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


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 10^5 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 (HRCT) [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 antigentic 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 plethysmograph (Vmax229, SensorMedics, Yorba Linda, USA), and dynamic lung volumes by mass flow sensors (6200 Autobox DL, 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-Giems.

Measurement of BALF lymphocyte subsets was performed by two-color direct immunofluorescence staining and flow cytometry. Briefly, 2.5x10^6 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%) 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.

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

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).

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

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)

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


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