Hypersensitivity Pneumonitis
Insights in Diagnosis and Pathobiology

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Hypersensitivity pneumonitis (HP) is a complex syndrome resulting from repeated exposure to a variety of organic particles. HP may present as acute, subacute, or chronic clinical forms but with frequent overlap of these various forms. An intriguing question is why only few of the exposed individuals develop the disease. According to a two-hit model, antigen exposure associated with genetic or environmental promoting factors provokes an immunopathological response. This response is mediated by immune complexes in the acute form and by Th1 and likely Th17 T cells in subacute/chronic cases. Pathologically, HP is characterized by a bronchiolocentric granulomatous lymphocytic alveolitis, which evolves to fibrosis in chronic advanced cases. On high-resolution computed tomography scan, ground-glass and poorly defined nodules, with patchy areas of air trapping, are seen in acute/subacute cases, whereas reticular opacities, volume loss, and traction bronchiectasis superimposed on subacute changes are observed in chronic cases. Importantly, subacute and chronic HP may mimic several interstitial lung diseases, including nonspecific interstitial pneumonia and usual interstitial pneumonia, making diagnosis extremely difficult. Thus, the diagnosis of HP requires a high index of suspicion and should be considered in any patient presenting with clinical evidence of interstitial lung disease. The definitive diagnosis requires exposure to known antigen, and the assemblage of clinical, radiologic, laboratory, and pathologic findings. Early diagnosis and avoidance of further exposure are keys in management of the disease. Corticosteroids are generally used, although their long-term efficacy has not been proved in prospective clinical trials. Lung transplantation should be recommended in cases of progressive end-stage illness.

Keywords: hypersensitivity pneumonitis; extrinsic allergic; immune response; diagnosis

Hypersensitivity pneumonitis (HP) is a complex syndrome caused by exposure to a wide variety of organic particles small enough to reach the alveoli (<5 μm). In susceptible individuals, these antigens provoke an exaggerated immune response of the small airways and lung parenchyma (1). The causative antigens include fungi; bacterial, protozoal, animal, and insect proteins; and low–molecular-weight chemical compounds (Table 1). HP may occur in a variety of occupational, home, and recreational environments (1).

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EPIDEMIOLOGY

The prevalence varies considerably around the world, depending on disease definition, diagnostic methods, type and intensity of exposure, geographical conditions, agricultural and industrial practices, and host risk factors. Furthermore, the definite prevalence of HP is uncertain, primarily because cases may go undetected or are misdiagnosed. In addition, there is no consistent, standardized epidemiological approach for assessing the various forms of HP. High attack rates may be found among exposed individuals during sporadic outbreaks and in occupational settings. Studies on incidence are scanty. In a large, general-population–based study, the incidence of HP was approximately 1 per 100,000 in the UK population (2). The disease is uncommon in children, and a recent report in Denmark showed an incidence of 2 per year and a prevalence of 4 per 1,000,000 children (3).

PATHOGENESIS

An intriguing question regarding HP is why, given the universal and wide distribution of the offending antigens, only few individuals develop the disease. A two-hit hypothesis has been suggested, wherein preexisting genetic susceptibility or environmental factors (i.e., the first hit) increases the risk for the development of HP after antigen exposure (the second hit). Antigen exposure acts as the inducing factor, and genetic or environmental factors act as promoting risk factors (Figure 1).

ANTIGENS

HP is seen worldwide, and the most commonly implicated antigens are thermophilic actinomycete species, fungi, and bird proteins. Thermophilic actinomycete (i.e., *Saccharopolyspora rectivirgula*) and a variety of fungi (i.e., *Aspergillus* species and *Penicillium* species) are implicated in HP in a variety of occupations, such as farming, but also may be responsible for the disease acquired in home environments (e.g., summer-type HP in Japan) (4). A complex mixture of high– and low–molecular-weight proteins from avian serum, feces, and feathers produce the "bird fancier's lung" (BFL), also called pigeon-breeder's lung. Pigeons, parakeets, budgerigars, and other small cage birds are usually involved, but the disease may also occur in individuals using feather-down duvets and pillows and even by indirect contact to birds in consorts (e.g., handling others' clothing).

Increasing evidence shows that colonization of heated water by *Mycobacterium avium* complex causes HP (for instance, in hot tub and warm water therapy pool users) (5). Nontuberculous mycobacteria (NTM) have a competitive advantage over many other bacterial species in these environments because of their thermotolerance and disinfectant resistance. Some NTM, like *Mycobacterium immunogenum*, have the ability to colonize contaminated metalworking fluids and have been associated with HP

TABLE 1. ETIOLOGIC AGENTS OF HYPERSENSITIVITY PNEUMONITIS

Disease	Antigen	Source
Fungal and bacterial		
Farmer's lung	*Saccharopolyspora rectivirgula*	Moldy hay, grain, silage
Ventilation pneumonitis; humidifier lung; air conditioner lung	*Thermoactinomyces vulgaris, Thermoactinomyces sacchari, Thermoactinomyces candidus, Klebsiella oxytoca*	Contaminated forced-air systems; water reservoirs
Bagassosis	*T. vulgaris*	Moldy sugarcane (i.e., bagasse)
Mushroom worker's lung	*T. sacchari*	Moldy mushroom compost
Enoki mushroom worker's lung (Japan)	*Penicillium citrinum*	Moldy mushroom compost
Suberosis	*Thermoactinomyces viridis, Aspergillus fumigatus, Penicillium frequentans, Penicillium glabrum*	Moldy cork
Detergent lung; washing powder lung	*Bacillus subtilis* enzymes	Detergents (during processing or use)
Malt worker's lung	*Aspergillus fumigatus, Aspergillus clavatus*	Moldy barley
Sequoiosis	*Graphium, Pullularia, and Trichoderma* spp., *Aureobasidium pullulans*	Moldy wood dust
Maple bark stripper's lung	*Cryptostroma corticale*	Moldy maple bark
Cheese washer's lung	*Penicillium casei, A. clavatus*	Moldy cheese
Woodworker's lung	*Alternaria* spp., wood dust	Oak, cedar, and mahogany dust, pine and spruce pulp
Hardwood worker's lung	*Paecilomyces*	Kiln-dried wood
Paprika slicer's lung	*Mucor stolonifer*	Moldy paprika pods
Sauna taker's lung	*Aureobasidium* spp., other sources	Contaminated sauna water
Familial HP	*B. subtilis*	Contaminated wood dust in walls
Wood trimmer's lung	*Rhizopus* spp., *Mucor* spp.	Contaminated wood trimmings
Composter's lung	*T. vulgaris, Aspergillus*	Compost
Basement shower HP	*Epicoccum nigrum*	Mold on unventilated shower
Hot tub lung	*Mycobacterium avium complex*	Hot tub mists; mold on ceiling
Wine maker's lung	*Botrytis cinerea*	Mold on grapes
Woodsman's disease	*Penicillium* spp.	Oak and maple trees
Thatched roof lung	*Saccharomonospora viridis*	Dead grasses and leaves
Tobacco grower's lung	*Aspergillus* spp.	Tobacco plants
Potato riddler's lung	*Thermophilic actinomycetes, S. rectivirgula, T. vulgaris, Aspergillus* spp.	Moldy hay around potatoes
Summer-type pneumonitis	*Trichosporon cutaneum*	Contaminated old houses
Dry rot lung	*Merulius lacrymans*	Rotten wood
Stipatosis	*Aspergillus fumigatus; T. actinomycetes*	Esparto dust
Machine operator's lung	*Mycobacterium immunogenum; Pseudomonas fluorescens*	Aerosolized metalworking fluid
Residential provoked pneumonitis Amebae	*Aureobasidium pullulans*	Residential exposure
Humidifier lung	*Naegleria gruberi, Acanthamoeba polyphaga, Acanthamoeba castellani, Bacillus* sp., others	Contaminated water from home humidifier, ultrasonic misting fountains
Shower curtain disease	*Phoma violacea*	Moldy shower curtain
Animal proteins		
Pigeon breeder's or pigeon fancier's disease	Avian droppings, feathers, serum	Parakeets, budgerigars, pigeons, chickens, turkeys
Pituitary snuff taker's lung	Pituitary snuff	Bovine and porcine pituitary proteins
Fish meal worker's lung	Fish meal	Fish meal dust
Bat lung	Bat serum protein	Bat droppings
Furrier's lung	Animal fur dust	Animal pelts
Animal handler's lung; laboratory worker's lung	Rats, gerbils	Urine, serum, pelts, proteins
Insect proteins		
Miller's lung	*Sitophilus granarius* (i.e., wheat weevil)	Dust-contaminated grain
Lycoperdonosis	Puffball spores	Lycoperdon puffballs

Definition of abbreviation: HP = hypersensitivity pneumonitis.
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in automotive plants and metalworking operations, such as metal cutting, machine finishing, and machine tooling (6).

Some low–molecular-weight chemicals, such as isocyanates, may sometimes cause HP. Isocyanates are not antigenic by themselves but may combine with host proteins forming haptens. Isocyanates are used for the large-scale production of polyurethane polymers, widely used in the manufacture of polyurethane foams, paints, and plastics.

PROMOTING AND PROTECTING FACTORS

Genetic Susceptibility

Studies regarding genetic susceptibility are few. Given its role in regulating the immune response, a focus has been placed on the major histocompatibility complex (MHC). The high level of polymorphism and heterozygosity within the MHC genomic region provide the immune system with a selective advantage against the diversity of pathogens but has the added risk of generating diverse immunopathological disorders. Class II MHC molecules appear to be the primary susceptibility locus in HP, and, accordingly, polymorphisms associated with HLA-DR and DQ have been associated with increased risk for HP in populations with different genetic backgrounds (7, 8).

Likewise, the immunoproteasome catalytic subunit, β type 8 (PSMB8), which participates in the degradation of ubiquitinated proteins generating peptides presented by MHC class I molecules, is also implicated. Patients with HP had a significant increase of the PSMB8 KQ genotype frequency compared with matched control subjects (9). Likewise, polymorphisms of the transporters associated with antigen processing (TAP) genes have been shown to increase the susceptibility for HP (10). TAP transports peptides for loading onto class I MHC molecules

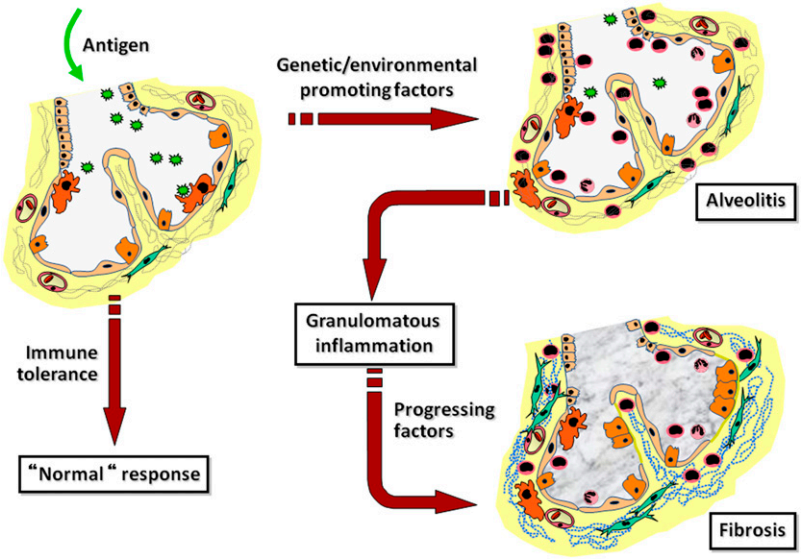

Figure 1. Proposed mechanisms in the pathogenesis of hypersensitivity pneumonitis. Most exposed individuals develop an immune tolerance, and the antigen inhalation may result at most in a mild increase of local lymphocytes, without clinical consequences. The coexistence of genetic or environmental promoting factors provokes the development of an exaggerated immune reaction that results in marked lung inflammation. The generation of the granulomatous inflammation requires, among others, the expression of Th1 cytokines, including tumor necrosis factor-α, IL-12, and interferon-γ, as well as a toll-like receptor 9–mediated dendritic cell response, which is believed to promote Th1 skewing and prevent Th2 skewing during the development of the adaptive immune response. Subsequently, in the presence of progressing factors (i.e., further exposure) or genetic predisposition, critical immunopathological changes occur in the lung microenvironment inducing the expansion and activation of the fibroblast population and the accumulation of extracellular matrix.

that present them to cytotoxic T cells at the cell surface. HP susceptibility was associated with the allele Gly-637 and the genotypes Asp-637/Gly-637 and Pro-661/Pro-661 of the subunit TAP1 (10). TNF-α gene, also located within MHC, has been explored with contradictory results. Thus, although patients with farmer's lung display high frequency of TNFA2 (-308) allele, which increases its biological activity, in patients with BFL this allele is similar to control subjects (8, 11). Two studies performed in patients with HP with different ethnic backgrounds demonstrated that promoter variants in tissue inhibitor of metalloproteinase-3 (TIMP-3) have a protective effect (12, 13). However, the mechanisms by which TIMP-3 polymorphism may decrease the risk to develop HP remain unclear.

In general, case-control studies evaluating gene polymorphisms in HP have been performed in small cohorts, and at present, with the exception of the MHC, there are no genetic factors consistently associated with this disease.

Environmental Promoting Factors

Many individuals suffering from acute HP report initial symptoms suggestive of respiratory viral infection, and many of them have common respiratory viruses in the lower respiratory tract (14). Interestingly, mice infected with parainfluenza virus develop an exacerbated inflammatory response to HP antigens that persists for up to 30 weeks after the viral infection (15).

A cross-sectional analysis of a large, heterogeneous farming population found that high pesticide exposure, both organochlorine and carbamate, was strongly associated with a diagnosis of farmer's lung, indicating that pesticide exposure may be an overlooked risk factor for farmer's lung (16).

The Paradoxical Role of Cigarette Smoking

HP is less frequent in smokers than in nonsmokers under the same risk of exposure (1). Moreover, when exposed to an environment with high levels of HP antigens, smokers have lower levels of specific antibodies to the causative antigen. The mechanisms by which cigarette smoke protects from HP are unclear, but experimental approaches attribute this effect to nicotine (17). Mice challenged with *S. rectivirgula* and simultaneously treated with nicotine showed a significant decrease of lung inflammation. Nicotine affects macrophage activation, decreases lymphocyte proliferation, and impairs T-cell function (17, 18). Activation of the nicotinic acetylcholine receptor α7 reduces the secretion of several proinflammatory cytokines by macrophages, whereas on lymphocytes it decreases the reactivity of the Th1 and Th17 lineages, increasing the Th2 response (18). Importantly, evidence indicates that although HP develops more frequently in nonsmokers, when HP occurs in smokers, they may develop a chronic clinical course with more recurrent episodes and a significantly poorer survival rate compared with nonsmoker patients (19). In a murine model of BFL it was found that in short-term exposure (4 wk), cigarette smoke decreased inflammation and lymphocyte proliferation, whereas long-term exposure to cigarette smoke (17 wk) enhances lung inflammation with fibrosis (20).

Immune Tolerance as Protective Factor

Many exposed individuals develop a mild lymphocytic alveolitis but remain asymptomatic, suggesting the development of a tolerant response to HP antigens (1). Although the mechanisms are unclear, tolerance may be mediated by regulatory T cells (T_{reg}), a unique population of $CD4^+$ T cells that play a pivotal role in the maintenance of the balance between the tissue-damaging and protective effects of the immune response. T_{reg} cells function as suppressors of Th1 and Th2 cell immune responses, and, for example, mice lacking them display overwhelming autoimmune disease (21).

It has been shown that although T_{reg} from asymptomatic exposed subjects suppress T-cell proliferation similar to normal unexposed individuals, these cells obtained from patients with HP (from blood and bronchoalveolar lavage [BAL]) were unable to suppress activated T-cell proliferation (22). Also, in experimental HP it was demonstrated that T_{reg} cells play a protective antiinflammatory role (23).

IMMUNOPATHOLOGICAL MECHANISMS OF DISEASE

Immune complexes mediate the acute form of HP. Subacute and chronic forms of HP are provoked by T lymphocytes through a Th1 immune response under the specific "master regulator" transcription factor, T-bet (1, 24). On interaction with the HP antigen presented by alveolar macrophages and dendritic cells (DC), $CD4^+$ T cells can differentiate into a variety of effector subsets. IL-12 and IFN-γ polarize lymphocytes toward the Th1 cell differentiation program. Data in experimental models of HP suggest that the expression of CD34 and the toll-like

receptor-9 are critical for efficient trafficking of DC through the lungs and for development of a Th1 granulomatous inflammatory response (25, 26). Likewise, apoptosis of lung epithelial and immune cells promotes immune responses against HP antigens by enhancing maturation and chemokine production of $CD11c^+$ DCs (27).

$CD4^+$ T cells can differentiate, in addition to the classical Th1 and Th2 cells, into a variety of other effector subsets, such as Th17 cells, follicular helper T cells, and induced T_{reg} cells. Interestingly, microarray analysis in human HP revealed that in addition to Th1 factors, IL-17 and IL-17–associated transcripts were also up-regulated (28). Furthermore, it was shown that in chronic exposure to *S. rectivirgula*, $CD4^+$ T cells were not polarized to Th1 but rather to Th17 with differential expression of IL-17A and IL-22 (29). Moreover, this study established an important role for $CD4^+$ Th17 cells in the subsequent development of lung fibrosis. Likewise, genetic deletion or antibody-mediated depletion of IL-17 resulted in decreased inflammation and protection against the disease (30). Thus, a Th-17 polarization with up-regulation of its signature cytokines appears to play an important role in the pathogenesis of HP.

The immunopathological processes contributing to disease chronicity and eventually to the development of fibrosis is beginning to be elucidated. Patients with chronic HP show an increase of $CD4^+$ T cells and of the $CD4^+/CD8^+$ ratio and exhibit skewing toward Th2 activity (31). Th2-biased immune response was also profibrotic in a murine model of chronic HP (32). By contrast, increase of γδ T cells seems to have an antifibrotic and protective effect, partially involving the inhibition of αβ T cells by the regulatory IL-22 (31, 33). Thus, attenuation of IL-22 activity, either by mutating the receptor or inhibiting its signaling, accelerated lung fibrosis.

Interestingly, patients with HP exhibit increased frequency of fetal microchimerism (i.e., the persistence of foreign cells) that show a multilineage capacity, because the microchimeric cells in HP lungs were either macrophages, $CD4^+$, or $CD8^+$ T cells (34). The presence of microchimerism appeared to increase the severity of the disease.

Finally, the role of other inflammatory cells in the fibrotic process is unclear. Some evidence suggests that patients with chronic fibrotic HP have an increase of neutrophils loaded with matrix metalloproteinase-8 and -9 (35).

CLINICAL BEHAVIOR

Although numerous antigens induce HP, the clinical features are similar and have been conventionally classified into acute, subacute, and chronic forms. Unfortunately, this classification scheme is inadequate because there are no widely accepted criteria to distinguish the various forms, little information exists concerning the latency between exposure and symptom onset, and it is uncertain that they represent different stages of the disease.

Reexamination of data from a large prospective multicenter cohort using cluster analysis showed that most of the cases examined fit best into a two-cluster model. This study showed that subacute HP is particularly difficult to define because the features in this subset overlap with both the "acute" and "chronic" components. Patients in cluster 1 had more recurrent systemic symptoms (chills, body aches) and normal chest X-rays, whereas those in cluster 2 showed significantly more features of chronic and severe disease (i.e., clubbing, hypoxemia, restrictive patterns on pulmonary function tests, and fibrosis on high-resolution computed tomography [HRCT] scan) (36). Cluster 1 looks most like the classical acute form of HP and tends to occur in individuals exposed to thermophilic actinomycete species or fungi (e.g., farmer's lung). Conversely, cluster 2 favors the classical chronic form of HP and tends to occur in individuals with bird antigen exposure (Table 2). Importantly, it is emphasized that the words acute and chronic do not describe pathogenic pathways and do not imply that chronic HP follows acute HP, which remains uncertain (36).

TABLE 2. DIFFERENCES IN CLINICAL, PHYSIOLOGIC, RADIOLOGIC, BRONCHOALVEOLAR LAVAGE, HISTOLOGIC, AND PROGNOSTIC FEATURES BETWEEN MICROORGANISMS AND SOLUBLE AVIAN PROTEINS EXPOSURES

Antigen	Microorganisms: Thermophilic Actinomycetes, Fungi (e.g., Farmer's Lung; Water Damage)	Soluble Avian Proteins (e.g., BFL)
Exposure	Usually short and massive: ~ 750,000 actinomycetes spores per min	Recurrent: breed dozens of pigeons in a loft. Insidious: prolonged and low level (i.e., few birds in the domestic environment or down products)
Clinical behavior	Primarily acute/subacute: higher frequency of fever and recurrent episodes More recurrent systemic symptoms (chills, body aches)	Recurrent BFL: cough and mild exertional dyspnea, low-grade fever Insidious BFL: progressive dyspnea; clubbing
Lung function tests	Mild restrictive abnormalities that resolve Airflow obstruction (usually mild) seen in chronic disease	Restrictive pattern Hypoxemia at rest or exercise common
Lung imaging studies	Chest X-ray: frequently normal HRCT: ground glass opacities, predominating in the lower lobes, fine nodular shadowing Most frequent long-term sequelae: mild emphysema often sparing the upper parts of the lung	Chest X-ray: frequently abnormal HRCT: irregular reticular opacities, traction bronchiectasis and honeycombing superimposed to subacute changes (e.g., ground-glass opacities or nodules)
BAL	Neutrophilia Lymphocytosis (> 50%) with decreased CD4/CD8 ratio (< 1)	Eosinophilia or neutrophilia Lymphocytosis (< 50%) with increased (> 1.0) CD4/CD8 ratio
Lung biopsy	Small, poorly-formed noncaseating granulomas located near bronchioles Peripheral airways: proliferative bronchiolitis obliterans, characterized by fibroblast proliferation, and an organizing intraluminal exudate that occludes bronchioles from within	Ill-formed granulomas (may be difficult to identify) Fibrotic pattern: NSIP-pattern or UIP-like pattern. Peripheral airways: constrictive bronchiolitis
Outcome	Usually resolves Chronic exposure may lead to chronic bronchitis or emphysema	Poor, often progress to fibrosis

Definition of abbreviations: BAL = bronchoalveolar lavage; BFL = bird fancier's lung; HP = hypersensitivity pneumonia; HRCT = high-resolution computed tomography; NSIP = nonspecific interstitial pneumonia; UIP = usual interstitial pneumonia.

Data from References 4, 36, 38, 42, 77.

ACUTE HP

Acute HP is characterized by an influenza-like syndrome occurring a few hours after a (usually) substantial exposure. Symptoms gradually decrease over hours/days but often recur with reexposure. Acute episodes can be indistinguishable from an acute respiratory infection caused by viral or mycoplasmal agents. In farmers, the differential diagnosis must include the organic dust toxic syndrome, which is usually associated with unloading silos. Occasionally, respiratory symptoms in acute HP are mild or absent, and the disease can behave as a nonspecific febrile disorder. Furthermore, acute and subacute HP can be associated with wheezing, bronchial hyperresponsiveness, and a normal chest radiograph. In these cases, the differential diagnosis includes asthma, mainly in occupational settings. In general, the acute form is nonprogressive and intermittent, with spontaneous improvement after antigen avoidance. Importantly, some patients with recurrent acute episodes of farmer's lung may develop an obstructive lung disease with centrilobular emphysema instead of fibrosis (37).

SUBACUTE HP

Subacute HP may result from repeated low-level exposure to inhaled antigens. It is characterized by an insidious onset of dyspnea, fatigue, and cough that develops over weeks to a few months. Patients may have fever mainly at the onset of the illness. The subacute form may represent patients with acute episodes in which respiratory symptoms are mild or absent and thus behaving initially as a nonspecific febrile disorder until respiratory symptoms become visible. In general, subacute HP is a progressive disease, with coughing and dyspnea becoming persistent. The differential diagnosis includes infectious pneumonia or noninfectious interstitial lung disease (ILD), such as sarcoidosis. Sarcoidosis is a multisystem disorder with protean clinical manifestations, which affects several tissues. Lymph nodes are usually involved, and in contrast to HP, lung granulomas are well formed and distributed in a lymphangitic pattern, which is a distinctive feature of this disease. Other disorders that should be considered in the differential diagnosis of HP include organizing pneumonia, nonspecific interstitial pneumonia (NSIP), lymphocytic interstitial pneumonia, and drug-induced lung disease.

CHRONIC HP

Unrecognized and untreated acute/subacute episodes may evolve to chronic HP. However, many patients with chronic HP have no recognizable acute episodes and present as a slowly progressive (insidious) chronic respiratory disease. This presentation is common in patients with bird antigen exposure. The clinical presentation is characterized by progressive dyspnea, cough, fatigue, malaise, and weight loss. Digital clubbing may be present and predicts clinical deterioration (1). Often, these patients develop progressive fibrosis, and in advanced forms the disease may mimic idiopathic pulmonary fibrosis (IPF) or fibrotic NSIP (38).

ACUTE EXACERBATIONS

Some patients with chronic HP may experience an accelerated respiratory deterioration with the presence of new bilateral ground-glass opacities on HRCT scan (39, 40). These patients usually require assisted ventilation and have a poor prognosis. Although the precipitating factors are unknown, acute exacerbation seems to occur mainly in smoker men with fewer lymphocytes and increased neutrophils in BAL fluids, with advanced fibrosis and worse pulmonary function at the time of diagnosis (39). Histology reveals organizing diffuse alveolar damage superimposed on fibrotic lung disease.

HRCT

Acute HP

HRCT is useful in separating the clinical forms of HP. HRCT may be normal in patients with symptomatic acute HP (41). When abnormal, the predominant findings are ground-glass opacities or poorly defined small nodules (42, 43). Diffuse areas of dense air-space consolidation may be associated with ground-glass opacities (43).

Subacute/Chronic HP

Because of the considerable overlap in clinical cases of subacute and chronic HP, the HRCT patterns are more variable. Ground-glass opacities or poorly defined small nodules are commonly found in subacute HP (Figure 2A). In addition, mosaic perfusion is observed in patients with extensive bronchiolar obstruction and is secondary to shunting of blood away from poorly ventilated regions of lung. Patchy areas of air trapping on expiratory scans, often in a lobular distribution, and representing indirect signs of small airways obstruction, are seen in subacute and chronic HP (Figures 2B and 2C) (43, 44).

Distinctive HRCT findings in chronic HP are the combination of reticular, ground-glass, and centrilobular nodular opacities associated with signs of "fibrosis" (i.e., interlobular septal thickening, lobar volume loss, traction bronchiectasis, and honeycombing) (Figure 3) (42, 45). The reticulation can have a predominantly subpleural or peribronchovascular distribution but often tends to spare the lung bases. The HRCT findings in chronic HP may mimic those of IPF. The features that best differentiate chronic HP from IPF and NSIP are the presence of lobular areas with decreased attenuation and air trapping, centrilobular nodules, and the lack of lower zone predominance (45). Patients with IPF are more likely to have basal predominance with honeycombing compared with those with chronic HP.

A small percentage of patients with subacute and chronic HP show thin-walled cysts, usually in areas of ground-glass attenuation, resembling those observed in lymphocytic interstitial pneumonia (45, 46). Furthermore, some patients with chronic farmer's lung, including lifelong nonsmokers, are more likely to develop emphysema than fibrosis (37). Interestingly, combined

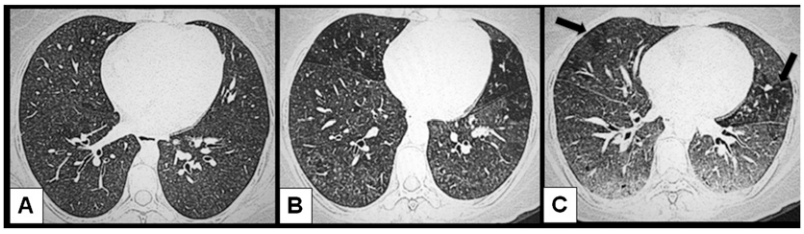

Figure 2. (*A*) A 40-year-old woman exposed to birds. High-resolution computed tomography (HRCT) scan obtained through lower lungs shows numerous ill-defined nodules. (*B*) A 53-year-old woman exposed to birds. HRCT images show patchy ground-glass opacities, ill-defined nodules, and patchy areas of mosaic perfusion. (*C*) Same patient as in *B*. Expiratory image demonstrating the prominence of the attenuation differences supporting the presence of air trapping (*arrows*).

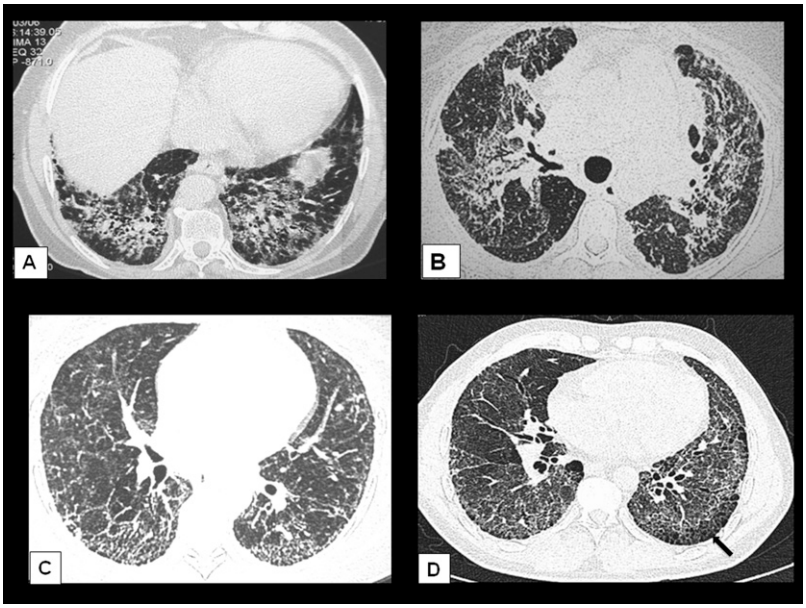

Figure 3. Chronic hypersensitivity pneumonitis. High-resolution computed tomography scans obtained in four different patients (*A*) Bronchiolocentric septal thickening, patchy areas of ground-glass opacities, and consolidation with traction bronchiectasis and architectural distortion. (*B*) Irregular reticular opacities with traction bronchiectasis and architectural distortion with a central distribution. Areas of ground-glass opacities are also present. (*C*) Subpleural predominant distribution of scattered nodules, ground-glass and reticular opacities, and traction bronchiectasis. (*D*) Irregular reticular and ground-glass opacities with architectural distortion, traction bronchiectasis, and honeycombing (*arrow*) in a peripheral distribution simulating the UIP-like pattern. Scattered ill-defined nodules are also present.

pulmonary fibrosis and emphysema, a pathological process described widely in IPF, has also been reported in chronic HP that morphologically has a usual interstitial pneumonia (UIP)-like pattern (47).

PULMONARY FUNCTION TESTS

In acute HP, lung function may be normal (41). However, abnormal lung function is common in most patients characterized by a restrictive functional pattern with decreased capacities and compliance and moderate to severe reduction of the carbon monoxide diffusing capacity (D_{LCO}). Hypoxemia is common; however, patients with mild/moderate disease may be normoxemic at rest, but develop hypoxemia with exercise. Importantly, these abnormalities are neither specific nor diagnostic for HP because similar changes are found in most ILDs. Thus, the importance of pulmonary function tests is to determine the severity of the physiologic impairment at diagnosis and during follow-up (1). Serial follow-up lung function studies in chronic HP are scanty. In some patients with farmer's lung, a common functional impairment can be an obstructive defect with decreased flow rates resulting from emphysema.

BAL

BAL is a highly sensitive method to detect lung inflammation in a patient suspected of having HP. An increase in the total cell count with a remarkable elevation in the percentage of T lymphocytes, often over 50%, characterizes HP (Figure 4A). This increase is unusual in other diseases generally considered in the differential diagnosis, such as IPF (48, 49). However, in patients with HP who are smokers or have chronic, fibrotic parenchymal abnormalities, the BAL lymphocyte count is lower. An increase in BAL lymphocytes may be found in asymptomatic exposed individuals, which may represent a "normal" inflammatory response or the presence of a low-intensity alveolitis without clinical consequences (50).

The evaluation of $CD4^+$ and $CD8^+$ T-cell subsets is not recommended for clinical practice, because a growing body of evidence has shown that these subsets and the $CD4^+/CD8^+$ ratio diverge according to a number of situations, including the type of inhaled antigen, the intensity of exposure, the smoking habit, and the clinical stage (1, 31, 49).

Small numbers of B-lymphocytes, plasma cells, and mast cells, and high levels of immunoglobulins M, G, and A and immunoglobulin-free light chains are also found in BAL fluids, mainly when BAL is performed a few days after the last antigen exposure (51, 52). It has been suggested that an increase in mast cells may distinguish HP and cryptogenic organizing pneumonia from a number of other ILDs, although validation studies are insufficient (53). An increase in BAL neutrophils may also be observed in patients with acute HP.

The proteomic differences in BAL fluids of patients with HP showing UIP-like or NSIP-like patterns have been evaluated (54). Surfactant protein A, Ig heavy chain α, heat shock glycoprotein, haptoglobin β, and Ig J chain were significantly higher in the patients with UIP pattern, whereas glutathione s-transferase, vitamin D–binding protein, and β-actin were significantly higher in the patients with NSIP pattern. Diagnostic and pathological consequences of these findings are presently unknown.

HISTOPATHOLOGY

Patients with acute HP rarely undergo biopsy. A retrospective study of selected cases of acute HP showed interstitial inflammation in a peribronchiolar pattern, loose histiocytic aggregates, prominent increase of interstitial neutrophils, and fibrin deposition (55). In some cases intraalveolar fibrin accumulation was marked, consistent with acute fibrinous and organizing pneumonia.

Subacute HP, independent of the etiologic agent, is characterized by a granulomatous interstitial bronchiolocentric pneumonitis. The inflammation is composed mainly of lymphocytes, with fewer plasma cells and histiocytes, and only occasional eosinophils and neutrophils (Figure 4B) (56). Typically, the granulomas are small, nonnecrotizing, poorly formed, and loosely arranged (Figure 4C). Associated lymphoid hyperplasia in the form of peribronchiolar lymphoid aggregates is present in most patients (56). With the exception of patients with hot tub lung, well-formed granulomas are uncommon. Isolated multinucleated giant cells containing various nonspecific cytoplasmic inclusions are usually seen. Importantly, granulomatous features may be absent in as many as 30% of surgical lung biopsies from patients with HP (56). In this context, detection of microgranulomas may improve by staining with cathepsin K, a cysteine protease expressed at high levels in activated macrophages and

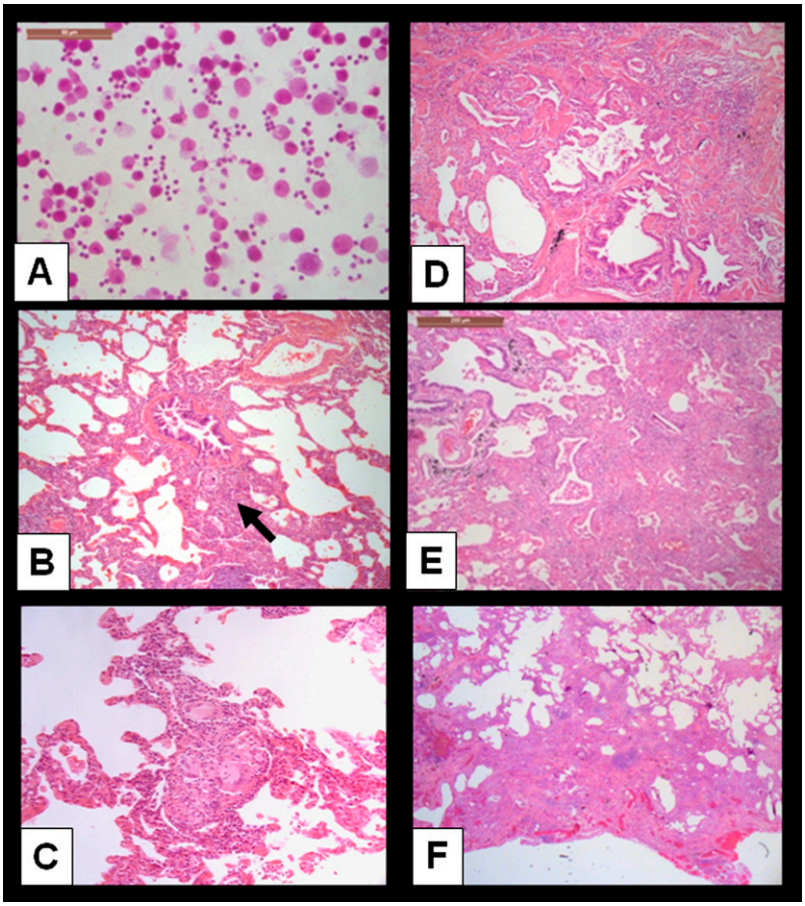

Figure 4. (*A*) Bronchoalveolar lavage of a patient with subacute hypersensitivity pneumonitis (HP) showing a marked increase in lymphocytes. (*B*) Photomicrograph (hematoxylin and eosin [H&E]) of surgical lung biopsy from same patient illustrated in Figure 2A, showing two key histopathologic features of HP: the lymphocytic interstitial pneumonitis and a poorly formed granuloma around a small airway (*arrow*). (*C*) High magnification photomicrograph of a typical interstitial HP granuloma. (*D*) Chronic HP: Photomicrograph (H&E) of surgical lung biopsy showing fibrosis, architectural remodeling in peribronchiolar pattern. (*E*) Chronic HP: Photomicrograph (H&E) of surgical lung biopsy showing architectural remodeling with chronic inflammation, giant cell with a cholesterol cleft and a distinct centrilobular fibrosis. (*F*) Chronic HP: Photomicrograph (H&E) of surgical lung biopsy showing fibrosis, architectural remodeling with septal and subpleural fibrosis as seen in usual interstitial pneumonia (UIP) but without the honeycombing and fibroblastic foci required for diagnosis of UIP.

epithelioid cells. Intense expression of cathepsin K was found in epithelioid and giant cells in all cases containing granulomas, including HP, whereas diseases characterized by large collections of alveolar macrophages, such as desquamative interstitial pneumonia and respiratory bronchiolitis-ILD, were negative (57). This finding suggests that cathepsin K may represent a sensitive and specific marker to detect granulomas, mainly in chronic HP.

Chronic HP presents with fibrotic changes and architectural distortion superimposed on subacute changes (Figures 4D–4F). The pathological patterns may mimic UIP-like pattern, NSIP, organizing pneumonia, or airways-centered interstitial fibrosis (58, 59). The UIP-like pattern includes patchy fibrosis, subpleural honeycombing, and fibroblast foci. Occasionally these lung specimens may lack typical subacute changes and can be indistinguishable on pathologic grounds from idiopathic UIP (58–60). Histopathological evidence supporting HP includes bronchiolocentric accentuation of the inflammation, peribronchial fibrosis, bronchiolar epithelial hyperplasia, and the presence of granulomas or multinucleated giant cells (often containing cholesterol clefts) (56, 58–60). Peribronchiolar metaplasia is frequent in HP and occurs in a minority of patients with UIP/IPF (60). In an autopsy study, centrilobular fibrosis involving peribronchiolar alveolar ducts with extensions into the perilobular areas giving the appearance of bridging fibrosis distinguished chronic HP from IPF (61).

Although histologic changes associated with HP are relatively uniform in distribution, infrequently, lung biopsy shows discordant findings that included typical HP findings in one specimen and UIP-like pattern or nonspecific fibrotic changes in others (60). This observation indicates that, as in IPF, biopsy should be taken from two different lobes.

Cigarette smokers with chronic fibrotic HP may also have emphysematous lesions (61), and, importantly, some of them may also develop lung cancer, primarily squamous cell carcinoma (62). In general, lung cancers were observed in patients with a UIP-like pattern, and the tumors were located mainly adjacent to honeycomb changes.

A recent study reported a curious finding of the coexistence of histopathological features of HP and pulmonary alveolar proteinosis (63). The HRCT appearances were varied and the linkage between both (if any) is unclear.

ANTIGEN DETECTION, SPECIFIC ANTIBODIES, AND T-CELL CHALLENGE TESTING

Specific circulating antibodies are evidence of sensitization but not of disease and should be seen as a marker of exposure. However, a positive test, in the appropriate clinical setting, supports the diagnosis of HP. False-negative results may be seen in acute and chronic HP cases. Several serological techniques, including electrosyneresis, enzyme immunoassay, and fluoroenzymeimmunoassay, have been successfully used to detect HP antigens (64, 65). Peptide nucleic acid–fluorescence *in situ* hybridization (PNA-FISH) and DNA-FISH assays were found useful for detection of *M. immunogenum* and of *Pseudomonas* in sputum (66). IgG antibody against avian antigens, quantified by fluorometry, provided a good discriminator of disease. Levels below 10 mg/L were insignificant, whereas increasing titers were associated with disease (67). Increasing antibody titer reflected the likelihood of HP, and decreasing titers confirmed antigen avoidance.

Because it can be difficult to reveal specific antibodies in a number of patients with chronic fibrosis, it has been proposed

that the evaluation of the proliferation indices of peripheral blood mononuclear cells stimulated with the specific antigen can be used for diagnosis purposes (68). However, experience with this test is scanty.

INHALATION CHALLENGE

A natural challenge at the workplace or home, or a "provoked" inhalation challenge under standardized conditions after a period of avoidance, can recreate the symptoms and laboratory and functional abnormalities of a mild/moderate acute episode. Typically, a positive challenge is characterized by cough and dyspnea, fever, and decrease of FVC and oxygen saturation a few hours (8–12 h) after exposure. Because the magnitude of the attack is unpredictable, the patients should be monitored closely for at least 24 hours. If the inhalation challenge is positive it can confirm, in the appropriate clinical setting, the diagnosis of HP, although false-negative results may occur (69, 70). However, because of a lack of standardized antigens (imprecise mixtures of antigen and nonspecific irritants) and challenge techniques and given the risk of a severe attack, the challenge should only be performed in selected patients by qualified personnel in specialized centers with experience with this procedure (1).

DIAGNOSTIC DILEMMA

HP represents a diagnostic challenge because of the absence of any unique features that distinguish it from other ILDs. The diagnosis of HP relies on a high level of clinical suspicion, the recognition of antecedent antigen exposure, and a constellation of clinical, radiologic, laboratory, and pathologic findings. Importantly, several issues often delay or prevent the diagnosis of HP, including: failure to consider the diagnosis leading to inadequate questioning of the patient and family about potential exposures (direct or indirect); dismissal of the relationship between the exposure and the illness (in particular, presence of any history of water damage either in the home or work environment should be examined); presence of negative serum precipitins accepted as ruling out the possibility of HP; presence of normal lung function or chest imaging studies; absence of an offending antigen (seen in approximately 40% of the cases) leading to consideration of another ILD, in particular IPF or NSIP; transbronchial lung biopsy read as "negative" or "inadequate"; or incomplete evaluation of the lung biopsy, usually because subtle findings are assumed to be insignificant and not features of HP.

In general, the diagnosis depends on the clinical presentation and type of exposure (Table 2). Massive exposure and the presence of a flu-like syndrome with substantial improvement in a few hours/weeks can be quite helpful in the diagnosis acute HP. Patchy ground-glass opacities on HRCT scan and increased BAL neutrophils and lymphocytes are also important diagnostic clues.

The diagnosis of subacute/chronic HP is most troublesome. An algorithmic approach for the diagnosis of subacute/chronic HP is included in Figure 5. Evidence of exposure and specific serum antibodies, an ILD clinical behavior, BAL lymphocytosis, and ground-glass opacities, poorly defined centrilobular nodules, and mosaic attenuation and air trapping on HRCT scan are very useful findings supporting the diagnosis. Lung biopsy, if performed, shows a granulomatous interstitial bronchiolocentric pneumonitis. Recurrent chronic HP has similar findings to subacute HP, but reticular opacities superimposed to subacute changes on HRCT scan and fibrotic changes on lung biopsy are usually seen. Insidious chronic HP may lack subacute features and may represent an unsolvable diagnostic problem.

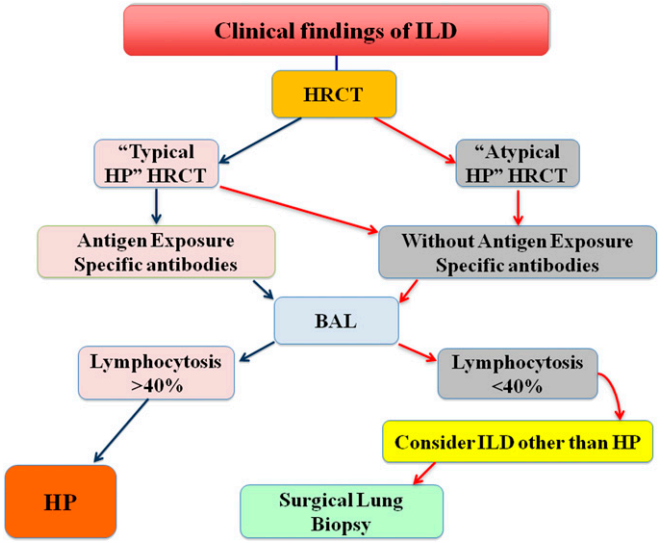

Figure 5. Algorithmic approach for the diagnosis of subacute/chronic hypersensitivity pneumonitis (HP). The algorithm takes into consideration two important initial findings for the suspicion of subacute or chronic HP, clinical and functional features of an interstitial lung disease (ILD), and the antecedent of exposure based in the history and the presence of specific antibodies. Both the exposure and circulating specific antibodies are necessary primarily in regions/areas with high prevalence of antigen exposure, for example in countries where keeping birds at home is a common hobby. Bronchoalveolar lavage (BAL) cellular analysis is not required in all cases; however, the presence of a "typical" high-resolution computed tomography (HRCT) scan and BAL lymphocytosis (*blue arrows*) makes the diagnosis of HP confident. Typical HRCT features include ground-glass opacities, poorly defined small nodules, mosaic perfusion, and patchy areas of air trapping on expiratory scans (*see* Figure 2). Any other combination makes the specific diagnosis more difficult and, in the absence of other diagnostic clues, lung biopsy is recommended (*red arrows*). Although transbronchial biopsy is often performed with BAL, it is uncommon for the biopsies to yield diagnostic features of HP; consequently, in the vast majority of cases, surgical lung biopsy is required.

TREATMENT

Early diagnosis and antigen avoidance are key actions in the management of HP. Although some patients may remit despite subsequent exposure, sustained antigen inhalation is associated with an adverse outcome in most cases.

Improvements in the industrial and agricultural work conditions are essential to reduce occupational exposure; sometimes moving the worker from exposure may be necessary. Also, it is important to minimize microbial or avian-antigen exposure by having a clean environment at home. Home and workplace inspections are useful to guarantee environmental control. The use of air-purifying respirators may be indicated for patients unable or unwilling to separate from the antigen to minimize exposure. However, patients usually complain of mask discomfort and refuse to use it for long periods.

Pharmacological therapy consists primarily of systemic corticosteroids, although their long-term efficacy has not been proved in prospective clinical trials. In patients with subacute disease, if antigen exposure is avoided, 3 to 6 months of prednisone can be enough for disease remission. However, in patients with subacute progressive and chronic disease, corticosteroids may need to be sustained for prolonged time. An empiric scheme may consist of 0.5 mg/kg/d of prednisone for 4 to 6 weeks followed by a gradual reduction until a maintenance dose of approximately 10 mg/d is reached (1). Complete withdrawal of corticosteroids

is recommended in the absence of clinical and/or functional response. Corticosteroids are also useful in NTM-related HP (i.e., hot tub), especially in severely affected patients. Antimycobacterial therapy does not appear to be required. Progressive lung scarring that characterizes chronic advanced HP has no effective therapy, and lung transplantation should be recommended.

Treatment of pediatric HP has been extrapolated from adults. In a small cohort of children, monthly courses of high doses of intravenous methylprednisolone were used (3). In addition, oral prednisolone was used in most cases, and according to severity, other immunosuppressive drugs such as azathioprine or cyclosporine were added. Most children improved, and no mortality was observed (3). Experience in adults, however, is scanty. Inhaled corticosteroids have occasionally been used to reduce the severe side effects of prolonged systemic steroid therapy; however, evidence of efficacy is lacking (1).

PROGNOSIS

There are few population-based studies regarding HP mortality. Data obtained from the National Center for Health Statistics multiple cause-of-death data files for the period 1980 to 2002 for US residents aged 15 years or older revealed an increase in HP mortality from 0.09 to 0.29 per million, although it is unclear what factors accounted for this increase (71). By contrast, the mortality rate was stable over a 40-year period (1968–2008) in England and Wales, although it increased over time in the older population (72).

In the clinical setting, most of our knowledge about outcome derives from research in pigeon breeder's disease or farmer's lung, but whether these observations are relevant to other causes is uncertain. In general, patients with acute disease, if correctly and timely diagnosed and treated, have a good prognosis, and patients usually improve. By contrast, patients with subacute/chronic HP (in particular those with bird fancier's disease) often progress to irreversible pulmonary fibrosis and may die within a few years after diagnosis (73). Actually, the finding of fibrosis at lung biopsy or HRCT scan indicates a poor prognosis (65, 66, 73–75). Moreover, patients with UIP-like and fibrotic NSIP patterns show a survival rate similar to that observed in IPF (73, 74). Pulmonary hypertension occurs in approximately 20% of patients with chronic HP and is associated with a greater risk of death (76).

Author disclosures are available with the text of this article at www.atsjournals.org.

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