

DIAGNOSIS:

FOLLOW UP:

* Duration and intensity of exposure. ** Only if it is necessary for diagnosis.

Some example of exposure to occult antigens:

Bird droppings on the window sills and patio, attracting birds to come close to their normal day-to day activities (e.g., feeding), using feather duvets, feather/down pillows/blankets, jackets; cleaning the lawn /yard known to have bird feathers/droppings (maintenance of bird feeders in the yard/on lawn of patient, shooting birds as a hobby and then skinning the birds feathers , keeping feathers at home and/or any hobbies /work associated with birds (including veterinarians involved with the health and care of birds), using hot tubs that are often considered 'normal' activities of daily living and /or enjoyment; visible /unseen moulds, nests in attics, etc are among other occult potential, sources for avian and fungal antigens attributable to manifest ILD/HP.

Determination of specific IgG antibodies

The technique for IgG antibody determination is described in our previous study (2). Briefly, specific IgG was measured by ELISA with avian sera, bloom and feathers extract and fungus as the antigen. Wells of high-binding microtiter plates (Costar, Cambridge, MA) were incubated with 2 µg protein/well in 0.1M Na₂CO₃/NaHCO₃ buffer (pH 9.6) at 4°C overnight. 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. Specific IgG assays were performed in duplicate and plates were washed 4 times between steps. Serum samples at an appropriate dilution and standard curve were incubated 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 then added and plates were incubated for 2 hours at 37°C. The reaction was developed with 3,3', 5,5'-tetramethylbenzidine (Sigma Chemicals) and 3% H₂O₂ for 20 minutes at room temperature in the dark and stopped with 2M H₂SO₄. Optical density at 450 nm was measured with a microplate reader (Titertek Multiskan Plus MKII).

Results were expressed as absorbance units at 450 nm (A₄₅₀ 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: pigeon serum >0.284 A₄₅₀ nm, parakeet serum >0.180 A₄₅₀ nm, canary serum >0.336 A₄₅₀ nm, hen serum >0.445 A₄₅₀ nm, parrot serum >0.94 A₄₅₀ nm, and goose feathers

Specific inhalation challenge (7,9)

Bronchial challenge tests were carried out in the hospital on an outpatient basis after obtaining written consent from the patient. These tests were performed only in patients with FVC >50% and DLCO >40% of predicted values. In all cases, bronchial challenge with a placebo solution was carried out 1 day before testing with the suspected antigen. Patients were requested to inhale 2 mL of the suspected antigen at a 1/100 (0.01 mg/mL) dilution using a De Vilbiss 646 nebulizer (De Vilbiss, Somerset, PA) or a dosimeter (Mefar, Ele H₂O, Medicali, Brescia, Italy), which releases the solution during the first second of each inspiration. FVC, FEV₁, DLCO, and the patient's temperature were recorded at baseline, 20 minutes after inhalation, and every hour

thereafter for the next 8 hours. Leukocyte count, chest X-ray, and O₂ saturation measurements were performed before and 8 hours after inhalation. . When the test proved negative, inhalation of a 1/10 (0.1 mg/mL) antigen dilution was performed the next 3 days using the same procedure.

The test was considered positive when any of the following responses was elicited as compared to baseline values (11): 1) FVC decrease >15% and/or DLCO decrease >20%; 2) 10%–15% FVC and/or 15%–20% DLCO decrease plus at least one of the following clinical criteria with respect to baseline: a) white blood cell increase >20%; b) O₂ Hb saturation decrease >3%; c) rise in body temperature >0.5°C; d) evident clinical symptoms (cough, dyspnea); e) significant radiologic changes (infiltrates >10%); and 3) FVC decrease <10%; and/or DLCO <15%, but with evidence of three or more of the previously mentioned clinical criteria.

Table 4. Studies on survival in chronic HP

Study	Clinical forms	Nº of patients	5 year mortality	Median survival	Survival predictors
Perez Padilla et al.(1) Am Rev 1995	Chronic HP Bird fanciers lung	78		11,2 years	Age, gender, Histologic fibrosis Radiographic fibrosis
Vourlekis et al.(2) Am J Med 2004	Subacute and chronic HP	72		12,8 years	Age FEV1/FVC TLC RV. DLCO Histologic fibrosis
Sahin et al.(3) Radiology 2007	Chronic HP with fibrosis	24		3,9 years	Histologic fibrosis
Hanak et al.(4) Chest 2008	HP (does not specify)	69			FVC, Crackles Radiographic fibrosis
Lima et al.(5) Resp Med 2009	Subacute and chronic	103	27%		Age Oxygen saturation during exercise Airtrapping/mosaic patten on HRCT
Fernández Perez et al.(6) Chest 2015	Chronic HP with identified antigen and	142		18,2 years and 9,3 years respectively	Age FVC, TLC Smoking habbit Pulmonary fibrosis Identifying incitin antigen
Mooney et al.(7) Chest 2015.	HP (acute, subacute and chronic)	176	25%		Radiographic fibrosis Crackles FVC FEV1/FVC Cigarette smoking
Ojanguren et al. (8) Allergy, 2019	crohnic	160	31	7 years	Sex Age TLC, DLCO Linfocytes in pulmonary lavage

(1) Perez-Padilla R, Salas J, Chapela R, Sánchez M, Carrillo G, Perez R. Sansores R, Gaxiola M, Selman M. Mortality in Mexican patients with chronic pigeon breeder's lung compared with those with usual interstitial pneumonia. Am Rev Respir Dis 1993; 148:49–5.

- (2) Vourlekis JS, Schwarz MI, Cherniack RM, Curran-Everett D, Cool CD, Tuder RM, King TE Jr, Brown KK. The effect of pulmonary fibrosis on survival in patients with hypersensitivity pneumonitis. *Am J Med.* 2004 15; 116:662-8.
- (3) Sahin H, Brown KK, Curran-Everett D, Hale V, Cool CD, Vourlekis JS, Lynch DA. Chronic hypersensitivity pneumonitis: CT features comparison with pathologic evidence of fibrosis and survival. *Radiology* 2007; 244:591–598.
- (4) Hanak V, Golbin JM, Hartman TE, Ryu JH. High-resolution CT findings of parenchymal fibrosis correlate with prognosis in hypersensitivity pneumonitis. *Chest.* 2008; 134:133-8.
- (5) Lima MS, Coletta EN, Ferreira RG, Jasinowodolinski D, Arakaki JS, Rodrigues SC, Rocha NA, Pereira CA. Subacute and chronic hypersensitivity pneumonitis: histopathological patterns and survival. *Respir Med.* 2009; 103:508-15.
- (6) Fernández Pérez ER, Swigris JJ, Forssén AV, Tourin O, Solomon JJ, Huie TJ, Olson AL, Brown KK. Identifying an inciting antigen is associated with improved survival in patients with chronic hypersensitivity pneumonitis. *Chest.* 2013; 144:1644-51.
- (7) Mooney JJ, Elicker BM, Urbania TH, Agarwal MR, Ryerson CJ, Nguyen ML. Radiographic fibrosis score predicts survival in hypersensitivity pneumonitis. *Chest.* 2013; 144:586-92.
- (8) Ojanguren I, Morell F, Ramón MA, Villar A, Romero C, Cruz MJ, Muñoz X. Long-term outcomes in chronic hypersensitivity pneumonitis. *Allergy.* 2019;74:944-52.