EXPERT REVIEWS

Pulmonary alveolar proteinosis: time to shift?

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Pulmonary alveolar proteinosis (PAP) is categorized into hereditary, secondary and autoimmune PAP (aPAP) types. The common pathogenesis is the ability of the alveolar macrophages to catabolize phagocytized surfactant is affected. Hereditary PAP is caused by mutations involving the GM-CSF signaling, particularly in genes for the GM-CSF receptor and sometimes by *GATA2* mutations. Secondary PAP occurs in hematologic malignancies, other hematologic disorders, miscellaneous malignancies, fume and dust inhalation, drugs, autoimmune disorders and immunodeficiencies. aPAP is related to the production of GM-CSF autoantibodies. PAP is characterized morphologically by the inappropriate and progressive 'occupation' of the alveolar spaces by an excessive amount of unprocessed surfactant, limiting gas exchange and gradually exhausting the respiratory reserve. Myeloid cells' immunity deteriorates, increasing the risk of infections. Treatment of PAP is based on its etiology. In aPAP, recent therapeutic advances might shift the treatment option from the whole lung lavage procedure under general anesthesia to the inhalation of GM-CSF 'as needed'.

Keywords: autoimmune pulmonary alveolar proteinosis • granulocyte macrophage-colony stimulating factor • granulocyte macrophage-colony stimulating factor receptors a and b • hereditary pulmonary alveolar proteinosis • inhaled granulocyte macrophage-colony stimulating factor • lung and systemic infections • pulmonary alveolar proteinosis • secondary pulmonary alveolar proteinosis • surfactant • whole lung lavage

First described by Rosen *et al.* in 1958, 27 biopsy-proven patients 'histologically characteristic and similar from one patient to another', with an 'obscure' etiology disease [1]. Pulmonary alveolar proteinosis (PAP) is currently categorized into three etiologic forms: hereditary PAP, secondary PAP and autoimmune PAP (aPAP) [2,3]. In all the above conditions, the common pathogenesis is that the ability of the alveolar macrophages to catabolize phagocytized surfactant through different mechanisms is affectd. PAP of any etiology is characterized morphologically by inappropriate and progressive 'occupation' of the alveolar spaces by an excessive amount of unprocessed surfactant, limiting gas exchange and gradually exhausting respiratory reserve, leading to respiratory failure and, if untreated, death. [1,4]. In addition, myeloid cells' immunity deteriorates, increasing the risk of severe lung and systemic infections [2,5,6]. Alveolar interstitial wall inflammation and/or fibrosis in the absence of a superimposed infection or inhaled dusts, almost always lack.

Hereditary PAP

GM-CSF receptor a/b related PAP

Mutations in *CSF2RB*, the gene encoding for the β-chain of the GM-CSF receptor [7,8], are monogenetic causes of PAP. They have been reported in a 36-year-old adult patient and in a 9-year-old child. In addition, in 20 cases aged between <1 year and 19 years, PAP was found to be caused by *CSF2RA* gene defects [9]. The protein encoded by the *CSF2RA* gene is the α-chain subunit of the heterodimeric GM-CSF receptor. This receptor regulates the production, differentiation and function of granulocytes and macrophages [10]. Interruption of GM-CSF signaling in the alveolar macrophage impairs the catabolism of surfactant and alveolar debris, thus expanding the intra-alveolar surfactant pool and filling up the alveoli with cellular debris. Additional and alternative factors not known in detail contribute to this process, explaining why subjects with the same disease-causing mutations have very different manifestations of their PAP. or, sometimes, lack the clinical syndrome of PAP (9). Subjects with PAP due to *CSF2RA* mutations are usually females (85%) and more than 55% have a history of consanguinity (9). Although it is a hereditary disease, the age at symptom onset is on average 4 years, ranging between 0.2 and 19 years. Usually, it takes about 1 year until the diagnosis is established (diagnostic latency

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0–5.8 years). At presentation, about 70% of the children have dyspnea, more than 15% have tachypnea, 55% have hypoxemia and about one third have global respiratory failure; about 15% are endotracheally intubated and ventilated. Cough was reported to be relatively rare (≥26%) (9). The overall outcome reported so far is favorable, that is, the majority (>90%) of patients were alive at the last follow-up; however, only 30% were free of respiratory symptoms. The majority (>70%) were managed by therapeutic whole lung lavages (WLLs; see below for technique). In these children and young adults, several comorbidities were identified, including failure to thrive (55%), hepatomegaly (5%) and pectus excavatum (10%) (9).

GATA2 deficiency

About 20% of the patients with disease-causing mutations in *GATA2*, a zinc finger transcription factor essential for hematopoiesis and lymphatic angiogenesis, have PAP. The additional phenotype of the disease is very wide, including viral and bacterial infections, cytopenias, myelodysplasia, myeloid leukemias and lymphedema. The disease may present at any time in life, mostly in adolescence to early adulthood period [11]. Symptomatic treatment by WLL is possible and the causative treatment is possibly by bone marrow transplantation.

Surfactant dysfunction disorders

Disorders associated with mutations in the surfactant genes, *SFTPB*, *SFTPC*, *ABCA3* and *TTF1*, primarily manifest during the neonatal period and later during infancy and childhood [12–19]. In particular, during presentation in the neonatal period and in infancy, these diseases have histopathological pattern similar to that of PAP. Clinical and radiographic appearance may resemble aPAP [18,19]. In addition to filling of the alveolar spaces with periodic acid-Schiff–1 positive material in these entities, the interstitial tissue is frequently broadened [18]. In agreement with this, WLL treatment does not appear to be helpful (own observations). The prognosis of these diseases is often very limited, with death occurring during early infancy. PAP has also been described in Niemann Pick disease. In particular, in Niemann Pick Type C2 (NPC2) patients, PAP is a presenting feature; WLL has been tried without long-term success [20].

Secondary PAP

Secondary PAP is related to several nosologic conditions [21] such as hematological malignancies [22–34] and other hematological disorders [35–42], miscellaneous malignancies [43–48], inhalation of dusts (both organic and inorganic) and fumes [49–61], drugs [62–67], autoimmune disorders [68–74] and immunodeficiencies with or without associated chronic lung infections (Table 1) [11,75–78]. In all the above conditions, the common denominator for the development of PAP relates to an acquired presumed or real inability (transient or permanent) of the alveolar macrophages to handle and catabolize surfactant. Alveolar macrophages may present in reduced numbers with a reduced functional ability to metabolize phagocytized surfactant, including an acquired loss of GM-CSF signal perception, or in normal numbers but 'engulfed and occupied' by a different phagocytized material and others. In some instances, secondary PAP and aPAP may overlap, since some patients developing PAP after dust exposure may also present high titers of anti GM-CSF autoantibodies and vice versa. Patients diagnosed with aPAP may present in their history significant and compatible dust exposure [79,56]. Secondary PAP presents the same imaging and histopathology hallmarks of aPAP, but prognosis may be even worse depending on the associated condition.

Autoimmune PAP

aPAP, the most common form of PAP (>90% of patients), is caused by the inappropriate production of IgG class autoantibodies against GM-CSF, a 23 kDa hematopoietic cytokine [80,81]. Anti GM-CSF autoantibodies show high affinity and avidity for their target, neutralizing its entire bioactivity on the alveolar macrophages and, thereby, their ability to catabolize phagocytized surfactant, leading to its accumulation in the alveolus and related airspace-occupying consequences.

Till 1996, aPAP was considered idiopathic, after which a new era in its history unfolded when two independent groups of investigators working with GM-CSF–deficient mice made a serendipitous observation of the death of their animals from a disease phenotypically identical to PAP and, therefore, the stimulating role of GM-CSF on alveolar macrophages' ability to catabolize surfactant became evident [82,83]. Soon after, it also became evident that several lung and systemic infections caused increased mortality in GM-CSF–deficient mice, discovering in this way the second major characteristic of PAP patients, that is, their susceptibility to severe microbial infections [84–89]. In 1999, another group of investigators discovered the presence of high titers of GM-CSF neutralizing autoantibodies and a few years later, their pathogenetic role in the disease was demonstrated by elegant experimental studies on primates [80,90].

aPAP is an ultra-rare disease with a worldwide distribution [4,59,91–93], and its incidence is estimated to be 0.2 cases per million per year [94] and its prevalence is 3.7 patients per million population. It affects males more frequently with a 1.3–2:1 male/female ratio for smokers and a 1:1 ratio for nonsmokers, and appears more frequently in the fourth to fifth decade of life [4,59,91,92]. Clinically, the disease presents insidiously with progressively deteriorating exertional dyspnea and chronic cough. In some patients, the disease presents as an unexpected finding on a chest radiogram performed for other reasons, since one-third of the cases are asymptomatic. Fever is uncommon and should alert the physicians for the coexistence of lung or systemic opportunistic infections. On physical examination, finger clubbing is usually absent, though it is reported in some patients [92]; in the absence of lung infection, the chest auscultation is usually normal. Pulmonary function tests in a symptomatic patient present a restrictive pattern with low diffusing capacity of the lung for carbon monoxide (DLCO) and hypoxemia deteriorating on exercise. Pulmonary hypertension is not a characteristic of the disease. Disease progression as well as

the response of patients to treatment modalities may be extremely variable (see below). Occasionally, spontaneous remission may be observed [95,96].

Perilous lung and systemic opportunistic infections constitute a real thread of life in aPAP patients and may influence the sudden slow progression of the disease [97]. Infection-related deaths in PAP are estimated to be around 20% [4]. Several microbial pathogens have been involved in lung and systemic infections in aPAP patients, such as bacteria [91,97–104], mycobacteria [91,97,98,105–109], fungi [91,97,98,110–113], *Pneumocystis jirovecii* spp. [91,97,98,114,115]. and viruses (Table 2) [91,97,98,116–118]. Infections occur because GM-CSF is necessary for the maturation and integrity of the immune status in differentiated myeloid cells including alveolar macrophages. On the myeloid cells, GM-CSF acts through an increase in the production of the transcription factor PU.1 [2]. PU.1 is necessary for several antimicrobial cell activities such as chemotaxis, cellular adhesion, receptor signaling, cytokines and chemokines production, reactive oxygen species production, microbial Fc receptor-mediated phagocytosis, intracellular killing and others [6]. Analogous functional antimicrobial defects have also been described on the circulating neutrophils, adding to the defects in intra-alveolar and systemic immunity [119].

The diagnosis of aPAP presents no significant difficulties, though the disease frequently remains undiscovered and is confused with other disorders because of its rarity. On chest radiograms, aPAP presents with bilateral diffuse airspace opacities with ground-glass appearance more or less confluent and symmetrical (Figure 1). The extension of the infiltrates in most patients is disproportionate to symptom perception that is usually mild or even absent: 'clinicoradiologic discrepancy'. Reticular or reticulonodular pattern, as well as airspace consolidation with air bronchograms may also be observed in some patients [120]. The computed tomographic appearance seems even more characteristic showing the so-called 'crazy-paving' pattern, a network of septal lines of the dimensions of the secondary pulmonary lobule upon a 'ground-glass' background (Figure 2). The above pattern is characteristic but not pathognomonic of the disease, since it may be observed in other lung disorders such as alveolar hemorrhage, diffuse alveolar damage, *P. jirovecii* species pneumonia or other lung infections, bronchoalveolar carcinoma, lymphangitic carcinomatosis, drug-induced and radiation pneumonitis, hypersensitivity pneumonia, cryptogenic organizing pneumonia and few others. Both macroscopic and microscopic appearances of bronchoalveolar lavage fluid significantly add to diagnosis and may obviate surgical biopsy, which was once considered the gold

Table 1. Causes of secondary pulmonary alveolar proteinosis.

Hematological disorders/malignancies [22–34]

Myeloid disorders	*Lymphoid disorders*
• Myelodysplastic syndrome, • chronic myeloid leukemia, • overlap myeloproliferative neoplasm, • chronic myelomonocytic leukemia, • primary myelofibrosis, • acute myeloid leukemia, • polycythemia vera, • essential thrombocytosis	• Acute lymphoid leukemia, • lymphoma (Hodgkin's and non-Hodgkin's), • adult T cell leukemia/lymphoma, • thymic alymphoplasia, • cutaneous T cell lymphoma, • chronic lymphocytic leukemia

Miscellaneous hematologic conditions [35–42]	*Non-hematologic malignancies [43–48]*
• Fanconi's anemia, • aplastic anemia, • congenital dyserythropoietic anemia, • multiple myeloma/plasmacytoma, • idiopathic thrombocytopenic purpura, • IgG monoclonal gammopathy, • status post-hematopoietic stem cell or bone marrow transplantation	• Lung cancer, • melanoma, • mesothelioma, • glioblastoma, • thymoma

Dust/fume inhalation [49–61]	*Drugs [62–67]*
• Silica, • cotton-linen, • cement, • fibrous insulation material, • titanium, • aluminum, • indium, • ONB, • flour, wood, • chlorine gas, NO_2, gasoline, plastics, • fentanyl patch smoke	• Sirolimus, • leflunomide, • imatinib mesylate, • mycophenolate mofetil, • cyclosporine, • dasatinib, • busulfan

Autoimmune disorders/others [68–74]	*Immunodeficiencies [11,75–78]*
• Sjögren's syndrome, • GPA, • juvenile dermatomyositis, • Behçet's disease, • ANCA-related vasculitis, • amyloidosis, • Hermansky–Pudlak syndrome	• HIV infection, • DCML syndrome, GATA2 deficiency, • hypogammaglobulinemia/ agammaglobulinemia, • IgA deficiency

ANCA: Antineutrophil cytoplasmic antibody; DCML: Dendritic cell, monocyte, B and NK lymphoid; GPA: Granulomatosis with polyangiitis; ONB: Orasol Navy Blue.

Table 2. Opportunistic infections in autoimmune pulmonary alveolar proteinosis.

Bacteria [91,97–103]	Fungi [91,97,98,110–113]
Nocardia spp., *Streptococcus pneumoniae*, *Legionella pneumophila*	*Aspergillus* spp., *Cryptococcus* spp., *Histoplasma capsulatum*, *Pneumocystis jirovecii* [91,97,114,115]
Mycobacteria [91,97,98,105–109]	**Viruses** [91,97,98,116–118]
Mycobacterium tuberculosis, *Mycobacterium avium* complex, *Mycobacterium kansasii*	Epstein–Barr virus, cytomegalovirus, human parainfluenza virus

standard for diagnosis. In most of the patients, bronchoalveolar lavage fluid macroscopically presents a viscid milky appearance and cytology preparations on light microscopy stained with May–Grünwald-Giemsa show a confused background of granular cellular and acellular debris (Figure 3) that stains with periodic acid-Schiff which also shows numerous large, engulfed, foamy or degenerated, 'exploded' macrophages (Figure 4). On biopsy specimens, if necessary for diagnosis, the above-described material is included within the alveolar structures with intact and non-inflammatory walls. Immunohistochemistry discloses surfactant proteins.

Treatment modalities

Treatment of PAP is certainly related to its etiology. Hereditary disease requires mechanical removal of inappropriate surfactant accumulation through WLL procedures as needed [121–123]. Secondary PAP treatment requires the resolution of the etiologic determinant plus the administration of WLL if needed, until the achievement of the primary objective and the remission of the disease. In aPAP, both WLL and the exogenous administration of GM-CSF singularly administered or in combination may benefit patients, and the definition of the best treatment modality is under development.

Whole lung lavage

Nothing sounds more rational than treating an 'alveolar filling' disease through an 'alveolar emptying' procedure. Indeed, in the early 1960s, Dr. José Ramirez-Rivera conceived of a radical technique combining the blind instillation of warm saline in the lungs through a transtracheal endo-bronchial catheter with physical positioning and violent coughing four-times a day for 2–3 weeks to remove the 'white viscid material' that accumulated in the alveolar spaces of PAP patients. This was called 'segmental flooding' and was the first therapy in the history of PAP that resulted in reproducible and clinically significant amelioration in the signs and symptoms of the disease [124]. This initially distressing procedure was further developed by Ramirez-Rivera and his co-workers into a trial of WLL under local anesthesia [125–127]. In the last 50 years, the initial WLL technique has been greatly refined and declared as 'the standard of care' for the treatment of PAP [4]. Although official international guidelines are missing, the decision to perform WLL, in the first place, is mostly based on the availability of the technique and the presence of a serious symptomatic disease. The procedure is now performed in an operating room or in an intensive care unit bed under general anesthesia and muscle paralysis, the patient being intubated by a double-lumen endobronchial tube, the appropriate placement of which is checked by bronchoscopy. The patient is placed in the lateral decubitus position with the dependent lung being ventilated with a high inspiratory oxygen fraction and the non-dependent lung being actively deflated and degassed and then washed. Lavage is performed by the injection of tidal volumes of saline warmed at 37°C that is then drained by gravity through a large-bore tubing system. Manual or mechanical percussion is usually performed to ameliorate drainage. Normally, the initially milky outflow gets less and less opaque to become finally clear after a mean time of a few hours and the instillation of 15–25 l of saline for a single lung lavage. Lavage of the second lung may be performed on the same day, but is usually scheduled in the next 24–48 h. The procedure is safe in the hands of an experienced team and no death or major morbidity has been reported in the last years. However, complications do occur, such as difficulties in oxygenation during the procedure, pneumothorax, pleural effusions, convulsions and fever. The patient is closely monitored and supported and may be extubated a few hours later [4,94,128–130].

In infants and small children, different techniques must be used, since due to size limitations, double-lumen endobronchial tube cannot be placed. Some use bronchoscopic lavage washing segment by segment and others use extracorporal membrane oxygenation to wash via a tracheal tube. However, the bronchoscopic techniques may lead to local irritation and injury and the extracorporal membrane oxygenation procedures are quite invasive and cannot be repeated frequently if needed for extensive time periods. We have developed a technique which – having obtained some experience – is feasible in infants and small children [123,131]. Lavage of one lung is done through a pulmonary artery catheter with a balloon, placed into one lung, whereas the other is ventilated through an endotracheal tube. Such a technique may be used over years, until the child is large enough to be lavaged via a double-lumen tube [128].

WLL is so far considered as the 'standard of care' for the treatment of PAP. However, due to the rarity and the nature of the disease, randomized prospective trials are lacking in literature, and therefore, our opinion on the true impact of WLL on the natural history of PAP is based on several differently designed, mostly retrospective studies, in which many indices of response to treatment are not uniformly quantified and, therefore, are not reliably documented (Table 3). The mortality rate in PAP approached 30% prior to the broad application of WLL. In the 2002 study of Seymour and Presneill where

410 cases of PAP (up to 1998, the year when GM-CSF appeared as a treatment option for idiopathic PAP) were thoroughly examined, a significant 5-year survival benefit of 94 ± 2% was reported for patients having being treated with WLL, compared with 85 ± 5% for those not treated with WLL [4]. Furthermore, according to literature reports, PAP patients undergoing WLL show significant amelioration in radiographic, functional and gas exchange parameters such as chest x-ray findings, Forced vital capacity and alveolar-arterial oxygen gradient, and O_2, as early as 1 week after the procedure. DLCO and exercise limitation improve in a slower and steeper way; however, improvement of all parameters is continuous and sustained in patients presenting with disease remission during follow-up of at least 1–3 years [128,132]. The duration of such benefit varies from 15 months to 3 years between studies. As a result, the number of patients remaining free of disease at 3 years post-WLL ranges from <20 to 70%. [4,128]. A significant number of patients will need repeated WLL sessions. In the study of Campo *et al.* in a referral center in Italy, one-third of patients underwent multiple WLLs, whereas at the Royal Brompton Hospital, the median number of WLLs needed for long-term remission was estimated at 4. An interesting finding from the German cohort of 70 PAP patients is that smokers require on average five WLL sessions to achieve remission, compared to almost half this number for non-smokers. In the same cohort, the number of WLL sessions required to reach remission ranged from 1 to 16 [59,129,133]. In cases where WLL under general anesthesia is too dangerous to perform, successful attempts of WLL through multiple segmental or lobar lavage by fiberoptic bronchoscopy have been reported [134–136].

Inhaled GM-CSF

Soon after the recognition of the role of GM-CSF in the etiology of aPAP, several groups of investigators attempted the administration of GM-CSF through different routes, extrapulmonary and inhaled, in experimental animals, co-opting for the inhaled route because of its efficacy [137]. Different groups of contemporary investigators attempted the subcutaneous administration of GM-CSF in humans, which proved somewhat unexpectedly effective in most but not all patients (Table 4) [138–148]. Again, around the same time, a single case report, also in humans with aPAP, proved the effectiveness of the inhaled route of GM-CSF administration [149]. Subsequent studies confirmed the effectiveness of the inhalational route of administration in aPAP, as well as its superiority (Table 4) [150–152]. In addition to the above studies, few other case reports confirmed the effectiveness of inhaled GM-CSF (iGM-CSF) in treating aPAP. They comprise a total of seven patients who received iGM-CSF in a dose of 250–500 μg/day, and all but one responded [149–158]. These case reports add to the existing literature and further confirm that iGM-CSF is an effective treatment for aPAP. From the above inhalational studies [149–158], it appears that GM-CSF administration for 12–24 weeks and occasionally for longer periods, and by using slightly different dosage schedules, proved safe in all, but was effective only in a proportion of patients, thus dividing them into responders and non-responders, with the only therapeutic alternative for non-responders being the WLL approach.

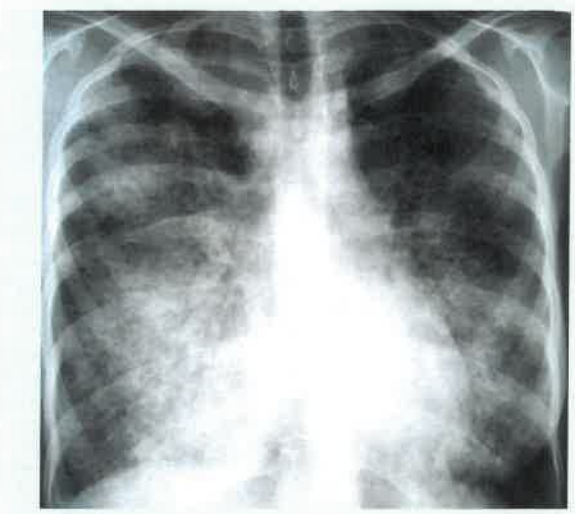

Figure 1. Posteroanterior chest radiogram showing bilateral airspace infiltrates asymmetrically distributed in a young lady with autoimmune pulmonary alveolar proteinosis. No lymphadenopathy, cardiomegaly or pleural fluids are evident.

More recently, in a study including six patients with aPAP, our group of investigators attempted a different schedule of GM-CSF inhalation, focusing on the effectiveness and safety of a very long-term administration of the drug, in order to obviate non-responders (Table 4) [159]. The reasons for the modification of the already published schedules were as follows: the extreme severity of the disease of two patients leaving in the antechamber of intubation, the non-availability of a reliable WLL clinic in Greece and the ineffectiveness of WLL performed in centers

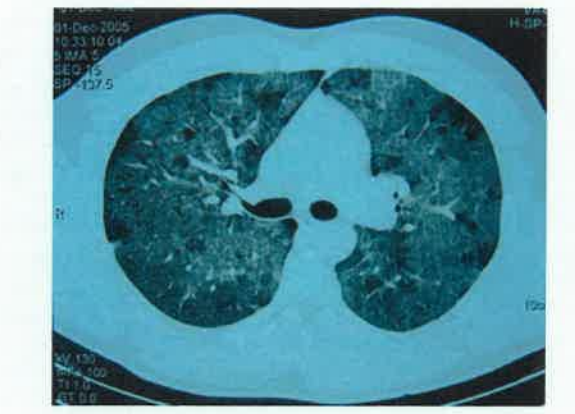

Figure 2. A computed tomography scan of the chest, showing 'the crazy-paving' pattern of airspace opacities in a young male with autoimmune pulmonary alveolar proteinosis.

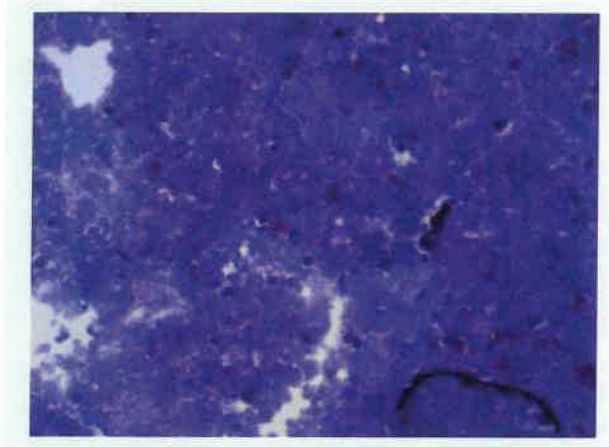

Figure 3. Bronchoalveolar lavage cytology preparations on light microscopy stained with May–Grünwald-Giemsa, showing a confused background of granular cellular and acellular debris.

of excellence abroad since two other patients had undergone 64 and 14 WLL procedures, respectively, and still needed more. We followed a specific treatment protocol according to which 250 mcg of iGM-CSF was administered once daily for 4 days followed by 4 days of abstinence (4 days on and 4 days off). This therapeutic scheme was continued until the achievement of disease remission (defined as the two of the following three criteria: absence of dyspnea on exertion, oxygen desaturation during 6 minute walking test lower than 4% and significant improvement of infiltrates on chest x-ray). When remission was achieved, the dose of iGM-CSF was decreased. All patients achieved disease remission (all responders) and surprisingly, they continued to improve (according to the results of the radiological and functional examinations) even with lower doses of iGM-CSF, allowing us to further reduce dosage

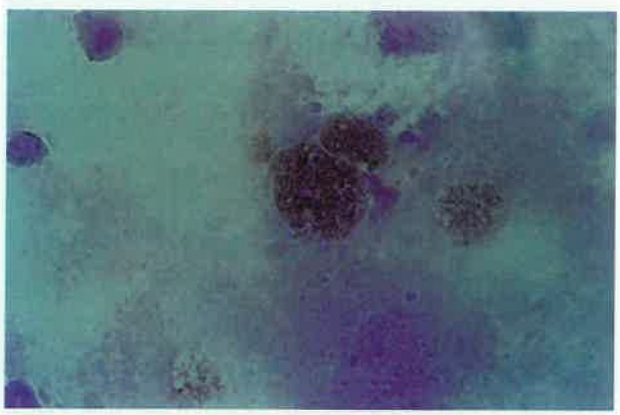

Figure 4. Bronchoalveolar lavage cytology preparations on light microscopy stained with periodic acid-Schiff, showing numerous large, engulfed, foamy or degenerated, 'exploded' macrophages upon a granular background.

and define the lowest effecting dose for each patient. If the decrease in administered dose of the iGM-CSF resulted in disease deterioration (it was observed in two patients), the patients were advised to return to the previous dose that proved to be effective. To date, all patients are in remission and receive the lowest effective dose. Finally, in order to investigate the safety of the very long-term administration of the iGM-CSF, especially its bone marrow overstimulation, specific tests were performed including complete blood cell counts, assessment of $CD34^+$ cells, detection of granulocyte macrophage progenitor colony-forming units and burst forming unit erythroid before and after therapy with iGM-CSF. The aforementioned tests did not reveal any abnormality in any of our patients.

From the above study, it became evident for the first time that: iGM-CSF may prove effective in all, but 'needs time'; 'de-escalation' of the inhaled dose is possible after 'remission attainment', maintaining or even further extending improvement; the definition of a 'lowest effective dose' in aPAP patients is achievable, minimizing costs; and finally the eventually needed 'very long-term' administration appears safe because no stimulatory activity on hematopoiesis was observed in any patient [159]. Actually, in an era where new efficacious treatments of aPAP such as iGM-CSF are emerging, the future of what is considered so far as the 'standard of care' for the treatment of PAP should be re-examined. Its role in the modification of response to iGM-CSF and in combination treatment strategies should be extensively studied based on the evidence that the partial restoration of the alveolar microenvironment following WLL could facilitate the action of iGM-CSF accelerating time to remission and anticipating dose de-escalation [159,160]. Furthermore, despite evidence for its efficacy in the treatment of aPAP, the administration of iGM-CSF as a sole therapy or in combination with WLL remains still 'off label'. This is most probably due to the lack of randomized controlled trials related to the rarity of the disease, which are needed to obtain official approval. The 'off-label' status of iGM-CSF in combination with its high cost creates many ethical, legal and policy problems, all of which are being faced only by patients and physicians and not by health authorities as they ought to. It is our belief and suggestion that the already gathered scientific evidence should encourage conducting properly designed studies empowered not only to prove the efficacy of iGM-CSF but also to justify the official approval of the drug as the mainstay of treatment in aPAP.

Other treatments

Although aPAP is caused by anti–GM-CSF autoantibodies, conventional immunosuppression has not been effective. Rituximab is a chimeric monoclonal antibody targeted against protein CD_{20} on the surface of B cells. Thus, rituximab, depleting the B cells, could reduce the production of anti–GM-CSF autoantibodies and be effective in treating aPAP. In the study of Kavuru *et al.* [161], 10 aPAP patients were treated with rituximab, which resulted in significant improvement in oxygenation and a reduction in anti–GM-CSF IgG levels in the bronchoalveolar lavage fluid, although the serum levels of

Table 3. Comparison between published cohorts of patients with pulmonary alveolar proteinosis treated with whole lung lavage.

Study (year)	Patients lavaged/ total number of patients (%)	Median n of WLL performed (range)	Median duration of clinical benefit from WLL	Responders (free of recurrence)	Ref.
Seymour *et al.* (2002)	146/231 (54%)	2 (1–22)	15 months	20% beyond 3 years	[4]
Briens *et al.* (2002)	25/41 (61%)	Not reported	Not reported	92% (time not reported)	[92]
Inoue *et al.* (2008)	Not reported/248	Not reported	Not reported	Not reported	[91]
Xu *et al.* (2009)	140/241 (59%)	1.06 (not reported)	Not reported	Not reported	[93]
Bonella *et al.* (2011)	63/70 (90%)	3.9 (1–16)	Not reported	52% (time to remission 3.7 ± 3.4 years)	[59]

WLL: Whole lung lavage.

anti–GM-CSF autoantibodies were not reduced. Similar is the rationale for using plasmapheresis in aPAP, that is, depletion of circulating anti–GM-CSF autoantibodies. There are only few case reports describing this treatment modality with conflicting results [162–164]. However, we remain skeptical if a patient with a condition such as PAP that predisposes to severe lung infections should receive an immunosuppressive therapy, and similar criticism on the use of immunosuppressive therapy in these patients was already considered earlier when a low-intensity regimen was adopted to reduce complications [162,163].

Pulmonary macrophage transplantation therapy is on the horizon and has been established in mouse models which develop a myeloid cell disorder identical to hereditary PAP in children with *CSF2RA* or *CSF2RB* mutations [165].

In conclusion, in aPAP, recent therapeutic advances might shift the treatment option from the WLL procedure under general anesthesia to the inhalation of GM-CSF 'as needed'. As already expressed in a previous editorial, ' the sleeping beauty, aPAP, may need no more... water bucket challenge, WLL, but just a kiss from her... beloved drug, inhaled GM-CSF' [166].

Expert commentary

WLL is considered so far as the 'standard of care' for the treatment of PAP. However, due to the rarity and the nature of the disease, randomized prospective trials are lacking in literature, and therefore, our opinion on the true impact of WLL on the natural history of PAP is based on several differently designed, mostly retrospective studies, in which many indices of response to treatment are not uniformly quantified and, therefore, are not reliably documented. Soon after the recognition of the role of GM-CSF in the etiology of aPAP, several groups of investigators attempted the administration of GM-CSF through different routes, extrapulmonary and inhaled, initially in experimental animals and subsequently in humans, co-opting for the inhaled route because of its efficacy. From the inhalational studies, it appeared that GM-CSF administration for 12–24 weeks and occasionally for longer periods, and by using slightly different dosage schedules, proved safe in all, but was effective only in a proportion of patients, thus dividing them into responders and non-responders, with the only therapeutic alternative for non-responders being the WLL.

More recently, a different schedule of GM-CSF inhalation, focusing on the effectiveness and safety of a very long-term administration of the drug in order to obviate non-responders was attempted. From this last study, it became evident for the first time that: iGM-CSF may prove effective in all, but 'needs time'; 'de-escalation' of the inhaled dose is possible after 'remission attainment'; maintaining or even further extending improvement, the definition of a 'lowest effective dose' in aPAP patients is achievable minimizing costs; and finally the eventually needed 'very long-term' administration appears safe because no stimulatory activity on hematopoiesis was observed in any patient.

Five-year view

Despite evidence for its efficacy in the treatment of aPAP, the administration of iGM-CSF as a sole therapy or in combination with WLL still remains 'off label'. This is most probably due to the lack of randomized controlled trials related to the rarity of the disease, which are needed to obtain official approval. The 'off-label' status of iGM-CSF in combination with its high cost creates many ethical, legal and policy problems, all of which are being faced only by patients and physicians and not by health authorities as they ought to. It is our belief and suggestion that the already gathered scientific evidence should encourage conducting properly designed studies empowered not only to prove the efficacy of iGM-CSF but also to justify official approval of the drug as the mainstay of treatment in aPAP.

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Table 4. Published studies of GM-CSF treatment in pulmonary alveolar proteinosis.

Study (year)	Patients (n)	Route of administration	GM-CSF dosage schedule	Treatment duration	Time to perceptible response	Responders	Ref.
Kavuru *et al.* (2000)	4	Subcutaneous	250 µg/day, if needed increase to 5 µg/kg/day in the fifth week, and if needed, increase to 7–9 µg/kg/day in the ninth week	12 weeks	8–12 weeks	3/4 (75%)	[140]
Seymour *et al.* (2001)	11	Subcutaneous	3 µg/kg/day, increase to 5 µg/kg/day on the sixth day; if no hematologic response, increase to 7.5–10–15–20–30 µg/kg every other 3 days at least	10–26 weeks	6–10 weeks	6/14 (43%)	[139]
Tazawa *et al.* (2005)	3	Inhalational	125 µg/kg/day b.i.d. every other week	24	Not available	3/3 (100%)	[150]
Venkateshiah *et al.* (2006)	25	Subcutaneous	250 µg/day, increase to 5 µg/kg/day in the second month, increase again to 9 µg/kg/day in the third month. If no response, increase to 12–18/µg/kg/day	12–52 weeks	8–12 weeks	12/25 (48%)	[142]
Case reports	8	Subcutaneous	3–8 µg/kg/day	8–12 weeks	8–12 weeks	7/8 (88%)	[138,141,143–148]
Wylam *et al.* (2006)	12	Inhalational	250 µg b.i.d. every other week; if no response, after 12 weeks, increase to 500 µg/kg/day b.i.d. every other week	>12 weeks	4 weeks	11/12 (92%)	[151]
Tazawa *et al.* (2010)	39	Inhalational	125 µg b.i.d. on days 1–8, 0 µg on days 9–14 for six 2-week cycles, then 125 µg/kg/day on days 1–4, 0 µg on days 5–14 for six 2-week cycles	24 weeks	12 weeks	24/39 (62%)	[152]
Case reports	7	Inhalational	250–500 µg/day in alternate weeks	24–56 weeks	12–56 weeks	6/7 (86%)	[149,153–158]
Papiris *et al.* (2014)	6	Inhalational	250 µg/day, days 1–4; 0 µg days 5–8; and upon remission, de-escalation to 250 µg/day, days 1–3; 0 µg days 4–8 with further de-escalation to 250 µg/day days 1–2; 0 µg days 3–8 in case of persistence of remission	14–65 months	14–38 months (remission)	6/6 (100%)	[159]

b.i.d.: Two-times a day.

includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

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Key issues

- Pulmonary alveolar proteinosis (PAP) is categorized as hereditary, secondary and autoimmune PAP (aPAP).
- Hereditary PAP is caused by mutations involving the GM-CSF signaling, particularly in genes for the GM-CSF receptor and sometimes by mutations in *GATA2*, NPC, SFTPB, SFTPC, ABCA3 and TTF1.
- Secondary PAP occurs in hematologic malignancies, other hematologic disorders, miscellaneous malignancies, fume and dust inhalation, drugs, autoimmune disorders and immunodeficiencies.
- aPAP is related to production of GM-CSF autoantibodies.
- Anti–GM-CSF autoantibodies have high affinity and avidity for their target, neutralizing its entire bioactivity on the alveolar macrophages and, thereby, their ability to catabolize phagocytized surfactant, leading to its alveolar accumulation and related airspace-occupying consequences.
- aPAP is an ultra-rare disease with a worldwide distribution.
- Treatment of PAP certainly relates to its etiology.
- Hereditary disease due to mutations in the GM-CSF receptor require mechanical removal of the inappropriate surfactant accumulated through whole lung lavage procedures as needed.
- Secondary PAP treatment requires the resolution of the etiologic determinant plus the administration of whole lung lavage, if needed, until the achievement of the primary objective and the remission of the disease.
- In aPAP, both whole lung lavage and the exogenous administration of GM-CSF, singularly administered or in combination, may benefit patients, and the definition of the best treatment modality is under development.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1. Rosen SH, Castleman B, Liebow A. Pulmonary alveolar proteinosis. N Engl J Med 1958;258(23):1123-42
2. Trapnell B, Whitsett J, Nakata K. Pulmonary alveolar proteinosis. N Engl J Med 2003;349(26):2527-39
3. Bonella F, Theegarten D, Guzman J, Costabel U. Alveolar lipoproteinosis syndromes. Eur Respir Mon 2011;54: 171-86
4. Seymour JF, Presneill JJ. Pulmonary Alveolar Proteinosis. Progress in the first 44 years. Am J Respir Crit Care Med 2002; 166(2):215-35

• **This is an excellent review describing the history of pulmonary alveolar proteinosis (PAP).**

5. Trapnell BC, Carey BC, Uchida K, Suzuki T. Pulmonary alveolar proteinosis, a primary immunodeficiency of impaired GMCSF stimulation of macrophages. Curr Opin Immunol 2009;21(5):514-21
6. Carey B, Trapnell BC. The molecular basis of pulmonary alveolar proteinosis. Clin Immunol 2010;135(2):223-35
7. Suzuki T, Maranda B, Sakagami T, et al. Hereditary pulmonary alveolar proteinosis caused by recessive CSF2RB mutations. Eur Respir J 2011;37:201-4
8. Tanaka T, Motoi N, Tsuchihashi Y, et al. Adult-onset hereditary pulmonary alveolar proteinosis caused by a single-base deletion in CSF2RB. J Med Genet 2011;48:205-9
9. Hildebrandt J, Yalcin E, Bresser HG, et al. Characterization of GSF2RA mutation related juvenile pulmonary alveolar proteinosis. Orphanet J Rare Dis 2014;9(1): 171
10. Conti L, Cardone M, Varano B, et al. Role of the cytokine environment and cytokine receptor expression on the generation of functionally distinct dendritic cells from human monocytes. Eur J Immunol 2008;38:750-62
11. Spinner MA, Sanchez LA, Hsu AP, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood 2014;123(6):809-21
12. Kröner C, Reu S, Teusch V, et al. Genotype alone does not predict the clinical course of SFTPC deficiency in paediatric patients. Eur Respir J 2015. [Epub ahead of print]
13. Thorwarth A, Schnittert-Hübener S, Schrumpf P, et al. Comprehensive genotyping and clinical characterisation reveal 27 novel NKX2-1 mutations and expand the phenotypic spectrum. J Med Genet 2014;51(6):375-87
14. van Hoorn J, Brouwers A, Griese M, Kramer B. Successful weaning from mechanical ventilation in a patient with surfactant protein C deficiency presenting with severe neonatal respiratory distress. BMJ Case Rep 2014;2014:pii: bcr2013203053
15. Griese M, Haug M, Brasch F, et al. Incidence and classification of pediatric diffuse parenchymal lung diseases in Germany. Orphanet J Rare Dis 2009;4:26
16. Schuerman FA, Griese M, Gille JP, et al. Surfactant protein B deficiency caused by a novel mutation involving multiple exons of the SP-B gene. Eur J Med Res 2008;13: 281-6
17. Brasch F, Schimanski S, Mühlfeld C, et al. Alteration of the pulmonary surfactant system in full-term infants with hereditary ABCA3 deficiency. Am J Respir Crit Care Med 2006;174:571-80
18. Hartl D, Griese M. Interstitial lung disease in children – genetic background and associated phenotypes. Respir Res 2005;6:32
19. Latzin P, Tredano M, Wüst Y, et al. Anti-GM-CSF antibodies in paediatric pulmonary alveolar proteinosis. Thorax 2005;60:39-44
20. Griese M, Brasch F, Aldana VR, et al. Respiratory disease in Niemann-Pick type C2 is caused by pulmonary alveolar proteinosis. Clin Genet 2010;77:119-30

21. Patel SM, Sekiguchi H, Reynolds JP, Krowka MJ. Pulmonary alveolar proteinosis. Can Respir J 2012;19(4):243-5
22. Chaulagain CP, Pilichowska M, Brinckerhoff L, et al. Secondary pulmonary alveolar proteinosis in hematologic malignancies. Hematol Oncol Stem Cell Ther 2014;7(4):127-35
23. Kajiume T, Yoshimi S, Nagita A, et al. A case of myelodysplastic syndrome complicated by pulmonary alveolar proteinosis with a high serum KL-6 level. Pediatr Hematol Oncol 1999;16(4):367-71
24. Green D, Dighe P, Ali NO, Katele GV. Pulmonary alveolar proteinosis complicating chronic myelogenous leukemia. Cancer 1980;46(8):1763-6
25. Pollack SM, Gutierrez G, Ascensao J. Pulmonary alveolar proteinosis with myeloproliferative syndrome with myelodysplasia: bronchoalveolar lavage reduces white blood cell count. Am J Hematol 2006;81(8):634-8
26. Bousser J, Zittoun R, Siguier F, et al. [Myelomonocytic leukemia and pulmonary alveolar proteinosis]. Sem Hop 1973;49(7): 507-11
27. Tsutsumi Y, Tanaka J, Saito S, et al. Myelofibrosis after essential thrombocythemia complicated by alveolar proteinosis. Leuk Lymphoma 2003;44(6):1049-52
28. Lakshminarayan S, Schwarz MI, Stanford RE. Unsuspected pulmonary alveolar proteinosis complicating acute myelogenous leukemia. Chest 1976;69(3): 433-5
29. Birsak CA, van Rossem RN, Nijhuis-Heddes JM, Maartense E. Pulmonary alveolar proteinosis: a complication in patients with hematologic malignancy. Neth J Med 2000;56(5):193-7
30. Pamuk GE, Turgut B, Vural O, et al. Pulmonary alveolar proteinosis in a patient with acute lymphoid leukemia regression after G-CSF therapy. Leuk Lymphoma 2003;44(5):871-4
31. Lugassy G, Michaeli J, Hurwitz N, et al. Pulmonary alveolar proteinosis associated with recurrence of Hodgkin's disease in the lung. Isr J Med Sci 1990;26(8):446-9
32. Jean R, Nezelof C, Bonnet H, et al. Proteinosis and pulmonary cytomegalic inclusions associated with thymic alymphoplasia. Arch Fr Pediatr 1968;25: 1009-21
33. Chung JH, Pipavath SJ, Myerson DH, Godwin D. Secondary pulmonary alveolar proteinosis: a confusing and potentially serious complication of hematologic malignancy. J Thorac Imaging 2009;24(2): 115-18
34. Breslow A, Snow P, Rosenberg MH. Pulmonary alveolar proteinosis and chronic lymphatic leucemia. Med Ann Dist Columbia 1965;34:209-12
35. Eldar M, Shoenfeld Y, Zaizov R, et al. Pulmonary alveolar proteinosis associated with fanconi's anemia. Respiration 1979; 38(3):177-9
36. Carden MA, Barman A, Massey G. Pulmonary alveolar proteinosis in association with congenital dyserythropoietic anemia: a case report. Case Rep Pediatr 2012;2012:624740
37. Ishii H, Tazawa R, Kaneko C, et al. Clinical features of secondary pulmonary alveolar proteinosis: pre-mortem cases in Japan. Eur Respir J 2011;37(2):465-8
38. Meijer WG, Van Marwijk Kooy M, Laddé BE. A patient with multiple myeloma and respiratory insufficiency due to accumulation of paraprotein in the alveolar space. Br J Haematol 1994;87(3):663-5
39. Proks C, Zamrazil V, Kuzma J. Kahler's myeloma with cryoglobulinemia and pulmonary alveolar paraproteinosis. Neoplasma 1966;13(2):217-22
40. Mork JN, Johnson JR, Zinneman HH, Bjorgen J. Pulmonary alveolar proteinosis associated with IgG monoclonal gammopathy. Arch Intern Med 1968; 121(3):278-83
41. Tomonari A, Shirafuji N, Iseki T, et al. Acquired pulmonary alveolar proteinosis after umbilical cord blood transplantation for acute myeloid leukemia. Am J Hematol 2002;70:154-7
42. Pidala J, Khalil F, Fernandez H. Pulmonary alveolar proteinosis following allogeneic hematopoietic cell transplantation. Bone Marrow Transplant 2011;46:1480-3
43. Bisetti A, Barbolini G. [Alveolar proteinosis secondary to pulmonary adenocarcinoma (secondary pneumopathy with amylaceous bodies). Anatomoclinical and histochemical study of a case]. Arch De Vecchi Anat Patol 1965;46(1):199-236
44. Su KC, Lay SL, Perng RP, et al. Lung cancer may develop subsequently or coincidently with pulmonary alveolar proteinosis. Lung Cancer 2007;58(1):144-8
45. Schiller V, Aberle DR, Aberle AM. Pulmonary alveolar proteinosis. Occurrence with metastatic melanoma to lung. Chest 1989;95(2):466-7
46. Cheewakriangkrai R, Litwin A, Nava ME, Ramnath N. Unusual thoracic problems in patients with malignancies: case 2. Malignant mesothelioma in a patient with long-standing pulmonary alveolar proteinosis. J Clin Oncol 2005;23(12): 2858-9
47. García Río F, Alvarez-Sala R, Caballero P, et al. Six cases of pulmonary alveolar proteinosis: presentation of unusual associations. Monaldi Arch Chest Dis 1995; 50(1):12-15
48. Matsunaga K, Nagata N, Iwata Y, et al. [Pulmonary alveolar proteinosis noted in a patient with thymoma]. Nihon Kokyuki Gakkai Zasshi 2008;46(6):497-500
49. Xipell JM, Ham KN, Price CG, Thomas DP. Acute silicoproteinosis. Thorax 1977;32(1):104-11
50. Thind GS. Acute pulmonary alveolar proteinosis due to exposure to cotton dust. Lung India 2009;26(4):152-4
51. Kosacka M, Dyla T, Jankowska R. Alveolar proteinosis after professional exposure to cotton and linen dust, successfully treated with whole lung lavage-a case report. Pneumonol Alergol Pol 2004;72(5-6):217-20
52. McCunney RJ, Godefroi R. Pulmonary alveolar proteinosis and cement dust: a case report. J Occup Med 1989;31(3):233-7
53. McDonald JW, Alvarez F, Keller CA. Pulmonary alveolar proteinosis in association with household exposure to fibrous insulation material. Chest 2000; 117(6):1813-17
54. Keller CA, Frost A, Cagle PT, Abraham JL. Pulmonary alveolar proteinosis in a painter with elevated pulmonary concentrations of titanium. Chest 1995;108(1):277-80
55. Miller RR, Churg AM, Hutcheon M, Lom S. Pulmonary alveolar proteinosis and aluminum dust exposure. Am Rev Respir Dis 1984;130(2):312-15
56. Cummings KJ, Donat WE, Ettensohn DB, et al. Pulmonary alveolar proteinosis in workers at an indium processing facility. Am J Respir Crit Care Med 2010;181(5): 458-64
57. Ballantyne B. Pulmonary alveolar phospholipoproteinosis induced by Orasol Navy Blue dust. Hum Exp Toxicol 1994; 13(10):694-9
58. Jeanmart M, Arbib F, Brambilla C. [Recurrent lung disease in a baker]. Rev Mal Respir 1999;16(5):849-581
59. Bonella F, Bauer PC, Griese M, et al. Pulmonary alveolar proteinosis: New insights from a single-center cohort of 70 patients. Respir Med 2011;105(12): 1908-16

60. Dawkins SA, Gerhard H, Nevin M. Pulmonary alveolar proteinosis: a possible sequel of NO2 exposure. J Occup Med 1991;33(5):638-41

61. Chapman E, Leipsic J, Satkunam N, Churg A. Pulmonary alveolar proteinosis as a reaction to fentanyl patch smoke. Chest 2012;141(5):1321-3

62. Pedroso SL, Martins LS, Sousa S, et al. Pulmonary alveolar proteinosis: a rare pulmonary toxicity of sirolimus. Transpl Int 2007;20(3):291-6

63. Wardwell NR Jr, Miller R, Ware LB. Pulmonary alveolar proteinosis associated with a disease-modifying antirheumatoid arthritis drug. Respirology 2006;11:663-5

64. Wagner U, Staats P, Moll R, et al. Imatinib-associated pulmonary alveolar proteinosis. Am J Med 2003;115(8):674

65. Hasan A, Ram R, Swamy T. Pulmonary alveolar proteinosis due to mycophenolate and cyclosporine combination therapy in a renal transplant recipient. Lung India 2014; 31(3):282-4

66. Yoshimura M, Kojima K, Tomimasu R, et al. ABL tyrosine kinase inhibitor-induced pulmonary alveolar proteinosis in chronic myeloid leukemia. Int J Hematol 2014; 100(6):611-14

67. Aymard JP, Gyger M, Lavallee R, et al. A case of pulmonary alveolar proteinosis complicating chronic myelogenous leukemia. A peculiar pathologic aspect of busulfan lung? Cancer 1984;53(4):954-6

68. Park E, Kim HR, Kim HJ, Lee SH. Pulmonary alveolar proteinosis as an unusual pattern of lung involvement in Sjögren syndrome. Rheumatol Int 2012; 32(9):2945-8

69. Shah SK, Phan NB, Goyal G, Sharma G. Pulmonary alveolar proteinosis in a 67-year-old woman with Wegener's granulomatosis. J Gen Intern Med 2010; 25(10):1105-8

70. Samuels MP, Warner JO. Pulmonary alveolar lipoproteinosis complicating juvenile dermatomyositis. Thorax 1988;43:939-40

71. Uchiyama M, Nagao T, Hattori A, et al. Pulmonary alveolar proteinosis in a patient with Behcet's disease. Respirology 2009;14: 305-8

72. Merino-Angulo A, Perez-Marti M, Diaz de Otazu R. Pulmonary alveolar phospholipoproteinosis associated with amyloidosis. Chest 1990;98(4):1048

73. Onoda S, Nishino J, Yoshida H, et al. [ANCA-related-vasculitis with pulmonary alveolar proteinosis and hemophagocytic syndrome]. Nihon Naika Gakkai Zasshi 2006;95(10):2087-9208; Article in Japanese

74. Ozyilmaz E, Gunasti S, Kuyuku Y, et al. Hermansky Pudlak Syndrome and Pulmonary Alveolar Proteinosis at the same patient: first case report in the world literature. Sarcoidosis Vasc Diffuse Lung Dis 2013;30(3):217-20

75. Ruben FL, Talamo TS. Secondary pulmonary alveolar proteinosis occurring in two patients with acquired immune deficiency syndrome. Am J Med 1986; 80(6):1187-90

76. Ferreira PG, Carvalho L, Gamboa F. A novel immunodeficiency syndrome as a rare cause of secondary pulmonary alveolar proteinosis: a diagnosis after 5 decades. Rev Port Pneumol 2014;20(5):273-8

77. Patiroglu T, Akyildiz B, Patiroglu TE, et al. Recurrent pulmonary alveolar proteinosis secondary to agammaglobulinemia. Pediatr Pulmonol 2008;43:710-13

78. Webster JR Jr, Battifora H, Furey C, et al. Pulmonary alveolar proteinosis in two siblings with decreased immunoglobulin A. Am J Med 1980;69(5):786-9

79. Costabel U, Nakata K. Pulmonary alveolar proteinosis associated with dust inhalation: Not secondary but autoimmune? Am J Respir Crit Care Med 2010;181:427-8

80. Kitamura T, Tanaka N, Watanabe J, et al. Idiopathic Pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte macrophage colony stimulating factor. J Exp Med 1999;190:875-80

81. Uchida K, Nakata K, Suzuki T, et al. Granulocyte/macrophage colony-stimulating factor autoantibodies and myeloid cell in healthy individuals. Blood 2009;113: 2547-56

82. Dranoff G, Crawford AD, Sadelain M, et al. Involvement of granulocyte-macrophage colony stimulating factor in pulmonary homeostasis. Science 1994;264:713-16

•• **A new era in autoimmune PAP (aPAP) history unfolded when two independent groups of investigators working with GM-CSF–deficient mice made a serendipitous observation discovering the death of their animals from a disease phenotypically identical to PAP and, therefore, the stimulating role of GM-CSF on alveolar macrophages' ability to catabolize surfactant.**

83. Stanley E, Lieschke GJ, Grail D, et al. Granulocyte/macrophage colony stimulating factor deficient mice show no major perturbation of hemopoiesis but develop a characteristic pulmonary pathology. Proc Natl Acad Sci USA 1994;91:5592-6

•• **A new era in aPAP history unfolded when two independent groups of investigators working with GM-CSF–deficient mice made a serendipitous observation discovering the death of their animals from a disease phenotypically identical to PAP and, therefore, the stimulating role of GM-CSF on alveolar macrophages' ability to catabolize surfactant.**

84. Seymour JF, Lieschke GJ, Grail D, et al. Mice lacking both granulocyte colony-stimulating factor (CSF) and granulocyte-macrophage CSF have impaired reproductive capacity, perturbed neonatal granulopoiesis, lung disease, amyloidosis, and reduced long-term survival. Blood 1997;90:3037-49

85. LeVine AM, Reed JA, Kurak KE, et al. GM-CSF-deficient mice are susceptible to pulmonary group B streptococcal infection. J Clin Invest 1999;103:563-9

86. Paine R 3rd, Preston AM, Wilcoxen S, et al. Granulocyte-macrophage colony-stimulating factor in the innate immune response to Pneumocystis carinii pneumonia in mice. J Immunol 2000;164: 2602-9

87. Riopel J, Tam M, Mohan K, et al. Granulocyte-macrophage colony stimulating factor-deficient mice have impaired resistance to blood-stage malaria. Infect Immun 2001;69:129-36

88. Gonzalez-Juarrero M, Hattle JM, Izzo A, et al. Disruption of granulocyte macrophage-colony stimulating factor production in the lungs severely affects the ability of mice to control Mycobacterium tuberculosis infection. J Leukoc Biol 2005;77:914-22

89. Ballinger MN, Paine R 3rd, Serezani CH, et al. Role of granulocyte macrophage colony-stimulating factor during gram-negative lung infection with Pseudomonas aeruginosa. Am J Respir Cell Mol Biol 2006;34:766-74

90. Sakagami T, Uchida K, Suzuki T, et al. Human GM-CSF autoantibodies and reproduction of pulmonary alveolar proteinosis. N Engl J Med 2009;361: 2679-81

•• **The pathogenetic role of GM-CSF neutralizing autoantibodies in the aPAP was demonstrated by this elegant experimental study on primates.**

91. Inoue Y, Trapnell BC, Tazawa R, et al. Characteristics from a large cohort of patients with autoimmune alveolar proteinosis in Japan. Am J Respir Respir Crit Care Med 2008;177:752-62
92. Briens E, Delaval P, Mairesse MP, et al. Lipoproteinose alveolaire pulmonaire (Pulmonary alveolar proteinosis). Rev Mal Respir 2002;19:166-82
93. Xu Z, Jiyong J, Wang H, et al. Pulmonary alveolar proteinosis in China: a systematic review of 241 cases. Respirology 2009;14: 761-6
94. Borie R, Danel C, Debray MP, et al. Pulmonary Alveolar Proteinosis. Eur Respir Rev 2011;20:98-107
95. Campo I, Kadija Z, Mariani F, et al. Pulmonary alveolar proteinosis: diagnostic and therapeutic challenges. Multidiscip Respir Med 2012;7:4
96. Martines-Lopez MA, Gomez-Cereno G, Villasante C, et al. Pulmonary alveolar proteinosis: prolonged spontaneous remission in 2 patients. Eur Respir J 1991;4:377-9
97. Punatar AD, Kusne S, Blair JE, et al. Opportunistic infections in patients with pulmonary alveolar proteinosis. J Infect 2012;65:173-9
98. Jones CC. Pulmonary alveolar proteinosis with unusual complicating infections. Am J Med 1960;29:713-22
99. Andriole VT, Balla M, Wilson GL. The association of nocardiosis and pulmonary alveolar proteinosis: a case study. Ann Intern Med 1964;60:266-75
100. Carlsen TT, Hill RB, Rowlands DT. Nocardiosis and pulmonary alveolar proteinosis. Ann Intern Med 1964;60: 275-81
101. Burbank B, Morrione TG, Cutler SS, et al. Pulmonary alveolar proteinosis and nocardiosis. Am J Med 1960;28:1002-7
102. Clague HW, Harth M, Hellyer D, et al. Septic arthritis due to Nocardia asteroides in association with pulmonary alveolar proteinosis. J Rheumatol 1982;9(3):469-72
103. Sánchez-Cuéllar S, García-Pérez FJ, Reina MT. [Streptococcus pneumoniae infection: cause or consequence of pulmonary alveolar proteinosis?]. Arch Bronconeumol 2008;44(11):645-6
104. Garmilla Ezquerra P, Gómez Roman J, Garcia de la Fuente C, Nan Nan D. Alveolar proteinosis in an immunocompetent patient with previous Legionella and Nocardia infections. Rev Clin Esp 2014;214(1):e1-3
105. Witty LA, Tapson VF, Piantadosi CA. Isolation of mycobacteria in patients with pulmonary alveolar proteinosis. Medicine Baltimore 1994;73(2):103-9
106. Lathan Jr SR, Williams JD Jr, McLean RL, et al. Pulmonary alveolar proteinosis. Treatment of a case complicated by tuberculosis. Chest 1971;59(4):452-4
107. Bakhos R, Gattuso P, Arcot C, Reddy VB. Pulmonary alveolar proteinosis: an unusual association with Mycobacterium avium-intracellulare infection and lymphocytic interstitial pneumonia. South Med J 1996;89(8):801-2
108. Couderc LJ, Bernaudin JF, Epardeau B, Caubarrere I. Pulmonary alveolar proteinosis and disseminated Mycobacterium avium infection. Respir Med 1996;90(10):641-2
109. Reyes JM, Putong PB. Association of pulmonary alveolar lipoproteinosis with mycobacterial infection. Am J Clin Pathol 1980;74(4):478-85
110. Kobayashi T, Ebina M, Oshima M, et al. [Case of alveolar proteinosis associated with pulmonary aspergillosis]. Nihon Naika Gakkai Zasshi 1999;88(1):135-7
111. Bjorkholm B, Elgefors B. Cerebellar aspergilloma. Scand J Infect Dis 1986;18: 375-8
112. Bergman F, Linell F, Bergman F, Linell F. Cryptococcosis as a cause of pulmonary alveolar proteinosis. Acta Pathol Microbiol Scand 1961;53:217-24
113. Hartung M, Salfelder K, Hartung M, Salfelder K. Pulmonary alveolar proteinosis and histoplasmosis: report of three cases. Virchows Arch A Pathol Anat Histol 1975; 368(4):281-7
114. Tran Van Nhieu J, Vojtek AM, Bernaudin JF, et al. Pulmonary alveolar proteinosis associated with Pneumocystis carinii. Ultrastructural identification in bronchoalveolar lavage in AIDS and immunocompromised non-AIDS patients. Chest 1990;98:801-5
115. Kotov PV, Shidham VB. Alveolar proteinosis in a patient recovering from Pneumocystis carinii infection: a case report with a review of literature. Cytojournal 2006;3:22
116. Edwards C, Primhak R, Cohen MC. Pulmonary alveolar proteinosis associated with Epstein-Barr virus infection. Eur Respir J 2010;36(5):1214-16
117. Ranchod M, Bissell M. Pulmonary alveolar proteinosis and cytomegalovirus infection. Arch Pathol Lab Med 1979;103(3):139-42
118. Butnor KJ, Sporn TA. Human parainfluenza virus giant cell pneumonia following cord blood transplant associated with pulmonary alveolar proteinosis. Arch Pathol Lab Med 2003;127(2):235-8
119. Uchida K, Beck DC, Yamamoto T, et al. GM-CSF autoantibodies and neutrophil dysfunction in pulmonary alveolar proteinosis. N Engl J Med 2007;356:567-79
120. Frazier AA, Franks TJ, Cooke EO, et al. Pulmonary Alveolar Proteinosis. Radiographics 2008;28:883-99
121. Paschen C, Reiter K, Stanzel F, et al. Therapeutic lung lavages in children and adults. Respir Res 2005;6:138
122. Suzuki T, Sakagami T, Young LR, et al. Hereditary pulmonary alveolar proteinosis: pathogenesis, presentation, diagnosis and therapy. Am J Respir Crit Care Med 2010;182:1292-304
123. Griese M, Ripper J, Sibbersen A, et al. Long term follow-up and treatment of congenital alveolar proteinosis. BMC Pediatr 2011;11:72

- **An excellent article on the treatment of congenital PAP and the development of a lung lavage technique, especially for infants.**

124. Ramirez J, Schultz RB, Dutton RE. Pulmonary alveolar proteinosis: a new technique and rationale for treatment. Arch Intern Med 1963;112:419-31
125. Ramirez J, Kieffer RF Jr, Ball WC Jr. Bronchopulmonary lavage in man. Ann Intern Med 1965;63:819-28
126. Ramirez RJ, Campbell GD. Pulmonary alveolar proteinosis: endobronchial treatment. Ann Intern Med 1965;63:429-41
127. Ramirez J. Pulmonary alveolar proteinosis: treatment by massive bronchopulmonary lavage. Arch Intern Med 1967;119:147-56
128. Beccaria M, Luisetti M, Rodi G, et al. Long-term durable benefit after whole lung lavage in pulmonary alveolar proteinosis. Eur Respir J 2004;23:526-31
129. Morgan C. The benefits of whole lung lavage in pulmonary alveolar proteinosis. Eur Respir J 2004;23:503-5. Editorial
130. Michaud G, Reddy C, Ernst A. Whole lung lavage for pulmonary alveolar proteinosis. Chest 2009;136:1678-81
131. Reiter K, Schoen C, Griese M, Nicolai T. Whole-lung lavage in infants and children with pulmonary alveolar proteinosis. Paediatr Anaesth 2010;20(12):1118-23
132. Lee KN, Levin DL, Webb RW, et al. Pulmonary alveolar proteinosis. High resolution CT, chest radiographic and functional correlates. Chest 1997;111: 989-95

133. Campo I, Mariani F, Rodi G, et al. Assessment and management of pulmonary alveolar proteinosis in a reference center. Orphanet J Rare Dis 2013;8:40

134. Cheng SL, Chang HT, Lau HP, et al. Pulmonary alveolar proteinosis: treatment by bronchofiberscopic lobar lavage. Chest 2002;122:1480-5

135. Doğru D, Yalçin E, Aslan AT, et al. Successful unilateral partial lung lavage in a child with pulmonary alveolar proteinosis. J Clin Anesth 2009;21:127-30

136. Froudarakis ME, Koutsopoulos A, Mihailidou HP. Total lung lavage by awake flexible fiberoptic bronchoscope in a 13 year old girl with PAP. Respir Med 2007;101: 366-9

137. Reed JA, Ikegami M, Cianciolo ER, et al. Aerosolized GM-CSF ameliorates pulmonary alveolar proteinosis in GM-CSF-deficient mice. Am J Physiol 1999;276:L556-63

138. Seymour JF, Dunn AR, Vincent JM, et al. Efficacy of granulocyte-macrophage colony stimulating factor in acquired alveolar proteinosis. N Engl J Med 1996;335:1924-5

• **This paper presents for the first time the efficacy of GM-CSF in PAP.**

139. Seymour JF, Presneill JJ, Schoch OD, et al. Therapeutic efficacy of granulocyte-macrophage colony-stimulating factor in patients with idiopathic acquired alveolar proteinosis. Am J Respir Crit Care Med 2001;163:524-31

140. Kavuru MS, Sullivan EJ, Piccin R, et al. Exogenous granulocyte-macrophage colony-stimulating factor administration for pulmonary alveolar proteinosis. Am J Respir Crit Care Med 2000;161:1143-8

141. Schoch OD, Schanz U, Koller M, et al. BAL findings in a patient with pulmonary alveolar proteinosis successfully treated with GM-CSF. Thorax 2002;57:277-80

142. Venkateshiah SB, Yan SB, Bonfield TL, et al. An open label trial of granulocyte-macrophage stimulating factor therapy for moderate symptomatic pulmonary alveolar proteinosis. Chest 2006;130:227-37

•• **A very important study of the efficacy of GM-CSF in PAP.**

143. Barraclough RM, Gillies AJ. Pulmonary alveolar proteinosis: a complete response to GM-CSF therapy. Thorax 2001;568:664-5

144. Acosta O, Marañés I, Pérez A, et al. Therapeutic efficacy of GM-CSF in pulmonary alveolar proteinosis [in Spanish]. Arch Bronconeumol 2002;384:191-3

145. de Vega MG, Sánchez-Palencia A, Ramírez A, et al. GM-CSF therapy in pulmonary alveolar proteinosis. Thorax 2002;57(9):837

146. Khanjari F, Watier H, Domenech J, et al. GM-CSF and proteinosis. Thorax 2003; 58(7):645

147. Valdés L, Pose A, Alvarez D, Ventura M. GM-CSF: a therapeutic alternative to the bronchoalveolar lavage in the treatment of alveolar proteinosis [in Spanish]. Med Clin (Barc) 2003;120(3):117

148. Abdul Rahman JA, Moodley YP, Phillips MJ. Pulmonary alveolar proteinosis associated with psoriasis and complicated by mycobacterial infection: successful treatment with granulocyte-macrophage colony stimulating factor after a partial response to whole lung lavage. Respirology 2004;93:419-22

149. Wylam ME, Ten RM, Katzmann JA, et al. Aerosolized GM-CSF improves pulmonary function in idiopathic pulmonary alveolar proteinosis [abstract]. Am J Respir Crit Care Med 2000;161:A889

150. Tazawa R, Hamano E, Arai T, et al. Granulocyte-macrophage colony–stimulating factor and lung immunity in pulmonary alveolar proteinosis. Am J Respir Crit Care Med 2005;171:1142-9

151. Wylam ME, Ten R, Prakash UBS, et al. Aerosol granulocyte-macrophage colony-stimulating factor for pulmonary alveolar proteinosis. Eur Respir J 2006;27:585-93

•• **An excellent paper on the efficacy of inhaled GM-CSF in PAP.**

152. Tazawa R, Trapnell BC, Inoue Y, et al. Inhaled granulocyte/macrophage colony stimulating factor as therapy for pulmonary alveolar proteinosis. Am J Respir Crit Care Med 2010;181:1345-54

•• **An excellent paper on the efficacy of inhaled GM-CSF in PAP, presenting the largest series till date.**

153. Arai T, Hamano E, Inoue Y, et al. Serum neutralizing capacity of GM-CSF reflects disease severity in a patient with pulmonary alveolar proteinosis successfully treated with inhaled GM-CSF. Respir Med 2004;98(12): 1227-30

154. Price A, Manson D, Cutz E, Dell S. Pulmonary alveolar proteinosis associated with anti-GM-CSF antibodies in a child: successful treatment with inhaled GM-CSF. Pediatr Pulmonol 2006;414:367-70

155. Robinson TE, Trapnell BC, Goris ML, et al. Quantitative analysis of longitudinal response to aerosolized granulocyte-macrophage colony-stimulating factor in two adolescents with autoimmune pulmonary alveolar proteinosis. Chest 2009; 135(3):842-8

156. Rodríguez Portal JA, Rodríguez Becerra E, Sánchez Garrido A. Response to inhaled granulocyte-macrophage colony-stimulating factor in a patient with alveolar proteinosis [in Spanish]. Arch Bronconeumol 2009;453: 150-2

157. Yu Hy, Sun Xf, Wang Yx, et al. Whole lung lavage combined with Granulocyte-macrophage colony stimulating factor inhalation for an adult case of refractory pulmonary alveolar proteinosis. BMC Pulm Med 2009;14:87

158. Yamamoto H, Yamaguchi E, Agata H, et al. A combination therapy of whole lung lavage and GM-CSF inhalation in pulmonary alveolar proteinosis. Pediatr Pulmonol 2008;438:828-30

159. Papiris SA, Tsirigotis P, Kolilekas L, et al. Long-term inhaled granulocyte macrophage-colony stimulating factor in autoimmune pulmonary alveolar proteinosis: effectiveness, safety, lowest-effective dose. Clin Drug Invest 2014;34:553-64

• **A very important paper presenting for the first time the efficacy of ultra long-term treatment with inhaled GM-CSF in aPAP.**

160. Hoffman RM, Dauber JH, Rogers RM. Improvement in alveolar macrophage migration after therapeutic whole lung lavage in pulmonary alveolar proteinosis. Am Rev Respir Dis 1989;139:1030-2

161. Kavuru MS, Malur A, Marshall I, et al. An open-label trial of rituximab therapy in pulmonary alveolar proteinosis. Eur Respir J 2011;38(6):1361-7

162. Luisetti M, Rodi G, Perotti C, et al. Plasmapheresis for treatment of pulmonary alveolar proteinosis. Eur Respir J 2009; 33(5):1220-2

163. Kavuru MS, Bonfield TL, Thomassen MJ. Plasmapheresis, GM-CSF, and alveolar proteinosis. Am J Respir Crit Care Med 2003;167(7):1036-7

164. Garber B, Albores J, Wang T, Neville TH. A plasmapheresis protocol for refractory pulmonary alveolar proteinosis. Lung 2015. [Epub ahead of print]

165. Suzuki T, Arumugam P, Sakagami T, et al. Pulmonary macrophage transplantation therapy. Nature 2014;514:450-4

166. Papiris SA, Tsirigotis P, Kolilekas L, et al. Autoimmune pulmonary alveolar Proteinosis: the sleeping beauty and the kissing drug. Pneumon 2014;34:553-64