVC (left) and BALF total CD8 + lymphocyte (right) with increasing exposure in Suberosis (<10 years, n=16, 10-20 years, n=29; >20 years, n=29). With increasing exposure duration, significantly lower numbers of total BALF lymphocytes and CD4 + cells were also found (not shown).

fanciers[3]. In Suberosis, the significantly longer exposure found in our series suggests a protracted low level antigenic exposure [12], also supported by the predominance of subacute and chronic forms we found.

In our group of patients with BFD and Suberosis with different clinical presentations, ground-glass opacifications were the predominant abnormal HRCT pattern (66.7% and 62.7% respectively), similarly to the reported in acute farmer's lung [23] and subacute and chronic BFD [24,25]. We did not find any significant difference between individual HRCT patterns and different clinical presentations in both HP.

In up to a fifth of the patients, chest X-rays and HRCT scans were normal, especially in Suberosis. In other series, Hansell et al [26] found that 18% of patients with HP had normal HRCT and Remy-Jardin et al [25] found 30% of normal chest x-rays in subacute BFD. However it was within the Suberosis group that we found the most significant correlations between radiology and other disease characteristics: BALF total lymphocyte and CD8+ lymphocyte counts were significantly lower in patients with normal HRCT, suggesting a less active alveolitis. These may be considered milder forms of HP, in which radiological evaluation may fail to show abnormalities. Perhaps the use of a more sensitive exam, like 99mTc-DTPA clearance, could detect alveolar involvement in those patients[27,28]. The ground glass pattern on HRCT correlated significantly with the proportion of BALF lymphocytes, in accordance with what has been shown at the pathologic level, where ground glass opacifications are related with mononuclear cell infiltration of the alveolar walls [29].

Although in Suberosis the chronic forms were very representative (43.2% of cases) we only found evidence of honeycombing in 6 cases (11.8%). This figure is lower than in other chronic HP where honeycombing occurs between around 50% in BFD [25] and 68% of cases in a series with a wide range of etiologies [30]. Suberosis may be equivalent to summer-type hypersensitivity pneumonitis where it occurs only in 20% of cases [31]. Interestingly, BALF mast cell proportion in Suberosis correlated significantly with fibrosis HRCT pattern, supporting the described role of these cells in lung fibrogenesis [32,33].

In our series, the most sensitive functional abnormality was a reduction in lung diffusion (DLCO). This finding is in accordance with previous studies in Farmer's Lung, where this test was a good marker for the presence and intensity of the disease [34]. A restrictive syndrome was clearly predominant, but airflow obstruction occurred in 5.7% of Suberosis cases and 10% of BFD cases. In fact, patients with chronic HP may develop an airways obstruction syndrome indistinguishable from emphysema [18,25], that may be due to peri-bronchiolar inflammation, fibrosis and obliterative bronchiolitis [35]. Although this was not a longitudinal study, we found that FEV1/FVC ratio significantly decreased in Suberosis patients with longer duration of exposure. In farmer's lung there is also evidence for a reduction in FEV1/FVC ratio in the long-term [36].

In BFD, functional impairment was more common, and a restrictive pattern with reduced diffusion capacity was more pronounced than in Suberosis. These findings together with BALF data, showing a more intense lymphocytic alveolitis and a negative correlation between DLCO and total cell counts, suggest that, comparatively with Suberosis, BFD induce a more severe lung impairment and alveolitis.

Mean BALF lymphocyte counts above 50% in both Suberosis and BFD confirm the characteristic high intensity alveolitis of these HP. In fact, only 1 patient with Suberosis and 3 with BFD had lmphocytosis under 25%.

There are many studies on the surface phenotypes of BAL lymphocytes in HP [37-41] and some of them study the differences between various HP [9,31]. Our study includes a larger number of patients than previous reports, and we were able to analyze cases with different clinical presentations (acute, subacute and chronic). In our BFD group, a significantly higher CD4/CD8 ratio was found in BALF comparatively with Suberosis (median and [interquartile range]) 0.6 [0.4-1.7] *versus* 0.4 [0.2-0.6]. In accordance with our results, it has been suggested that pigeon breeder's disease have higher CD4/CD8 ratios than other forms of HP [3]. In fact in a large epidemiological study in Japan, Ando et al [9], report a CD4/C8 ratio of 2.0±0.5, in a group of 19 patients with bird fancier's disease.

In our series, we also found that BFD patients with an acute presentation had significantly higher proportion of CD8+ lymphocyte counts (54.5 ± 15.8 % *versus* 34.1± 14.2%), with no significant differences in the CD4/CD8 ratios (median [interquartile range]: 0.6 [0.3-0.8] *versus* 1.2 [0.6-2.5]. This pattern is somewhat contradictory with a series with 59 patients with acute forms of BFD from Drent et al [42] in which they showed a higher mean CD4/CD8 ratio. The technique employed for the identification of T-cell subpopulation (conventional indirect immunofluorescence technique in that study and flow cytometry in ours) may have account for some differences in these results.

In Suberosis, the CD4/CD8 ratio was significantly lower than BFD similar to what has been described in summer-type hypersensitivity pneumonitis [9]. Moreover, CD8+ lymphocyte counts, although still increased (49.3%) were significantly lower in non-exposed Suberosis patients. These findings are consistent with a study involving a small group of patients with farmer's lung, where CD8 + lymphocytes tended to fall in patients no longer in contact with the antigen [41]. In other cases of HP, Costabel et al also demonstrated a decrease in these cells after antigen avoidance [43].

In our series, smoking habits did not significantly

affect differential counts as well as BAL lymphocyte phenotypes in both HP. Although these results are not consistent with the reported influence of smoking in total cell counts and CD4/CD8 ratios in patients with Farmer's lung and ventilation pneumonitis [9], the small percentage of smokers in our series may justify our findings.

Some possible explanation for the differences in BALF lymphocyte phenotypes in Suberosis and BFD, may be the differences in clinical presentations, the existence of other inhalants in the cork industry (cork dust itself) and the type of exposure (continuous in the cork industry *versus* intermittent in bird breeders [44]), that may have an influence in the alveolar immune and inflammatory response.

Mast cells counts were significantly higher in BFD, probably reflecting the significantly higher percentage of acute forms in this group [32,45]. In our group of patients, average neutrophil counts (Table 2) were similar in both HP but were somewhat lower than those reported in the literature including patients with acute BFD [42] or Farmer's Lung [46]. However, the increase in neutrophils associated with acute forms of HP has been critically related to a very recent natural [42] or induced antigen exposure[47], that was not the case in our study. In our series, mean BALF neutrophils in patients with acute forms were not significantly different from subacute and chronic forms, but with a wide dispersion of values acute forms 7.3± 12.3% *versus* chronic forms 5.1± 5.9%.

Eosinophils, although mildly increased and especially in BFD, were also lower than the described in acute BFD with recent (< 1 week) exposure [42]. In our experience, higher BALF eosinophil counts are associated with cork worker's occupational asthma and not with HP [48].

In conclusion, in a large group of patients with Hypersensitivity Pneumonitis, our study demonstrates that there are significant differences in clinical presentation, radiological and lung function findings as well as BALF profiles between Suberosis and Bird Fancier's Disease. Despite their pathophysiological similarities, different antigenic exposures may account for different immune and inflammatory dynamics in the lung.

Suberosis is a form of HP with a less striking radiological and functional involvement than bird fancier's disease, and is characterized by a less intense lymphocytic alveolitis with a lower CD4/CD8 ratio. HRCT appears to be more useful in the evaluation of the radiological involvement of Suberosis. DLCO is a sensitive lung function test in the evaluation of HP, especially in bird fancier's disease.

Our patients with Suberosis, although with a long exposure, have a less severe Hypersensitivity Pneumonitis than BFD, perhaps due to an earlier recognition and referral of this occupational disease in our region, as opposed to bird fanciers that reach medical evaluation at a later and advanced stage. This issue may have practical consequences in the disease prognosis as it relates with the duration of exposure after symptoms develop [18].

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References

1. Wild L, Lopez M. Hypersensitivity Pneumonitis: a comprehensive review. *J Invest Allergol Clin Immunol.* 2001;11:3-15
2. Cormier Y, Laviolette M. Farmer´s Lung. *Semin Respir Med.* 1993;14:31-36
3. Calvert J, Baldwin C, Allen A, Todd A, Bourke S. Pigeon fanciers' lung: a complex disease? *Clinical and Experimental Allergy.* 1999;29:166-175
4. Schuyler M, Cormier Y. The diagnosis of Hypersensitivity Pneumonitis. *Chest.* 1997; 111:534-536
5. American Thoracic Society. Respiratory Health Hazards in agriculture. Am J Respir Crit Care Med. 1998;158:S1-S76
6. Silver SF, Muller NL, Miller RR, Lefcoe MS. Hypersensitivity pneumonitis: evaluation with CT. *Radiology.* 1989;173:441-445
7. Trentin L, Facco M, Semenzato G. Hypersensitivity Pneumonitis. In: Mapp C, ed. Occupational Lung Disorders. Vol. 4: *European Respiratory Monograph;* 1999:301-319
8. Costabel U, Guzman J. Bronchoalveolar lavage in interstitial lung disease. *Curr Opin Pulm Med.* 2001;7:255-261
9. Ando M, Konishi K, Yoneda R, Tamura M. Difference in the phenotypes of bronchoalveolar lavage lymphocytes in patients with summer-type hypersensitivity pneumonitis, farmer's lung, ventilation pneumonitis, and bird fancier's lung: report of a nationwide epidemiologic study in Japan. *J Allergy Clin Immunol.* 1991;87:1002-1009
10. Pérez-Padilla R, Salas J, Chapela R, Sanchez M, Carrillo G, Pérez R, Sansores R, Gaxiola M, Selman M. Mortality in mexican patients with chronic pigeon breeder´s lung compared with those with usual interstitial Pneumonia. *Am Rev Respir Dis.* 1993;148:49-53
11. Erkinjuntii-Pekkanen R, Rytkonen H, Kokkarinen J, Tukiainen H, Partanen K, Terho E. Longterm risk of emphysema in patient´s with farmer´s lung and matched control farmers. *Am J Respir Crit Care Med.* 1998;158:662-665
12. Ávila R, Lacey J. The role of Penicillium frequentans in Suberosis (Respiratory disease in cork workers). *Clinical Allergy.* 1974;4:109
13. Leblanc P, Belanger J, Laviolette M, Cormier Y. Relationship among antigen contact, alveolitis, and clinical status in farmer's lung disease. Arch Intern Med. 1986;146:153-157
14. Official Statement of the European Respiratory Society. Standardized lung function testing. *Eur Respir J.* 1993;6:1-100
15. Quanjer P. Working Party on " Standardization of lung function test". *Bull Eur Physiopatol Respir.* 1983;19 (suppl. 5):7-10

16. Klech H, Hutter C. Clinical guidelines and indications for bronchoalveolar lavage (BAL): Report of the European Society of Pneumology Task Force on BAL. *Eur Respir J.* 1990;3:937-974
17. Dauber J, Wagner M, Brunsvold S, Paradis I, Ernst L, Waggoner A. Flow cytometric analysis of lymphocyte phenotypes in bronchoalveolar lavage fluid: comparison of a two-color technique with a standard immunoperoxidase assay. *Am J Respir Cell Mol Biol.* 1992;7::531-541
18. Zacharisen MC, Schlueter DP, Kurup VP, Fink JN. The long-term outcome in acute, subacute and chronic forms of pigeon breeder's disease hypersensitivity pneumonitis. *Ann Allergy Asthma Immunol.* 2002;88:175-182
19. Bourke S, Banham S, Carter R, Lynch P, Boyd G. Longitudinal Course of Extrinsic Allergic Alveolitis in Pigeon Breeders. *Thorax.* 1989; 44:415-418
20. Schuyler M. Are polymorphisms the answer in Hypersensitivity Pneumonitis? *Am J Respir Crit Care Med.* 2001;163:1513-1514
21. Schaaf BM, Seitzer U, Pravica V, Aries SP, Zabel P. Tumor Necrosis Factor alpha-308 promotor gene polymorphism and increased tumor necrosis factor serum activity in Farmer's Lung patients. *Am J Respir Crit Care Med*. 2001;163:379-382
22. Camarena A, Juarez A, Mejia M, Estrada A, Carrillo G, Falfan R, Zuniga J, Navarro C, Granados J, Selman M. Major histocompatibility complex and tumor necrosis factor-alpha polymorphisms in pigeon breeder's disease. *Am J Respir Crit Care Med*. 2001;163:1528-1533
23. Cormier Y, Brown M, Worthy S, Racine G, Muller NL. High-resolution computed tomographic characteristics in acute farmer's lung and in its follow-up. *Eur Respir J*. 2000;16:56-60
24. Hansell DM, Wells AU, Padley SP, Muller NL. Hypersensitivity pneumonitis: correlation of individual CT patterns with functional abnormalities. *Radiology*. 1996;199:123-128
25. Remy-Jardin M, Remy J, Wallaert B, Muller NL. Subacute and chronic bird breeder hypersensitivity pneumonitis: sequential evaluation with CT and correlation with lung function tests and bronchoalveolar lavage. *Radiology.* 1993;189:111-118
26. Hansell DM, Moskovic E. High-resolution computed tomography in extrinsic allergic alveolitis. *Clin Radiol.* 1991;43:8-12
27. Bourke MP, Banham S, J.H. M, Boyd G. Clearance of 99mTc-DTPA in pigeon fancier's hypersensitivity pneumonitis. *Am Rev Respir Dis*. 1990;142:1168-1171
28. Schmekel B, Wollmer P, Venge P, Linden M, Blom-Bulow B. Transfer of 99mTc DTPA and bronchoalveolar lavage findings in patients with asymptomatic extrinsic allergic alveolitis. *Thorax*. 1990;45:525-529
29. Leung AN, Miller RR, Muller NL. Parenchimal opacification in chronic infiltrative lung diseases: CT-pathologic correlation. *Radiology*. 1993;188:209-214
30. Adler BD, Padley SP, Muller NL, Remy-Jardin M, Remy J. Chronic hypersensitivity pneumonitis: high-resolution CT and radiographic features in 16 patients. *Radiology.* 1992;185:91-95
31. Yoshizawa Y, Ohtani Y, Hayakawa H, Sato A, Suga M, Ando M. Chronic hypersensitivity pneumonitis in Japan: a nationwide epidemiologic survey. *J Allergy Clin Immunol.* 1999;103:315-320
32. Bjermer L, Engstrom-Laurent A, Lundgren R, Rosenhall L, Hallgren R. Bronchoalveolar mastocytosis in farmer's lung is related to the disease activity. *Arch Intern Med*. 1988;148:1362-1365
33. Delgado L, Cuesta C, Winck JC, Sapage JM, Moura e Sa J, Fleming Torrinha JA. [Suberosis: involvement of broncho-alveolar +mastocytes in the genesis of interstitial involvement]. *Arch Bronconeumol.* 1999;35:71-78
34. Cormier Y, Belanger J, Tardif A, Leblanc P, Laviolette M. Relationships between radiographic change, pulmonary function, and bronchoalveolar lavage fluid lymphocytes in farmer's lung disease. *Thorax.* 1986;41:28-33
35. Lama M, Pérez-Padilla R. Airflow Obstruction and Airway Lesions in Hypersensitivity Pneumonitis. *Clinics in Chest Medicine.* 1993;14
36. Erkinjuntii-Pekkanen R, Kokkarinen J, Tukianen H, Pekkanen J, Husman K, Terho E. Long-term outcome of pulmonary function in farmer´s lung: a 14 year follow-up with matched controls. *Eur Respir J.* 1997;10:2046-2050
37. Milburn HJ. Lymphocyte subsets in hypersensitivity pneumonitis. *Eur Respir J*. 1992;5:5-7
38. Suda T, Sato A, Ida M, Gemma H, Hayakawa H, Chida K. Hypersensitivity pneumonitis associated with home ultrasonic humidifiers. *Chest.* 1995;107:711-717
39. Baur X. Hypersensitivity pneumonitis (extrinsic allergic alveolitis) induced by isocyanates. *J Allergy Clin Immunol.* 1995;95:1004-1010
40. Semenzato G, Agostini C, Zambello R, Trentin L, Chilosi M, Pizzolo G, Marcer G, Cipriani A. Lung T cells in hypersensitivity pneumonitis: phenotypic and functional analyses. *J Immunol.* 1986;137:1164-1172
41. Cormier Y, Belanger J, Leblanc P, Hébert J, Laviolette M. Lymphocyte subpopulations in Extrinsic Allergic Alveolitis. *Ann NYAc Sci.* 1986;465:370-377
42. Drent M, van Velzen-Blad H, Diamant M, Wagenaar SS, Hoogsteden HC, van den Bosch JM. Bronchoalveolar lavage in extrinsic allergic alveolitis: effect of time elapsed since antigen exposure. *Eur Respir J*. 1993;6:1276-1281
43. Costabel U, Bross KJ, Marxen J, Matthys H. T-lymphocytosis in bronchoalveolar lavage fluid of hypersensitivity pneumonitis. Changes in profile of T-cell subsets during the course of disease. *Chest.* 1984;85:514-522
44. McSharry C, Anderson K, Boyd G. A review of antigen diversity causing lung disease among pigeon breeders. *Clin Exp Allergy.* 2000;30:279-289
45. Haslam PL, Dewar A, Butchers P, Primett ZS, Newman-Taylor A, Turner-Warwick M. Mast cells, atypical lymphocytes, and neutrophils in bronchoalveolar lavage in extrinsic allergic alveolitis. Comparison with other interstitial lung diseases. *Am Rev Respir Dis.* 1987;135:35-47
46. Cormier Y, Belanger J, LeBlanc P, Laviolette M. Broncho-alveolar lavage in farmers' lung disease: diagnostic and physiological significance. *Br J Ind Med.* 1986;43:401-405
47. Reynolds SP, Jones KP, Edwards JH, Davies BH. Inhalation challenge in pigeon breeder's disease: BAL fluid changes after 6 hours. *Eur Respir J.* 1993;6:467-476
48. Winck JC, Delgado L, Vanzeller M, Guimaraes T, Torres S, Sapage JM. Broncho-alveolar inflammation in cork worker's asthma. *Allerg Immunol* (Paris). 2002;34:199-203

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