Bird Fancier's Lung

A Series of 86 Patients

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Abstract: Bird fancier's lung (BFL) is one of the most common types of hypersensitivity pneumonitis. Nevertheless, the criteria for diagnosing this condition are not standardized. The current study is an in-depth investigation into the clinical characteristics of BFL in the largest series examined for this purpose by a single group, to our knowledge, taking into account the acute, subacute, or chronic clinical presentation.

From 1977 to 2003, BFL was diagnosed in 86 patients using a homogeneous protocol. Data from the clinical history and physical examination were analyzed, as well as the results from the following complementary examinations: laboratory analyses, specific serum IgG antibodies determination, chest X-ray, chest computed tomography (CT), pulmonary function testing, immediate hypersensitivity skin testing, delayed cutaneous hypersensitivity testing, bronchofibroscopy with bronchoalveolar lavage (BAL) and/or transbronchial biopsy, bronchial challenge testing, and surgical lung biopsy. In addition, clinical and epidemiologic characteristics were determined in a control group of 60 pigeon breeders who did not meet the diagnostic criteria of BFL.

Eighty-six patients (21 men and 65 women) with a mean age of 47 years were studied. Seven (8%) patients were younger than 15 years of age at the time of the diagnosis. In 3 cases, the disease was caused by exposure to feather-filled bedding. Nearly 1 in 5 patients was diagnosed in the chronic phase of the disease. The mean diagnostic delay was 1.6 years overall, and 3.2 years in patients diagnosed in the chronic phase of the disease. Among the 17% of patients with chronic disease, the mean interval from initiation of exposure to diagnosis was 16 years, a higher value than in the acute or subacute presentation forms. Dyspnea and cough were the most common clinical symptoms (98% and 82%, respectively), and nearly 25% had grade III or IV dyspnea at diagnosis. Only 18% of patients experienced chest tightness, a symptom classically considered to be frequent in this condition. Erythrocyte sedimentation rate was elevated (>30 mm/h) in 44% of patients. Urinary calcium was elevated in 20% of patients. Angiotensin-converting enzyme was not elevated in any of the patients in which it was measured. Lactate dehydrogenase increases were found in 51% of patients. Specific IgG antibodies to avian antigens were documented in 92% of BFL patients, but also in 87% of pigeon breeder controls.

The most frequent radiologic finding was an interstitial pattern in 79% of patients. Common chest CT features were ground glass areas (68%) and a mosaic pattern (61%); areas of emphysema were found in 7/41 (17%) patients, 5 of whom had never smoked. Two patients had a CT pattern of pulmonary fibrosis indistinguishable from idiopathic pulmonary fibrosis. Immediate hypersensitivity skin testing with bird sera and pigeon bloom was positive in 78% and 100% of BFL patients, respectively, and in 64% and 88% of control pigeon breeders, respectively. Almost one-third of the patients (29%) presented an anergic response on delayed cutaneous hypersensitivity testing. Restrictive ventilatory impairment was the most frequent functional pattern (77%), although 9% and 4% showed a pure obstructive and mixed pattern, respectively. The carbon monoxide diffusing capacity was decreased (<80% of the predicted value) in 85% of cases. Forty-one percent of patients had PaO2 <60 mm Hg at diagnosis when blood gas analysis was performed. Lymphocytosis (>20% lymphocytes) was documented in 83% of patients who underwent BAL, with a similar frequency in the 3 presentation forms: 70% acute, 89% subacute, and 85% chronic. In addition, inversion of the CD_4/CD_8 ratio (<1) was observed in 62% of the patients, but 38% of cases showed a CD_4 predominance. The characteristic triad of histopathologic findings in hypersensitivity pneumonitis was found in only 9% of patients undergoing transbronchial biopsy, but at least 1 of these findings was seen in 69%. Surgical lung biopsy was undertaken in 14/86 (16%) patients; the complete triad was observed in 50% and at least 1 finding in 100%. In 54/86 (63%) patients, the diagnosis was confirmed by bronchial challenge testing, a test with a sensitivity of 92% and specificity of 100%.

BFL is a potentially severe disease that can progress to respiratory failure secondary to pulmonary fibrosis or chronic obstructive pulmonary disease, as a form of chronic occupational respiratory disease. Respiratory symptoms in exposed patients, including children and adults who have only 1 pet bird at home, should raise the suspicion of BFL. Diagnosis in the chronic phase is frequent, and the delay to diagnosis was greatest in these cases. Elevated urinary calcium, lactate dehydrogenase, and erythrocyte sedimentation rate in a bird fancier may constitute a combined marker for suspected BFL. Chest CT frequently discloses emphysema and a pattern of idiopathic pulmonary fibrosis in some patients. An anergic response on delayed cutaneous hypersensitivity testing is not infrequent. The presentation with respiratory failure and the predominance of CD_4 T lymphocytes in some patients' BAL are

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both remarkable. Lymphocytosis on BAL also persists in the chronic phase of the disease. Bronchial challenge testing has a high diagnostic yield, and surgical lung biopsy is not needed to reach the final diagnosis in the vast majority of cases.

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Abbreviations: ACE = angiotensin-converting enzyme, BAL = bronchoalveolar lavage, BFL = bird fancier's lung, COPD = chronic obstructive pulmonary disease, CT = chest computed tomography, DCHT = delayed cutaneous hypersensitivity testing, DLCO = carbon monoxide diffusing capacity, ELISA = enzyme-linked immunosorbent assay, ESR = erythrocyte sedimentation rate, FEV_1 = forced expiratory volume in 1 second, FVC = forced vital capacity, HP = hypersensitivity pneumonitis, IHST = immediate hypersensitivity skin testing, LDH = lactate dehydrogenase, PFT = pulmonary function test, SLB = surgical lung biopsy, TBB = transbronchial biopsy, TLC = total lung capacity.

INTRODUCTION

The term hypersensitivity pneumonitis (HP) encompasses a group of lung diseases produced by inhalation of certain substances, mainly organic, that trigger an inflammatory reaction in susceptible individuals at the level of the alveoli, bronchioles, and pulmonary interstitium, with a marked lymphocytic-histiocytic or monocyte-macrophage component[80]. A great number of antigenic sources have been identified as the etiologic agents producing this group of diseases[24]. Among all the types of HP, bird fancier's lung (BFL) is one of the most frequently diagnosed entities in our setting and, together with farmer's lung, one of the leading diseases in this group. This considerable prevalence is due to the fact that the disease can occur not only in pigeon breeders and keepers of other species, who are regularly exposed to many birds[18], but also in persons who keep 1 or 2 birds in their home and experience continuous daily exposure[89].

Lengthy exposure to avian proteins originating in serum[57,104] and found in epithelial products, bloom (a waxy powder that coats the feathers)[5,57,104], or bird droppings[5,57], can trigger a type-III immune complex-mediated hypersensitivity reaction[13] and type I[71] and type IV[13,67] reactions, with predominant activation of alveolar macrophages and T lymphocytes[14]. These reactions are responsible for the inflammatory response, which leads to the development of the disease and the acute, subacute, or chronic clinical manifestations. In some cases the clinical symptoms are severe enough to trigger acute respiratory failure[117], as characteristically occurs in some types of HP, such as espartosis[30].

The criteria for diagnosing HP are not well standardized, and despite the recent efforts of expert panels to establish the diagnosis on clinical data alone[56], the definitive diagnosis must be supported by additional tests, some of them having an invasive nature. Progression of the disease over years can lead to chronic respiratory failure resulting from well-established pulmonary fibrosis[83] or chronic obstructive pulmonary disease (COPD)[12], thereby conferring a status of potentially severe disease on this entity.

Since Reed and Barbee[98] described the first 2 cases of BFL in 1965, knowledge of this condition has been based on case reports and limited series, including 2 series described by our group[70,119], or on discussion of specific clinical characteristics or the findings obtained from complementary examinations performed in some series. Although a few case-control epidemiologic studies have included a good number of BFL patients[16] the present study was designed to delve deeply into the clinical characteristics of BFL in what is to our knowledge, the largest series investigated for this purpose by a single group to date. In addition, we describe the findings and diagnostic yield of the most frequently used complementary tests for diagnosing this condition (for example, laboratory analyses, imaging and bronchofibroscopic techniques, pulmonary function tests (PFTs), immunologic tests, bronchial challenge testing). The results of some of these tests are compared with those obtained with the same examinations in an ad hoc constituted control group. From the clinical viewpoint, the potential severity of this entity is highlighted; in many cases the condition may already present as pulmonary fibrosis or COPD at the time of the diagnosis[16]. Lastly, a broad review of the related literature is provided, with a discussion of the current knowledge about this condition and the results obtained in our series.

PATIENTS AND METHODS

Study Population

Over a period of 30 years (1974–2003), 86 patients (21 men and 65 women) were studied with a homogeneous diagnostic protocol and met the criteria for BFL (16, 29, 32, and 9 patients diagnosed during the periods 1974–1980, 1981–1990, 1991–2000 and 2001–2003, respectively). Some of these patients had been attended initially at other hospitals in Catalonia and were later referred to our center to complete the diagnostic study.

Diagnostic Protocol

For BFL patients, we analyzed data from the clinical history and physical examination, as well as the following additional tests: laboratory analyses with hemogram, erythrocyte sedimentation rate (ESR), gammaglobulins, total G and E immunoglobulins, calcium level, calciuria/24 h, angiotensin-converting enzyme (ACE), and plasma lactate dehydrogenase (LDH) levels, as well as specific serum IgG antibodies, chest X-ray, chest computed tomography (CT), PFT-spirometry, static lung volumes and carbon monoxide diffusing capacity (DLCO), immediate hypersensitivity skin testing (IHST), delayed cutaneous hypersensitivity testing (DCHT), bronchofibroscopy with bronchoalveolar lavage (BAL) and/or transbronchial biopsy (TBB), and bronchial challenge testing. In the few cases in whom a definite diagnosis could not be reached, surgical lung biopsy (SLB) was performed after individualized assessment of the indication. In more than half these cases, however, SLB had been undertaken before referral to our institution; therefore, the complementary examinations in these patients were performed after their arrival to our service. Because the

patients in this series were diagnosed over a lengthy interval of time (30 yr) and because some of them had been first attended in other centers, not all patients underwent the same complementary examinations; hence, the results are presented according to the number of patients (n) who underwent each examination or procedure.

Diagnostic Confirmation

The diagnosis was established following the steps described in the algorithm shown in Figure 1[76]: current or prior exposure to birds together with consistent signs and symptoms (dyspnea, cough, fever, chest tightness, malaise) related with the exposure (easier to establish in the acute forms), consistent chest X-rays (reticular or micronodular interstitial pattern or ground glass areas), and consistent PFTs (restrictive and/or obstructive ventilatory impairment and/or decreased DLCO). At this point, the presence of specific IgG antibodies (precipitins or enzyme-linked immunosorbent assay [ELISA]) together with positive IHST and clinical improvement with discontinuation of exposure was considered diagnostic. If only 1 of these last 2 tests was positive, results consistent with HP from at least 1 of the following 4 examinations were required for the diagnosis: 1) characteristic chest CT features (air trapping, ground glass opacities, mosaic pattern, and/or fine centrilobular nodules)[22,43,99], 2) BAL specimen showing marked lymphocytosis and inversion of the CD_4/CD_8 ratio[115] (although CD_4 cells sometimes predominate), 3) TBB material exhibiting the following triad: interstitial lymphocytic-histiocytic infiltrate, bronchiolitis obliterans, and poorly formed granulomas[93] (in cases in which only lymphocytic-histiocytic infiltrate was confirmed, at least 1 of the other 2 described criteria was required), and/or 4) a positive bronchial challenge test.

In addition, SLB was performed in 14 patients, in whom the diagnosis could not be definitely established on the findings from these tests. As mentioned above, 8 (57%) of these patients underwent biopsy before referral to our service. The criteria considered consistent with the diagnosis were the presence of the triad described for TBB, particularly when there was predominantly centrilobular involvement[21]. Lastly, in 3 patients the previously established diagnosis was confirmed by histopathologic study of an explanted lung specimen following lung transplantation in our center.

Criteria Used to Categorize Clinical Presentation

Acute form: 1) when the symptoms (malaise, dyspnea, cough with or without expectoration, fever, and chest tightness) are evident and present within some hours (generally 4–8 h, up to a maximum of 24 h) following contact with birds (1 or more episodes), and improve with discontinuation of exposure; 2) when the diagnosis is made because of a recent acute episode, despite the fact that the patient had prolonged clinical symptoms.

Subacute form: when, over a period of weeks or months, overlapping symptoms occur, consisting of asthenia, constitutional syndrome, mild fever, weight loss, dry or productive cough, and/or dyspnea on exertion.

Chronic form: when a patient reporting current or past contact with birds presents persistent grade III/IV dyspnea[29] and CT or lung biopsy demonstrates diffuse interstitial lung disease with well-established fibrosis; that is, honeycombing or microcystic pattern with or without peripheral predominance in CT and usual interstitial pneumonia- or nonspecific

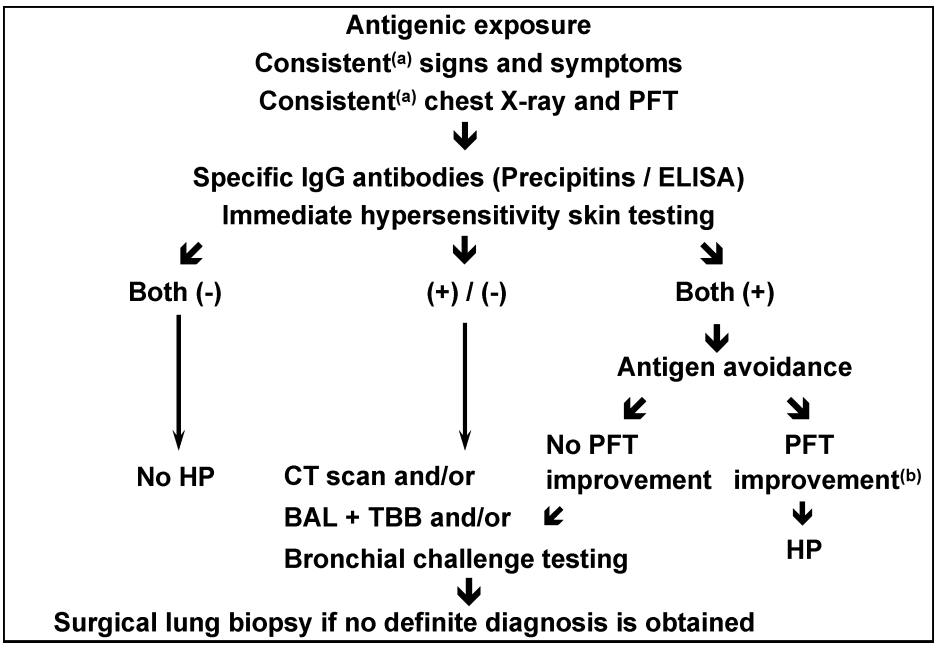

FIGURE 1. Diagnostic algorithm for hypersensitivity pneumonitis. (**a**) See Diagnostic Confirmation section in Patients and Methods. (**b**) PFT improvement: $\geq$20% increase of FVC, FEV_1, and/or DLCO in PFTs.

interstitial pneumonia-like lesions in lung biopsy. Nonsmoker patients with chronic symptoms of dyspnea, cough, and expectoration, whose PFTs are consistent with chronic airway obstruction (COPD-emphysema) are also included in this group; these patients can be considered as affected with an occupational COPD form.

Control Group of Pigeon Keepers

The control group for the study comprised 60 pigeon keepers who were members of various associations of carrier pigeon fanciers and were exposed to large numbers of these birds. The persons enrolled had responded to a survey sent by mail on avian exposure and the related symptoms, and were later visited in our service. None of them fulfilled the diagnostic criteria of BFL. The epidemiologic characteristics of the control group are shown in Table 1. The study protocol for this group of pigeon keepers was clinical history, PFT, chest X-ray, laboratory analyses (with the same determinations as in the group of patients with BFL), and IHST.

Population Exposed to Birds in Our Setting

To determine the percentage of the population exposed to birds in our setting, an ad hoc survey was made of workers at Hospital Vall d'Hebron (n = 400) during their mandatory medical check-up, asking each person whether they were currently or at any time in the past exposed to birds at home; level of exposure (h/wk) was also requested.

Preparation of Antigen Extracts

IHST and bronchial challenge tests were performed with avian sera and pigeon bloom extracts prepared in our laboratory. Blood for avian sera extracts was collected from several birds and centrifuged; the serum protein concentration was measured by bicinchoninic acid assay (Pierce Chemicals, Rockford, IL). Bloom was obtained by aspiration, collecting the feather dust of 20 pigeons from a filter fitted onto a mini-vacuum cleaner. Antigen extract from pigeon bloom was prepared by defatting the raw material with anhydrous ether at 37°C in a Soxhlet apparatus (Corning, Stone, Staffordshire, UK) for 4 hours[104]. The material was then dried and extracted (1:20 w/v) in 0.2 mol/L ammonium bicarbonate, pH 7.9, overnight at 4°C. The solution was centrifuged and the supernatant dialyzed overnight at 4°C against deionized water in a 3500 molecular weight cutoff membrane (Spectra/Por; Spectrum Medical Industries, Los Angeles, CA) until a clear extract was obtained. The material was lyophilized and the protein concentration measured by the bicinchoninic acid assay method[104]. The different lots of antigen extract were characterized by SDS-PAGE to assure that they had the same protein profile.

Determination of Specific IgG Antibodies

In the first 31 cases, specific precipitating antibodies were determined exclusively by countercurrent immunoelectrophoresis. Twenty μL of antigen solution (protein concentration: 100 mg/mL) were placed in agar plate wells and tested against 20 μL of undiluted serum from each patient. The agar plates underwent electrophoresis in a sodium barbital buffer (pH 8.4, $\mu = 0.05$) and then were left in 5% sodium citrate solution for 72 hours. Subsequently, plates were washed with PBS and stained with Coomassie

TABLE 1. Clinical and Epidemiologic Data

	BFL Patients (n = 86)	Pigeon Breeders (Control Group) (n = 60)	p Value
Mean age, yr (range)	47 (9–76)*	44 (22–69)	0.5300
Sex	21 M (25%), 65 F (75%)	60 M (100%)	0.0001
Smoking			
Smokers	12 (14%)†	25 (41%)	0.0055
Exsmokers	7 (8%)	8 (14%)	0.4160
Prior yr of exposure to birds; mean (range)	9.9 (0.1–50)	23 (3–53)	0.2920
Latency period, yr of exposure until symptoms; mean (range)	8.7 (0.1–49.8)	NA	NA
No. of birds; mean (range)	17 (1–200)	91 (20–200)	0.0001
Species of birds‡	Pigeons 56 (65%) patients, canaries 30 (35%), parakeets 27 (31%), hens 13 (15%), parrots 9 (10%), doves 8 (9%), goldfinches 7 (8%), ducks 5 (6%), turtledoves 3 (3%), chickens 2 (2%), "carolinas" (parrot-like) 2 (2%), partridges, storks, and quails, 1 patient each	Only pigeons 43 (72%), pigeons + other birds 17 (28%)	

Abbreviations: NA = not applicable.

*Seven patients aged <15 yr.

†The 12 smokers were 7/21 (33%) men and 5/65 (8%) women.

‡Fifty-one (59%) patients were exposed to more than 1 species of birds (pigeons + other birds in 35 of them), and 35 were exposed to a single species (20 to pigeons, 5 to parakeets, 3 to parrots, 3 to doves, 2 to canaries, 1 to turtledoves and 1 to hens); 11 (13%) had only 1 bird, and 32 (37%) had >10 birds; 3 patients mentioned that they had a feather-filled quilt or pillow in addition to a limited prior history of contact with birds.

Brilliant Blue (Pierce Chemical Co., Rockford, IL), and the precipitation lines were assessed by direct visualization.

In the remaining cases, specific IgG was measured by an ELISA technique based on the method of Metzger et al[64] modified with avian sera or bloom extract as the antigen. Wells of high-binding microtiter plates (Costar, Cambridge, MA) were incubated with 2 μg protein/well in 0.1M $Na_2CO_3/NaHCO_3$ buffer (pH 9.6) at 4°C overnight. The wells were then washed 3 times with washing buffer (0.1M phosphate buffered saline, pH 7.5/0.005% Tween 20) and blocked with phosphate buffered saline/1% bovine serum albumin for 1 hour at 37°C. Washing the plates 4 times between steps, the specific IgG assays were performed in duplicate by incubating the serum samples at an appropriate dilution and the standard curve for 2 hours at 37°C. A solution of horseradish peroxidase-labeled antihuman IgG (clone MH16-1ME, 0.5 mg/mL) diluted at 1:1000 was added and plates were incubated for 2 hours at 37°C. The reaction was developed with 3,3′,5,5′-tetramethylbenzidine (Sigma Chemicals), 3% H_2O_2 for 20 minutes at room temperature in the dark and stopped with 2M H_2SO_4. Optical density at 450 nm was measured with a microplate reader (Titertek Multiskan Plus MKII).

Results were expressed as absorbance units at 450 nm ($A_{450\ nm}$). Values above the mean plus 2 standard deviations of the results obtained in a control population of 30 healthy individuals previously studied in our laboratory were deemed positive. The following results were considered positive: pigeon serum >0.284 $A_{450\ nm}$, parakeet serum >0.180 $A_{450\ nm}$, canary serum >0.336 $A_{450\ nm}$, hen serum >0.445 $A_{450\ nm}$, and parrot serum >0.294 $A_{450\ nm}$.

For bloom extract, results were expressed as arbitrary ELISA units using a reference serum pool with an assigned value of 5×10^4 U/mL. The pool was obtained with serum samples from precipitin-positive symptomatic patients.

Pulmonary Function Testing

PFTs were performed using a MasterLab apparatus (MasterLab, Jaegger, Germany). All tests were done according to the European Respiratory Society guidelines[27,95] described in 1993, even those carried out before that year. Static lung volumes were measured using the plethysmography method, and DLCO was measured using the single breath-hold method[39]. The predicted spirometric values proposed by Morris et al[77] and by Roca et al[103] for the Mediterranean population were applied from 1974 to 1985 and from 1986 to the present, respectively. The predicted values used for static lung volumes and DLCO tests were those proposed by the European Respiratory Society[27,95].

A restrictive ventilatory pattern was defined as forced vital capacity (FVC) <85% of the predicted value with forced expiratory volume in 1 second (FEV_1)/FVC ratio >80%[95] in the absence of a static lung volume study, together with a total lung capacity (TLC) <80% of the predicted value[91], when these tests were performed. An obstructive ventilatory pattern was established on the basis of FEV_1/FVC ratio <70% together with FEV_1 <80% of the predicted value[95]. The concomitant presence of characteristic functional criteria for both patterns was defined as a mixed ventilatory pattern. The DLCO was considered decreased at values of <80% of the predicted value[91].

The dyspnea grade was determined following the New York Heart Association proposed grading criteria[29].

Bronchoscopy Techniques

BAL was performed according to the recommendations of the European Respiratory Society[54]. The TBB procedure used has been described by other authors[2].

Immediate Hypersensitivity Skin Testing

IHST was performed by intradermal injection in the forearm of 0.1 mL of solutions (1/100 w/v) of different avian extracts: pigeon bloom and pigeon, parakeet, canary, hen, and parrot sera. Based on the role of IgG in the mediation of immediate hypersensitivity[88], development of a wheal with a maximum diameter over 10 mm at 15 minutes (immediate reading) was the criterion defining a positive test[66,68].

For both the BFL patients and the control pigeon keepers, the reference results applied were taken from published values obtained by our group in a population of 20 BFL patients, 20 asymptomatic pigeon breeders, and 10 unexposed individuals[66].

Delayed Cutaneous Hypersensitivity Testing

DCHT was performed by intradermal injection in the forearm of 0.1 mL of each of the following antigen extract solutions: candidin 1/100 w/v (Leti Laboratories), tuberculin (PPD Evans RT-23) 0.1 mL = 2 UT (Medeva-Pharma S.A.), trichophyton mentagrophytes 100 μg/mL (Leti Laboratories), and varidase (streptokinase 40 IU/mL/streptodornase 10 IU/mL) (Lederle Laboratories). Development of a wheal with a maximum diameter >5 mm by 48 hours after extract injection was considered positive[84,85].

Two types of immune response were defined: *normal response,* when positive status (>5 mm wheal) to at least 1 antigen was established and *anergic response,* when tests to all 4 antigens were negative.

Bronchial Challenge Testing

Bronchial challenge tests were performed in the hospital setting after obtaining the patient's written consent. Using a De Vilbiss 646 nebulizer (De Vilbiss, Somerset, PA) and a Mefar MB3 dosimeter (Mefar, Ele H_2O, Medicali, Brescia, Italy), which releases the solution during the first second of each inspiration, the patient was requested to inhale 2 mL of the suspected antigen at a dilution of 1/100 (0.01 mg/mL)[73]. FVC, FEV_1, DLCO, and the patient's temperature were recorded at baseline, 20 minutes after the inhalation, and every hour thereafter for the next 8 hours. Blood cell count, chest X-ray, and O_2 saturation measurement were performed before and 8 hours after inhalation. In all cases, a bronchial challenge test with a placebo solution was carried out 1 day before testing with the suspected antigen.

The test was considered positive when any of the following responses was elicited:

1. FVC decrease >15% or DLCO decrease >20% as compared to baseline values;

2. 10%–15% FVC decrease plus at least 1 of the following criteria with respect to clinical status and basal analytic values[47,96]: a) white blood cell increase $\geq$20%, b) O_2 saturation decrease $\geq$3%, c) significant radiologic changes, d) rise in body temperature >0.5°C, and e) evident clinical symptoms (for example, cough, dyspnea);
3. FVC decrease <10% but with evidence of 3 or more of the previously mentioned clinical or analytic criteria[73].

When the test proved negative, inhalation of a new antigen dilution of 1/10 (0.1 mg/mL) was performed the next day following the same procedure.

To determine the validity, sensitivity, and specificity of bronchial challenge testing in our setting, we recruited a control group comprising 20 individuals exposed to birds and affected with other lung diseases, who showed a diffuse interstitial radiologic pattern. The final diagnoses of these

TABLE 2. Epidemiologic Data, Chest CT Findings, Pulmonary Function Tests, and PaO2 and Bronchoalveolar Lavage Results in Patients With BFL, by Clinical Presentation Phase

	BFL Patients No. (%)	Acute Phase No. (%)	Subacute Phase No. (%)	Chronic Phase No. (%)
Epidemiologic data				
No. of patients	86	34 (40)	37 (43)	15 (17)
No. of birds; mean (range)	17 (1–200)	21 (1–200)	14 (1–100)	13 (3–30)
Birds per yr*; mean (range)	206 (0.5–2000)	64 (0.5–1660)	279 (0.5–2000)	304 (9–1200)
Exposure time (yr); mean (range)	9.9 (0.1–50)	5.6 (0.1–20)	10.8 (0.2–50)	16 (3–40)
Diagnostic delay, yr of symptoms until diagnosis; mean (range)	1.6 (0–10)	0.8 (0–5)	1.7 (0.1–8)	3.2 (0.5–10)
Chest CT findings†				
Normal	1/41 (2)	1/12 (8)	0/20 (0)	0/9 (0)
Ground glass areas	28/41 (68)	8/12 (66)	13/20 (65)	7/9 (78)
Air trapping/mosaic pattern	25/41 (61)	7/12 (58)	15/20 (75)	3/9 (33)
Bronchiectasis	19/41 (46)	3/12 (25)	11/20 (55)	5/9 (55)
Nodular pattern	17/41 (41)	7/12 (58)	7/20 (35)	3/9 (33)
Septal thickening	12/41 (29)	0/12 (0)	8/20 (40)	4/9 (44)
Emphysema	7/41 (17)‡	3/12 (25)	2/20 (10)	2/9 (22)
Honeycombing	3/41 (7)	0/12 (0)	0/20 (0)	3/9 (33)
Pulmonary function test				
Spirometry pattern				
Normal	8/78 (10)	5/31 (16)	3/34 (9)	0/13 (0)
Restrictive	60/78 (77)	25/31 (81)	23/34 (68)	12/13 (92)
Obstructive	7/78 (9)§	1/31 (3)	5/34 (15)	1/13 (8)
Mixed	3/78 (4)	0/31 (0)	3/34 (9)	0/13 (0)
RV >140%	15/57 (26)‖	4/22 (18)	9/23 (39)	2/12 (17)
DLCO <80%	45/53 (85)	17/19 (89)	18/24 (75)	10/10 (100)
PaO2 <60 mm Hg	14/34 (41)**	5/13 (38)	4/14 (29)	5/7 (71)
Bronchoalveolar lavage				
% Lymphocytes >20%	30/36 (83)††	7/10 (70)	17/19 (89)	6/7 (85)
CD4/CD8 <1	8/13 (62)	2/3 (66)	4/7 (57)	2/3 (60)
CD4/CD8 >1.2	5/13 (38)	1/3 (33)	3/7 (43)	1/3 (33)
Polymorphonuclear >4%	26/36 (72)‡‡	6/10 (60)	15/19 (79)	5/7 (71)

*Birds per yr: exposure variable defined as the number of birds multiplied by the number of yr of exposure.

†In 6/41 (15%) patients undergoing CT, the chest X-ray showed no significant alterations; predominant location of the lesions was apical in 8 (19%) patients, basal in 15 (36%), and diffuse in 18 (44%); 2 patients, both in the chronic stage of the disease, presented a fibrosis pattern that was indistinguishable from the pattern in idiopathic pulmonary fibrosis.

‡Five patients had never smoked and 2 were smokers of (<20 cig/d; 1 patient had obstructive ventilatory impairment and the other 6 restrictive ventilatory impairment.

§Only 1 patient was an active smoker (<20 cig/d) and 1 was an exsmoker.

‖Chest CT was performed in 7 of these 15 patients; air-trapping was seen in 4/7 (57%) and emphysema in 1/7 (14%).

**Blood gases were measured only in patients with a high degree of dyspnea.

††Mean percentage of lymphocytes in BAL specimens from the 36 patients was 38% (range, 2%–85%); a percentage >60% was registered in 10/36 (28%) patients (4 acute, 5 subacute, and 1 chronic phase).

‡‡Mean percentage of neutrophils in BAL specimens from 36 patients was 11% (range, 1%–64%).

patients, confirmed by TBB or SLB in the cases of diffuse interstitial lung disease, were usual interstitial pneumonia in 8 patients, nonspecific interstitial pneumonia in 2, sarcoidosis in 8, and bronchiolitis obliterans and hemosiderosis in 1 patient each.

Statistical Analysis

The Fisher exact test and unpaired t-tests were used to compare the nominal and continuous variables, respectively. All p values are 2-tailed. The Wilson method was used to calculate the sensitivity and specificity indices of bronchial challenge testing.

RESULTS

Clinical and Epidemiologic Data (Tables 1 and 2)

Noteworthy clinical and epidemiologic data include the high percentage of women and the low percentage of smokers. The exposure exclusively to canaries in 2 patients and to hens in 1 patient is also worthy of mention. Other relevant aspects include the mean delay of 1.6 years to diagnosis, and initial diagnosis in the chronic phase of the disease in 17% of patients. It must be highlighted that the mean diagnostic delay was 2-fold higher in patients diagnosed in the subacute phase and more than 3-fold higher in those in the chronic phase, as compared to patients diagnosed in the acute phase.

Pulmonary Function Testing and Blood Gas Measurement (Table 2)

Pulmonary function was assessed in 78 (91%) patients. Spirometry was performed in all cases, static lung volume study in 57 (73%), and DLCO testing in 53 (68%). The distribution of the various ventilatory patterns as assessed by spirometry is described in Table 2. Mean FVC relative to the predicted value was 66% (range, 28%–104%), with values lower than 85% in 66 (76%) patients and lower than 50% in 18 (21%) patients. Mean TLC relative to the predicted value was 84% (range, 46%–127%), with values below 80% in 19 (33%) patients. Mean DLCO relative to the predicted value was 56% (range, 14%–131%), with values below 80% in 45 (85%) patients and below 50% in 27 (51%). Lastly, the presence of air trapping (residual volume >140%) was demonstrated in 26% of patients in whom lung volumes were determined.

Spirometry was also carried out in 51 (85%) of the 60 control pigeon keepers without BFL. The test was normal in all but 4 (8%) patients, who presented a slight restrictive ventilatory impairment, which, in the absence of radiologic alterations, may be attributable to excess weight (body mass index of the 4 patients was 33, 34, 35, and 39 kg/m^2, respectively).

The PaO2 at the time of diagnosis was determined in 34 (40%) patients. Respiratory failure (PaO2 <60 mm Hg)[127] was documented in 41% of them, and was more frequent in patients diagnosed in the chronic phase of the disease. The mean PaO2 value was 64 mm Hg (median, 63 mm Hg; range, 31–97 mm Hg). Hypercapnia was seen in only 1 (14%) of the 7 chronic-phase patients in whom this parameter was recorded, with a value of 62 mm Hg (this patient had a body mass index of 35 kg/m^2 and no other known respiratory or cardiologic disease). In the 13 acute-phase patients and the 14 subacute-phase patients in whom blood gases were measured, normocapnia was documented.

Bronchoalveolar Lavage (Table 2)

BAL was undertaken in 36 (42%) patients. Lymphocytosis (>20%) and inversion of the CD_4/CD_8 T-lymphocyte ratio (<1) were found in most of the patients and in a similar percentage in all 3 presentation forms of the disease. A predominance of CD_4 (T-lymphocyte ratio >1.2) was seen in about one-third of patients.

Physical Examination and Symptoms (Table 3)

Inspiratory crackles were the most frequent finding, but the physical examination was strictly normal in 15% of patients. The incidence of digital clubbing in the overall

TABLE 3. Physical Examination and Symptoms

	BFL Patients (n = 86) No. (%)	Pigeon Breeders (Control Group) (n = 60) No. (%)	p Value
Physical examination			
Crackles*	68/86 (80)	0	<0.0001
Wheezing	13/86 (15)	0	0.0015
Digital clubbing†	6/86 (7)	0	0.046
Cyanosis	4/86 (5)	0	0.126
Signs of CHF	3/86 (3)	0	0.210
Normal	13/86 (15)	60/60 (100)	<0.0001
Symptoms			
Dyspnea‡	84/86 (98)	6/60 (10)	<0.0001
Cough§	71/86 (82)	14/60 (24)	<0.0001
Fever‖	44/86 (52)	0	<0.0001
Asthenia	39/86 (46)	0	<0.0001
Expectoration	33/86 (38)	8/60 (13)	0.0013
Rhinitis	30/86 (35)	13/60 (22)	0.0986
Weight loss	28/86 (33)	0	<0.0001
Conjunctivitis	21/86 (25)	4/60 (7)	0.0066
Chest tightness**	15/86 (18)	0	0.0004

Abbreviations: CHF = congestive heart failure.

*Crackles were more frequent in patients in the chronic phase (14/15, 93%) than in patients in the acute phase (25/34, 73%) or subacute phase (29/37, 78%).

†Five of 6 (83%) patients with digital clubbing were in the chronic phase of the disease.

‡Dyspnea grade I in 13 (15.1%) patients, grade II in 51 (59.3%), grade III in 18 (20.1%), and grade IV in 2 (2.3%).

§Twelve of 15 (80%) patients diagnosed in the chronic phase did not have significant cough.

‖Fever was much more frequent in patients diagnosed in the acute phase (28/34, 82.3%) than among those diagnosed in the subacute (12/37, 32.4%) or chronic (4/15, 26.6%) phases.

**Eight of 15 (53%) patients who mentioned chest tightness presented in the acute form of the disease; among all patients in the acute phase, this symptom occurred in 8/34 (24%) cases.

population was low, although in the group with chronic disease, digital clubbing was seen in one-third of patients.

The most frequent clinical signs and symptoms were dyspnea, cough, fever, asthenia, and expectoration. Although none of the control pigeon keepers met the diagnostic criteria for BFL, some of them reported respiratory symptoms.

Laboratory Data and Determination of IgG Antibodies to Bird Antigens (Table 4)

The most frequent findings were hypergammaglobulinemia; elevated LDH, ESR and IgE; and hypercalciuria. Specific serum IgG antibody determinations were performed in 78 (91%) patients. Specific IgG antibodies against avian serum or bloom were frequently present not only in patients with BFL, but in control pigeon keepers as well.

Imaging Examinations (Chest X-Ray and Chest CT) (Tables 2 and 5)

The radiologic studies showed a clear predominance of interstitial pattern, more than half of reticular type. Chest CT disclosed a high frequency of ground glass areas in all 3 presentation forms of the disease. Air trapping was less frequently found in the chronic presentation. Two patients presented a pattern of pulmonary fibrosis that was indistinguishable from idiopathic pulmonary fibrosis; their final diagnosis was obtained after an SLB was performed. Emphysema was documented in 25% of acute, 10% of subacute, and 22% of chronic cases. Chest CT was not performed in the control group of pigeon keepers, and chest X-rays showed no significant findings.

TABLE 5. Radiologic Patterns and Predominant Location of the Lesions in Patients With BFL

Chest X-Ray (n = 82)	No. (%)
Radiologic pattern*	
Normal	10 (12)
Alveolar	1 (1)
Alveolar-interstitial	6 (7)
Interstitial†	65 (79)
Reticular	38 (58)
Reticulonodular	13 (20)
Nodular	10 (15)
Miliary	4 (6)
Predominant location of the lesions	
Apical	3 (4)
Basal	41 (50)
Diffuse	38 (46)

*The records mentioned volume loss in 13 (16%) cases.
†Percentage of cases with an interstitial pattern among patients in the acute, subacute, and chronic phases was 26/33 (79%), 25/34 (73%), and 14/15 (93%), respectively.

TABLE 4. Laboratory Data

	BFL Patients (n = 86) No. (%)	Pigeon Breeders (Control Group) (n = 60) No. (%)	p Value
Laboratory data			
Leukocytosis (≥9500/mm3)	19/82 (23)	4/28 (13)	0.4237
Lymphopenia* (≤20%)	15/64 (23)	8/28 (29)	0.6094
Eosinophilia (≥350/mm3)	12/55 (22)	2/24 (8)	0.2066
Elevated ESR (≥30 mm/h)	22/50 (44)	0/24 (0)	<0.0001
Hypergammaglobulinemia (≥18%)	31/47 (66)	8/26 (31)	0.0066
Elevated IgG (≥650 mg/dL)	16/53 (30)	6/58 (10)	0.0158
Elevated IgE (≥120 U/mL)	12/43 (28)	4/42 (9)	0.0501
Hypercalcemia (≥10.5 mg/dL)	3/39 (8)	0/22 (0)	0.547
Hypercalciuria† (≥250 mg/24h)	8/31 (26)	0/24 (0)	0.0071
Elevated ACE (≥130 U/L)	0/28 (0)	0/24 (0)	0.9999
Elevated LDH‡ (≥350 U/L)	21/41 (51)	0/42 (0)	<0.0001
IgG antibodies to bird antigens			
Total no. of positive results	72/78 (92)	52/60 (87)	0.3945
Positive results against serum of an avian species to which the patient was exposed§	62/67 (93)	—	—
Positive results against serum of an avian species to which the patient was not exposed‖	10/11 (91)	—	—

*Lymphopenia was slightly more frequent in acute-phase patients (7/27, 26%) compared with subacute (6/28, 21%) or chronic (2/11, 18%) patients.
†Hypercalciuria was observed in 1/7 (14%) patients in the acute phase, 6/16 (37%) in the subacute phase, and 1/1 (100%) in the chronic phase.
‡LDH was elevated in 7/17 (41%) patients in the acute phase, 10/20 (50%) in the subacute phase. and 4/4 (100%) in the chronic phase.
§Among the group of 17 cases in which 2 or more sera were tested, positive results against more than 1 type of serum were obtained in 15 (88%) of them.
‖Specific serum extracts were not available at the time of the study in these 11 cases; hence, 1 or more different antigenic extracts were tested.

TABLE 6. Immediate Hypersensitivity Skin Testing (IHST)

	BFL Patients* No. (%)	Pigeon Breeders (Control Group)† No. (%)	p Value
Bird serum	54/69 (78)	32/50 (64)	0.292
Pigeon bloom	17/17 (100)	44/50 (88)	0.452
Both	12/17 (71)	30/50 (60)	0.439

*At least 1 positive response was obtained in 59/69 (85%) patients tested: in 42 cases to various serum extracts, in 5 to pigeon bloom, and in 12 to serum and pigeon bloom; in the 19 patients who kept various different birds and underwent tests with different antigenic extracts, more than 1 positive response was elicited in 12 (63%) of them (10 patients with 2, and 2 patients with 3 positive results).

†At least 1 positive response was obtained in 46/50 (92%) control pigeon breeders tested.

Immediate Hypersensitivity Skin Testing (Table 6)

IHST was performed in 69 (80%) patients with BFL, and in 50 (83%) control pigeon keepers. In 50 (72%) of the BFL patients, the test was performed with antigen extract from serum or bloom from a single type of bird, in 14 (20%) with extracts from 2 types, and in 5 (7%) with extracts from 3 types. At least 1 positive response was obtained in 85% of BFL patients and in 92% of control pigeon keepers.

Delayed Cutaneous Hypersensitivity Testing

DCHT was performed in 56 (65%) patients. Sixteen of them (29%) had an anergic response and 17 (30%), 17 (30%), 4 (7%), and 2 (4%) had positive responses to 1, 2, 3, and 4 antigens, respectively. The percentages of positive results for candidin, tuberculin, trichophyton, and varidase were 28/56 (50%), 14/56 (25%), 13/56 (23%), and 17/56 (30%), respectively.

Transbronchial Biopsy and Surgical Lung Biopsy (Table 7)

TBB was carried out in 33 (38%) patients and SLB in 14 (16%). Among the 14 patients undergoing SLB during the diagnostic process, 2 (14%) had an acute presentation, 5 (36%) subacute, and 7 (50%) had chronic disease. Six (43%) of the 14 SLB procedures were indicated from our service; the other 8 had been performed in other centers before referral to our hospital. The biopsy specimen was obtained by thoracotomy in 9 patients and by thoracoscopy in 5. In 11 (79%) of these 14 patients, a bronchial challenge test had not been carried out during the process of diagnosis, either because it was not available at the center where the initial study was carried out or because the patient had a functional contraindication for the test. In the other 3 (21%) cases, the diagnosis of HP was later corroborated with bronchial challenge testing.

TABLE 8. Bronchial Challenge Testing: Sensitivity and Specificity

	Positive Results*	Antigens Used in the Positive Bronchial Challenge Tests (No. of Patients)
BFL patients†	54/59 (92%)	Pigeon serum: 37
Sensitivity	92% (83–96)	Parakeet serum: 8
Specificity	100% (88–100)	Canary, dove, and parrot serum: 2
Positive predictive value	100 (95–100)	Hen, turtledove, and goose serum: 1
Negative predictive value	80 (64–89)	
Pigeon breeders (control group)	0/20 (0%)	
Patients with ILD (not BFL) exposed to birds‡	0/20 (0%)	

Abbreviations: ILD = interstitial lung disease.

*See Patients and Methods section for criteria for positive results.

†Six patients underwent 2 bronchial challenge tests against various avian antigens; 1 of them showed a positive response against 2 different antigen extracts (pigeon and parakeet) and another presented a positive response following direct exposure to different birds in his pet shop (the patient did not want to undergo another bronchial challenge test in the hospital after the first was negative); the 5 (8%) patients with a negative bronchial challenge test were diagnosed with BFL by other means.

‡Eight patients with usual interstitial pneumonia, 2 with nonspecific interstitial pneumonia, 8 with sarcoidosis, 1 with bronchiolitis obliterans, and 1 with hemosiderosis.

TABLE 7. Transbronchial Biopsy (TBB) and Surgical Lung Biopsy (SLB) Findings of the Classic Diagnostic Triad for Hypersensitivity Pneumonitis*

	L-H Infiltrate	Poorly Formed Granuloma	Bronchiolitis Obliterans	1 Finding	2 Findings	3 Findings
TBB (n = 33)†	22 (67)	7 (21)	6 (18)	14 (42)	6 (18)	3 (9)
SLB (n = 14)‡	12 (86)	10 (71)	10 (71)	3 (21)	4 (29)	7 (50)§

Abbreviations: L-H = lymphocytic-histiocytic.

*No. (%).

†Other findings included alveolitis in 11 (33%) cases, giant cells in 8 (24%), interstitial fibrosis in 7 (21%), and foam cells in 6 (18%). TBB was reported as nonspecific in 3 (9%) patients.

‡Other SLB findings were alveolitis in 8 (57%) cases, giant cells in 6 (43%) and foam cells in 3 (21%).

§The complete triad was observed in SLB material from 1/2 (50%) patients in the acute phase, 3/4 (75%) patients in the subacute phase, and 3/8 (38%) chronic cases; these 7 patients corresponded to 2 of the 3 who also had a positive SBCT and to 5 of the 11 who did not undergo this test.

All 3 of the characteristic histologic features of HP (lymphocytic-histiocytic infiltrate, poorly formed granulomas, and bronchiolitis obliterans) were found more frequently in SLB than in TBB specimens. In fact, SLB confirmed the diagnosis of HP based on the complete triad in 50% of patients who underwent the procedure; that is, in 8% of the total patient series.

Bronchial Challenge Testing (Table 8)

Bronchial challenge tests were undertaken in 59 (69%) patients with BFL, 20 pigeon keepers, and 20 patients with diffuse interstitial lung disease (not HP) exposed to birds. Of the 86 patients with BFL, the diagnosis was confirmed in 54 (63%) based on the outcome of a bronchial challenge test. The sensitivity and specificity of this test were 92% and 100%, respectively. An example of a positive bronchial challenge test with pigeon serum is shown in Figure 2.

Control Group of Pigeon Keepers

The results regarding symptoms and the various examinations performed in the 60 pigeon breeders are presented in the corresponding tables (Tables 1, 3, 4, 6 and 8).

Population Exposed to Birds in Our Setting

Among the 400 individuals surveyed, 9% reported current contact with birds in their homes for more than 5 hours per week, and 24% reported close contact with birds at home for more than 1 year at some time in their lives.

DISCUSSION

Epidemiology and Demographic Data

The various forms of HP are encompassed in the group of more than 200 entities that make up the diffuse interstitial lung diseases. Development of clinically evident HP depends on individual susceptibility and on the type, intensity, and duration of exposure to the causal agent[89]. It is likely that certain differential factors, such as the climate, geographic location, local customs, or smoking habit, can lead to a differing prevalence of HP among countries. Specifically, in Spain, a 2004 report of a registry[128] of the incidence of diffuse interstitial lung diseases placed HP as the fifth most frequent (6.6% of cases) of these diseases after idiopathic pulmonary fibrosis, sarcoidosis, cryptogenic organizing pneumonia, and collagen-associated interstitial lung

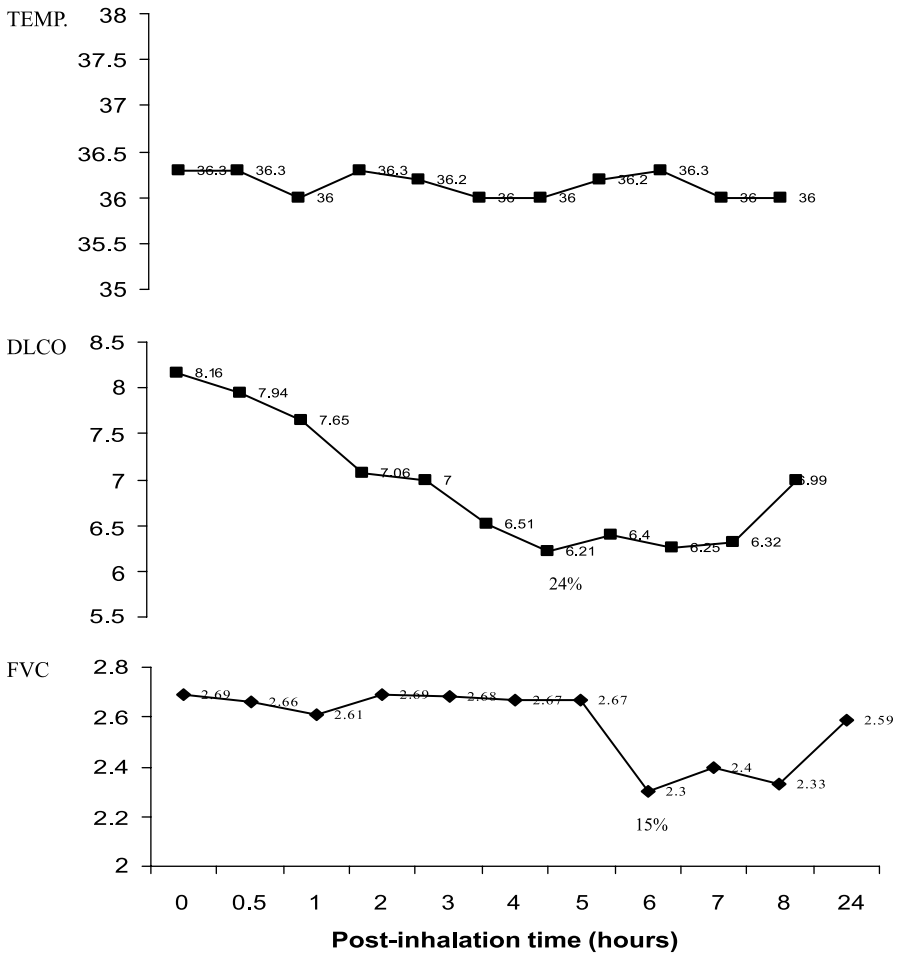

FIGURE 2. Positive result of a bronchial challenge test with pigeon serum: no changes in temperature but a decrease of 24% and 15% in DLCO and FVC, respectively, are observed.

disease. In our center, patients with HP accounted for 15% of the 500 patients with interstitial lung disease seen from 1995 to 2005 in the specialized outpatient clinic for these diseases[72]. These values are in keeping with those recorded in registries from other European countries, such as Belgium (12%) or Germany (11%)[118]. The prevalence of BFL ranges from 8% to 30% among pigeon keepers belonging to associations of pigeon fanciers[12,28], and sensitization may reach 32% among persons with high, continuous exposure[33]. In Spain, Rodriguez de Castro et al[105] estimated that 8% of individuals among a large sample of 343 pigeon fanciers had symptoms of BFL. Currently, however, the main cause of new BFL cases is likely to be exposure to pet birds at home, with a reported prevalence among persons exposed to parakeets of 0.5%–7.5%[46].

The series of 86 patients presented herein, the largest reported to date to our knowledge, reveals that BFL is not an unusual disease in our setting: an average of 3 patients per year are diagnosed with this condition in our service. The study also shows that the affected persons may be exposed to a large number of birds or to only 1 pet bird at home (see Table 1). These latter patients currently represent an increasingly larger percentage of BFL diagnoses in our experience. This is not surprising considering that in the survey performed in 400 of our hospital employees, 9% reported current contact with birds at home during more than 5 hours per week and 24% mentioned a prior history of exposure to birds lasting more than 1 year. The clear predominance of women in our series is also interesting. The proportion of men and women in the various published studies appears to be related to the type of avian exposure under consideration (with pigeon keepers being mainly men) and with certain social, economic, and cultural differences between countries (men predominate in the United States and Europe, and women in Mexico)[106]. The predominance of women in our series may be due to the fact that they spend more time at home than men and, therefore, are more prone to lengthy continuous exposure to pet birds, which are usually under their care. Low-level but continuous exposure to avian antigens can induce the development of this type of BFL[31]. Such contact can even favor progression to chronicity, since avian antigens may persist in the home environment for a lengthy period of time despite removal of the bird[28]. The number of hours per week a person is exposed to birds[105] as well as the concept of bird per number of years should be included in the routine case history of these patients, since these parameters provide a truer assessment of exposure to avian antigens[53]. In the current study we found that the latter marker of exposure increased progressively in the successive presentation forms of the disease (see Table 2).

Another noteworthy epidemiologic aspect of the current study was the large proportion of never-smokers in our series (78%). The literature has described a ''protective'' role of smoking in BFL[126]; in fact, among smokers there was a lower incidence of precipitating antibodies to pigeon antigens and lower titers of serum IgG and IgA antibodies to pigeon intestinal mucin and serum proteins by ELISA, compared with nonsmokers and exsmokers[7]. In addition, significantly greater elevations of circulating IgG antibodies against pigeon gammaglobulin have been found in nonsmoking pigeon keepers or those with low exhaled CO concentrations (<10 ppb) with respect to those who smoke or have higher exhaled CO levels (>10 ppb)[4]. Thus, it has been suggested that smoking depresses both the T-dependent and T-independent immune response against inhaled antigens[7]. Moreover, experimental studies in rats have shown that nicotine inhibits alveolar macrophages, leading to reductions in the release of tumor necrosis factor and decreases in mRNA expression of tumor necrosis factor, IL-10 and IFN-gamma following stimulation[10].

Our findings, in a series with 14% smokers, seem to concur with these results. However, it should be kept in mind that although our pigeon keeper control group included 41% smokers, other studies have had a lower percentage of smokers (35%) among pigeon keepers[105]. Moreover, to properly assess our series, we should take into account that adult women were predominant (75%). According to the Encuesta de Salud de Catalunya (Catalonian Health Survey) of 2002[35], the prevalence of smoking among the population over 15 years old is 32%, and the percentage is higher among men (38%) than women (26%). In the present series of BFL patients, 33% of the men and only 8% of the women were smokers. Thus, we can conclude that there were lower percentages of smokers among BFL patients than in the general population. This observation is even more compelling taking into consideration that the prevalence of smoking in our series is clearly lower than the observed rates among the Catalonian population aged 16–64 years at different time periods: in 1982 (M 58%, W 20%), 1986 (M 58%, W 23%), 1990 (M 50%, W 26%), 1994 (M 46%, W 26%) and 1998 (M 44%, W 31%)[86].

Seven patients in our series were under 15 years of age; BFL in children is infrequent, but not exceptional, being described in case reports[20] and even in some limited series[122]. In light of the potentially chronic and progressive nature of this disease, it is particularly necessary to maintain an elevated level of suspicion regarding BFL in children with respiratory symptoms or repeated episodes of fever, and contact with birds. Among adults, we believe it is advisable for bird-exposed individuals with any respiratory symptoms to undergo a yearly respiratory check-up, including a clinical examination and a pulmonary function study with DLCO testing, to facilitate an early diagnosis of the condition in susceptible people.

Etiology

The wide variety of bird species the patients in our series were in contact with exemplifies the known possibility that HP can develop following exposure to many species. Exposure to hens or exclusive contact with canaries has not been widely recognized as a cause of the disease, yet 1 and 2 cases of HP in our series, respectively, were exclusively due to these circumstances.

As an isolated factor, it does not seem that the number of birds involved in the exposure is decisive in triggering the condition. In fact, the control pigeon breeders in our study

possessed a much higher mean number of birds (91 birds) versus the patients (16 birds), although there may have been a component of self-selection in the controls over time; that is, persons noting symptoms may have given up the hobby of raising these birds. Previous studies have described a progressive course of the disease in patients exposed to a few or only 1 bird, and a more acute and episodic course in patients exposed to a large number of birds[44]. Nevertheless, later studies have shown that among patients who have reached the chronic phase, there are as many cases of recurrent acute episodes in patients intensively exposed to numerous birds as cases with a more insidious pattern in patients with low exposure to birds in their home[83]. It is likely, therefore, that the development and evolution of this disease depends on the interaction of several factors. In any case, the quantification of exposure according to the ''bird per years'' concept, which combines 2 of these conditioning factors (intensity and duration of exposure) seems highly useful for evaluating these patients. In the current study, there was a higher degree of exposure in the subacute and chronic presentation forms than in the acute form. But, we should not forget that there is a well-recognized individual predisposing factor to the development of HP. Along this line, some authors have suggested that major histocompatibility complex genetic polymorphisms or tumor necrosis factor-α polymorphisms could be related to this predisposition[15,108].

Several antigenic substances have been described as causing the development of BFL[61]. These include serum proteins (mainly gammaglobulins and albumin), pigeon IgA[41] and pigeon IgG (both of which are present in pigeon dropping extract and bloom)[57], and pigeon intestinal mucin, a glycoprotein and major carbohydrate antigen that is also present in these 2 antigen sources[5]. Thus, in the various studies published in this field, the antigen source used for the immunologic study of these patients has been serum[3,6,7,57,63,104], bloom[8,57,104], or pigeon dropping extracts[3,6]. Because extracts from droppings are likely to contain bacterial endotoxin, teichoic acid, fungal beta-1,4-glucan, and many other substances with nonspecific biological activity as well as microbes, which limit their application in serologic, skin, and inhalation challenge testing[61], we have excluded the use of these substances in our daily clinical practice. Instead, we use serum extract from several birds, since it is readily available and contains the major antigens[61], as well as bloom extract, which possesses some of the avian antigens found in serum and seems to contain other specific antigens that are not present in this source[57,104].

With regard to etiology, we make special mention of the 3 patients in our series who used a feather-filled quilt or pillow. Although all 3 of these patients referred to a very limited prior history of contact with birds, we assume that these feather-filled items were the antigenic source causing the symptoms. In fact, suspicion of bird-related products being responsible for BFL symptoms was first raised after the case of a man who had only occasionally been exposed to birds in the past, who described episodic and repetitive acute symptoms of dyspnea, cough, and malaise only after sleeping in a house where he used to go on weekends. After an exhaustive search for possible antigenic sources, the patient explained that he used to sleep with a feather pillow in that house; symptoms completely disappeared after removing that pillow from the room. It is likely that this form of exposure is underestimated in the series because this question is not usually asked. Nonetheless, in our experience and in other studies, it has been observed that this type of exposure can induce BFL or aggravate existing disease[50]. In reference to this point, when the causal antigen is unknown, it is better to seek an unusual source of exposure to a common antigen rather than to investigate a rare cause; that is, don't look for zebras when horses can do.

Diagnosis, Signs and Symptoms

Diagnosis of BFL is based on the presence of consistent clinical symptoms, confirmation that the patient has been exposed and is sensitized to a causal agent, consistent chest X-ray or CT imaging findings, evidence of increased lymphocytes on BAL study, demonstration of consistent histologic features, and/or a positive bronchial challenge test. According to some experts, these are the 6 major diagnostic criteria[110]. To confirm the diagnosis, at least 4 of the 6 criteria must be met, as well as at least 2 of the following 3 minor criteria: auscultation of bilateral crackles, decreased DLCO, and hypoxemia at rest or on exertion[110]. Confirmation of an improvement when exposure to the antigen is discontinued is also a criterion to take into consideration[89]. The diagnostic algorithm used in our center (see Figure 1) follows the criteria established by experts[76].

As has been indicated by these experts[89], although fulfillment of the established criteria constitutes a useful guide for diagnosis, their strict application is not justified in practice, since the diagnosis in patients with suspected BFL can be made on the basis of typical clinical characteristics, presence of exposure to a known antigen, and clinical improvement when contact with the antigen is avoided. To assess more precisely the diagnostic yield of these ''clinical characteristics,'' a multicenter study[56] was undertaken to investigate the clinical diagnosis of HP. The authors concluded that the diagnosis can be sustained in 98% of the cases when the following criteria are met: 1) exposure to aknown antigen, 2) demonstration of specific IgG in serum; 3) recurrent symptomatic episodes; 4) auscultation of crackles; 5) symptoms 4–8 hours following exposure; and 6) weight loss.

Before the complementary tests used in our series to diagnose BFL, which will be discussed later, immunologic tests had been performed to disclose activation of cellular immunity. These tests have proved effective in the diagnosis of BFL in studies by other authors[48], and in reports from our group in patients with BFL[69] and farmer's lung[67]. In the case of BFL[69], we found T lymphocyte activation in 83% of patients and only 14% of asymptomatic pigeon breeders. However, because of the technical difficulty of the tests and the time required to perform them, these tests have now fallen into disuse for routine diagnosis of these diseases. In fact, we prefer to use IHST in our diagnostic algorithm, as

this immunologic test showed even higher sensitivity and specificity than T lymphocyte activation in our previous studies, and it is much less time consuming[66].

Diagnostic delay is a crucial aspect in the evolution of this disease. The lengthy interval observed may be attributable to the still low index of suspicion for BFL and the added difficulty of a delay in the onset of symptoms following exposure to the causal agent. The most striking epidemiologic differences between patients diagnosed in the chronic phase (17%) compared with those diagnosed in the acute and subacute phases were a significantly greater diagnostic delay and mean exposure time before diagnosis (see Table 2). Hence we can conclude, as indicated in a prior study by our group[32], that the duration of exposure and the delay before diagnosis are decisive prognostic factors in the evolution of BFL.

Along this line, in a retrospective review of 72 patients with various forms of chronic HP diagnosed by surgical biopsy (29% with BFL), the evolution time of the symptoms was significantly higher ($p = 0.05$) among patients who had developed lung fibrosis (2.8 yr) relative to those without fibrosis (1.8 yr). Moreover, mean survival was 7.1 years in the fibrosis groups and more than 21 years in those without fibrosis[124]. A retrospective review of 18 patients with BFL concluded that the poorest prognosis is in patients initially diagnosed in the chronic stage of the disease, who rarely showed a clinical improvement despite discontinuation of antigenic exposure[131]. It is clear that all our efforts in the diagnostic process should be directed toward early recognition of this disease.

As to the physical examination, all the asymptomatic pigeon breeders and 15% of BFL patients presented a strictly normal examination at the time of diagnosis; hence, a normal examination does not exclude BFL. The typical feature of inspiratory crackles on pulmonary auscultation (80% of patients) in a large series such as this provides support for the idea suggested in the literature[56] that this finding is a predictive factor for HP in exposed patients; in fact, crackles were not detected in any of the control pigeon breeders. Digital clubbing at the time of the diagnosis was found in a small percentage of our patients (7%) and most of them (83%) were in the chronic stage of the disease. These findings differ from the results of another study in which digital clubbing was seen in 51% of the patients and the incidence was similar in the 3 stages of disease defined by the authors[107]. Nevertheless, the 2 studies agree on the fact that digital clubbing seems to be associated with a poor prognosis (in the aforementioned study[107], clinical deterioration was more frequent among patients with digital clubbing).

The prevalence of the various clinical manifestations of BFL in our patients, with a predominance of dyspnea, cough, and fever (see Table 3), did not differ substantially from those described in other series of BFL[30,73] or the overall values in HP[118]. Almost 25% of our patients presented grade III or IV dyspnea at the time of the initial diagnosis, a fact that provides additional evidence of the delay in diagnosing this condition. As is well recognized, fever was seen more often in patients diagnosed in the acute phase of the disease (82%), who present fever following episodic exposure to avian antigens, compared with subacute (32%) and chronic (27%) cases. Therefore, in many cases this manifestation can be very helpful for establishing an initial diagnosis suspicion. The observed presence of cough and expectoration in asymptomatic pigeon breeders (although infrequent), in the absence of alterations on spirometry and imaging studies, may be justified as a consequence of the irritating effect on the airways of massive exposure to avian feces, bloom, or epithelial products, which would manifest as simple chronic bronchitis[11]. Some authors have even suggested that symptoms of chronic bronchitis should be considered a minor part of the clinical spectrum of BFL[105]. This idea is sustained by the fact that such symptoms occurred in 15% of pigeon breeders who did not present other risk factors for their development, but did show a significantly higher level of specific IgG antibodies to pigeon serum compared to breeders without these symptoms[105].

There are few reports describing symptoms of rhinitis or conjunctivitis following antigenic exposure in patients with HP. In the current series, 35% and 25% of BFL patients who had been systematically questioned about these manifestations presented symptoms of rhinitis and conjunctivitis, respectively, as compared to 22% and 7%, respectively, of the control group. These rates do not differ substantially from those described by other authors in studies performed in pigeon breeders. Rodriguez de Castro et al[105] reported a 31% incidence of rhinitis in the 343 pigeon breeders studied in Gran Canarias (Spain), and Charitopoulus et al[17] reported 31% of rhinitis and 26% of conjunctivitis among 54 breeders investigated in Salónica (Greece). Other authors[121] have described specific IgE antibodies in 18% of parakeet fanciers and 25% of canary fanciers, all of whom had symptoms of rhinitis and/or bronchial asthma. These findings suggest an allergic origin of these symptoms in a percentage of the patients, which would be in agreement with the IgE elevation detected in 28% of our series. Reasons for the paucity of upper tract symptoms in HP are unknown. We might speculate with 2 hypotheses: the capacity of nasal mucus and cilia to prevent the deposition of larger antigenic particles, and the tendency for smaller antigenic particles to reach bronchi and alveoli where they remain for a longer time and, consequently, may induce a stronger immunologic response. Lastly, a late nasal response (and sometimes a dual response) has been described following nasal challenge with pigeon dropping extract in 53% of patients with allergic rhinitis regularly exposed to pigeons[90]. In this last study, however, precipitating antibodies to pigeon droppings were found in 80% of the patients, general malaise in 52%, elevated peripheral blood eosinophils in 28%, and increased body temperature in 36% of the cases following nasal challenge testing. These findings led the authors to suggest that there is also a nasal form of BFL. In fact, some authors have performed nasal biopsy to investigate the presence of granulomas as a method to diagnose some forms of HP[94] and BFL[123].

Laboratory Data

The analytical results obtained in this series were nonspecific, as also occurs in other forms of HP. The incidence of related findings (leukocytosis, hypergammaglobulinemia, eosinophilia) is similar to the values obtained in a previous study by our group performed in 25 patients[70]. ESR stands out as a nonspecific marker of the disease, with elevated values at the time of diagnosis in 44% of patients and no cases of elevated values among the asymptomatic pigeon breeders, a datum that has received little attention in the literature.

Although it is known to occur[26], the finding of peripheral lymphopenia in 23% of patients with BFL at the time of diagnosis is worthy of mention. We also observed this fact in sarcoidosis[75], another disease that shows histologic features of lung lymphocytic and granulomatous inflammation. In both entities, this finding can be attributed to a compartmentalization of the immune response during acute inflammation[49]. It is noteworthy that in the current study, we found a similar incidence of peripheral lymphopenia (29%) among the control pigeon breeders, who, as was mentioned, were exposed to a large number of pigeons. Previous studies have shown that lymphocytic alveolitis on BAL can occur in both asymptomatic exposed farmers[23] and asymptomatic pigeon breeders[101]. Thus, the explanation for the peripheral lymphopenia found in our controls could be the same as that described for BFL patients, namely, it is secondary to an accumulation of lymphocytes in the lung due to an intense antigenic exposure.

Given that BFL is a granulomatous disease like sarcoidosis, with a form of presentation sometimes similar to sarcoidosis, and that during the active phases of sarcoidosis levels of ACE are often elevated[75], it seems logical that this enzyme, which is synthesized by giant cells and epithelioid cells of the granulomas, might also be elevated in BFL. Nonetheless, ACE was not found to be elevated in any of the 28 BFL patients undergoing this test. These findings agree with a previous report[60], which concluded that the granulomatous reaction in HP can differ from that of sarcoidosis at the enzymatic level. In fact, granulomas are fewer in number and are anatomically less well formed in BFL than in sarcoidosis.

Plasma LDH values were elevated in approximately half of patients with BFL undergoing this test, whereas there were no elevations in the control breeders (see Table 4). Elevated LDH was found in up to 80% of cases in a series of 38 patients with farmer's lung[97]. A comparative study showed higher LDH levels in 10 patients with HP than in 36 patients with sarcoidosis or 47 with cryptogenic fibrosing alveolitis, although the authors had hypothesized that LDH might be a useful marker of activity in these diseases[59]. In our experience, LDH is elevated in a large percentage of diffuse interstitial lung diseases, which makes it a very nonspecific marker.

With respect to calcium metabolism, we observed a higher frequency of hypercalciuria (26%) than of hypercalcemia (8%). The same happens in sarcoidosis, in which hypercalciuria is found up to 6 times more often than hypercalcemia in the active phase, and is occasionally seen in the inactive phase of the disease[75]. None of the pigeon breeders in the control group presented hypercalcemia or hypercalciuria. Thus, elevated urinary calcium, LDH, and ESR in a bird fancier may constitute a combined marker for suspected BFL.

Determination of Specific IgG Antibodies

It is well recognized that specific IgG antibodies against avian antigens are a marker of exposure, but not of disease[89]. Using the ELISA technique described above with pigeon bloom and serum, we previously found 100% positive results for both extracts in patients with BFL and about 50% among asymptomatic pigeon breeders[104]. However, in the current series, which contains a much larger number of patients, positive results were documented in 92% of BFL patients, but also in 72% and 87% of the control breeders, using serum and bloom antigens, respectively. We believe that this higher frequency of positive findings among the controls with respect to the former study may be due to the fact that in the present series the number of birds the controls were exposed to was very high, at a mean of 91 pigeons. This hypothesis concurs with the results of another study[83] in which the presence of specific IgG antibodies in chronic BFL patients was much higher in those with elevated antigenic exposure and recurrent episodes of symptoms (87%) than in those with less intense exposure and more insidious symptoms (35%). Along this line, a significant association has been found between specific IgG antibody levels and the hours per week of exposure and number of birds with which the patient was in contact[105].

It is noteworthy that a high percentage of our patients presented specific IgG antibodies against the serum of bird species other than those they were in contact with. This suggests the existence of a cross reaction between serum antigens of different species, probably because they share common antigens. This has been suggested by other authors who reported that antigens from pigeon serum seem sufficient to recognize immune sensitivity to most of the common pet avian species[63].

Chest X-Rays and Computed Tomography

The chest X-ray can show a variety of findings in patients with HP, from completely normal features to an alveolar, interstitial, or alveolar-interstitial pattern. Chest X-rays performed in the current study disclosed a clear predominance of interstitial pattern (79%), mainly of a reticular type (58% of them), which was independent of the phase of the disease (see Table 5). It is noteworthy that in a series in which 60% of the patients present a subacute or chronic form of the disease, only 4% of the chest X-rays performed disclosed lesions in an apical location, when this is the site of involvement typically described in the more advanced forms of the disease[111]. In contrast, we found a predominance of basal or diffuse involvement, not only in the X-rays, but also the CTs, as has been reported in previous studies using chest CT scanning[78]. X-rays were normal in 12% of the cases, but CT scans were normal in only 2% of

patients. This illustrates, as would be expected, the higher sensitivity of chest CT relative to X-rays, a technique that can miss the alterations, even in patients with significant functional deterioration[116].

Moreover, CT provides much more precise information on the status of the lung parenchyma and airways. Although there is often some overlap of the findings occurring in each stage of HP, a characteristic feature in the acute phase is confluent micronodular opacities, and in the subacute phase, areas of ground glass, centrilobular nodules, air trapping, and a mosaic pattern. The chronic phase shows irregular subpleural linear opacities, distorted pulmonary architecture, and sometimes, honeycombing[45]. Specifically, the combination of a mosaic pattern with ground glass areas and centrilobular nodules is highly suggestive of the diagnosis[40]. Areas of ground glass and a reticular pattern have been correlated with restrictive abnormality on PFT[43], whereas air trapping in a mosaic pattern has correlated with an increase in the residual volume[43]. Our CT scanning results in 41 patients with BFL are highly representative of these features. Effectively, the 2 most common findings were areas of ground glass (68%), as has been observed in other series[65], and air trapping in a mosaic pattern (61%). It is interesting that these ground glass areas, classically considered to be representative of disease activity[9], were found in similar percentages in the 3 clinical forms of BFL. In contrast, air trapping in a mosaic pattern was much more common among patients diagnosed in the acute (58%) and subacute (75%) phases of the disease than among those in the chronic phase (33%). This is not surprising since an inverse correlation has been described between the fibrosis score on high-resolution chest CT and the extent of air trapping in patients with chronic HP or idiopathic pulmonary fibrosis[132]. We also observed that septal thickening and fibrosis (to a greater or lesser degree) were detected only in patients in the subacute (40%) or chronic phases (44%), and honeycombing was present only in chronic patients (33%). Previous studies in patients with pulmonary fibrosis have demonstrated the value of CT as a tool for differentiating between chronic HP and idiopathic pulmonary fibrosis[58]. In a series of 12 patients with chronic HP and 12 others with idiopathic pulmonary fibrosis, CT had a sensitivity for diagnosing these conditions of 50% and 92%, and a specificity of 75% and 83%, respectively[132]. Currently, however, it is known that HP can progress to a form of nonspecific interstitial pneumonia or to a form of idiopathic pulmonary fibrosis[82] (as was seen in the chronic phase of the disease in 2 of our patients in whom a SLB was performed); hence, a diagnosis based merely on clinical and radiologic evidence may be even less precise[1].

It is noteworthy that CT disclosed areas of emphysema in 7 (17%) of the patients studied, 5 never-smokers and 2 smokers of less than 20 cigarettes per day. Although only 1 of these patients showed a predominantly obstructive alteration on functional studies (the others were predominantly restrictive), this CT feature has been described mainly in smoker patients with other types of HP[22,37,58]; in patients with farmer's lung[22,37], in whom it is even more common than fibrosis in the long term[22]; and very occasionally in BFL[99]. Thus, the possibility of a diagnosis of HP should also be kept in mind when signs of emphysema are seen on the chest CT scan.

In conclusion, we think that CT scan might be placed in a prior step than it is in the HP diagnosis algorithm shown here, together with other initially noninvasive first choice complementary tests, such as chest X-ray and PFT. We support this hypothesis because of its easy availability, noninvasive nature, and usefulness in showing characteristic findings of all 3 BFL disease stages (acute, subacute, and chronic), which are frequently undetected in chest X-ray.

Immediate Hypersensitivity Skin Testing

The utility of IHST for diagnosing the various types of HP has been investigated by several authors. Special attention was first paid to the late reaction, which was thought to be more specific for the diagnosis[92]. Later, several studies reported positive immediate cutaneous reactions in specific skin tests performed in HP patients[38]. Among them, 2 from our group showed that specific skin tests are effective in discriminating between HP patients and asymptomatic bird fanciers[66] and farmers[68], particularly in the case of an immediate reaction. The sensitivity and specificity of the test were 90% and 85%, respectively, using bird serum extract in BFL, and 83% and 72%, respectively, using hay extract in farmer's lung. These immediate hypersensitivity reactions have been attributed to the role of an IgG subclass rather than IgE[66].

The high sensitivity of this diagnostic technique was again illustrated in the current study, in which a positive result was elicited in 85% of patients. Nevertheless, it was surprising to see the considerable percentage of asymptomatic pigeon breeders who also had a positive response to this test: 64% with pigeon serum extract and 88% with pigeon bloom extract. Conversely, in the study cited above[66], a positive response was elicited in only 5% (3/20) of asymptomatic bird fanciers (including very few pigeon breeders) and 0% (0/10) of unexposed individuals. Considering the following factors: 1) the immediate skin reaction[66] is attributed to an IgG subclass; 2) there seems to be a correlation between the titer of specific serum IgG antibodies and the diameter of the wheal in the specific skin test among patients with BFL[62]; and 3) the elevated percentage of asymptomatic control pigeon breeders with these antibodies in our series (87% for serum extract), it would be reasonable to attribute the positive results in the controls to their extremely high degree of exposure.

With regard to positivity against 1 or the other antigen and the fact that positive IHST status was higher in both BFL patients and controls with the use of bloom extract (100% and 88%, respectively) compared to serum (71% and 64%, respectively), a study by our group showed that values for specific IgG antibodies against bloom extract tripled those observed against serum extract in a group of pigeon breeders; moreover a higher number of breeders presented specific antibodies against bloom than against serum[104]. These results, which are similar to those of other authors[8], suggest that bloom extract is a more potent antigenic source than

serum extract[104]. Longbottom et al[57] identified 29 antigenic components in bloom and only 10 in serum and reported a certain antigenic similitude between the 2 sources, but suggested that some of these antigens are specific. Further investigation is needed to confirm the yield of this test in keepers of a small number of birds.

In our opinion, determining specific IgG antibodies and IHST are still essential in any HP diagnostic study, not only because a sensitizing agent must be identified, but also because of their previously described high diagnostic sensitivity and specificity[66,68,104]. The large number of positive results with these immunologic tests among our pigeon breeders control group suggests that maybe these tests are not so useful when studying highly exposed individuals. Nevertheless, taking into account that BFL is being increasingly diagnosed among individuals with pet birds at home, their use is still recommended.

Delayed Cutaneous Hypersensitivity Testing

In a previous study, we found that the mean diameter of the wheal obtained with DCHT was significantly smaller in a group of 13 patients with BFL and another group of 34 sarcoidosis patients compared with the results in a control group of 50 asymptomatic individuals[84]. We concluded that in BFL cellular immunity may be depressed, as occurs in sarcoidosis[75]; moreover, the immunosuppression persisted at 1 year. Findings in the current study complement and support the conclusions derived from that earlier study[84].

Pulmonary Function

PFTs in HP usually show restrictive ventilatory impairment with a reduction in lung volumes and frequent alterations in the CO diffusing capacity[102]. At times, however, an obstructive component may manifest, with a decreased FEV_1/FVC ratio, attributed to bronchiolitis (obstruction of the small airways)[74,89] or emphysema, particularly in the chronic stage of the disease[113]. The results of the current study demonstrate that a restrictive pattern (77%) is also the most common among BFL patients, as has been reported[65], although 9% and 4% of them showed a pure obstructive and a mixed ventilatory impairment, respectively.

Development of obstructive ventilatory impairment is infrequent in patients with BFL, but airway involvement, alone or in association with interstitial involvement, has been described previously[112]. In many cases, however, the obstruction has predominantly affected the small airways, with a decrease in the mid-expiratory flow[12]. The cases described in the present study are similar to the 6 cases of persistent obstruction reported in a prior follow-up study of 16 patients with BFL[131], most of whom had never been smokers, as was the case with our patients. The obstruction affected the central airways in all cases, with decreases in FEV_1/FVC ratio and FEV_1 values. After several years of follow-up, the functional evolution of these patients showed chronic obstruction. Another study reported an obstructive abnormality with FEV_1/FVC ratio <70% in patients with chronic HP (29% of them with BFL), particularly in the subgroup of cases with no pulmonary fibrosis[124]. As to air trapping, we believe that the higher rate of this finding in static lung volume determinations (26%) with respect to the obstruction documented by spirometry (9%) is an indication that air trapping in these patients is often secondary to small airway involvement alone. This is corroborated in the vast majority of cases by chest CT features of a mosaic air trapping pattern without signs of emphysema (see Table 2).

The lung function results we present indicate that the ventilatory impairment in patients with BFL, which is mainly restrictive, can be quite severe; the mean FVC value in the series was 66% of the predicted value. In addition, it has been demonstrated that persistent exposure to avian antigens leads to more accelerated functional deterioration in patients with BFL compared with healthy controls[109]. Moreover, the greater the development of fibrosis, the greater is the patient's restrictive alteration[124]. Most of our patients also presented a decrease in the DLCO, which was severe in many cases (<50% of the predicted value in 51% of patients). In fact, the DLCO demonstrated a higher sensitivity, being more frequently decreased (85% of cases) than the FVC (76% of cases), as has been reported[129]. It is clear that functional deterioration can be very severe in these patients and can lead to an irreversible clinical situation in which the only therapeutic option is lung transplantation, as occurred in 3 of our patients.

Blood gas assessment, which was performed only in patients with a higher degree of dyspnea (34 cases), revealed that 41% presented respiratory failure (PaO2 <60 mm Hg)[127] at the time of diagnosis. As expected, the highest percentage of cases with respiratory failure corresponded to patients in the chronic stage of the disease (71%), many of whom had advanced pulmonary fibrosis. Nevertheless, we should also mention the quite high percentage of acute (38%) and subacute (29%) patients showing respiratory failure on blood gas analysis, which may have occurred following a specific massive exposure or after continuous exposure to a less intense antigenic load. These findings suggest that BFL should be considered severe not only in cases where there is progressive clinical and functional deterioration, but also in cases of episodic symptoms, which can be life threatening, as we have described in patients with espartosis[30].

Bronchoalveolar Lavage

The characteristic profile of BAL material in patients with HP is that of lymphocytic alveolitis with a predominance of CD_8 T lymphocytes, in contrast to sarcoidosis, in which there is usually a predominance of CD_4 T lymphocytes[43]. However, this cellular profile depends on the time since the last antigenic exposure. CD_8 suppressor T cells predominate in BAL of patients recently exposed to avian antigens and gradually decrease in number with cessation of exposure[25]; the total of T lymphocytes remains elevated, however, because of a parallel increase in CD_4 T cells[36]. In addition, neutrophilia is characteristic during the acute postexposure phase[79], particularly during the first 48 hours[81]. Increased neutrophils are also seen in patients with progression to fibrosis[87]. In our series (n = 36), lymphocytosis was the most characteristic finding regardless of the phase of

the disease (see Table 2). Even though some chronic patients showed neutrophil values of up to 14% or 15% of the cell count, the percentages of lymphocytes were always higher than normal. Other studies have also reported frequent findings of lymphocytosis in BAL specimens of chronic patients, which in the study by Ohtani et al[82] reached more than 20% in 68% of the cases and more than 15% in 80% of BFL patients showing a histologic pattern of usual interstitial pneumonia, nonspecific interstitial pneumonia, or cryptogenic organizing pneumonia.

As to the T lymphocyte subpopulations, the finding of an inverted CD_4/CD_8 lymphocyte ratio in 8/13 (62%) of our patients is also in keeping with the known predominance of CD_8 T cells in BAL specimens of BFL patients. The presence of a CD_4/CD_8 lymphocyte ratio >1.2 does not exclude the diagnosis, however. In fact, 5 (38%) of our patients had this finding. If CD_8 T lymphocytes gradually decrease in number with cessation of exposure[25], it would be reasonable to expect that CD_4/CD_8 ratios >1.2 would be less frequent in patients with an acute presentation of the disease after high antigenic exposure. However, this was not so in our series, where the incidence of this finding did not significantly differ between the 3 forms of the disease (see Table 2). Nonetheless, the CD_4/CD_8 lymphocyte ratio was determined in only 13 patients, which limits the possibility of deriving conclusions from these data.

In any case, BAL is of considerable diagnostic value, particularly in typical cases. It is known that lymphocyte values above 60% together with detection of more than 1% of mast cells is diagnostic of HP[114].

Transbronchial Biopsy

With regard to TBB results, the previously described histopathologic features of HP are distinctive but not pathognomonic[93]. The fact that only 9% of our patients showed the histopathologic triad of characteristic findings in HP illustrates that this procedure rarely leads to a definite diagnosis in patients with suspected BFL, as is also the case in patients with farmer's lung[55]. However, the presence of at least 1 of these findings in 69% of cases indicates that the results of TBB can provide the basis to consider a diagnosis of BFL feasible and prompt additional tests before BFL is ruled out. It is important to remember that the diagnostic performance of TBB is highly dependent on the quality of the biopsies obtained. In this sense, the number of specimens obtained is also a determinant factor in the diagnostic performance of this test, with 4 to 6 samples being recommended[34].

Surgical Lung Biopsy

SLB is required to establish the diagnosis in very few cases of HP. It is generally not needed in the acute forms because the causal relationship is easier to establish and the diagnosis can be made on the basis of clinical criteria. In the subacute and chronic stages, however, the clinical symptoms can overlap, there is a weaker relationship in time between the symptoms and exposure, and the development of lung fibrosis may be indistinguishable from the features of usual interstitial pneumonia or nonspecific interstitial pneumonia on high-resolution CT[82]. Thus, SLB may be occasionally needed to reach a precise diagnosis.

It has been established that patients with HP in the chronic phase can develop fibrosis, which on histologic study presents as usual interstitial pneumonia-like lesions[19,52] or nonspecific interstitial pneumonia-like lesions[19,125]. More recently, however, and specifically in patients with chronic BFL who have already developed pulmonary fibrosis, another pattern has been added to those already included in the American Thoracic Society/European Respiratory Society consensus criteria for the classification of idiopathic interstitial pneumonia[120]: cryptogenic organizing pneumonia-like lesions[82]. Moreover, COPD-like and cellular nonspecific interstitial pneumonia-like lesions have been correlated with a better prognosis, and fibrotic nonspecific interstitial pneumonia-like and usual interstitial pneumonia-like lesions with a poorer one[82]. Therefore, some authors have concluded that HP in the chronic stage is likely to be underdiagnosed, with some cases classified as a form of idiopathic interstitial pneumonia, particularly patients having an insidious clinical course and lacking a suitable immunologic study or exhaustive clinical history[130]. It has been shown that a meticulous clinical history, with specific questions on antigenic exposure, and proper use of complementary diagnostic tests can help to decrease the need for SLB[42]. In fact, a previous study retrospectively assessing the presence of organic antigenic agents at work and at home in patients diagnosed with idiopathic interstitial pneumonia showed significant amounts of these agents in the majority of cases, suggesting that they may have had a causal effect in patients whose condition had been classified as idiopathic[51]. In a review of patients with nonspecific interstitial pneumonia[100], we observed a history of significant exposure to birds in 7/16 (44%) cases, although specific IgG antibodies against avian antigens were observed in only 25% of the patients undergoing this determination.

In the current series of 86 patients with BFL, only 14 (16%) underwent SLB and, as was mentioned above, 8 of the 14 (57%) SLBs had been performed before the patient was referred to our service. Thus, in our experience, the need to indicate SLB is exceptional. This may be because a large number of cases (69%) underwent bronchial challenge tests, which yielded a high percentage of positive results (92%) and allowed establishment of the definite diagnosis without the need for surgery. The fact that 12 of the 14 patients undergoing SLB (86%) were in the subacute or chronic phase of the disease indicates that in these subgroups, SLB may occasionally be needed. In this sense, the possibility that the cause of idiopathic pulmonary fibrosis or nonspecific interstitial pneumonia may be HP, whether BFL or another form, will now increase the indications for confirmational SLB, since the treatment is very different in these conditions, particularly the use of corticosteroids, which may be effective if the cause is HP. When performed, the diagnostic yield of SLB in the 14 patients was high (see Table 7).

Bronchial Challenge Testing

One of the most novel aspects of the current study is that in 54 of 86 (63%) patients the diagnosis of BFL was

confirmed by a positive bronchial challenge test, which, in a disease where the diagnostic criteria are sometimes imprecise, lends greater reliability to the diagnoses obtained. This test, known to be highly useful for diagnosing HP[79], has demonstrated a sensitivity and specificity of 100% in some studies for the specific diagnosis of BFL, even in cases in the chronic stage of the disease, when this test can be beneficial for the differential diagnosis with idiopathic lung fibrosis[81,96]. In the current study, which assesses the diagnostic yield of bronchial challenge testing in daily practice, the sensitivity of 92% and specificity of 100% confirm this test as a useful tool for BFL diagnosis. Another interesting aspect of this test is that a positive response was obtained not only to pigeon serum, but also to serum of other birds, such as parakeet, canary, dove, parrot, cockatoo, and in 1 case even to hen serum, an antigen that rarely produces this disease.

This kind of bronchial challenge testing must be performed in a hospital setting, by experienced staff and with the support of a laboratory where high-quality antigenic extracts can be prepared. Severe exacerbations are very rare and the procedure is safe under these conditions[89]. Thus, only respiratory departments of reference hospitals should perform these bronchial challenge tests.

These technical requirements and the need for a previous and accurate immunologic approach to identify patient sensibilization, justify why bronchial challenge testing is located at almost the last step of the diagnostic algorithm, despite its high sensitivity and specificity. It must be noted, however, that the diagnostic algorithm described here is not the result of our experience after 27 years of studying BFL patients, but the one that we have followed during all this time, which was based on previously reported data on HP diagnosis. In view of our results, which confirm those observed in smaller series[81,96], we think that bronchial challenge testing must be considered now as the ''gold standard'' among the noninvasive BFL diagnostic examinations. We emphasize that none of our patients had a severe reaction to the test, which, considering the large number of tests carried out, underscores the safety of bronchial challenge testing when the established protocol is followed.

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