Hypersensitivity Pneumonitis: Challenges in Diagnosis and Management, Avoiding Surgical Lung Biopsy

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Abstract

This review presents an update of the currently available information related to hypersensitivity pneumonitis, with a particular focus on the contribution of several techniques in the diagnosis of this condition. The methods discussed include proper elaboration of a complete medical history, targeted auscultation, detection of specific immunoglobulin G antibodies against the most common antigens causing this disease, skin tests, antigen-specific lymphocyte activation assays, bronchoalveolar lavage, and cryobiopsy. Special emphasis is placed on the relevant contribution of specific inhalation challenge (bronchial challenge test). Surgical lung biopsy is presented as the ultimate recourse, to be used when the diagnosis cannot be reached through the other methods covered.

Keywords

- hypersensitivity pneumonitis
- bronchoalveolar lavage
- specific inhalation challenge
- bronchial challenge test
- precipitins
- cryobiopsy
- skin test

Current guidelines emphasize the significant contribution of surgical lung biopsy (SLB) in establishing a definite diagnosis of interstitial lung disease (ILD).[1–3] This method is also recommended in the diagnosis of hypersensitivity pneumonitis (HP),[4] where it is often considered to have a pivotal role.[5] These recommendations and other factors have led to the notion that SLB is essential to confidently reach the diagnosis of HP. This idea may partially stem from an absence of alternative diagnostic techniques in many centers, such as serum-specific immunoglobulin G (ssIgG) antibody detection in serum, or practice of bronchoalveolar lavage (BAL), cryobiopsy, or specific inhalation challenge (SIC), or from clinicians being unaware of the importance of meticulous historytaking and expert interpretation of findings from high-resolution computed tomography (HRCT) of the lungs. Nonetheless, SLB is not free from associated risk, as was seen in a recent systematic review and meta-analysis of 2,148 patients with suspected ILD undergoing this surgery, which reported a postoperative mortality rate of 3.6% with significant heterogeneity between centers,[6] and in studies showing comparable morbidity and mortality rates between open lung biopsy techniques and video-assisted thoracoscopic surgery.[7,8]

Definition of Hypersensitivity Pneumonitis and Description of the Most Well-Known Types

The term HP refers to group of lung diseases resulting from inhalation of certain organic substances and chemicals. In some individuals, these agents produce an immunological inflammatory response that leads to bronchoalveolar and interstitial disease. To acquire this condition, an individual must be genetically predisposed to have an exaggerated

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immunological response to an antigenic substance and be exposed to the substance. As Hippocrates said, the reason for disease is partly from individuals, themselves (genetic), and partly environmental.

Many types of HP have been described. Some of the most common are caused by inhalation of proteins and less often, polysaccharides, found in the home or workplace. Inhalation of antigens derived from fungi, actinomycetes, or mycobacteria present in the patients' environment is another frequent cause of HP, whereas exposure to chemical substances, such as isocyanates and copper sulfate, is less common. The first type of HP reported in modern times was *farmer's lung*, described by Campbell[9] in 1932 and caused by inhalation of dust or mold from agricultural products (►**Fig. 1**). Another prominent disease in the HP group is bird fancier's lung or pigeon breeder's disease, resulting from exposure to avian antigens and first described by Reed and Barbee in a patient who had been repeatedly hospitalized for signs and symptoms attributed to pneumonia.[10] The most well-known types of HP are summarized in ►**Table 1** and some examples are shown in ►**Fig. 2**.

Diagnosis of Hypersensitivity Pneumonitis

In medical practice, a fundamental element in diagnosing disease is clinical historytaking. Sometimes, the physician's questions will easily uncover the reason for HP symptoms, as when patients mention that they keep birds or work in fields containing moldy straw or grains. But often, particularly in chronic disease, the culprit antigen is not so easily identified, and a thorough knowledge of the types of exposures that can cause HP is needed to approach the patient with targeted questions. It is essential for clinicians to be aware that the hidden cause of exposure is much less likely a rare or little-known substance than an unusual exposure to a well-known cause and direct their questioning accordingly. For example, bird droppings on window sills, use of feather/down duvets, pillows, or jackets, cleaning yards or patios containing bird feathers or droppings, keeping feathers or stuffed birds at home, nests in attics, bird droppings deposited at the entrance to air conditioning systems, and even walking daily under trees where starlings nest[11] are less evident sources of avian antigens with a potential to cause HP.[12,13] The use of hot tubs and humidifiers, and unseen molds can be the sources of fungal antigens. Clinicians and their patients may not be aware of such occult exposure occurring during day-to-day activities at home or work, or when spending regular periods in other environments (e.g., friends' and relatives' home, old buildings), and a routine initial clinical history taken by a general physician or pulmonologist may not address these factors. This may partially justify the lack of an attributable antigen reported in 25 to 60% of HP patients.[14,15] Since strict avoidance of the exposure source is an essential recommendation in HP, it is important to identify the etiology of the disease. In a recent study, mean survival in the HP cohort decreased from 18.2 to 9.3 years when the source was not detected.[15]

During the physical examination, auscultation may yield normal findings or the presence of inspiratory rales. In clinically advanced cases, "velcro" crackles are usually heard on inspiration. One characteristic, but uncommon sound is a high-pitched wheeze at the end of inspiration, known as "chirping" rales.[16] This was first described by Laënec as *le cri dún petit oisseau* and is likely an indication of bronchiolar inflammation or bronchiolar fibrosis.

HRCT (►**Fig. 1**) usually shows a ground glass pattern, typically in a mosaic pattern, due to areas of low attenuation consecutive to bronchiolitis. A micronodular pattern may also be seen. A pattern of fibrosis, sometimes mimicking a usual interstitial pneumonia (UIP) pattern, is not unusual and in

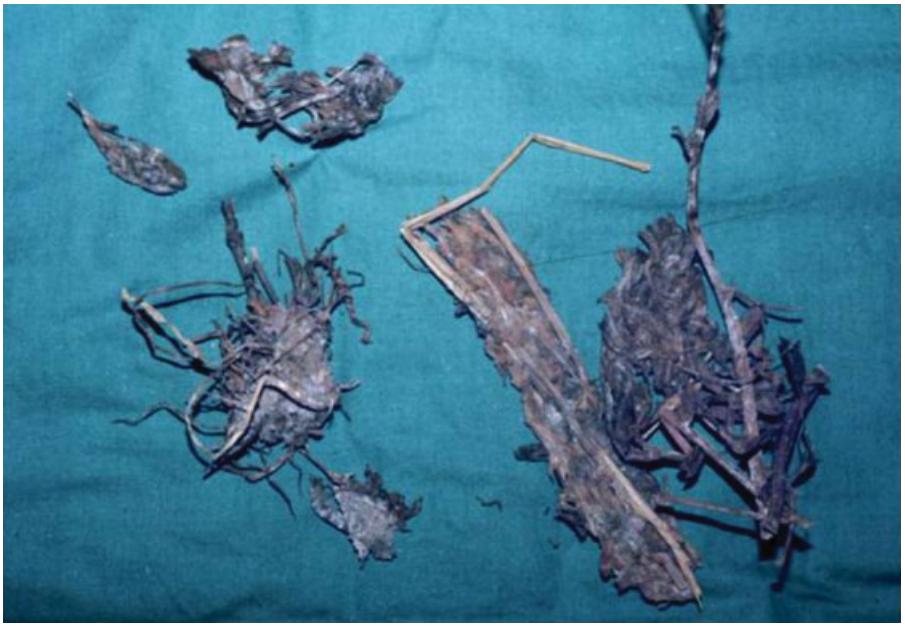

Fig. 1 Hay contaminated with *Sacaropolisporarectivirgula* and *Aspergillus* in a case of farmer's lung.

Table 1 Materials and antigens causing HP

Disease	Antigen source	Antigen
Farmer's lung	Moldy hay	*Saccharopolyspora rectivirgula*, *Thermoactinomyces vulgaris*, *Aspergillus flavus*, and *Aspergillus fumigatus*
Bird keeper's lung	Pigeon, parakeet, parrots, etc.	Avian serum proteins, intestinal mucin (glycoprotein), droppings, and dust (bloom)
Feather duvet lung	Feather duvets and pillows	Avian proteins and molds
Stipatosis	Esparto grass, stucco work	*Aspergillus*, *Penicillium*
Suberosis	Moldy cork	*Penicillium frequentans*, *Aspergillus* sp.
Air conditioner lung	Air conditioners, humidifiers	Thermophilic actinomycetes, thermotolerant bacteria, protozoa
Home ultrasonic humidifier HP	Contaminated humidifier water	*Cephalosporium acremonium* and *Candida albicans*
Dry sausage worker's lung	Mold from dried meat products	*Penicillium* and *Aspergillus*
Mollusk shell lung	Dust from shells, buttons, pearls	Proteins
Soybean worker's lung	Soy dust	Soy proteins
Machine operator's lung	Contaminated lubricants, refrigerating fluid	*Pseudomonas fluorescens*, *Aspergillus niger*, *Rhodococcus* sp., *Staphylococcus*, *Mycobacterium immunogenum*
Spa, hot tub, and shower lung	Mist and hot water spray	*Mycobacterium avium* complex and other mycobacterial species, *Cladosporium*
Hard metal lung disease (giant cell interstitial pneumonitis)	Cobalt + tungsten carbide in metal working	Cobalt + tungsten
Candida lung	Contaminated material, urine, etc.	*Candida* sp.
Steam iron lung	Contaminated mist from irons	*A. fumigatus*
Mushroom worker's lung	Cultivated mushrooms	*T. vulgaris* and *S. rectivirgula*
Compost lung	Moldy compost	*T. vulgaris*, *Aspergillus* sp.
Insecticide lung	Insecticides	Pyrethroids
Bagassosis	Moldy sugarcane (bagasse)	*T. vulgaris* and *T. sacchari*
Maple bark stripper's lung	Damp bark of maple trees	*Cryptostroma corticale*
Sequoiosis	Moldy redwood tree dust	*Graphium* sp. and *Aureobasidium pullulans*
Wood dust disease	Ramin (*Gonystylus balcanus*)	Wood
Malt worker's lung	Moldy barley and malt	*Aspergillus clavatus* and *A. fumigatus*
Miller's lung	Contaminated grain	*Sitophilus granarius* and *Sporobolomyces*
Wood worker's lung	Moldy wood pulp	*Alternaria* sp.
Cheese washer's lung	Moldy cheese	*Penicillium casei* and *Acarus siro*
Fish meal worker's lung	Fish	Fish meal dust
Fertilizer lung	Contaminated fertilizer	*Streptomyces albus*
Tobacco worker's lung	Tobacco	*Aspergillus* sp.
Furrier's lung	Fox and astrakhan fur	Animal fur dust
Coffee worker's lung	Coffee beans	Coffee dust
Pituitary snuff taker's lungs	Pituitary powder	Pituitary proteins
Thatched roof lung (new guinea)	Thatched roof of dried grasses, leaves	*Streptomyces olivaceus*
Detergent lung	Enzyme detergents	*Bacillus subtilis*
Paprika slicer's lung	Paprika dust	*Mucor stolonifer*

(*Continued*)

Table 1 (*Continued*)

Disease	Antigen source	Antigen
Contaminated water mist	Spray emitted from water-cooled machinery	Six different fungi
Sauna taker's lung	Contaminated sauna water	*Aureobasidium* sp.
Coptic lung (mummy handler's lung)	Cloth wrapping of mummies	
Rodent handler's lung	Rats	Proteins from urine
Bat lung	Bat droppings	Serum proteins
Summer-type alveolitis (Japan)	Damp interiors	*Trichosporon cutaneum*, *Cryptococcus albidus*, and *Cryptococcus neoformans*
Sericulturists' lung	Silk larvae	Proteins from larvae
Wine maker's lung	Mold on grapes	*Botrytis cinerea*
Saxophone player's lung	Mouthpiece and case	*Ulocladium botrytis* and *Phoma* sp.
Trombone player's lung	Biofilm within the instrument	*Mycobacterium chelonae*, *Abscesus*, *Fusarium* sp.
Baker's lung	Flour	*A. fumigatus*
Chemical agents		
Berylliosis	Neon lights, TV sets, etc.	Beryllium
Isocyanate lung	Polyurethane foams, glues, paints	Isocyanates
Epoxy worker's lung, plastic worker's lung	Plastics, resins, and epoxy	Phthalic anhydride
Vineyard sprayer's lung	Copper sulfate (Bordeaux mixture)	Copper sulfate
Dental technicians' lung	Dental prostheses	Methacrylate
Trichloroethylene HP	Degreasing	Trichloroethylene
In addition, many other sporadic cases of HP have been reported		

Abbreviation: HP, hypersensitivity pneumonitis.

some patients, merely, nonspecific fibrotic findings are found[12] (►**Fig. 3**).

Specific skin testing using antigen extracts of potential causative agents is a controversial diagnostic method in HP. It may not be mentioned at all in reviews of the disease,[17,18] be considered irritating and nondiscriminatory, and therefore, neither appropriate nor practical,[19] or simply be deemed unhelpful in the diagnosis.[20] Notwithstanding, properly performed skin tests using well-prepared antigen extracts have shown diagnostic value in some studies. Reading of immediate skin reactions (15 minutes) has proven useful in farmer's lung (diagnostic sensitivity 83%, specificity 72%, using hay extract)[21] and in bird breeders' disease (sensitivity 90%, specificity 85%, using bird serum extract).[22] Of note, however, if the asymptomatic control population comprises pigeon breeders with an extremely high degree of exposure, a considerable percentage may test positive (64%), whereas asymptomatic bird keepers of one or a few birds may elicit positive testing in only 5%.[22] One major difficulty in skin testing is that the antigenic substance used must be directly extracted from the original material in a laboratory with adequate equipment for this purpose, and then be diluted to the proper concentration a short time before the test is performed to preserve the antigenic potency of the material.[23] This implies the need for an organizational structure that may not be within the reach of many centers.

Determination of Specific Immunoglobulin G Antibodies

ssIgG antibodies against a battery of antigens that commonly cause HP should be routinely determined in the diagnostic workup of this condition, particularly when the clinical interview does not raise suspicion of an antigenic source. An immune response to inhaled antigens can be detected by the production of ssIgG in serum.[24,25] Nonetheless, the diagnostic value of this finding in HP patients is controversial. As is the case of specific inhalation challenge (SIC), the lack of standardized techniques and established physiologic ranges for ssIgG against possible causative antigens has generated concerns about diagnostic use of this technique. In any case, a major limitation of ssIgG measurement is that validated antigen preparations for most substances causing HP are not available. Moreover, specific antibodies cannot always be identified in HP patients, likely an indication that some antigens causing this condition are still unknown.[26] Various

Fig. 2 Sausages contaminated with *Penicillium* and *Aspergillus* in a case of dry sausage worker's lung.

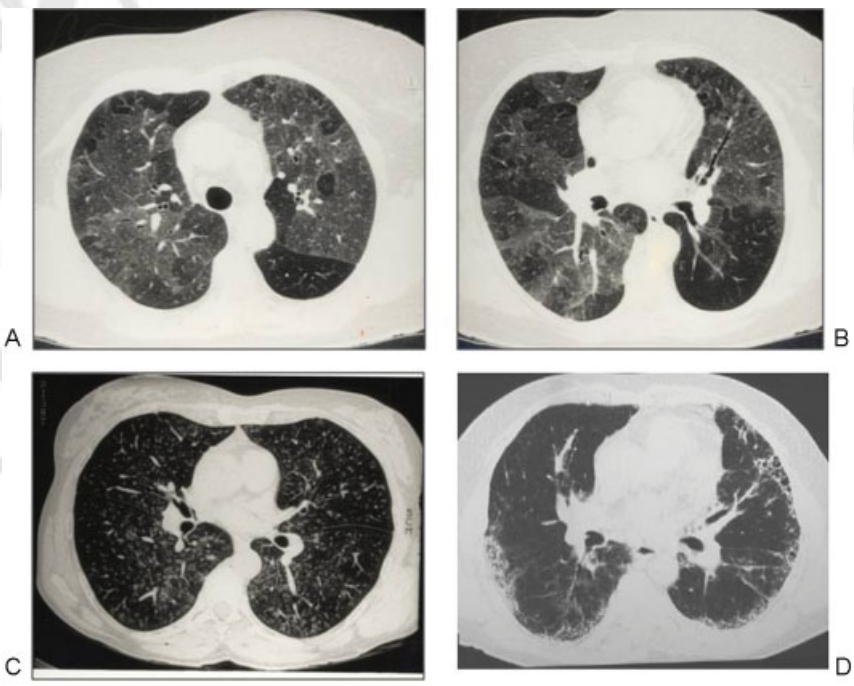

Fig. 3 High-resolution chest computed tomography scans. (**A** and **B**) Mosaic pattern; (**C**) micronodular pattern; and (**D**) usual interstitial pneumonia pattern.

methods for qualitative determination (e.g., precipitation, Ouchterlony double diffusion, immunoelectrophoresis) and quantitative determination (ELISA, ImmunoCAP, Immulite) of ssIgG antibodies are available, but the results obtained often differ considerably.[27] Antigens available for testing in most centers include, goose, pigeon, parrot, and parakeet, canary, and hen sera, dove feather antigen, *Aspergillus* spp., and *Penicillium* spp. *Mucor* spp. and *Rhizopus* spp. extracts. These antigens cover most cases of HP (bird fancier's lung, pigeon breeder's disease, farmer's lung, and humidifier lung). The selection of antigens to be tested often needs to be determined locally based on those that are most prevalent.

An elevated titer of antigen-specific IgG antibodies associated with consistent clinical features is strongly supportive of HP. Lacasse et al[28] reported that positive serum antibody testing is a significant predictor of HP (odds ratio: 5.3; 95% confidence interval: 2.7–10.4). However, it is well recognized that the presence of ssIgG antibodies to the inducing antigen demonstrates sensitization but is not necessarily diagnostic. In fact, only 1 to 15% of individuals exposed to HP antigens actually develop the disease, and many exposed individuals have a high titer of serum precipitating antibodies but remain asymptomatic. As was reported by Costabel et al,[29] 30 to 60% of healthy farmers produce precipitating antibodies to the

antigens to which they are exposed, and 10 to 15% of patients with farmer's lung do not develop serum precipitins. Thus, in addition, negative testing does not rule out the disease.

Despite these limitations, determination of ssIgG antibodies is useful for supporting the diagnosis of HP, and sometimes, the results are determinant for discovering new causes of the disease. For example, a study in suberosis patients found that in addition to contamination by *Penicillium glabrum* and *Aspergillus fumigatus*, cork itself, uncontaminated by fungi, can participate in the pathogenesis of HP. This cause was confirmed by positive specific skin test results, ssIgG antibody analyses, and SIC.[30]

Specific IgG antibodies should be routinely tested in individuals presenting with idiopathic pulmonary fibrosis (IPF). In a recent study, two-thirds of patients diagnosed with IPF based on the 2011 guidelines criteria[1] had been exposed to antigens known to cause HP, seen on ssIgG testing, and almost half the patients had a final diagnosis of chronic HP.[31] Clearly, the exposure history and specific antibody testing would be particularly relevant factors in regions with a high prevalence of certain exposures, such as countries where domestic bird keeping is a common hobby.[32]

Lymphocytic Activation Testing

The current experience with tests determining lymphocyte activation by antigens is scanty, but some promising results have been reported. In patients with chronic fibrosis and negative ssIgG antibody testing, evaluation of the proliferation indices of peripheral blood mononuclear cells stimulated with the specific antigen has been proposed to support the diagnosis of pigeon breeder's disease.[33] One study has shown that the leucocyte migration inhibition test, an indicator of lymphocyte stimulation, applied to the diagnosis of farmer's lung yielded positive findings in 19 of 20 (95%) patients with this condition, and only 11 of 25 (44%) control farmers without the disease ($p< 0.005$). The test proved to be more effective than specific antibody determination in the diagnosis of farmer's lung.[34] The same occurred when the test was used in bird breeder's disease: positive results were seen in 10 of 12 (83%) patients with this condition and only 1 of 7 (14%) control breeders ($p< 0.02$). The diagnostic yield of the test was better than that of precipitins detection by immunoelectrophoresis (positive in 11 of 12 [92%] bird breeder's disease patients and 6 of 7 [86%] asymptomatic breeders) and the Ouchterlony double diffusion technique (positive in 11 of 12 [92%] and 5 of 7 [71%], respectively).[35]

Bronchoalveolar Lavage

Reynolds and Newball reported the first description of BAL in 1974.[36] In the beginning, this technique was developed to analyze inflammatory and immunologic cells present in the lower respiratory tract in normal lung and in several types of ILD. In this ground-breaking study, BAL was performed in 32 control individuals and 26 ILD patients, most of whom had IPF or HP. By the 1980s, the main indication for BAL was ILD study,[37,38] but over the years, doubts have remained concerning its usefulness. In fact, BAL is not included in recent guideline recommendations for the diagnosis of IPF.[1,2] However, this technique continues to have an important role in the diagnostic workup of ILD in many centers, as will be discussed later.

First, we mention certain technical aspects that have made the technique reproducible. The lavage fluid used is isotonic saline solution at room temperature. The amount of saline instilled varies, with the general recommendations being between 150 and 200 mL. Quantities below 100 mL may contain an excessive amount of bronchial secretions and be unrepresentative of the alveolar space, whereas quantities above 250 mL may increase the risk of clinical complications. In diseases that diffusely affect the lung, BAL is usually performed in a region enabling recovery of the largest amount of solution, such as the middle lobe and lingula, where additionally, the repercussion on arterial oxygen levels is lower.

The lavage solution should be processed within 4 hours after recovery to ensure reliable cell analysis. If this time frame is not feasible, the solution should be maintained at 4°C until analysis. For processing, the sample is centrifuged at 300 to 600 $\times$ *g*, the cell pellet is resuspended in saline, and total cell count is determined using a Neubauer chamber. The cell percentages obtained can vary somewhat depending on the method used, but this should not have an impact on the diagnostic assessment. A minimum of 300 cells is needed to obtain reliable percentages.

In a healthy person, total cellularity in BAL ranges from 100,000 to 700,000 cell/mL, and this value may be fourfold higher in smokers. Therefore, although these values are generally accepted, it is useful for each center to have a control group of both nonsmokers and smokers for reference purposes. Study of the inflammatory cell profile in BAL specimens is useful in the diagnostic workup of ILD and may, in itself, lead to establishment of the diagnosis.[39,40]

Bronchoalveolar specimens from a nonsmoking healthy population, contain 80 to 90% macrophages, 10 to 15% lymphocytes, 1 to 3% polymorphonuclear cells, <1% eosinophils, and <1% mast cells (►**Table 2**). Thus, differential cell counts in BAL fluid comprising >15% lymphocytes, >3% neutrophils, or >1% eosinophils are considered to represent a lymphocytic, neutrophilic, or eosinophilic pattern, respectively.[41]

The presence of a lymphocytic BAL pattern is highly suggestive of granulomatous disease. In particular, a

Table 2 BAL results in nonsmoking healthy individuals[41–43]

Cell type	Percentage (%)
Alveolar macrophages	85
Lymphocytes	10–15
Neutrophils	<3
Eosinophils	<1
Squamous cells	<5

Abbreviation: BAL, bronchoalveolar lavage.

lymphocyte value greater than 25% is characteristic of HP and sarcoidosis, although it has also been described in other conditions (e.g., berylliosis, drug toxicity, nonspecific interstitial pneumonia, organizing pneumonia, lymphocytic pneumonia, lymphoma, radiation pneumonitis, patients receiving amiodarone and methotrexate, Wegener granulomatosis, Crohn disease, and primary biliary cirrhosis).[42]

It has been reported that a differential of $\geq$50% lymphocytes is highly suggestive of HP or cellular nonspecific interstitial pneumonia (NSIP), particularly if the CD4:CD8 T-lymphocyte ratio is < 2 in HP.[43] Inversely, as the disease progresses to fibrosis, the lymphocyte count in BAL decreases.[31,44] BAL analysis shows a >20% lymphocyte value in 80% of patients with chronic HP,[12,45] whereas this percentage is seen in only 15% of chronic HP patients with criteria for IPF.[31] In any case, evidence supporting a diagnosis of chronic HP should be actively sought in any IPF patient with elevated lymphocyte counts. Despite the findings of some studies, CD4/CD8 ratio determination is not currently recommended in HP, as it varies according to the intensity of exposure and disease stage; thus, a normal or elevated ratio does not exclude the disease.[32,45]

With regard to other cell types, elevated neutrophil counts in the absence of infection, with or without elevated eosinophils, is found in BAL specimens from 70 to 90% of IPF patients and has been related to the extension of fibrosis in some studies.[42,43,46] Eosinophilia in BAL at percentages higher than 25% is suggestive of eosinophilic pneumonia.[39,42] In patients with IPF, HP, and some types of drug-induced pneumonitis, eosinophil values higher than 1% have been described.[42] The presence of mast cells in BAL has been reported in IPF, HP,[47] and sarcoidosis, and has been related to the extension of the fibrotic process and the rate at which it progresses.[48,49] Finally, detection of plasma cells, foamy cells, and an elevated lymphocyte count is also highly suggestive of HP, particularly in the acute phase of the disease,[50] although these findings have also been described in patients with lung disease secondary to drug toxicity.[51,52]

Conflicting results have been reported in the differential diagnosis of IPF versus other idiopathic interstitial lung diseases such as NSIP. In the study by Ryu et al,[53] lymphocyte count was higher in NSIP patients (29%) than in those with IPF (<5%), whereas neutrophil count was clearly higher in IPF (7%) than in NSIP (3%). In contrast, one study reported that BAL findings had no diagnostic or prognostic value in a cohort of patients with IPF and idiopathic NSIP.[54] Along this line, in a recent study including 76 patients with diagnostic criteria of IPF (without SLB), Ohshimo et al[55] reported that a cutoff of 30% lymphocytes in BAL had high discriminatory power for establishing an alternative diagnosis of HP in all cases. The change in diagnosis was later validated by SLB or follow-up findings over time. In another recent study including 46 patients with HRCT criteria of IPF, lymphocyte values higher than 20% were only found in patients ultimately diagnosed with chronic HP, but these accounted for only 3 of the 20 cases with a final diagnosis of chronic HP.[31] These findings contrast with the results of Gaxiola et al[56] in 8 of 10 HP patients with a UIP-like pattern on SLB. Mean lymphocyte value in BAL specimens from these patients was 36 ± 23%, a value not far from the mean of 19% found by Ohtani et al in chronic HP/UIP patients.[44]

Exclusion of an alternative diagnosis in patients with IPF is essential for estimating the prognosis and deciding on the treatment strategy. Hence, we believe that the BAL should have a relevant role in the study of IPF and the diagnosis of HP.

Transbronchial Lung Biopsy and Cryobiopsy

Taking advantage of the bronchoscopy procedure for BAL, many centers simultaneously perform transbronchial biopsy (TBB), even though this technique is known to have a low diagnostic yield in ILD.[57] TBB does have a certain utility in granulomatous diseases such as HP and sarcoidosis, as well as in lymphangitic carcinomatosis, diffuse alveolar damage, alveolar proteinosis, and eosinophilic pneumonia. The low effectiveness of the technique in interstitial disease is related to the patchy, heterogeneous nature of the lung involvement and the small size of the specimen, which may miss the affected parts. Furthermore, the forceps used in the procedure make preservation of the lung tissue structure difficult. According to some studies, the diagnostic yield of this technique in ILD is around 30%.[58,59]

Over the last few years, lung biopsy using cryoprobes has become a consolidated, reliable technique showing high diagnostic performance in ILD. In 2009, Babiak et al[60] described the potential utility of cryobiopsy in interstitial disease, reporting that the information from analysis of the specimens obtained provided a definite diagnosis in a significant number of cases.

Briefly, a flexible probe, 2.4 mm in diameter and 900 mm long, connected to a cryotherapy unit, is inserted in the fiberscope working channel. The tissue sample around the probe tip is then frozen and removed. Following the introduction of this technique in interstitial disease, several groups have investigated its performance.[61] It is now considered a viable alternative to TBB, providing a larger specimen, better preservation of the lung parenchyma architecture, a higher diagnostic yield, and a relatively low incidence of complications.[62–65] Other authors have additionally shown that cryobiopsy is less costly than SLB and have suggested that it should be included in the diagnostic algorithm of ILD before the use of SLB.[66] Based on the currently available data, it seems reasonable to say that TBB should not be recommended in the study of ILD, since the diagnostic yield of cryobiopsy is so much higher.

Specific Inhalation Challenge

Most related guidelines and review articles state that the diagnosis of HP should be based on clinical, radiologic, and laboratory criteria.[17,24,29] To our mind, as HP is a disease with a clearly immunologic basis, more diagnostic value should be placed on immunologic tests, such as SIC. These techniques are especially relevant considering that the typical triad of pathologic changes seen in biopsies (lymphocytic infiltrate, bronchiolitis, and poorly formed granulomas) is observed in

Table 3 Sensitivity and specificity of SIC

	Patients	SIC results
Hendrick et al (1980)[70]	29 suspected HP; 2 controls	Specificity: 95% Sensitivity: 48–85%
Ramírez-Venegas et al (1998)[71]	1 chronic HP; 17 other interstitial lung diseases; 5 controls	Specificity: 82–86% Sensitivity: 76–100%
Ohtani et al (2000)[72]	11 chronic HP	Not specified
Morell et al (2008)[12]	59 HP; 30 healthy pigeon keepers; 20 other interstitial lung diseases	Specificity: 100% Sensitivity: 92%

Abbreviations: HP, hypersensitivity pneumonitis; SIC, specific inhalation challenge.

less than 30% of patients.[32] In fact, in the clinical practice of many centers, the presumptive diagnosis is made on findings of an elevated lymphocyte percentage in BAL, which is evidence of an immunologic change.[25,67]

In this context, it would be logical to assume that SIC findings could be a fundamental element in the diagnosis of HP.[68] Nonetheless, various authors believe that because the technique lacks standardization, both in the inhalation protocols and the criteria used to define a positive response, and because of the risk of severe reactions, it should only be performed in selected patients by qualified personnel in specialized centers.[18,29,69] This opinion may stem from the scarcity of related information, with few published studies investigating the utility of the test in the diagnosis of HP. The articles include samples of 11 to 59 patients, are focused only on avian antigens, and the inhalation method, final dose used, and criteria for positive status differ between studies.[12,70–72] Despite this heterogeneity, the sensitivities and specificities reported are quite satisfactory (►**Table 3**), thus suggesting that SIC could be a valid option for use in the diagnosis of this disease.

In a recent study by Muñoz et al,[73] SIC showed good diagnostic yield in 113 patients with suspected HP. The overall sensitivity and specificity were 73 and 84%, respectively, when tests against all causative agents were analyzed together, and 85 and 86% when evaluating the results only in patients exposed to avian or fungal antigens. The exposure method consisted in administration of an extract of the suspected causative agent using a nebulizer. Patients were requested to inhale 2 mL of the suspected antigen at a dilution of 1/100 (0.01 mg/mL). Forced vital capacity (FVC), forced expiratory volume in 1 second, diffusing capacity for carbon monoxide (DLco), and the patient's temperature were recorded at baseline, 20 minutes after inhalation challenge, every hour thereafter for the next 8 hours, and at 24 hours. Blood cell count, chest X-ray, and O_2 saturation measurement were performed before and 8 hours after inhalation. In all cases, SIC with a placebo solution (saline) was performed 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 with baseline values; (2) 10 to 15% FVC decrease plus at least one of the following criteria with respect to clinical status and baseline analytical values: (a) white blood cell increase of 20%, (b) O_2 saturation decrease of 3%, (c) significant radiologic changes,[71] (d) rise in body temperature of more than 0.5°C, or (e) evident clinical symptoms (e.g., cough, dyspnea); (3) FVC decrease <10% but with evidence of three or more of the previouslymentioned clinical or analytical criteria. 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. Using this method, only nine patients (8%) experienced SIC-related reactions, which were transient, and only three patients required administration of oral corticosteroids.[73]

The results of this study seem to show that SIC should not be considered a test restricted to certain patients and only in centers interested in this subject. If adequate antigens are available[74] and a proper protocol is followed, it is a relatively simple procedure that can be widely practiced. When SIC is performed in the manner described, the yield is high and there are few adverse effects. Furthermore, false diagnoses of IPF can be ruled out with this test, as was recently shown in a series of 46 patients. The study patients met the criteria for IPF, but SIC results showed that 42% of the total were actually affected with HP.[31] Differentiation between these conditions is clinically relevant because each requires a different treatment.[70]

Surgical Lung Biopsy

In our opinion, SLB should only be indicated as a last resort when the clinical interview, physical examination, and assemblage of findings from the diagnostic tests mentioned earlier (see diagnosis discussed previously) do not suffice to establish the diagnosis. SLB is an invasive procedure associated with considerable discomfort and a postoperative mortality rate of 3.6%, according to a meta-analysis including 2,148 ILD patients.[6] The study reported a higher surgical risk in immunocompromised patients and those with ventilation dependence or severe respiratory dysfunction. The diagnostic yield does not seem to be influenced by the technique used (video-assisted vs. open lung biopsy).[75]

Pathology

In reference to the pathologic findings on SLB in patients with HP (►**Fig. 4**), Myers[5] reported the classic triad, consisting of nonnecrotizing granulomas, bronchiolitis with lymphocytic inflammation, and interstitial inflammation

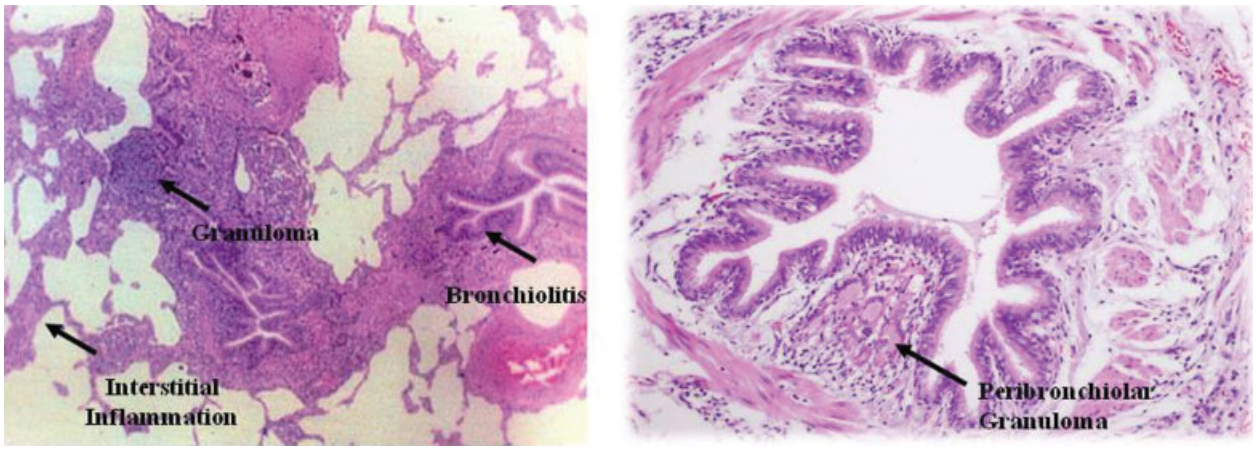

Fig. 4 Pathologic findings in hypersensitivity pneumonitis.

with lymphocytes and plasma cells. These typical findings are not always seen altogether. Bronchiolitis consists of variable degrees of peribronchial fibrosis and metaplasia (hyperplasia of the bronchial epithelium). Sometimes, granulomas are not seen; hence, the interstitial inflammation has the appearance of NSIP. The features of obliterative bronchiolitis may also be found. Multinuclear giant cells may be detected instead of granulomas, a finding that has diagnostic significance. Late-stage fibrotic HP may be indistinguishable from the characteristic HP images.[5]

The pathologic criteria described by Colby for diagnosing subacute HP by SLB[31] include cellular bronchiolitis or centrilobular scarring with interstitial lymphoplasmacytic infiltrate and poorly formed nonnecrotizing granulomas. Features considered consistent with chronic HP are interstitial fibrosis with pattern of NSIP, organizing pneumonia or UIP, and features that are atypical for UIP, such as prominent peribronchiolar metaplasia, marked interstitial lymphoplasmacytic infiltrates, lymphoid aggregates without germinal centers, relatively prominent organizing pneumonia, granulomas, and constrictive or obliterative bronchiolitis. Prominent centrilobular and bronchiolocentric lesions are not typical for IPF and support a chronic HP diagnosis.

Pulmonary fibrosis, the most advanced stage of chronic HP, may mimic the histological features of UIP. As described by Katzenstein et al,[76] the main difference between UIP and fibrotic HP seen in on autopsy is that honeycombing changes predominated in the upper lobes in less than half the HP patients.

In conclusion, HP is an interstitial disease of the lungs whose diagnosis depends on identifying exposure to a known antigen by meticulous, targeted historytaking and the combined results of radiologic, laboratory, and pathology techniques. HRCT images should be examined for characteristic features or (at least) abnormal findings consistent with the clinical symptoms. In all patients, testing should be done to determine specific IgG antibodies against antigens present in the causative substance, and BAL material should be analyzed to detect lymphocyte increases. Because of its high yield and low rate of complications, cryobiopsy is recommended to investigate the typical triad of pathologic findings in HP or lesions consistent with the symptoms of pneumonitis. SIC can be used to confirm the causal relationship with the suspected antigen and in some cases, can lead to the diagnosis. With the application of this diagnostic workup, SLB will not be needed in most affected patients and its practice can be avoided to their benefit.

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