Diagnosing Pulmonary Alveolar Proteinosis*
A Review and an Update

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Abbreviations: GM-CSF=granulocyte/macrophage colony-stimulating factor; HRCT=high-resolution CT; IPF=idiopathic pulmonary fibrosis; LDH=lactate dehydrogenase; PAP=pulmonary alveolar proteinosis; PAS=periodic acid-Schiff; PCP=Pneumocystis carinii pneumonia; SCID=severe combined immunodeficient; SP-A, SP-D=surfactant proteins A and D

Pulmonary alveolar proteinosis (PAP) was first described in 1958 as "a remarkable disease of the lung that consists of the filling of the alveoli by a periodic acid-Schiff (PAS)-positive proteinaceous material, rich in lipid." Since that original description, PAP has remained an uncommon but fascinating disease of uncertain etiology. Although the basic clinical and radiologic features of PAP can be indistinguishable from those of other respiratory disorders, certain features on high-resolution CT (HRCT) scanning of the chest strongly suggest PAP. An elevated serum lactate dehydrogenase level along with an elevated shunt fraction while breathing 100% oxygen can also be helpful in diagnosing PAP. More recently, measurement of levels of lung surfactant proteins A and D in both serum and BAL fluid in patients with PAP has been investigated.

Open lung biopsy has been the traditional means of making the definitive diagnosis of PAP, but BAL and transbronchial biopsies have largely supplanted this more invasive procedure, particularly in conjunction with CT scanning. In this review, we discuss the various approaches to making the diagnosis of PAP. In addition, we summarize recent experimental developments in the pathogenesis of PAP involving granulocyte/macrophage colony-stimulating factor (GM-CSF)-deficient and severe combined immunodeficient (SCID) mice. Management of PAP, which consists primarily of whole lung lavage, is not discussed herein but has been reviewed recently.

HISTORY AND CLINICAL FEATURES

PAP has been reported in a wide range of ages, from neonatal onset to a 72-year-old patient. Most of those affected are between 20 and 50 years of age. A genetic basis to some familial cases of PAP has been suggested. The male-to-female ratio in several series has ranged from 2:1 to 4:1. The primary symptom in patients eventually diagnosed as having PAP is shortness of breath with exercise. The other most common symptom is a mild cough, usually nonproductive, but occasionally with sputum described as "white and gummy" or "chunky" in consistency. Weight loss, malaise, and fatigue also can be present. Complaints of chest pain and hemoptysis are rare. Fever usually implies a superinfection or a different disease, although a low-grade fever is present in a minority of patients. Physical exam findings, from fine crackles in the affected areas of the lung to clubbing, occur in a minority of patients and are nonspecific as well.

Pulmonary alveolar proteinosis has been described in two forms: a "primary" or "idiopathic" form occurring in the absence of an identifiable associated disease or exposure, and a "secondary" form provoked by or associated with another condition. Secondary PAP has been described in several clinical settings that can be grouped into three main categories: (1) infections of the lung; (2) hematologic

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malignancies and other conditions altering a patient's immune status; and (3) exposure to inhaled chemicals and minerals.16

Historically, infection with Nocardia asteroides has been frequently noted in patients with PAP,19 although this association is made less frequently in recent years.20 Other reports have occasionally associated PAP with Mycobacterium tuberculosis infection.21,22 More recently, a high incidence of isolation of Mycobacterium avium-intracellulare was found in patients with PAP (eight of 19 subjects, or 42% culture-positive prevalence from therapeutic lavage fluid).23 Another group of investigators has noted an association of PAP with Pneumocystis carinii pneumonia (PCP) in both HIV- and non-HIV-immunosuppressed patients.24

Secondary PAP has also been associated with hematologic disorders such as lymphoma and leukemia, particularly myeloid disorders (eg, chronic myelogenous leukemia and acute myelogenous leukemia).25,26 PAP has also been reported in a few patients with HIV infection.27,28

The other important history to obtain is that of exposure to inorganic dusts or fumes, which can presumably lead to secondary PAP. Pathologic features characteristic of PAP have been reported to occur in humans after exposure to a number of inhaled substances, including silica,29 aluminum dust,30 insecticides,31 and titanium.32

Although primary and secondary PAP have a similar histologic appearance, there may be subtle differences. Singh and colleagues26 found that the intra-alveolar material from 17 patients with primary PAP (13 from lung tissue and four from BAL material) stained uniformly for surfactant-specific apoprotein by immunoperoxidase methods, whereas the staining was generally focal or weak in material from five patients having PAP associated with chemotherapy. Staining overlapped in some cases.

LABORATORY DATA

The most common abnormality among routine laboratory data is an elevation in the serum lactate dehydrogenase (LDH) level. The elevation of the LDH level is usually mild, approximately 25% above normal, but can be marked, up to two to three times normal. This elevation in serum LDH level in patients with PAP has been reported in 14 of 18 patients in one series2,33 and in 10 of 16 patients in another series.34 The serum LDH isoenzyme distribution in PAP is normal.34

Recently, serum levels of lung surfactant proteins A and D (SP-A and SP-D) have been found to be markedly elevated in patients with PAP as compared with levels in healthy volunteers.3,4,7 High levels of serum SP-A were also found in patients with idiopathic pulmonary fibrosis (IPF) as compared with only mild elevations in patients with pneumonia, tuberculosis, or panbronchiolitis. Serum SP-D was elevated approximately five- to seven-fold in patients with IPF, PAP, and interstitial pneumonia associated with various rheumatologic or collagen diseases. Thus, in patients in whom the diagnosis of PAP is being considered but in whom further testing such as bronchoscopy is not possible, measurement of serum SP-A or SP-D levels may help narrow the diagnosis to PAP or IPF in the appropriate clinical setting.

RADIOLoGY STUDIES

Routine chest radiographs in patients with PAP typically show bilateral air-space disease of an ill-defined nodular or confluent pattern, usually worse in the bases. An interstitial pattern can also be present. The abnormalities are often more pronounced in the perihilar regions, suggestive of the “butterfly” or “bat wing” appearance of pulmonary edema, except that other radiographic signs of left heart decompensation such as cardiomegaly, Kerley B lines, fissural thickening, and pleural effusions are absent (Fig 1). Lymphadenopathy is rarely present.13,16,35

The clinical and chest radiographic features of PAP can be indistinguishable from those of other
common pulmonary disorders, such as noncardiogenic pulmonary edema, pulmonary infection (bacterial, viral, fungal, or PCP), malignancy (in particular, bronchiolo-alveolar carcinoma in a patient with few symptoms), pneumocystis, sarcoidosis, and pulmonary interstitial diseases.

To further define the nonspecific chest radiographic findings in PAP, CT scanning, especially with high-resolution techniques, is used to characterize lung disease morphologic features. Routine chest CT scans in patients with PAP show air-space filling with a variable and patchy distribution. The air-space opacification is often sharply demarcated from surrounding normal lung tissue, creating a "geographic" pattern. HRCT scanning further shows that this opacification is often more of a "ground-glass" appearance, reflecting the presence of the phospholipid/proteinaceous material of PAP within the alveoli. Intralobular and interlobular septa typically show thickening, often in polygonal shapes that have been called "crazy-paving" (Fig 2). Following therapeutic whole lung lavage, HRCT scans show the decrease in alveolar filling and septal thickening (Fig 3). Although the patterns of PAP on HRCT are not necessarily pathognomonic—similar patterns can be seen in patients with PCP and sarcoidosis—the combination of the classic air-space and interstitial findings described above with the history and clinical presentation can be very suggestive of the diagnosis of PAP.

PULMONARY FUNCTION TESTING

The most common physiologic abnormalities in patients with PAP are mild pulmonary restriction, as indicated by mildly decreased functional lung volumes, and a reduction in the diffusing capacity for carbon monoxide, probably reflecting alveolar filling. This disorder does not cause airflow obstruction. The arterial Po2 and O2 saturation are characteristically reduced. Arterial pH is typically normal with a reduced PCO2 indicating a compensated respiratory alkalosis.13,15,16

The shunt fraction, while breathing 100% oxygen, is typically elevated in PAP. In a study by Martin and colleagues,2 12 patients with a diagnosis of PAP had an average shunt fraction while breathing 100% oxygen of 20%, significantly higher than a comparison group of 35 patients with other forms of diffuse lung disease who had an average shunt fraction of 8.9%.

SPUTUM ANALYSIS

In the 1960s, the diagnosis of PAP by sputum examination was suggested by Vidone and colleagues18 in their review of several cases in which the sputum contained PAS-positive material similar to the intra-alveolar material found on subsequent open lung biopsy specimens.19 They noted, however, that sputum examination could lead to false-negative conclusions. PAS-positive sputum, furthermore, was found in various other pulmonary diseases, including chronic bronchitis, bronchiectasis, pneumonia, and primary and secondary malignancies.

Expectorated sputum samples from three individuals with PAP were analyzed for SP-A in one recent report and found to have levels 400 times higher than those in control subjects.30 The 20 control subjects had other pulmonary diseases, specifically chronic bronchitis, bronchial asthma, emphysema,
IPF, pneumonia, or lung carcinoma. Because the authors did not specify how many of the control subjects had IPF and because serum levels of SP-A appear to be elevated in patients with either IPF or PAP, it is unknown whether sputum SP-A levels can distinguish IPF from PAP. Moreover, many patients with PAP have no cough or only a nonproductive cough, thereby severely limiting the usefulness of sputum analysis to diagnose PAP. 13,20

**Bronchoalveolar Lavage**

BAL typically yields a “milky” or “muddy” effluent. The differential cell count of BAL fluid does not appear to be helpful in making the diagnosis of PAP, as both macrophage predominance37 and lymphocyte predominance38 have been reported in small series of patients. The CD4/CD8 ratio among the lymphocytes in the BAL fluid was similarly variable with both low and high ratios found.38 Under light microscopy, the BAL fluid contains large amounts of amorphous, lipoproteinaceous material that is characteristically eosinophilic, granular, and brightly positive with a PAS stain (Fig 4).34,37 Ultrastructurally, the BAL sediment is identical to the intra-alveolar material seen in biopsy specimens.

Electron microscopy of the intra-alveolar material reveals degenerating cell debris and osmophilic material that forms “myelin figures” similar to condensed surfactant (Fig 5).17,20 Electron microscopy can thus provide ultrastructural confirmation of the light microscopy diagnosis of PAP. However, this expensive and time-consuming process is not available to many clinicians and is unnecessary in most cases to establish the diagnosis.

Martin and colleagues33 developed and tested criteria to diagnose PAP in “segmental lavage” (BAL) fluid. Both experienced and general surgical pathologists were able to separate five cases of untreated PAP from 33 “control” subjects with other diseases. BAL fluid from five patients in remission (most previously treated by therapeutic BAL) were also distinguishable histologically, but there was a significant rate of false-positive diagnoses of “PAP in remission” among the control patients with other diseases. The authors conclude that PAP can be diagnosed reliably from BAL fluid if the histologic findings are considered together with the clinical setting.

Biochemical analyses of lavage fluid from patients with PAP have shown a preponderance of phospholipids and protein, consistent with large amounts of pulmonary surfactant within alveoli.40 Recently, immunologic studies performed on the lavage fluid from a small number of PAP patients showed a marked (10- to 50-fold) elevation in levels of SP-A and SP-D as compared with findings from normal volunteers.5,6 Another investigator reported the elevation of tumor markers, including carcinoembryonic antigen and CA 19-9, in the serum and BAL fluid from six patients with PAP.41 The specificity of these findings for PAP as opposed to other diffuse lung disorders remains to be determined. One recent study suggested that elevated pulmonary SP-D levels in BAL fluid, as opposed to levels in the serum, could be highly specific for PAP. In this study, the mean level of BAL fluid SP-D in nine patients with PAP was approximately 20-fold higher than that measured in 60 patients with sarcoidosis, 33 with IPF, and seven with interstitial pneumonia associated with various rheumatologic or collagen diseases.7

![Figure 4. Panel A: appearance in BAL fluid. Material in a cell block made from BAL sediment has the same histologic appearance as the intra-alveolar material found in biopsy specimens except for internalized airway inflammatory cells (hematoxylin-eosin, ×165). Panel B: smears of BAL fluid are rich in aggregates of acellular material interspersed with airway inflammatory cells (Papanicolaou stain, ×330).](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21743/ on 04/28/2017)
TRANSBRONCHIAL BIOPSY AND OPEN LUNG BIOPSY

The basic pathologic characteristic of PAP, as seen in tissue from transbronchial or open lung biopsy specimens, is the accumulation of granular, PAS-positive, lipoproteinaceous material within the air spaces of otherwise preserved alveolar lung tissue (Fig 6). This material contains small clefts and artifactual cracks that impart a “dry” appearance. Unless superinfection is present, there are very few inflammatory cells, including viable macrophages. Alveolar septa are normal except for reactive type II pneumocytes; secondary lobular septa may be thickened owing to reactive fibroblasts.

A retrospective review by Rubinstein and colleagues42 showed that the diagnosis of PAP was reliably established in five of six patients in whom transbronchial biopsy was performed. Transbronchial biopsy yielded inadequate material in one patient and was subsequently followed by open lung biopsy. In two cases, transbronchial biopsy specimens revealed the diagnosis of PAP, but the clinicians thought the diagnosis was not secure and these patients proceeded to have a confirmatory open lung biopsy.

Figure 5. Ultrastructural appearance of BAL fluid. Scanning magnification reveals large and small aggregates of cell debris, other proteinaceous material, and osmophilic surfactant vesicles (arrows). At high magnification, the characteristic lamellar structure of the surfactant vesicles is evident (inset) (original magnification, ×6,000; inset magnification, ×31,000).

Figure 6. Biopsy specimen appearance of PAP. The alveoli are filled with eosinophilic material that is usually finely granular but is occasionally condensed into larger irregular bodies. Alveolar septa characteristically show only slight reactive thickening or hyperplasia of type II pneumocytes (hematoxylin-eosin, ×82.5).
Thus, transbronchial biopsy specimens can effectively make the diagnosis of PAP and obviate the need for an open lung biopsy. The question of whether the diagnosis of PAP can be reliably made by BAL without transbronchial biopsy was not addressed specifically in this study as none of the patients of Rubinstein et al. had BAL performed strictly for diagnostic purposes.

**Recent Developments in the Possible Pathogenesis of PAP**

The origin of PAP remains unknown, but its pathogenesis is believed to involve excessive secretion and/or disrupted clearance of surfactant. More specifically, Claypool and colleagues have suggested that alveolar macrophages in PAP are defective in the processing and clearing of surfactant. Recently, it has been shown that experimental mice, deficient in the gene for GM-CSF, develop alveolar accumulations of surfactant substances similar to that seen in PAP. The absence of macrophage activation for surfactant clearance by locally synthesized GM-CSF could be the mechanism involved. Other recent reports have shown the spontaneous development of lung surfactant accumulations, similar to that seen in human PAP, in SCID mice. Nonfunctional T and B lymphocytes in SCID mice may affect alveolar macrophage surfactant clearance or alveolar type II pneumocytes directly. These murine models may prove to be useful in determining the pathogenesis of PAP, and in turn lead to new diagnostic and therapeutic interventions.

**Conclusion**

The diagnosis of PAP can be difficult to make. Symptoms and physical signs are nonspecific, often leading to substantial delay in making the correct diagnosis. Routine laboratory data are usually unremarkable, with the exception of a mild elevation in the LDH level in most but not all cases. Pulmonary function testing typically reveals a decreased diffusing capacity for carbon monoxide arterial Po2 with mild restriction but no specific pattern of abnormality. An elevated intrapulmonary shunt fraction while breathing 100% oxygen is usually present. Routine chest radiographs are usually abnormal but not specific. HRCT scan findings, while not pathognomonic, can be suggestive of this specific disorder. Although the HRCT pattern can be similar to that found in certain cases of PCP and sarcoidosis, the clinical presentation can usually distinguish these entities.

Analysis of serum and/or sputum for levels of specific lung SP-A and SP-D can help narrow the diagnosis to PAP or IPF. Such tests, however, are currently experimental and their clinical value needs to be confirmed in larger populations.

At present, in our opinion, BAL alone is sufficient to diagnose most cases of PAP. Examination of the BAL fluid by light microscopy reveals the characteristic granular, amorphous PAS-positive lipoproteinaeous material. This intra-alveolar material can be confirmed to represent lung surfactant material by electron microscopy or specific immunochemistry methods, although these tests are probably unnecessary in most cases.

Actual lung tissue, obtained via transbronchial biopsy or open lung biopsy, remains the "gold standard" of diagnosis but is not necessary except in problematic cases. When several clinical features (eg, symptoms, laboratory test results, chest radiographs, and HRCT findings) suggest this diagnosis, BAL alone is generally sufficient to exclude other conditions and make the diagnosis of PAP. In the future, additional tests on the BAL fluid, particularly for the presence of specific pulmonary surfactant apoproteins such as SP-D, may further increase the specificity in diagnosing PAP.

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