Pulmonary Alveolar Proteinosis

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Abstract

The term pulmonary alveolar proteinosis (PAP) comprises a heterogeneous group of rare disorders characterized by abundant deposition of surfactant- and lipoproteins in the alveoli. The autoimmune form accounts for 90 % of cases and is characterized by the presence of GM-CSF autoantibodies. Secondary PAP is associated with several underlying conditions, mainly hematologic malignancies, infections and inhalation exposure, and is GM-CSF antibody negative. Several conditions can mimic PAP, in particular the radiological findings: the crazy paving pattern on high resolution computed tomography (HRCT) is common also to infections, neoplasms, and other interstitial lung diseases. Bronchoalveolar lavage (BAL) typical findings and the detection of serum GM-CSF antibodies are usually sufficient for the diagnosis of PAP. Whole lung lavage (WLL) is still the gold standard for treatment of PAP and is followed by complete remission in about 50 % of cases. Inhalative treatment with GM-CSF alone or in combination with WLL could represent the future approach for patients with autoimmune PAP refractory to WLL alone. The anti CD-20 antibody rituximab represents a further promising approach for autoimmune PAP. The treatment of secondary PAP should be focused on the underlying disease.
Keywords: *alveolar proteinosis, granulocyte-macrophage colony stimulating factor autoantibody, whole lung lavage.*

Rezumat

Proteinoza alveolară pulmonară
Termenul proteinoză alveolară pulmonară (PAP) cuprinde un grup heterogen de afecțiuni rare caracterizate prin depunerea abundentă de surfactant și lipoproteine în alveole. Forma autoimună reprezintă 90% din cazuri și este caracterizată prin prezența de autoanticorpi GM-CSF. PAP secundar este asociat cu mai multe condiții care stau la bază, în principal boli maligne hematologice, infecții și expunerea inhalatorie, și este anticorp GM-CSF negativă. Mai multe boli pot imita PAP, în special aspectele radiologice: modelul de „pavaj" pe tomografia computerizata de înaltă rezoluție (HRCT) este un aspect comun, de asemenea, în infecții, neoplasme și alte boli pulmonare interstițiale. Aspectul tipic al lavajului bronhoalveolar (BAL) și detectarea anticorpilor serici GM-CSF sunt de obicei suficiente pentru diagnosticul de PAP. Lavajul Pulmonar Total (LPT) este încă standardul de aur pentru tratamentul PAP și este urmat de remisiune completă în aproximativ 50% din cazuri. Tratamentul prin inhalare cu GM-CSF singur sau în combinație cu LPT poate reprezenta viitoarea abordare pentru pacienții cu PAP autoimună refractară la LPT singur. Anticorpii Anti-CD 20 rituximab reprezintă o abordare promițătoare viitoare pentru PAP autoimună. Tratamentul PAP secundară trebuie să se concentreze asupra bolii de bază.
Cuvinte-cheie: *proteinoză alveolară, granulocyte-macrophage colony stimulating factor autoantibody, lavaj pulmonar total*

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Running head: Pulmonary Alveolar Proteinosis: recent advances

1. Introduction

Pulmonary alveolar proteinosis (PAP) includes several heterogeneous disorders defined as alveolar filling syndromes. Primary autoimmune PAP is associated with the presence of antibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF), secondary PAP is associated with several underlying conditions. The primary and secondary forms are practically indistinguishable on radiology and histopathology. The primary and secondary forms have in common that they are disorders of impaired surfactant homeostasis.

In this review we illustrate the clinical features, pathogenesis, diagnosis of the different forms of PAP and other conditions mimicking PAP. The clinical management of the patients is also described, focusing specifically on whole lung lavage (WLL) and GM-CSF substitution therapy. Outcome and prognosis of the secondary forms are worse than of the primary form and are related to the underlying condition.

2. Definition, epidemiology and classification

PAP, first described in 1958 by Rosen and Castelman[1], is a rare diffuse parenchymal lung disease characterised by abundant accumulation of surfactant-derived phospholipids and lipoprotein components within the alveoli. PAP is a chronic disease leading to a progressive impairment of gas exchange and respiratory insufficiency. PAP is best viewed as a syndrome composed of a heterogeneous group of disorders[2].

The prevalence of PAP is currently estimated on the basis of about one thousand reported cases[3-7]. In Japan, due to the existence of a national registry, the prevalence of alveolar proteinosis has been estimated to be 6.2 per 1.000.000[4]. The median age at onset is 51 years in the Japanese cohort[4], 10 years older than reported in Caucasians[3,5,6,8]. A few cases have been reported in infants and children. The reported male to female ratio varies from 2:1[4,7] to 3:1[3], and smokers are predominantly affect-

ed (reported rate: 56-80%)[3,4,6]. Secondary PAP is more rare than the primary autoimmune form accounting for only 10 % of all reported cases[2,9,10].

The classification of PAP has emerged on the basis of the important progresses in our understanding of the pathogenesis (Table 1)[9,11].

Primary PAP disorders are caused by disrupted GM-CSF signaling. GM-CSF plays a critical role in the regulation of surfactant homeostasis, alveolar macrophages maturation, function, lung host defense, and innate immunity[9].

Autoimmune PAP is characterized by the loss of GM-CSF signaling due to the presence of neutralizing anti GM-CSF antibodies[9,12,13]. GM-CSF is essential for normal surfactant turnover by activating the alveolar macrophages and increasing their rate of surfactant clearance[14]. In-vivo and in-vitro data showed that GM-CSF binding to specific receptors on alveolar macrophages stimulates the terminal differentiation of the macrophages through the nuclear transcription factor PU.1[15]. This GM-CSF signaling is the critical process for the catabolism of surfactant by alveolar macrophages. It is likely that the anti-GM-CSF antibody is pathogenic in the development of the disease through its ability to inhibit the activity of endogenous GM-CSF, leading to a state of functional GM-CSF deficiency[14]. The summary of current evidence suggests that adult idiopathic PAP is an autoimmune disease caused by decreased availability of functional GM-CSF due to GM-CSF blocking activity of a neutralizing autoantibody[9,12,13].

This breakthrough has important implication for the treatment of PAP. The supplementation with exogenous GM-CSF is likely to be useful to treat only the GM-CSF antibody positive form, but not the other form of primary PAP, in which the loss of GM-CSF signaling is due to mutations in the α- or β-chain of the GM-CSF receptor.

Hereditary PAP occurs in neonates and children and is caused by mutations in genes encoding for the GM-CSF receptor[9,16] . The GM-CSF receptor β-chain plays a critical role in surfactant homeostasis in humans[9]. Hereditary PAP associated with absence of GM-CSF receptor β-chains on blood leukocytes was reported in infants presenting with respiratory failure[17]. A point mutation within CSF2RB encoding the GM-CSF receptor β-chain has been sporadically detected in children with PAP[18]. Hereditary PAP caused by abnormalities or absence of the GMCSF receptor α-chain has also been reported[16,19]. The largest series comprises 8 children with CSF2RA mutations, 6 of them being symptomatic[16]. This hereditary form of PAP presents as insidious, progressive dyspnea in children that can be treated successfully by whole lung lavage[16].

Secondary PAP develops in association with inhalation of dusts and fumes, with infections such as histoplasmosis, mycobacteriosis and pneumocystosis, with malignancies, particularly lymphoma and leukemia, and finally in association with immunodeficiency (Table 1). The pathogenesis of secondary PAP is poorly understood. The associated diseases presumably cause the syndrome by reducing either the number or certain functions of alveolar macrophages, thereby impairing alveolar-macrophage mediated surfactant clearance[20,21]. Another pathogenetic hypothesis is based on an

Table 1 Classification of pulmonary alveolar proteinosis syndromes according to the pathogenesis

Clinical type	Pathogenesis	
Primary PAP Autoimmune Hereditary	Impaired GM-CSF signaling: GM-CSF autoantibody GM-CSF receptor α/β chain mutations	
Secondary PAP	**Reduction in number and function of alveolar macrophages**	
Inhalation exposure	Inorganic dust	Aluminum Cement Silica Titanium Indium Tin
	Organic dust	Sawdust Fertilizer/agricultural dust Bakery flour
	Fumes	Synthetic plastic Gasoline
	Others	Varnish Chlorine Petroleum Cleaning products
Infections	Cytomegalovirus *Mycobacterium tuberculosis* Nocardia *Pneumocystis jiroveci* HIV	
Hematologic disorders	Myelodysplastic syndrome Acute lymphatic leukemia Acute myeloid leukemia Chronic myeloid leukemia Hairy cell leukemia Hodgkin's disease Non-Hodgkin's lymphoma Multiple myeloma Essential thrombocythemia Polycythemia vera Amyloidosis Fanconi's anemia	
Other malignancies	Adenocarcinoma Glioblastoma Melanoma	
Immunologic diseases	Monoclonal gammopathy Selective IgA deficiency Severe combined immunodeficiency	
Miscellaneous	Membranous nephrophaty Dermatomyositis Lung transplantation Lysinuric protein intolerance	
PAP-like diseases SP-B and SP-C mutations ABCA3 mutations NKX2-1 mutations	**Impaired surfactant production** SP-B and SP-C deficiency Abnormal surfactant Disrupted surfactant homeostasis	

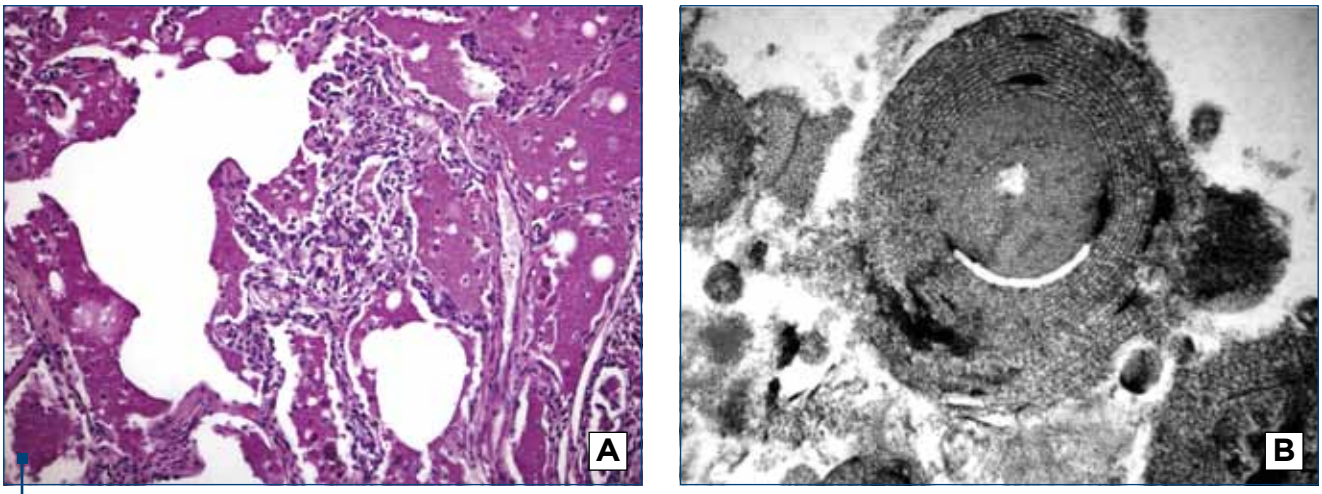


Figure 1: *Pathology of pulmonary alveolar proteinosis: (A) light microscopy shows intraalveolar eosinophilic material without inflammation or fibrosis (Haematoxylin-Eosin stain, 200x). (B) Characteristic myelin-like-multilamelled structures at tissue electron microscopy.*

acquired loss of GM-CSF signaling. In children with acute myeloid leukemia and PAP, the loss of GM-CSF stimulation of alveolar macrophage-mediated surfactant clearance was due to defective expression of the GM-CSF receptor[22].

Hematological disorders constitute 90% of all secondary PAP causes[10,23]. Among these, myelodysplastic syndrome (MDS) is the most frequent accounting for 65% of secondary PAP[10,23]. A reduction of the number of alveolar macrophages has been described as the most probable mechanism associated with the development of PAP in hematological malignancies and immunodeficiency[9,20,21].

With respect to dust and fume exposure, PAP developed in rats exposed to inhaled silica although the mechanism was not determined[24]. Patients with secondary PAP have been considered autoantibody-negative, primarily based on studies of the large cohort of Japanese patients[4]. However, the secondary cases in the Japanese cohort, which were all GM-CSF autoantibody negative, have been limited to those with hematologic or autoimmune comorbidity. A recent report by Cummings et al[25] about the occurrence of autoimmune alveolar proteinosis in indium workers supports the hypothesis that an inhaled agent may be the trigger for the development of autoimmune PAP. The mechanism by which dust exposure may induce GM-CSF antibody formation needs further investigation. Obviously it is essential to obtain a detailed occupational and environmental history in every patient newly diagnosed with PAP.

With regard to other forms of secondary PAP, lysinuric protein intolerance is a very rare disease caused by mutations in the SLC7A7 gene, mainly occurring in Finnish children[26]. PAP and interstitial lung disease represent the major cause of an unfavorable clinical course and fatal outcome[27].

Accurate diagnosis of secondary PAP is important since its prognosis is worse than that of autoimmune PAP[2,10]. Ishii et al observed a median survival time of only 20 months[10]. In our cohort of 70 patients we registered a death rate of 50 % in patients with secondary PAP most of them secondary to hematological disorders[5].

Several conditions are due to impaired surfactant production and can mimic PAP. PAP-like diseases include recessive mutations in the genes encoding for SP-B[28-30], SP-C[30-32], ABCA3[33-35] or Thyroid Transcription Factor Gene NKX2[36]. SP-B and SP-C deficiency caused by SP-B and SP-C mutations presents with a wide range of pulmonary manifestations, from unexplained acute respiratory failure in full-term neonates to extensive fibrosis with poor prognosis. ABCA3 is an integral membrane lipid transporter located on the limiting membrane of lamellar vesicles in alveolar type 2 cells. Alterations in the gene encoding ABCA3 result in various clinical presentations ranging from respiratory failure and death in neonates to interstitial lung disease in adolescents[9,33-35].

The recently described mutations in the gene encoding thyroid transcription factor, NKX2-1, result in neurologic abnormalities, hypothyroidism, and neonatal respiratory distress syndrome (RDS) that together are known as the brain-thyroid-lung syndrome[36,37]. Lung histopathology demonstrated evidence of disrupted surfactant homeostasis in the majority of cases[36].

3. Pathology

PAP belongs to the alveolar filling disorders, like alveolar hemorrhage or microlithiasis, and is characterized by alveolar spaces filled with a characteristic eosinophilic acellular, finely granular material that stains with periodic acid-Schiff (PAS) stain and is diastase-negative[38,39]. Lung involvement is usually diffuse, but sometimes a patchy distribution can be found. Typically there is little inflammation or interstitial fibrosis. Histopathologically, hyperplastic Type II pneumocytes, foamy macrophages, cholesterol clefts and ghost cells can be found (Figure 1A)[1,39]. On electron microscopy, the abnormal material consists predominantly of unusual tubular, myelin-like, multilamelled structures, which are similar to the tubular myelin found in normal lungs but without the intersecting membranes of normal tubular myelin (Figure 1B). Structures that relate to cell debris are also present. Lamellar bodies of normal lungs are only minor components[3,9,12].

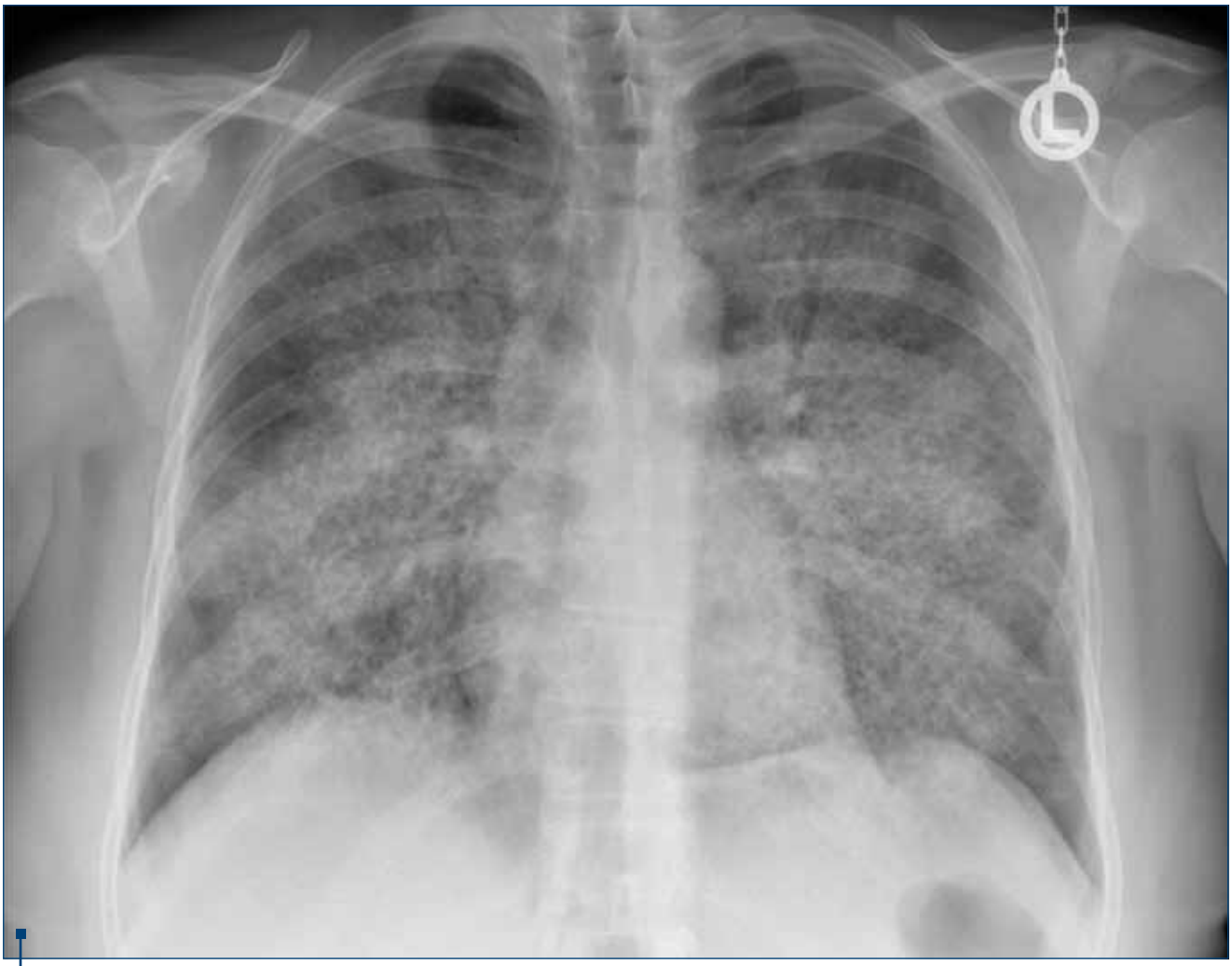


Figure 2: *Chest radiograph of pulmonary alveolar proteinosis: bilateral symmetrical alveolar opacities creating a "butterfly" appearance.*

Biochemically total phospholipids are increased in the BAL, with a relative decrease in phosphatidylcholine and phosphatidyIglycerol, and a relative increase in sphingomyelin and phosphatidylinositol. Surfactant proteins A, B and D are increased with a relative abundance of surfactant protein A isoforms[3,9,40].

4. Clinical presentation

The majority of patients (70-90%) presents with slowly increasing dyspnea on exertion and cough[4,41,42]. Less frequently (30-50%) fever, weight loss, fatigue and chest pain are seen. The physical examination may reveal inspiratory crackles and clubbing (15-20%). Cyanosis or evidence of cor pulmonale is rare (<5%)[3,4,41-43]. A Disease Severity Score (DSS) ranging from 1 (less severe) to 5 (most severe) based on the presence of symptoms and degree of reduction in PaO2, has been proposed to stratify the patients[4,44]. Its utility needs further investigations.

5. Diagnostic procedures

Radiology

The chest radiograph is not pathognomonic, but may be distinctive showing diffuse bilateral symmetrical alveolar infiltrates with air bronchograms. The shadowing may be cloudy and butterfly or batwing like, when a more prominent involvement of the perihilar regions occurs (Figure 2). Less commonly, unilateral infiltrates or a reticulonodular pattern may be seen. Lymphadenopathy and pleural lesions are rare. Kerley B lines are absent initially but may develop later. Cavitation has not been reported in noninfectious alveolar proteinosis[45,46].

The HRCT shows airspace filling in variable and patchy distribution (Figure 3). The distinctive features are: ground-glass opacities (GGO) sharply demarcated from normal lung, creating a 'geographical' pattern; GGO with intralobular lines and interlobular septal thickening, often in polygonal shapes, called 'crazy paving'; areas of consolidation with air bronchograms, surrounded by GGO.

Ishii et al recently compared HRCT scan findings between autoimmune PAP and secondary PAP[47]. Although the major HRCT scan finding was GGO in both in patients with autoimmune and secondary PAP, the appearance of the GGO was distinctive: a patchy geographic pattern of crazy paving with lower lung field predominance was typical for autoimmune PAP (71%), whereas a diffuse pattern with even distribution was more common in secondary PAP (62%). Some cases showed overlapping features[47].

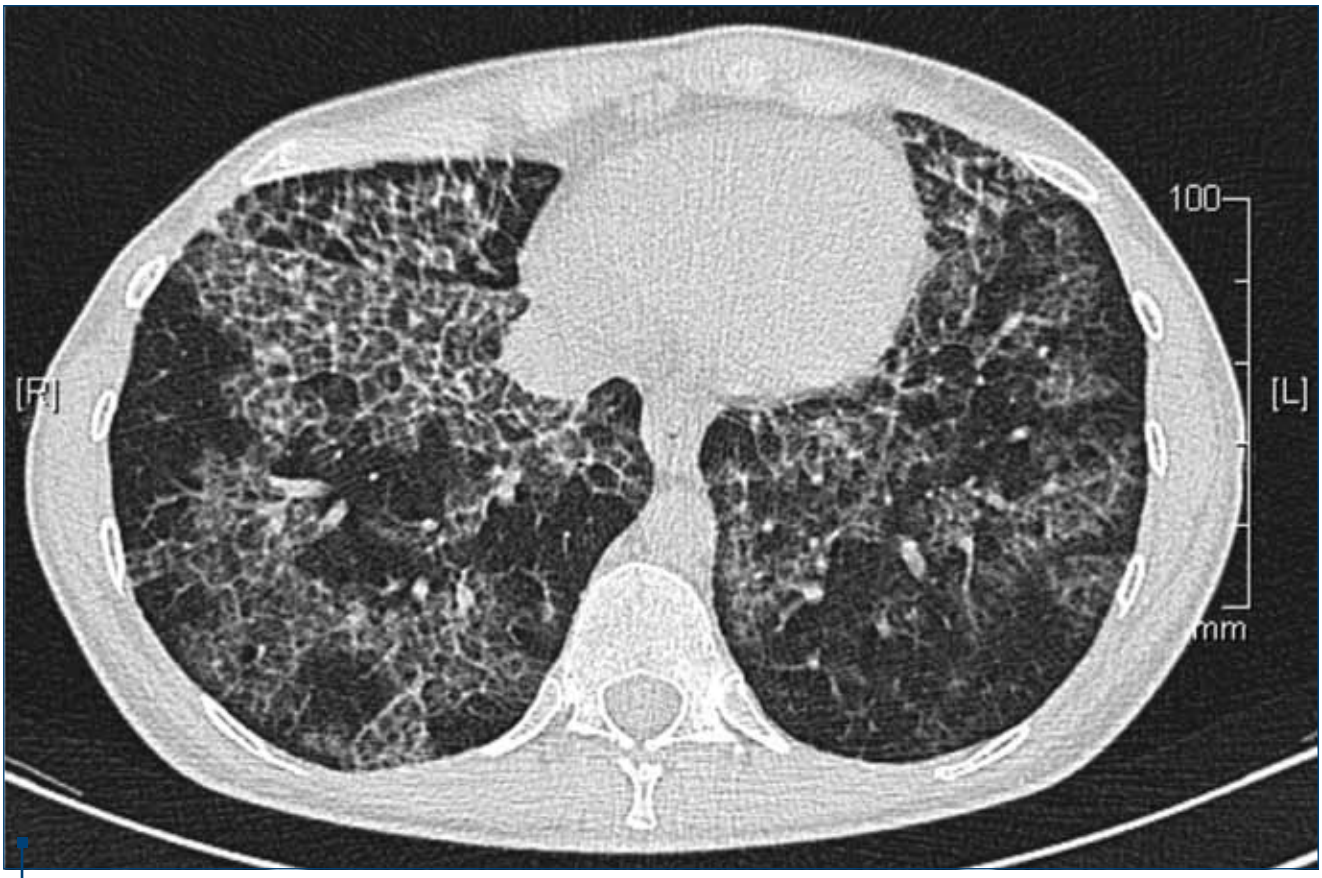


Figure 3: *High resolution computed tomography of pulmonary alveolar proteinosis: characteristic "crazy paving" pattern in a "geographic" distribution.*

The crazy paving pattern on HRCT of the lungs was initially described in cases of alveolar proteinosis. In reality, this pattern can be found in a variety of idiopathic, infectious, sanguineous, neoplastic and inhalational disorders of the lung (Table 2). Thus, the crazy-paving pattern is not specific for PAP[48,49]. Knowledge of the many conditions underlying this pattern can be useful in preventing diagnostic errors. Differences in the location of the opacities or attenuation in the lungs as well as presence of additional radiologic findings, together with a detailed history and clinical presentation, can often be useful in addressing the appropriate diagnosis.

One of the most frequent cases of PAP differential diagnostic in patients with haematological disorders is represented by *Pneumocystis jirovecii* pneumonia, presenting in severely immunocompromised patients[50,51]. Chest radiographs are normal in up to 18% of patients. The bilateral, perihilar reticular and poorly defined GGO often progress to alveolar consolidation in 3–4 days[52,53]. HRCT usually reveals scattered GGO that can be associated with interlobular septal thickening and rarely appear as crazy paving pattern[54,55]. Histological features underlying the ground-glass appearance include the foamy nature of the intra-alveolar exudates with alveolar filling, the infectious agents can be identified by special stains in BAL or through immunohistochemistry[48].

Bronchoalveolar lavage (BAL)

The diagnosis can usually be established by BAL, obviating the need for transbronchial or open biopsy in many instances[3,4,38]. In reference centres with large experience with BAL, PAP diagnosis through BAL has been reported in up to 74 % cases[5]. On gross examination, the BAL fluid has a characteristic milky appearance (Figure 4A). On light microscopy, the striking features are acellular globules that are basophilic on May-Grünwald-Giemsa and positive with PAS staining, few and foamy macrophages and large amounts of cell debris showing weak PAS staining (Figure 4B and C).

Electron microscopy is not usually required to establish the diagnosis but, if performed, the BAL sediment shows characteristic myelin-like-multilamelled structures, debris and foamy macrophages. When electron microscopy evidences the presence of inhaled crystals and particles, energy-dispersive X-ray spectroscopy (EDS), a technique used for the elemental analysis or chemical characterization of a sample can also be performed to identify the spectrum of elements included in the particle[25].

Lung function tests

Pulmonary function tests characteristically show a restrictive pattern and a reduced diffusing capacity[3], but not infrequently they can be normal. Hypoxemia at rest is present in about one-third and during exercise in more than one half of patients[4]. Hypoxemia is caused by ventilation–perfusion inequality and intrapulmonary shunting, resulting in a widened alveolar-arteriolar diffusion gradient[3]. Desaturation at rest and widening of the alveolar-arteriolar diffusion gradient are principal indications to perform whole lung lavage[56].

Table 2 Causes and frequency of crazy paving pattern on HRCT (modified from Bonella et al[11]).

Disease	Characteristics	Distribution	Frequency (%)*
Alveolar proteinosis	GGO opacities with geographical distribution of a crazy paving pattern	Variable, mostly distinguishable from healthy parenchyma	100
Acute interstitial pneumonia	GGO opacities, traction bronchiectasis, microcysts	Mostly symmetrical, basal, geographic.	31
Acute respiratory distress syndrome	Consolidation, GGO, geographical crazy paving, pleural effusion possible	Asymmetrical, mostly basal, dependent areas	21
Cardiogenic pulmonary edema	GGO, crazy paving, pleural effusion, cardiomegaly	Symmetrical, peripheral	14
Drug-induced pneumonia	GGO, crazy paving	Variable	12
Chronic eosinophilic pneumonia	GGO with crazy paving, sometimes mediastinal adenopathy	Bilateral, patchy, middle and upper lobe.	8
Organizing pneumonia	Peripheral parenchymal consolidations with air bronchogram with or without surrounding ground-glass-like opacities	Peribronchial, peripheral, mostly basal	8
Pneumocystis jirovecii pneumonia	GGO with geographical appearance of a crazy paving pattern, mediastinal adenopathy, pleural effusion possible	Perihilar, bilateral, symmetrical, mostly in the middle-upper lobe	7
Alveolar hemorrhage	GGOopacities or attenuation, crazypaving rare	Variable	n.a.
Bronchioloalveolar carcinoma	Consolidation or GGOopacities, pseudo-cavitation possible, pleural effusion possible.	Uni- or bilateral, patchy, asymmetrical, mostly peripheral and subpleural	n.a.

GGO= ground glass opacity

Laboratory blood tests

Serological diagnosis of PAP by demonstration of autoantibodies against GM-CSF has an excellent sensitivity and specificity for the autoimmune variant of primary PAP. Anti-GM-CSF antibodies can be detected in healthy individuals at very low levels (<3mg/ml or <1:400 by titer assay)[57] and in patients with malignancies[58], inflammatory conditions[59], or secondary alveolar proteinosis due to dust exposure[25]. The prognostic value of GM-CSF antibodies needs to be further investigated[4,5]

Serum lactate dehydrogenase (LDH) is increased in 82 % of PAP patients[3], with a normal isoenzyme pattern[60]. LDH has a rapid kinetic and has been found to reflect the dynamic changes in disease severity during treatment after therapeutic lavage or spontaneous resolution[3,61].

Elevation of serum and BAL tumor antigen biomarkers such as carcinoembryonic antigen (CEA) and CYFRA 21-1 may also reflect the severity of disease[62,63]. Serum levels of SP-A and SP-D can be increased and correlate with the severity of disease[61], but are not specific for PAP[40].

At present, the most promising diagnostic and prognostic biomarker for PAP is Krebs von den Lungen 6 (KL-6), a mucin-like glycoprotein used in Japan routinely to assess ILD severity[64]. Serum and BAL levels are extremely high in PAP, higher than in patients with other interstitial lung disease[65]. Recently, Inoue et al[4] reported a good correlation of KL-6 with the disease severity score in 284 patients with autoimmune PAP[4] and Bonella et al[56] found a predictive value of serum KL-6 for disease progression and to identify a group of patients needing repeated whole lung lavage.

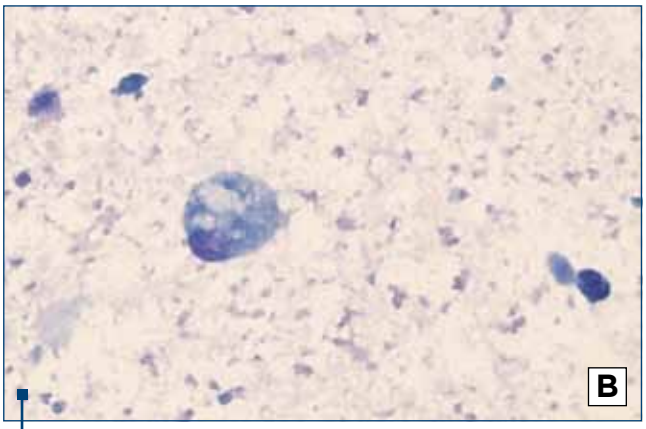
Serum levels of KL-6, SP-D, SP-A, and CEA are elevated to a similar degree in autoimmune and secondary PAP[4,10].

Diagnostic algorithm for PAP

Slowly developing dyspnea, a 'butterfly' pattern of acinar shadowing on the chest radiograph and characteristic findings on HRCT (crazy paving) pattern should be sufficient to suggest a diagnosis of PAP (Table 3). GM-CSFautoantibody testing, with a reported sensitivity and specificity for autoimmune PAP close to 100%[66] is essential to confirm the diagnosis of autoimmune PAP [6, 13, 57, 67-69]. Other serum biomarkers like LDH, CEA, CYFRA 21-1, SP-A, SP-D or KL-6 are not yet validated for diagnostic purposes. Bronchoscopy with bronchoalveolar lavage, cytological analysis and transbronchial biopsy should be performed once the diagnosis has been suspected on the basis of the radiological findings (Figure 5).The diagnosis is usually confirmed by the characteristic BAL findings but special stains and cultures should be performed to rule out infection by common and opportunistic microbial pathogens[38].

6. Clinical course and treatment of PAP

Spontaneous remission has been reported in 5-10 % of patients with PAP[3-6]. Treatment is indicated when respiratory symptoms impair the quality of life or when lung function deteriorates, but established criteria do not exist.



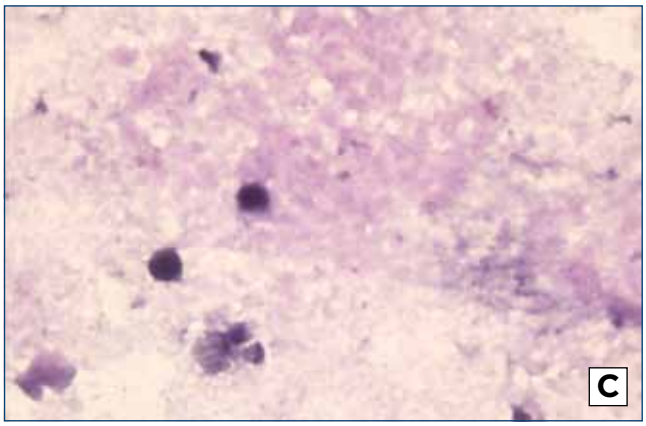


Figure 4: *BAL findings in PAP. Macroscopic appearance of BAL during a whole lung lavage (A). Microscopic aspects: (B) foamy macrophages and acellular particles on MGG staining, and (C) PAS positive staining.*

The treatment of choice is whole lung lavage (WLL), which is almost always effective [2, 70-72]. WLL is adopted as an institutional procedure in only a limited number of specialized clinical centers. The WLL technique is not standardized and attempts to draft WLL guidelines are ongoing[73]. Since its introduction in the 1960s by Ramirez et al[74], the technique has been improved through the application of manual or mechanical chest percussion[75], also in combination with postural changes[76]. After intubation with a double-lumen endobronchial tube, aliquots of warmed saline (usually of the tidal volume or 1 Liter) are infused in the nondependent lavaged lung; the dependent lung is ventilated. At the end of each aliquot infusion, the drainage limb is clamped. After passive recovery of the fluid over the drainage limb, the next washing cycle begins. Generally an instillation-drainage cycle takes up to 5 minutes and the recovery of the fluid should be at least 80%[71]. A further modification of the technique was introduced by Bingisser et al[77] in one patient and recently has been extensively studied by Bonella et al[71]. By applying manual ventilation with tidal volume about at half of the procedure a larger amount of proteins can be removed and the time between two WLL can be prolonged[71,77].

Clinically significant improvement in radiologic appearance, PaO_2, lung volumes and DLCO is seen in 84 % of patients following the first therapeutic lavage[3]. In secondary PAP WLL, although feasible, usually provides only transient benefit[70].

The treatment with exogenous GM-CSF, still considered experimental, has shown encouraging results in patients affected by autoimmune PAP. Two prospective, open-label, uncontrolled trials[78,79] and several anecdotal reports have shown that daily subcutaneous administration of recombinant human GM-CSF is effective in about 50 % of patients with autoimmune PAP. The administration of aerosolized GM-CSF seems to be more effective, as shown in a retrospective case series[80], and recently in a controlled prospective trial of 50 patients with a response rate of 62%[81]. It is unclear, whether the pretreatment blood levels of GM-CSF antibodies are able to predict a response to such treatment since two groups reported conflicting data[61,79,82].

A combined therapy with WLL and plasmapheresis is able to reduce the titer of GM-CSF antibodies[83,84], but the data on clinical efficacy are controversial[83,84].

B-lymphocyte depletion is also a promising option for autoimmune PAP[72]. Rituximab is a humanized monoclonal antibody that by binding CD20 selectively decreases the B-cell pool. In a open label proof-of-concept Phase II clinical trial Rituximab was administered intravenously (1 g in two Infusions fifteen days apart) in 10 patients with autoimmune PAP and high GM-CSF autoantibody titer[85]. A clinical amelioration, a consistent depletion of B cells and a reduction of GM-CSF neutralizing antibodies in BAL fluid were achieved[79].

Features	Specific	Nonspecific
Clinical findings	None	Dyspnea, fatigue, cough, chest pain
Chest radiograph	Butterfly pattern of acinar shadowing	Peripheral shadowing
HRCT findings	Ground glass opacification with geographical distribution of a crazy pattern	Reticulo-nodular pattern
BAL findings	Macroscopic: milky fluid; Cytology: foamy macrophages, PAS positive noncellular globules, large amount of cell debris	PAS negativity of noncellular material
Biomarkers	GM-CSF Ab positivity	LDH, CEA, CYFRA 21-1, SP-A, SP-D or KL-6 increased

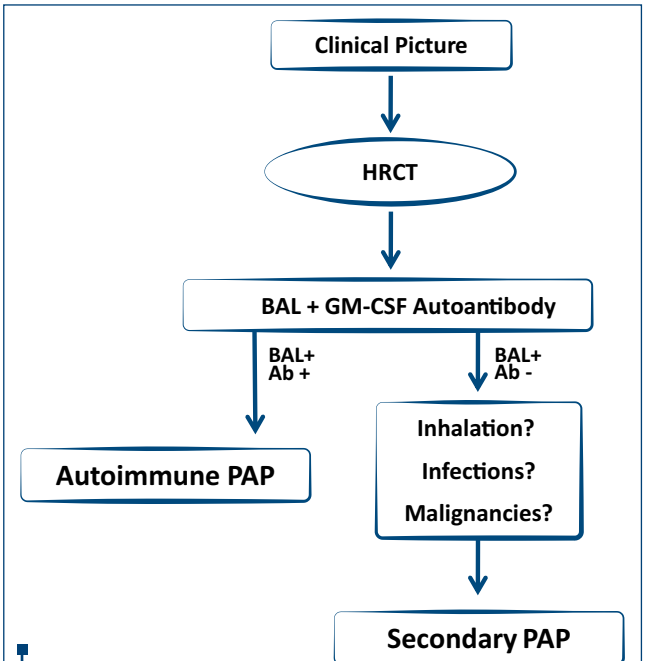


Figure 5: *Proposed algorithm for the diagnosis of PAP in adults.*

7. Outcome and prognosis

Prognosis of PAP has improved considerably with introduction of whole lung lavage. 25~50 % of patients achieve a remission after one WLL[4-6], but smokers require 2-4 whole lung lavage to reach remission[5].In the era of therapeutic lavage infectious complications like nocardiosis, cryptococcosis, mucormycosis have became rare. Nowadays is death an extremely rare occurrence.

Lung transplantation may be an option for patients developing progressive interstitial pulmonary fibrosis, although recurrence of PAP has been reported in one patient 3 years after double-lung transplantation[86].

For secondary PAP associated with hematologic malignancy, the prognosis is linked to the underlying disease and is generally worse than in autoimmune PAP [2, 5, 10, 23]. The probability of survival at two yearshas been reported to be 46% in cases with secondary PAP complicating hematological disorders[23].

8. The EuPAPNet project and the need of an international PAP register

Pulmonary Alveolar Proteinosis (PAP) is a neglected respiratory disorder. The EuPAPNet (European Network for PAP) project started in 2010 and was aimed at creating for the first time an international network for PAP, among four centers from three countries (Italy, Germany and the Netherlands) with special interest in this disease (for details see http://www.alveolarproteinosis.eu). The project was founded by the European Agency for Rare Disease (E-RARE project) based on cohorts already available in the centers, it is expected the enrollment of one of the largest series of PAP patients ever reported, in order to better understand about the epidemiology of the different forms of PAP in Europe.

Within the activities of this project the EuPAPNet database has been established, as a multi-institutional web-based system, to collect data to include retrospectively and prospectively cases of PAP from each EuPAPNet centre[5,6]. The aim is to create a database containing useful information to correlate clinical data with the analysis of biomaterial obtained from PAP patients. The overall perspective of the program is to gain more information about the clinical presentation and the natural history of patients with different forms of PAP in patients of all age groups, including neonates, children and adults.

In EuPAPNet project, patients have been added to a functional SNP-array, containing genetic variations with proven and presumed functionality, to identify genomic regions and genes in humans which provide candidates for PAP susceptibility; furthermore pediatric PAP forms have been identified and characterized genetically.

Through a proof-of-concept approach it has been found that some biomarkers, like serum KL-6 and YKL-40, are valid to predict outcome disease outcome and response to whole lung lavage in PAP[56].

Finally, as WLL is not yet a procedure standardised and is still suffering from lack of information, both clinically and technically, an international survey was launched, to answer many open questions related to lavage in PAP[73] . The questionnaire was not limited to “lavagers” perform-

ing WLL, but also to physicians performing lobar or segmental bronchoscopic therapeutic lavages.

In summary the transnational collaboration in EuPAPNet project is a unique opportunity to ensure a translation of basic research into clinical management of PAP patients, and in turn to create the basis for the implementation of the database in a European-wide basis. This would result in the organization of a European registry for PAP, with the aim of both improving the knowledge of the PAP epidemiology, and, most importantly, of creating awareness among pulmonologists about this disorder and its management.

9. Conclusion

Basic and translational research in the last decade has allowed an improvement in the diagnosis and classification of alveolar proteinosis disorders. The primary autoimmune form of PAP accounts for 90 % of cases and is characterized byincreased GM-CSF antibody serum levels. Whereas WLL is still the treatment of choice, the discovery of the pathogenetic role of GM-CSF and its antibodies has provided the basis for the experimental therapy with exogenous GM-CSF for autoimmune PAP. Additional studies are needed to define clinical and radiological features as well as pathogenetic mechanisms in secondary PAP. ■

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