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Review

Pulmonary alveolar proteinosis: Clinical and morphological overview of a rare disease associated with macrophage dysfunction

Brigita Javorská[1], Róbert Slivka[1], Barbora Durcová[1,2], Adela Vrbenská[3], Jozef Škarda[4], Janka Vecanová[5], Natália Hvizdošová[5], Mária Makovická[6,7], Vojtěch Kamarád[6] and Jozef Muri[6,8]

[1] *Second Department of Pneumology and Phthisiology, National Institute for Tuberculosis, Lung Diseases and Thoracic Surgery in Vyšné Hágy, Vysoké Tatry, Slovakia*
[2] *First Department of Pneumology and Phthisiology, National Institute for Tuberculosis, Lung Diseases and Thoracic Surgery in Vyšné Hágy, Vysoké Tatry, Slovakia*
[3] *Department of Pathology, National Institute for Tuberculosis, Lung Diseases, and Thoracic Surgery in Vyšné Hágy, Vysoké Tatry, Slovakia*
[4] *Institute of Clinical and Molecular Pathology and Medical Genetics, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic*
[5] *Department of Anatomy of the Faculty of Medicine of Pavol Jozef Šafárik University in Košice, Slovakia*
[6] *Institute of Histology and Embryology, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic*
[7] *Cancer Research Institute, Biomedical Research Centre of the Slovak Academy of Sciences, Bratislava, Slovakia*
[8] *Centre for Thoracic Surgery, National Institute for Tuberculosis, Lung Diseases, and Thoracic Surgery in Vyšné Hágy, Vysoké Tatry, Slovakia*

Abstract. Pulmonary alveolar proteinosis (PAP) is a rare disease characterised by excessive accumulation of surfactant components in alveolar macrophages, alveoli, and peripheral airways. The accumulation of surfactant is associated with only a minimal inflammatory response but can lead to the development of pulmonary fibrosis. Three clinical forms of PAP are distinguished – primary, secondary and congenital. In recent years, significant findings have helped to clarify the ethiology and pathogenesis of the disease. Apart from impaired surfactant protein function, a key role in the development of PAP is played by signal pathway of granulocyte and macrophage colonies stimulating growth factor (GM-CSF) which is necessary for the functioning of alveolar macrophages and for surfactant homeostasis. Surfactant is partially degraded by alveolar macrophages that are stimulated by GM-CSF. The role of GM-CSF has been shown especially in primary PAP, which is currently considered an autoimmune disease involving the development of GM-CSF neutralising autoantibodies. Clinically, the disease may be silent or manifest with dyspnoeic symptoms triggered by exertion and cough. However, there is a 10 to 15% rate of patients who develop respiratory failure. Total pulmonary lavage is regarded as the standard method of treatment. In addition, recombinant human GM-CSF has been studied as a prospective therapy for the treatment of PAP.

Key words: Pulmonary alveolar proteinosis — Surfactant — Macrophages — GM-CSF signalling

Correspondence to: Jozef Muri, Institute of Histology and Embryology, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic
E-mail: muri.jozef@gmail.com

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Abbreviations: ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid; CSFR, colony-stimulating factor receptor; CT, computed tomography; GGO, ground glass opacity; GM-CSF, granulocyte and macrophage colonies stimulating growth factor; HRCT, high resolution computed tomography; PaO2, partial pressure of oxygen; PAP, pulmonary alveolar proteinosis; PAP-PF, pulmonary alveolar proteinosis associated pulmonary fibrosis; PAS positivity, periodic acid Schiff positivity; SP, surfactant protein; TLCO, carbon monoxide transfer factor; TTF1, transcription termination factor 1; WLL, whole-lung lavage.

Introduction

Pulmonary alveolar proteinosis (PAP) is a rare disease characterised by excessive accumulation of surfactant components in the respiratory section of the lungs, alveoli, and alveolar macrophages. This excessive surfactant accumulation is associated with a minimal inflammatory response but can lead to the development of pulmonary fibrosis. The disease leads to impaired respiratory gas exchange and the gradual development of restrictive ventilatory disorder, with variable clinical manifestations, ranging from asymptomatic cases with mild reduction in lung diffusion capacity and spontaneous regression to severe courses with respiratory failure (Seymour and Presneill 2002). PAP was first described by Rosen and colleagues in 1958. The disease incidence is worldwide, regardless of gender. The prevalence is reported to be between 3.7 and 40 cases *per* million population. Smokers account for 53–85% of PAP patients. The incidence of the disease may be underestimated, mainly due to its gradual onset and non-specific respiratory symptoms. The incidence of PAP increases with age, with two peaks – the first between 45–54 years and the second in patients over 75. Literature data confirm the important role of aging and oxidative stress in the pathogenesis of lung diseases, not excluding PAP. Cells change their phenotype and secretome with age, referred to as SPRA (secretory phenotype related to ageing). This may lead to a reduced ability of macrophages to migrate, to a weakening of their phagocytic activity or to their unfavourable metabolic reprogramming. Age is also associated with the possibility of prolonged exposure to toxic nox. Numerous oxygen free radicals can be generated by external noxious stimuli, leading to an imbalance in the redox state, which can directly or indirectly induce respiratory diseases (Li et al. 2022). Inhaled pollutants alter the function of various cell populations (Gualtieri et al. 2011; Longhin et al. 2013), including alveolar macrophages which play a key role in the development of PAP (Ma et al. 2017). In addition to industrial pollution, important sources of pollutants include smoking and, more recently, the increasingly popular use of electronic cigarettes associated with EVALI (E-cigarette or Vaping Use-Associated Lung Injury). The five-year survival rate of PAP is approximately 75% (Borie et al. 2011). Currently, PAP is classified according to the pathophysiological mechanism of onset as primary, secondary, congenital and unclassified PAP (Table 1). Primary PAP may be autoimmune or hereditary. Primary autoimmune PAP accounts for more than 90% of all PAP cases. Secondary PAP accounts for about 5–10% of PAP cases. Congenital PAP is the rarest type of PAP and accounts for about 2% of cases. The diagnosis of PAP is based on the characteristic findings on CT (computed tomography) or HRCT (high resolution computed tomography) of the lungs and the characteristic findings in bronchoalveolar lavage fluid (BALF). The characteristic findings on CT, HRCT and in BALF will be explained in more detail in the following sections of the article. Histological confirmation of the diagnosis is rarely required but may be necessary in individual cases (Trapnell et al. 2019). In all forms of PAP, impaired surfactant degradation by macrophages, caused by impaired macrophage stimulation or function, may play a key role in the pathogenesis. The aim of our article is to provide a comprehensive and current overview of pulmonary alveolar proteinosis,

Table 1. Classification of PAP according to the pathophysiological mechanism of onset

Primary PAP Disruption of GM-CSF/CSFR signaling	Primary autoimmune PAP – positive antibodies against GM-CSF Primary hereditary PAP – negative antibodies against GM-CSF, GM-CSF receptor mutation
Secondary PAP	Haematological malignancies Chronic infections (HIV, Nocardia, Pneumocystis jiroveci) Chronic inflammation Immunodeficiencies, Lung transplantation, Bone marrow transplantation Drug-induced damage
Congenital PAP Surfactant production disorder	Mutations in SFTPB (SP-B), SFTPC (SP-C), ABCA3, TTFI (thyroid transcription factor I)
Unclassified PAP	–

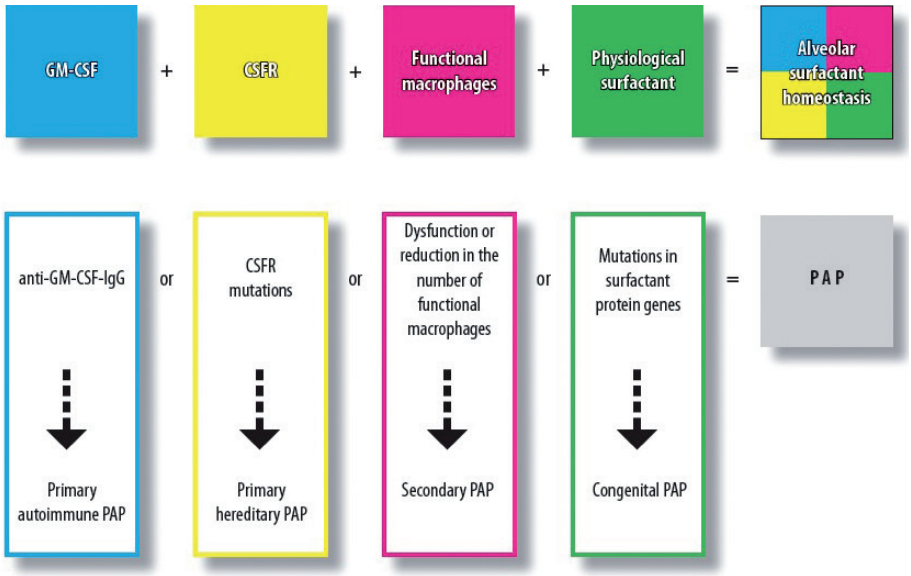

Figure 1. Key pathomechanisms leading to each type of PAP, anti-GM-CSF-IgG – IgG class autoantibodies with high affinity for GM-CSF.

a disease that may escape attention due to its non-specific symptomatology and rare occurrence, leading to delayed monitoring and treatment of affected patients. At the same time, we present a disease in which macrophage dysfunction may be a key pathomechanism. The role of macrophages in the pathogenesis of various diseases is currently receiving considerable attention in the scientific community. Therapeutic strategies targeting macrophage signal transduction, differentiation, migration, and polarisation may be of benefit not only to patients with such a rare disease as PAP, but also to patients with common civilization diseases such as metabolic syndrome (syndrome X, Reaven's syndrome), diabetes mellitus, or oncological patients. Understanding the role of macrophages in the pathogenesis of various diseases and the potential to influence them to improve treatment options is a challenge that needs to be addressed.

Pathophysiological mechanisms of the development of PAP and characteristics of its types

Surfactant, the accumulation of which in the lungs is the main consequence of PAP, is produced by type II pneumocytes and primarily composed of proteins and lipids, especially phosphatidylcholine. The main surfactant proteins are SP-A, SP-B, SP-C and SP-D, encoded by the genes SFTPA, SFTPB, SFTPC, and SFTPD. The role of surfactant is to reduce the surface tension in the alveoli, thereby preventing their collapse during expiration. Surfactant also plays a role in protection against infection. Under physiological conditions, most of the surfactant is reabsorbed by type II pneumocytes, and some is phagocytosed and degraded by alveolar macrophages (Whitsett et al. 2010). The presence of granulocyte and macrophage colonies stimulating growth factor (GM-CSF) or functional signal transduction between GM-CSF and its receptor, CSFR (colony-stimulating factor receptor), is critical for alveolar macrophage maturation and their ability to remove surfactant. GM-CSF is a glycoprotein and a hematopoietic growth factor. It appears to be a key cytokine in the pathogenesis of a large group of PAP cases. GM-CSF is produced by various cells in the human body, including pneumocytes, macrophages, lymphocytes, and mesothelial cells. GM-CSF binds to membrane receptors on target cells, which in humans consist of two subunits, α and β. Impaired signal transduction through the GM-CSF/CSFR axis is typical of primary PAP. This disorder may be caused by a deficiency of a functional signaling molecule GM-CSF or a dysfunction of its receptor (CSFR). The deficiency of a functional signaling molecule due to the presence of autoantibodies is the cause of primary autoimmune PAP. In primary autoimmune PAP, IgG class autoantibodies with high affinity for GM-CSF (anti-GM-CSF-IgG) are produced, blocking its activity. Due to reduced stimulation, alveolar macrophages are unable to adequately remove surfactant. Neutrophils and lymphocytes are also functionally suppressed, contributing to susceptibility to infections, especially opportunistic ones. Receptor dysfunction for GM-CSF due to mutations in the respective genes is the cause of primary hereditary PAP. In patients with a mutated CSFR, concentrations of the signaling molecule (i.e., GM-CSF) are elevated and anti-GM-CSF-IgG antibodies are absent. Quantitative and functional alterations of macrophages

accompanying some oncological, haematological, and other diseases lead to secondary PAP. Mutations in genes encoding surfactant proteins can be detected in congenital PAP. Homozygous mutations of the SFTPB gene for SP-B have been confirmed in neonates with severe respiratory failure and death shortly after birth. Heterozygotes for this gene mutation have normal lung function. An autosomal dominant mutation of the SFTPC gene for SP-C is associated with interstitial lung impairment in all age groups. Other significant molecules whose mutations are related to PAP are ABCA3 and TTF1. ABCA3 (ATP-binding cassette sub-family A member 3) belongs to a family of ATP-binding membrane transporters involved in the transmembrane transport of endogenous lipids. The transcription factor TTF1 (transcription termination factor 1) is essential for proper lung development. TTF1 deficiency causes hypothyroidism, brain abnormalities, and acute or chronic lung disease in neonates (Iwatani et al. 2000; Salvaterra and Campo 2020). The key pathomechanisms leading to each type of PAP are summarized in Figure 1.

Primary autoimmune PAP

This is the most common form of PAP, accounting for 90–95% of cases. More than 90% of patients are middle-aged adults. A characteristic finding is an elevated serum concentration of IgG class antibodies against GM-CSF. These autoantibodies may also be present at lower serum concentrations in other forms of PAP, but in primary autoimmune PAP, they reach concentration above 5 μg/ml. The concentration of autoantibodies does not correlate with the severity of the disease. Elevated concentrations of these autoantibodies can also be detected in BALF. The previously suggested association between the autoantibody production and cigarette smoke inhalation or infection has not been confirmed. Primary autoimmune PAP is rarely associated with other autoimmune diseases. One of the largest published studies of this disease, from Japan in 2008, reported on 248 patients. The average age of the patients was 51 years. Smokers accounted for 56% of the patients included in the study and were predominantly male. In the group of smokers, the disease was twice as common in men, which is due to the distribution of smokers between genders. In non-smokers, the distribution of primary autoimmune PAP was equal between the genders (♂: ♀ 1:1) (Inoue et al. 2008; Iftikhar et al. 2021). Clinically, the disease may be silent or manifest with dyspnoeic symptoms triggered by exertion and cough. Pulmonary function tests may show a decrease in the transfer factor for carbon monoxide (TLCO) and an increased alveolar-arterial oxygen gradient. Chest radiograph findings may be diffuse and bilateral. The radiograph image may mimic the finding of a pulmonary oedema.

Primary hereditary PAP

Primary hereditary PAP is caused by mutations in the genes for the α or β subunit of the CSFR (Suzuki et al. 2016). The inheritance pattern is autosomal recessive. The variable clinical manifestations in family members with identical mutations suggest that the course of the disease may also be influenced by other factors. Primary hereditary PAP is clinically and histologically indistinguishable from primary autoimmune PAP. In laboratory tests, anti-GM-CSF-IgG autoantibodies are not present at critical serum concentrations.

Secondary PAP

This form accounts for 5–10% of cases of PAP in adults. It may be associated with various oncological diseases, haematological diseases, immunodeficiency syndromes, chronic inflammatory diseases, or chronic infections. Among haematological diseases, secondary PAP has been described mainly in chronic myeloid leukaemia and myelodysplastic syndrome. Less commonly, it may be associated with acute myeloid leukaemia, acute lymphoblastic leukaemia, lymphomas or myeloma. In solid malignancies, secondary PAP is less frequent, but has been described in lung carcinoma, mesothelioma, and glioblastoma. Immunodeficiency syndromes associated with secondary PAP mainly include conditions following organ transplantation and AIDS. Rare cases have also been reported in connection with severe combined immunodeficiency (SCID), agammaglobulinemia, adenosine deaminase deficiency, as well as in connection with common variable immunodeficiency and DiGeorge syndrome. Secondary PAP has also been observed in dermatomyositis and rheumatoid arthritis. In addition to HIV (human immunodeficiency virus) infection, secondary PAP may also occur in cytomegalovirus infection or *Pneumocystis jiroveci* infection. Drug-induced secondary PAP is rare but has been described in association with the use of androgenic anabolic steroids, busulfan, cyclosporine, dasatinib, fentanyl, imatinib, leflunomide, mycophenolate mofetil, antineoplastic drugs, and sirolimus. The onset of secondary PAP has been observed in association with exposure to silica, titanium, kaolin, aluminium dust, indium, and, rarely, cellulose fibres (Delaval et al. 2005; Cummings et al. 2010).

Congenital PAP

Congenital, or inherited PAP accounts for less than 1% of cases of PAP. It can occur in neonates and children, as well as in adults. It is associated with varying degrees of surfactant accumulation in the alveoli and is always accompanied by pulmonary fibrosis.

Unclassified PAP

This type accounts for less than 1% of cases. These are cases in which there is no evidence of elevated serum concentrations of autoantibodies against GM-CSF, no disease associated with the secondary form of PAP, and no genetic defects causing PAP.

Clinical presentation of PAP

In the early stages, PAP is usually clinically silent or associated with vague and non-specific symptoms. At the time of diagnosis, the most common symptom is dyspnoea or a combination of dyspnoea and cough. In addition to gradually worsening exertional dyspnoea and cough, patients may have fever, weight loss, or chest pain. Haemoptysis, associated with a possible secondary infection, may be the first sign of PAP. Opportunistic infections such as nocardiosis, cryptococcosis, mucormycosis, histoplasmosis, and aspergillosis are common in patients with PAP. Herpetic, mycobacterial and cytomegalovirus infections, or infections caused by *Pneumocystis jiroveci*, may also occur. These infections are more likely to develop due to the often-accompanying immunodeficiency, may be extensive and may extend beyond the respiratory system, e.g., with concomitant CNS involvement. PAP may progress to pulmonary alveolar proteinosis-associated pulmonary fibrosis (PAP-PF). The pathogenesis of PAP-PF is poorly understood. There is no clear predisposing factor for PAP to PAP-PF transition. For example, it has been suggested that accumulated intra-alveolar material could alter local cytokine expression, thereby promoting proinflammatory and profibrotic pathways (Hu-Wang et al. 2023). However, it is not clear why PAP to PAP-PF transition also occurs in patients with early diagnosis and treatment of PAP and, conversely, PAP-PF is relatively rare in patients with long-standing disease. The above suggests that several factors other than PAP status and duration contribute to PAP progression to PAP-PF (Nam et al. 2018). Clinical correlates of PAP-PF include auscultatory findings of crepitus, clubbing of the fingers, cyanosis, and signs of cor pulmonale (Trapnell and Luisetti 2015).

Diagnosis of PAP

The diagnosis of PAP is based on the patient's medical history, laboratory test results, lung radiographs, pulmonary function tests, characteristic findings in BALF and, in some cases, histological findings from a lung biopsy. The diagnosis of primary autoimmune PAP is based on the triad of: 1) "crazy paving" pattern of lung involvement on HRCT imaging defined as interlobular and intralobular septal thickening with the presence of ground-glass opacities (GGO), 2)

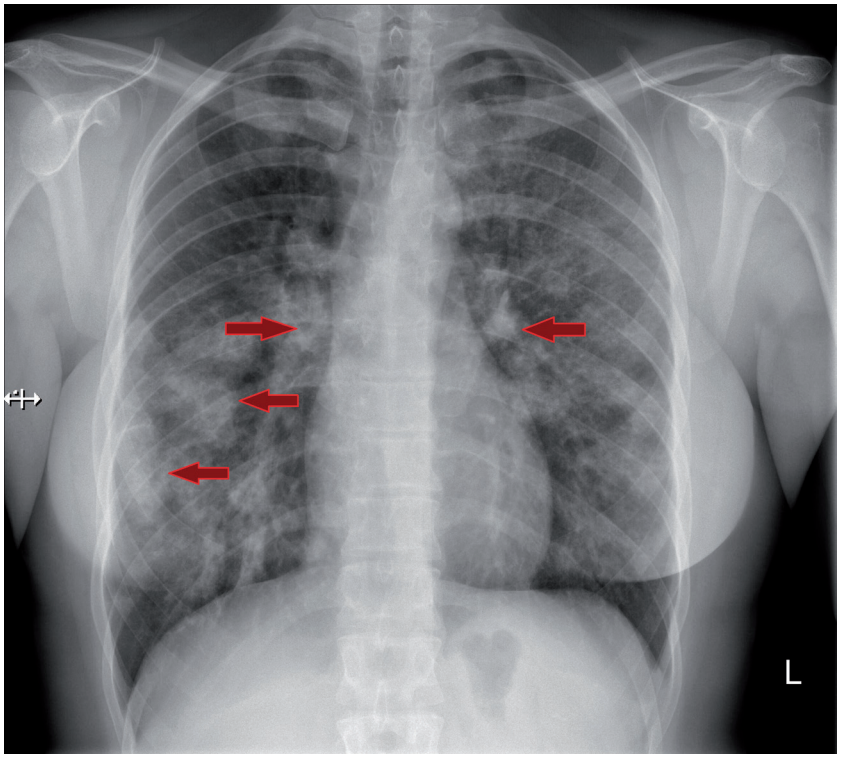

Figure 2. Chest radiograph finding in a patient with PAP showing diffuse patchy infiltrates with perihilar and more peripheral distribution, sometimes even confluent (Department of Radiological Diagnostics, National Institute for Tuberculosis, Lung Diseases and Thoracic Surgery in Vyšné Hágy, Vysoké Tatry, Slovakia).

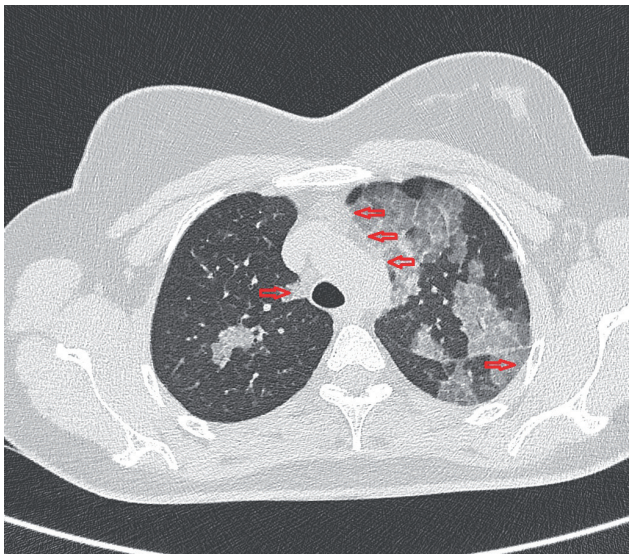

Figure 3. Findings on lung HRCT in a patient with PAP – bilateral, predominantly subpleural-localised ground-glass opacities (GGO) with smooth thickening of the interlobular septa, showing a crazy paving pattern (Department of Radiological Diagnostics, National Institute for Tuberculosis, Lung Diseases and Thoracic Surgery in Vyšné Hágy, Vysoké Tatry, Slovakia).

a milky appearance of BALF, and 3) elevated serum levels of autoantibodies against GM-CSF.

Laboratory tests

Among the laboratory parameters, it is important to measure the levels of autoantibodies against GM-CSF in the serum and BALF. The concentration of autoantibodies is determined by the ELISA method, which has a 100% sensitivity and specificity in the case of primary autoimmune PAP when it exceeds 5 μg/ml. As mentioned above, serum concentrations of autoantibodies do not correlate with the duration of the disease or the degree of lung dysfunction. The levels of the biomarkers SP-A, SP-B, SP-C, KL-6 (Krebs von den lungen 6 glycoprotein), CEA (carcinoembryonic antigen), cytokeratin 19, CYFRA 21-1 (cytokeratin fraction 21-1), NSE (neuron specific enolase) are often elevated in PAP patients and some of them correlate with the severity of the disease. However, none of these are specific or diagnostic for PAP. Higher KL-6 levels are associated with the progressive form of the disease, whereas patients with stable or regressing disease have low KL-6 levels.

Chest radiograph and CT, HRCT of the lungs

Chest radiographs of patients with PAP show diffuse, often bilateral, symmetrical infiltrates of the lung parenchyma with a perihilar distribution (Fig. 2). In more severe cases, these infiltrates may be confluent. The radiographic appearance of PAP may occasionally resemble pulmonary oedema. Unilateral lesions are less common. Thickening of the interlobular septa occurs due to the accumulation of lipoprotein material in the lung interstitium. The main imaging method used to diagnose PAP is lung HRCT. The typical finding on HRCT is the "crazy paving" pattern (Fig. 3). This consists of bilateral, predominantly subpleural-localised GGO with smooth thickening of the interlobular septa. Focal consolidation with air bronchograms may also be present. However, the crazy paving pattern of lung involvement is not specific to PAP and may be seen in other lung diseases such as lung infections, malignancies, pulmonary oedema, alveolar haemorrhage, organising pneumonia, or acute respiratory distress syndrome (ARDS). The presence of fibrotic changes indicates a poorer prognosis for the disease. Findings that are not typical of PAP and should prompt the consideration of alternative diagnoses include pleural effusion, pulmonary nodules, and mediastinal lymphadenopathy (Holbert et al. 2001).

Pulmonary function testing

In the early stages of the disease, lung volumes are generally normal. In later stages, a restrictive ventilatory defect may be present. TLCO is reduced in most cases, reflecting impaired gas exchange across the alveolar-capillary barrier. The alveolar-arterial oxygen gradient is often elevated and is a better measure of disease activity than TLCO.

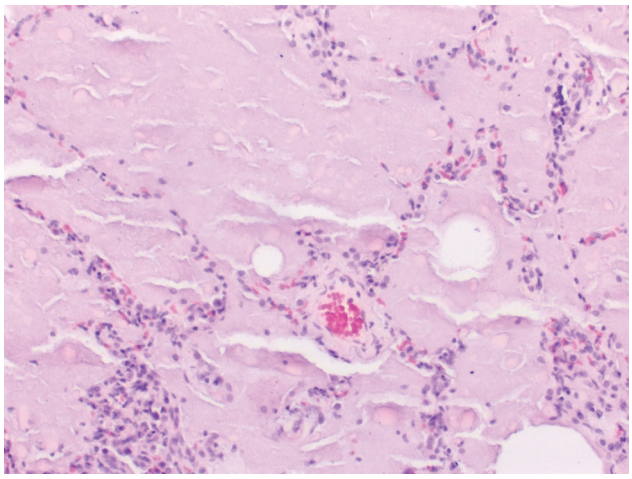

Figure 4. Histopathological findings in a lung biopsy in pulmonary alveolar proteinosis – view to thin alveolar lung septa with concentrated pinkish material. HE: 100× (Department of Pathology, National Institute for Tuberculosis, Lung Diseases and Thoracic Surgery in Vyšné Hágy, Vysoké Tatry, Slovakia and Institute of Histology and Embryology, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic).

Bronchoscopy and bronchoalveolar lavage

Examination of the BALF is helpful in establishing a definitive diagnosis. If the BALF is typically milky and the patient has corresponding clinical findings and a lung involvement pattern on HRCT, the diagnosis of PAP can be made without histological verification. In addition to its milky appearance, BALF is more viscous and forms a distinct sediment after centrifugation. Biochemically, there is an increase in the concentration of phospholipids and surfactant proteins A, B, C, and D. The BALF contains foamy macrophages, which are characteristic of PAP, and an increased number of lymphocytes.

Histopathological findings in lung biopsy in PAP

Histopathological examination of lung biopsies shows filling of alveolar spaces and terminal bronchioles with eosinophilic, acellular, granular material that is also PAS (periodic acid Schiff positivity)-positive (Fig. 4). The alveolar architecture is well preserved, but in advanced disease, the appearance of interstitial pneumonia and fibrosis may be present. Immunohistochemically, the presence of surfactant proteins can be demonstrated. The alveoli often contain numerous large foamy macrophages, as well as visually empty spaces left by cholesterol crystals. There is typically a sharp demarcation between affected and healthy lung tissue. The same material may also fill the bronchioles. Mild type II pneumocyte hyperplasia and a small inflammatory infiltration may be observed. Interstitial changes, especially fibrosis, may also be seen in the histological findings of PAP (Costabel and Guzman 2005).

Differential diagnosis of PAP

In the differential diagnosis of PAP, it is often necessary to distinguish between pneumonic infiltrate, diffusely disseminated malignancy, pulmonary oedema, diffuse alveolar haemorrhage, ARDS, non-fibrotic form of hypersensitivity pneumonitis, NSIP (non-specific interstitial pneumonia), LIP (lymphocytic interstitial pneumonia), DIP (desquamative interstitial pneumonia), alveolar sarcoidosis, radiation, and drug-induced pneumonitis. Differential diagnosis can be challenging in acute silicosis, which has a similar radiological appearance, is associated with milky BALF containing foamy macrophages, and can also be confirmed histologically with amorphous PAS-positive material in the alveoli (Costabel et al. 2005).

Treatment of PAP

The treatment of a patient with PAP depends on whether the disease is primary or secondary. Treatment also depends on the degree and severity of lung involvement and the clinical manifestations of the disease. The general recommendation is to stop smoking. In the secondary form of PAP, the underlying cause must be diagnosed and treated. If the disease progresses to a fibrotic form with a significant reduction in respiratory function, lung transplantation may be considered. However, recurrence of the disease in transplanted lungs has been reported in the literature. Asymptomatic diseases should be monitored. If there is progressive lung involvement with functional deterioration, or if the progression of findings is confirmed radiologically, therapeutic intervention is recommended (Parker and Novotny 1997). Summary of PAP treatment is included in Table 2.

Table 2. Treatment of primary and secondary PAP

Primary PAP	WLL GM-CSF supplementation Therapy targeting GM-CSF antibodies Rituximab Plasmapheresis Lung transplantation Gene therapy
Secondary PAP	Treatment of the underlying disease Termination of exposure to the causative agent WLL

Whole lung lavage (WLL) in the treatment of PAP

It is used in both primary and secondary forms of the disease. It was first performed by Juan Ramirez-Rivera et al. (1963) using a plastic catheter inserted into one of the principal bronchi. The lavage fluid was a saline solution warmed to body temperature, administered at a rate of 50–60 drops *per* minute in a total volume of 100 ml. This procedure was repeated four times a day for 2–3 weeks, resulting in improved radiological findings, elevated TLCO, and even documented regression of histopathological findings. However, the method was considered lengthy and imperfect. A year later, Ramirez-Rivera performed the procedure using a double-lumen endotracheal tube and a saline solution containing heparin or N-acetylcysteine as lavage fluid, at a volume of 3 liters *per* procedure. Over the next few years, whole lung lavage improved, particularly with better general anaesthesia, larger volumes of lavage fluid used, and the transition to bilateral sequential whole lung lavage in one session. Saline solution has become the preferred lavage fluid (Awab et al. 2017). Patients who underwent the procedure showed clinical improvement, improvement in radiological and functional findings, and at the cellular level, improved alveolar macrophage migration and phagocytic activity (Hoffman et al. 1989). The main indication for WLL is clinically restrictive dyspnoea. The methodology and indication

criteria for WLL may vary between individual departments. The most commonly used objective parameter to indicate WLL is a decline in lung function, a decrease in PaO2 (partial pressure of oxygen), and worsening radiological findings. Although the procedure has been safely performed repeatedly bilaterally in one session, most centers prefer sequential procedures with an interval of approximately 21 days between lavages of the right and left lungs. This interval represents the average time for the patient's lung function and oxygenation to improve, increasing the safety of contralateral lung lavage. Bridging with LMWH (low-molecular-weight heparin) depends on the risk of a thromboembolic event. If possible, the procedure should be postponed if the patient has had a myocardial infarction in the past 6 weeks and has had a bare-metal stent or drug-eluting stent implanted in the past 6 months with the use of clopidogrel or newer antiplatelet drugs (Douketis et al. 2012). In severe cases, WLL has been performed in patients on ECMO (extracorporeal membrane oxygenation) with no reported bleeding complications (Cooper et al. 1976). WLL is currently performed under general anaesthesia with intubation *via* a double-lumen endotracheal tube. The patient lies on the side of the lung to be lavaged. The anti-Trendelenburg position is used to improve fluid distribution in the lavaged part of the lung. The more affected lung, as described by CT or HRCT imaging, is lavaged first. If the CT/HRCT scan is unclear, a ventilation-perfusion scan may be used to determine the degree of involvement. Lavage is performed with a saline solution warmed to body temperature. Initially, the fluid has a macroscopically milky turbid appearance. Lavage is continued until the fluid is clear. The total volume of saline solution instilled can be up to 40 liters, but the average is 15 +/− 6.8 liters *per* lung (Campo et al. 2016). Calculated *per* body weight, this is approximately 250 ml/kg (Paschen et al. 2005). After completion of the lavage, the remaining fluid is removed from the lungs by bronchoscopy. The process can be supported pharmacologically with diuretics (Beccaria et al. 2004). If general anaesthesia is contraindicated, fibrobronchoscopic lavage of individual lung lobes can be performed under local anaesthesia. Clinical improvement is seen approximately 12 hours after WLL, and the effect lasts for an average of 15 months (Vašáková et al. 2002). Within a week of the procedure, spirometric parameters improve, the ventilation-perfusion ratio is adjusted, and PaO2 increases. TLCO values improve only after 6 months, although normalisation of values is rare. WLL has been shown to have a beneficial effect on prolonging patient survival (Homolka et al. 2001). The most common complications of WLL are fever, hypoxaemia, dyspnoea, and pneumonia. Pleural effusion and pneumothorax have been reported as less frequent. Rare complications reported in the literature including one cardiac arrest and one sudden death related to WLL, can be mentioned (Bhagwat et al. 1970).

Treatment of PAP with GM-CSF

First used in the late 1990s, recombinant GM-CSF was administered subcutaneously. Later, an inhaled form of GM-CSF was found to have a better response. Inhalation has the advantage of having a local effect without significant effects on the bone marrow. The effect of inhaled GM-CSF is comparable to WLL in indicated cases and is a promising pharmacological approach. However, neither the optimal dose nor the required duration of therapy with inhaled recombinant GM-CSF has been established. A prospective study of the effect of recombinant GM-CSF (sargramostin), published in 2024, showed an additive improvement in lung function in patients receiving sargramostin after WLL (Campo et al. 2024).

Rituximab in the treatment of primary autoimmune PAP

The production of autoantibodies against GM-CSF can be affected by the use of rituximab. Rituximab is a chimeric monoclonal antibody that targets the CD20 antigen on B-lymphocytes. Initially, rituximab was used to treat B-cell lymphomas. Later, its beneficial effects were also observed in the treatment of autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and granulomatosis with polyangitis (formerly Wegener's granulomatosis). Malur and colleagues published in 2012 the results of treatment with rituximab in nine patients with primary autoimmune PAP. This therapy resulted in a decrease in anti-GM-CSF antibodies in BALF, and seven of the nine patients showed clinical improvement, improvement in HRCT findings, and improvement in total lung capacity.

Plasmapheresis in the treatment of primary autoimmune PAP

Another option for the elimination of autoantibodies against GM-CSF is plasmapheresis (Kavuru et al. 2003). Bonfield et al. published in 2002 a study in which a patient unresponsive to WLL and subcutaneous GM-CSF treatment showed a decrease in anti-GM-CSF autoantibody titer after plasmapheresis, accompanied by an improvement in disease symptoms, oxygen saturation, and regression of radiological findings. However, the therapeutic effect of plasmapheresis may be ambivalent, as suggested by the work of Luisetti et al., who found in 2009 a decrease in anti-GM-CSF autoantibody titer after plasmapheresis, but this decrease did not lead to clinical improvement.

Statins in the treatment of PAP

Successful use of statins in severe autoimmune PAP was reported by McCarthy in 2018. Cholesterol is the main

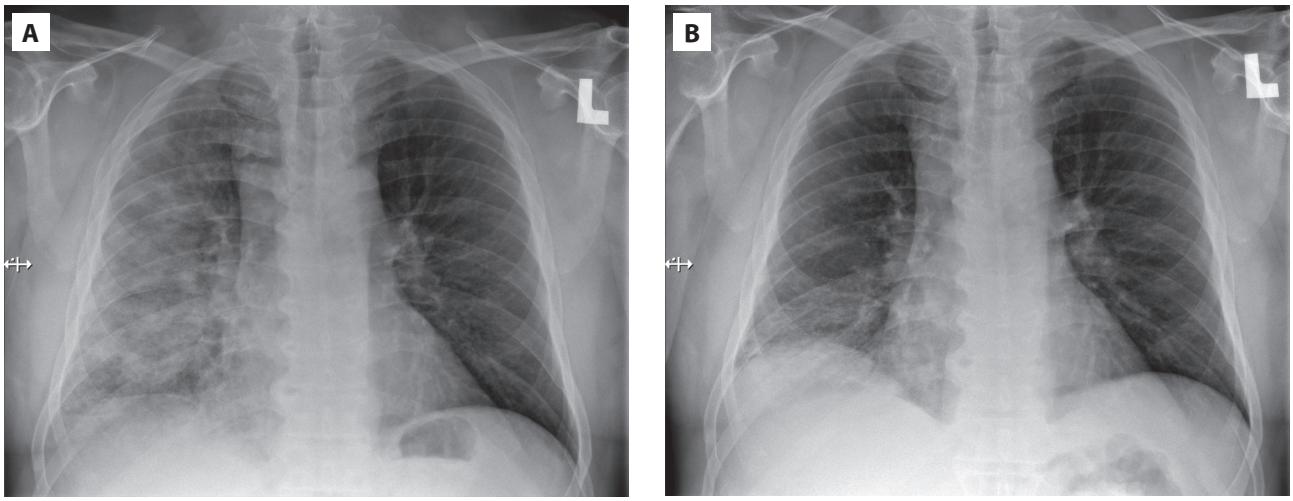

Figure 5. Chest radiograph finding in a patient with PAP before WLL (**A**) and after WLL (**B**) – visible regression of diffuse patchy infiltrates and increased transparency of the lung parenchyma, mainly in the right lung (Department of Radiological Diagnostics, National Institute for Tuberculosis, Lung Diseases and Thoracic Surgery in Vyšné Hágy, Vysoké Tatry, Slovakia).

material accumulating in alveolar macrophages in patients with PAP. Reduced clearance of cholesterol in macrophages appears to be a primary defect in their function, playing a key role in the pathogenesis of PAP (Sallese et al. 2017). GM-CSF regulates cholesterol clearance in macrophages. Statins inhibit cholesterol synthesis and reduce cholesterol levels in alveolar macrophages. Clinical and CT findings as well as lung function improved during statin treatment. Oral statin therapy is considered a promising new treatment for PAP, both in patients with hypercholesterolaemia (McCarthy et al. 2018) and in those without hypercholesterolaemia (Shi et al. 2022).

Lung transplantation

Lung transplantation offers hope for patients with progressive disease who have minimal or no response to treatment. However, cases of disease relapse in transplanted lungs have been reported.

Glucocorticoids in the treatment of PAP

Glucocorticoid treatment is not recommended. Increased mortality associated with a higher risk of infectious complications has been observed in patients treated with glucocorticoids.

Bone marrow transplantation

In an attempt to induce functional signal transduction through the defective receptor for GM-CSF, haematopoietic stem cell transplantation has been tried in mouse models. Despite the positive results achieved, this approach requires aggressive immunosuppressive therapy and myeloablation, which increases the risk of developing respiratory infections, graft-*versus*-host reactions, and the risk of toxicity and adverse effects of immunosuppressants. Interestingly, both lung and bone marrow transplantation are considered potential causes of secondary PAP.

Research on macrophage as a therapeutic option for hereditary PAP

Research is complicated by the low prevalence of the disease. Access to primary macrophages from affected patients is a challenge. To overcome this obstacle, induced pluripotent stem cells (iPSCs lines) derived from PAP patients are being used. Two concurrent publications report the successful generation of suitable iPSCs lines to study the impact of CFS2RA (colony stimulating factor 2 receptor subunit alpha) mutations on the onset and progression of PAP. Macrophages with CFS2RA mutations showed multiple defects, such as reduced CD11b (cluster of differentiation molecule 20 – B lymphocyte cell-surface antigen) activation, decreased phagocytic activity, and impaired surfactant clearance. All these defects were reversed by transducing the diseased iPSC cell lines with a lentiviral vector carrying the correct CSF2RA sequence (Lachman et al. 2014; Suzuki et al. 2014). Another research perspective is to focus on cholesterol homeostasis in macrophages. The possible use of statins has been mentioned previously. Another possible medication is Pioglitazone. Pioglitazone is a PPARγ (peroxisome proliferator-activated receptor γ agonist) that is used to treat, for example, type 2 diabetes and Alzheimer's

disease. Pioglitazone may increase cholesterol efflux from alveolar macrophages and alleviate pulmonary symptoms in patients with autoimmune PAP who have not responded to conventional therapy (Huang et al. 2023). A clinical trial investigating the efficacy of pioglitazone in the treatment of PAP is ongoing (NCT03231033) (Dupin et al. 2020).

Prognosis of PAP

The course of the disease can vary from spontaneous remission to a stable state, to progression leading to fatal lung dysfunction. Spontaneous remission is documented in about 10% of cases. Secondary PAP has the poorest prognosis, mainly due to the nature of the underlying disease. The median survival of patients with secondary PAP is less than 2 years. Data on the prognosis of the autoimmune form of PAP are unclear. Based on the registry of primary autoimmune PAP in Japan, no deaths were reported during a five-year follow-up period. PAP can be complicated by infections, especially opportunistic ones, which are responsible for about one-fifth of deaths. Pulmonary fibrosis can occur in all forms of PAP but is rare in the autoimmune form of the disease.

Our experience with PAP

Between years 2000–2023, eight patients with PAP (five men and three women), were hospitalized at the Second Department of Pneumology and Phthisiology at NÚTPCHaHCH Vyšné Hágy, Slovakia. Five patients had histological verified PAP and in three patients, PAP was diagnosed on the basis of the coincidence of characteristic findings in chest CT and BALF. The molecular-genetic background of the disease was not further determined. In our affected group there were six non-smokers and two active smokers. Two patients were asymptomatic and in symptomatic the most common clinical symptoms were dyspnoea, cough and fevers. In objective findings, two patients had clubbed fingers (digiti Hippocratici) and in two patients crepitus was audible in auscultatory respiratory examination. Five patients had hypoxemic respiratory insufficiency at the time of admission. In pulmonary function tests two patients had restrictive lung disease, in the other cases the findings in the pulmonary function tests were within normal limits. In terms of further therapeutic management, five patients are only dispensarized and three needed WLL. WLL in treated patients led to improved radiological findings (Fig. 5), improved diffusing lung capacity, and we also observed an improvement in the rate of restrictive lung disease. One patient underwent this procedure repeatedly, a total of 9 times. In his case, it was an example of a transition from PAP to PAP-PF and he finally died because of infectious complications by advanced disease. All living patients are dispensed by the pneumology outpatient clinics and are referred to our Institute if their condition worsens. One patient had spontaneous regression of the disease, which is rare.

Conclusion

PAP is a rare disease, but it must be considered in the differential diagnosis of lung diseases. Due to its low prevalence, there are no clear recommendations for diagnosis and treatment. Early diagnosis and appropriate follow-up management give patients a chance for disease control and significant clinical improvement. In recent years, we have gained a wealth of knowledge about the pathogenesis of the disease and the role of GM-CSF signaling, leading to a potential therapy using inhaled GM-CSF. The possibility of gene therapy targeting alveolar macrophages is also an important area of research. The lack of randomised trials in PAP patients makes research challenging, so patients should be centralized in specialized national centres. For us, pulmonary alveolar proteinosis is also a disease model where macrophage dysfunction is a potential key pathomechanism. The role of macrophages in the pathogenesis of various diseases is currently receiving considerable attention in the scientific community. It is known that macrophages, due to their functional and phenotypic plasticity, play a crucial role in the development of many diseases, not only rare ones such as pulmonary alveolar proteinosis, but also common civilisation diseases such as metabolic syndrome (syndrome X, Reaven syndrome), diabetes mellitus and its complications, or oncological diseases. Therapeutic strategies targeting macrophage signal transduction, differentiation, migration, and polarisation offer a potential solution for patients with these diseases.

Conflict of interest. The authors have no conflict of interest to report.

Publication ethics. Photographs of human materials and human tissues used in the study were obtained in accordance with the ethical standards of the institutional and national research committee and the Declaration of Helsinki of 1975, as revised in 2013. Informed consent was obtained from all individual participants included in the study. Patients provided signed informed consent for the publication of their data and photographs.

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