Pulmonary Alveolar Proteinosis

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Abstract

PAP is an ultra-rare disease in which surfactant components, that impair gas exchange, accumulate in the alveolae. There are three types of PAP. The most frequent form, primary PAP, includes autoimmune PAP which accounts for over 90% of all PAP, defined by the presence of circulating anti-GM-CSF antibodies. Secondary PAP is mainly due to haematological disease, infections or inhaling toxic substances, while genetic PAP affects almost exclusively children. PAP is suspected if investigation for interstitial lung disease reveals a crazy-paving pattern on chest CT-scan, and is confirmed by a milky looking bronchoalveolar lavage that gives a positive periodic acid-Schiff (PAS) reaction indicating extracellular proteinaceous material. PAP is now rarely confirmed by surgical lung biopsy.

Whole lung lavage (WLL) is still the first line treatment, with an inhaled GM-CSF as second line treatment. Inhalation has been found to be better than subcutaneous injections. Other treatments, such as rituximab or plasmapheresis, seem to be less efficient or ineffective. The main complications of PAP are due to infections by standard pathogens (Streptococcus, Haemophilus, and Enterobacteria) or opportunistic pathogens like mycobacteria, Nocardia, Actinomyces, Aspergillus or Cryptococcus are. The clinical course of PAP is unpredictable and spontaneous improvement can occur. The 5-year actuarial survival rate is 95%.

Key words: Granulocyte-macrophage colony-stimulating factor, infection, pulmonary alveolar proteinosis, rituximab, whole lung lavage.

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1. Introduction

Pulmonary alveolar proteinosis (PAP), first described by Rosen and Castleman in 1958,[1] is an ultra-rare disease in which the alveolar macrophages are dysfunctional, causing surfactant proteins and lipids to accumulate in the pulmonary alveoli, so hindering gas exchange.[2] Most patients present with increased dyspnea on exertion but appear to be normal on clinical examination when assessed for interstitial lung disease (ILD). Diagnosis is usually based on the presence of a crazy paving pattern on a chest CT-scan plus PAS (periodic acid Schiff) positive eosinophilic material in a bronchoalveolar lavage (BAL). The serum contains anti-GM-CSF antibodies in 90% of cases, leading to a diagnosis of autoimmune PAP. The evolution of PAP varies from spontaneous resolution to death due to respiratory failure or a lung infection. The classification, pathophysiology and therapeutic management of PAP has progressed in recent years, particularly as a result of randomized trials of inhaled GM-CSF.

2. Classification of PAPs

Cases of PAP are assigned to one of three groups:[2]

- Primary PAP (>90%), caused by disruption of granulocyte–macrophage colony-stimulating factor (GM-CSF) signalling. These, in turn can be either:
    - Autoimmune PAP (> 90% of all PAP), with serum anti-GM-CSF (granulocyte macrophage colony-stimulating factor);
    - Hereditary PAP, due to mutations in the genes encoding GM-CSF receptor subunits (eg: CSF2RA or CSF2RB);
- Secondary PAP (5-10%), caused by reduced numbers and/or functions of alveolar macrophages due to:
    - Haematological disease, particularly myelodysplastic syndromes,

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- Chronic infections,
- Inhalation of toxic substances,
- Malignancy
- Immune deficiency
- Chronic inflammation
- Mutations affecting mononuclear phagocytes numbers or function (eg: GATA2 mutations)

Treatment of the associated disease is pivotal in the management of most cases of secondary PAP. Whole lung lavage (WLL) can be used, however the benefit could be only transient.

- Congenital PAP (2%), caused by disorders of surfactant production, and so observed mainly in children, resulting from:
  - Mutations causing a lack of surfactant proteins,
  - Mutations producing a deficient lipid transporter ATP-binding cassette subfamily A member 3 (ABCA3)
  - Mutations affecting lung development.

In PAP patients with no proven serum GM-CSF auto-antibodies and without any sign of disease known to cause secondary PAP, assessment of serum GM-CSF level and GM-CSF signalling pathway integrity could help to differentiate hereditary, congenital and unclassifiable PAP and trigger further testing such as GM-CSF receptor chains or surfactant mutation. More details can be found in the recent and excellent review of Trapnell et al.[2]

As the vast majority of PAP cases are autoimmune PAP, this review will focus only on them.

3. Epidemiology of autoimmune PAPs

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The serum of patients suffering from autoimmune PAP (>90% of all PAP cases) contains anti-GM-CSF antibodies.[2] PAP is an extremely rare disease whose prevalence varies from country to country. The reported range is from 4 to 40 cases per million inhabitants and its incidence is 0.2 per million inhabitants per year.[3] Autoimmune PAP is predominantly a male disease, with a sex ratio > 2 and a mean age at diagnosis of around 50 years.[4-8] Tobacco is a suspected risk factor and 60% of patients are smokers.[6, 8] Exposure to dust and vapours is implicated in the genesis of secondary PAP, but many patients with autoimmune PAP are also exposed to these factors: 26% in the Japanese Inoue series and 54% in the German Bonella series.[4, 6]

4. Pathophysiology of autoimmune PAP

Our understanding of PAP pathophysiology has advanced considerably over the past few years. Figure 1 illustrates the pathogenesis of autoimmune PAP.

4.1. PAS-positive deposits in alveolar spaces

PAP was first recognized as a distinct disease by Rosen et al. in 1958.[1] It was defined by its pathology: alveolar periodic acid-Schiff (PAS)-positive proteinaceous deposits with no cellular infiltrate and normal interalveolar septa. This was “so characteristic and similar from one case to another that it seems highly unlikely that it could have escaped description previously”.[1, 8] But it was more than 15 years before this PAS-positive material was identified as surfactant.[8]

4.2. Surfactant

Surfactant is a mixture of proteins and lipids (mainly phosphatidylcholine) synthesized and secreted by type II alveolar epithelial cells [2]. The four main surfactant proteins (SP-A, -B, -C and -D) are encoded by the SFTPA, B, C and D genes. The surfactant lipids are stored as intracytoplasmic lamellar bodies. Surfactant components are recycled by type II alveolar epithelial cells or catabolized by alveolar macrophages. The lipids and proteins of the surfactant

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help reduce the surface tension of the alveoli (prevent tele-expiratory collapse) and are involved in the innate defence of the lungs, particularly SP-A and SP-D, which are collectins.[2] Lipidomic analysis of BAL has shown an increase in free cholesterol (by 60-fold), cholesteryl esters (by 24-fold) and in ceramide (more than 130-fold increase) and other sphingolipids.[9] The surfactant lipid class phosphatidylcholine expanded 17-fold, lysophosphatidylcholine expanded 54-fold, and the surfactant proteins A, B, and C expanded 144-, 4-, and 17-fold, respectively.[9] These increases were similar between the different kind of PAP analysed (auto-immune, secondary to myeloid leukemia and genetic). Logically, statin therapy has been shown to improve patients with autoimmune PAP, as well as to reduce alveolar macrophage cholesterol accumulation and PAP lung disease in a mice model of PAP (Csf2rb−/− mice).[10] In other studies also targeting cholesterol homeostasis, PPARγ-agonist therapy has been shown to increase cholesterol clearance in macrophages and to reduce disease severity in PAP mice.[11]

4.3. GM-CSF and alveolar macrophages

The granular and monocyte growth factor (GM-CSF) is a major factor in the pathophysiology of autoimmune PAP. Stanley et al. found that the haematological profile of GM-CSF (GM-CSF -/-) knockout mice showed little change but that their pulmonary system was severely affected; their alveoli were filled with PAS-positive eosinophilic material, lamellar bodies and foamy alveolar macrophages indicating an accumulation of surfactant.[13] These mice also showed signs of subclinical bacterial and fungal infections. As this mouse disorder was similar to human PAP, the authors concluded that GM-CSF is probably involved in the pathophysiology of PAP. Shibata et al. subsequently showed that the alveolar macrophages of these GM-CSF mice -/- also lacked PU.1, a transcription factor that drives the differentiation of alveolar macrophages and is activated by GM-CSF.[14] They restored the PU.1 concentration to normal by viral transfection and found that the alveolar macrophages had recovered their adhesive and phagocytotic capacities, their responsiveness to toll-like receptors (TLRs), and their ability to

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break down surfactant. Then Bonfield et al. showed that the alveolar macrophages of autoimmune PAP patients had the same defects (decreased PU.1, adhesion and phagocytosis) and that incubating these macrophages with GM-CSF in vitro restored their PU.1 and other functions.[15] Similarly, treating autoimmune PAP patients with GM-CSF (subcutaneous injections) restored the PU.1 concentration in their alveolar macrophages. Another study showed that injecting healthy primates with anti-GM-CSF antibodies from the sera of patients with PAP reproduced in them most features of the disease.[16]

Lastly, alveolar macrophages are not the only cells that are abnormal in patients with autoimmune PAP, the phagocytotic, adhesive and bactericidal capacities of their circulating neutrophils are also altered.[17]

5. Diagnosis

The diagnosis of PAP mainly relies on chest CT-scans and BAL after exclusion of other causes of ILD. The presence of anti-GM-CSF autoantibodies in the serum leads to the diagnosis of autoimmune PAP.

5.1. Clinical manifestations

Around one third of PAP patients are asymptomatic and any symptoms that are present are non-specific. Dyspnea is the most common symptom, followed by a chronic cough and chronic bronchitis.[4-6, 18] Chest pain and hemoptysis are rare and suggest complications, particularly superimposed infections.[4-6, 18] Systemic symptoms (fatigue and/or weight loss), often lasting many months before evaluation or initial diagnosis, could also lead to PAP diagnosis.[2]

The clinical examination is often normal.[8] Crackles are found in 50% of cases, digital clubbing is uncommon (20% of cases).[5]

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5.2. Complementary examinations

5.2.1. Imaging

A chest X-ray generally reveals non-specific features (Figure 2 and 3), such as symmetrical bilateral lung opacity, but multiple presentations are possible.[8] Abnormalities are often more pronounced around the hilum; they appear like "butterfly wings" or "bat wings" without other signs suggesting heart failure (cardiomegaly, Kerley lines B, pleural effusion). Pleural effusion is evocative of complications. The poverty of the clinical examination often contrasts with the profusion of radiological lesions.[8]

A chest CT-scan is essential for diagnosing PAP (Figure 2 and 3) and typically shows areas of "ground-glass" superimposed on smooth thickening of the inter- and intralobular septal lines resulting in a "crazy paving" appearance. The distribution of these changes is usually patchy or geographic (juxtaposition of healthy and diseased lobules).[8, 19] Crazy paving is not pathognomonic of PAP but is sufficiently evocative to lead to a BAL and a check for a milky appearance and amorph PAS material.[19, 20] The differential diagnoses of crazy paving are shown in Table 1.[18, 20] Pulmonary fibrosis seems to be uncommon in patients with autoimmune PAP; it may appear only in advanced or end-stage disease.[2]

5.2.2. Bronchoalveolar lavage (BAL)

BAL is the second essential test for PAP diagnosis. Bronchoscopy is macroscopically normal. The BAL fluid is classically described as "milky" (Figure 4) and contains large amounts of PAS-positive acellular eosinophilic lipoprotein.[3, 5, 8, 18] There are also foamy macrophages with PAS-positive intracellular inclusions. Electron microscopy of BAL shows concentric lamellar phospholipid structures (lamellar bodies).[3, 13]

5.2.3. Serum anti-GM-CSF antibodies

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High titers of anti-GM-CSF antibodies are found in the serum and BAL of patients with autoimmune PAP. These are polyclonal immunoglobulins (IgG, mainly IgG1 or IgG2). They bind to different epitopes of GM-CSF to block its interaction with its receptor.[21] There may be neutralizing and non-neutralizing anti-GM-CSF antibodies, depending on the epitope they target. Anti-GM-CSF antibodies can be determined by ELISA or by measuring the ability of antibodies to neutralize the effect of recombinant cytokine GM-CSF on GM-CSF-dependent TF1 erythroblasts cell line growth. Practically, serial dilutions of patients serum, negative (no anti-GM-CSF antibody) and positive control serum (from an acknowledged patient with autoimmune PAP) are incubated with TF1 cells and 1 ng/mL GM-CSF (Cellgenix®, Freiburg, Germany) for 2 days. The proliferation of TF1 cells is measured by the incorporation of tritiated thymidine (1mCi/mL added in culture medium for the last 18h hours) for each serum dilution and the values plotted against serum dilution. In the case of PAP patients serum, this typically results in a sigmoid-shaped curve for which can be calculated a dilution inhibiting 50% of TF1 cells proliferation (IC50). The titer of anti-GM-CSF antibodies is calculated as the reciprocal of this IC50. The presence of anti-GM-CSF autoantibodies in the serum indicates that the disorder is autoimmune PAP. The serum concentration of these antibodies is not correlated with disease severity.[6] Serum anti-GM-CSF antibodies are not pathognomonic of PAP, as they can be found in healthy subjects and in subjects with other ILD.[21, 23, 24] Nevertheless, the anti-GM-CSF antibody concentrations are significantly higher in patients with PAP.[22, 25] An anti-GM-CSF antibody concentration > 5.0 µg/mL measured by ELISA has both a 100% sensitivity and specificity for diagnosis of autoimmune PAP.[25] In addition, the antibody detection in the BAL is almost 100% specific for autoimmune PAP.[22] The BAL anti-GM-CSF antibody concentration can be used to monitor disease progression and could be used to predict the need for a WLL or the response to GM-CSF treatment.[23, 26, 27] However, the anti-GM-CSF antibodies in BAL are not yet determined routinely.

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5.2.4. Surgical lung biopsy

Surgical lung biopsy, once considered to be the gold-standard, is no longer required to diagnose PAP. An evocative chest CT-scan combined with a typical BAL is sufficient in almost all PAP cases. Pathological analysis shows pulmonary alveoli and terminal bronchioles filled with PAS-positive acellular eosinophilic material which can be associated with a slight interstitial lymphocytic infiltration.[8] A major pathological feature is that the pulmonary architecture is generally preserved except in the case of associated pulmonary fibrosis, a late complication of PAP.[15] Electron microscopy shows lamellar bodies in macrophages and type II alveolar epithelial cells.[8]

5.2.5. Pulmonary function tests (PFTs) and gas exchange

PFTs show a restrictive pattern with a disproportionate decrease in carbon monoxide diffusion capacity (DLCO) but only a moderate decrease in functional volumes.[6, 8] Briens et al. found that the average DLCO was 51 ± 13% of predicted values (% pred.),[5] while Inoue et al. found 69 ± 27% pred.[6] Radiological abnormalities are correlated with decreased PFT values, both volumes and diffusion capacities.[8, 28]

Hypoxemia could be assessed by pulse oximetry (SpO2), more accurately by arterial blood gases analyses (PaO2). Alveolo-arterial difference in oxygen (A-aDO2) has been described to be a strong marker of PAP severity,[2] explaining its use as primary endpoints in recent large clinical trials.[29, 30]

Desaturation on exercise, assessed by the 6 minutes walking test (6MWT), is also a reliable and reproducible marker of severity of PAP.

5.2.6. Other complementary examinations

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The serum lactate dehydrogenase (LDH) level is 2 to 3 times normal in half of PAP cases.[8] A Japanese study found that the severity of PAP was correlated with the serum LDH level and the concentrations of Krebs von den Lungen 6 (KL-6), carcinoembryonic antigen (ACE), SP-A and SP-D. Patients are often hypoxemic at diagnosis, with an average PaO2 of 59 ± 16 mmHg, but the delay in diagnosis may explain these differences.[8]

Although PAP is an autoimmune disease (anti-GM-CSF antibodies), it is rarely (< 2% of cases) associated with other autoimmune diseases.[8]

6. Evolution and complications of Autoimmune PAP

The evolution of autoimmune PAP is unpredictable. Spontaneous improvement is possible (17-25% of cases), but no predictive factors have been identified and secondary aggravation is possible.[5, 6, 8] Spontaneous resolution has also been described after cessation of exposure to a wide range of airborne contaminants, such as tobacco or dust, which probably influence the pathophysiology of autoimmune PAP.[5, 8]

Actuarial survival at 5 years was 88 ± 4% in 2002,[8] with most deaths (72%) linked to a progression to chronic respiratory failure.[8] The survival at 5 years is currently around 95%.[3, 6]

Secondary infections, the most common and threatening complication, occur in 5-13% of patients, and account for 18–20% of deaths.[2, 6, 8] The usual pathogens, Streptococci, Haemophilus spp., and Enterobacteriaceae, can be involved, but infections may be due to opportunistic pathogens such as mycobacteria, Nocardia spp., Actinomyces spp., Aspergillus spp. and Cryptococcus. They should be systematically looked for in cases of worsening and also during WLL.[8, 18, 31] Table 2 lists the main opportunistic pathogens associated with PAP. These infections may precede or follow PAP diagnosis. Half of the infected PAP patients showed no sign of fever.[31] The other symptoms of infection found by Punatar et al. included

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cough (64%), dyspnea (62%) and weight loss (23%). Infected sites were predominantly pulmonary (75-86% of cases) and brain abscesses were reported during nocardiosis (19% of cases). A quarter of the mycobacterial infections, and half the fungal infections resulted in disseminated (lymph node, hepatic, medullary, cerebral or ocular) infections.[31]

Secondary pulmonary fibrosis can also occur, as can honeycombing during follow-up.[32-34]

7. PAP: disease monitoring

Follow-up of patients with autoimmune PAP should be done regularly and be supervised by physicians experimented in this disease. Respiratory symptoms (cough, dyspnea) and general symptoms (fever, weight loss, and fatigue) have to be assessed at each appointment. Lung function parameters (especially DLCO), 6MWT, PaO2 and A-aDO2 should be monitored regularly as their degradation may lead to therapeutic changes, especially WLL. Serial thoracic imaging is mandatory, at least by chest X-ray. CT-scan delivers more ionizing radiation but is much more effective for monitoring the disease, evaluating treatment response and diagnosing complications such as infections. Like most chronic pulmonary diseases, PAP will benefit from the increasing use of ultra-low-dose CT,[35] which is almost as informative as standard CT for an exposure dose similar to that of a chest radiograph. CT scoring systems have been proposed to enable more quantitative and reproducible evaluation, and may also serve as endpoints in clinical trials.[36-38] Several serum biomarkers, including KL-6, LDH, YKL40, CCL-2, CCL-18, CEA, CA-19.9, CYFRA 21-1, NSE, have been shown to correlate variably with disease severity but are not specific and are therefore not used in daily clinical practice.[39]

8. Treatment of Autoimmune PAP

The therapeutic management of severe and/or disabling autoimmune PAP includes WLL, GM-CSF injections and inhalations, plasmapheresis and rituximab. In contrast, mild forms of the

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disease simply require monitoring, particularly because of the possibility of spontaneous improvement.[2, 5, 6, 18] Lung transplantation is also an option in selected cases.

8.1 Whole lung lavage (WLL)

This is still the gold-standard treatment for autoimmune PAP.

8.1.1 WLL technique

Ramirez et al. first described the technique of "segmented endobronchial flooding" as a way to physically remove accumulated alveolar material in 1963.[8, 40] Many improvements have been made since this date. WLL is now performed under general anesthesia. Patient are intubated with a selective double lumen intubation tube. Left selective probes should be preferred, regardless of the side to be washed, as positioning is easier (longer main left bronchus, without the lumen of the right upper bronchus). The correct positioning of this intubation probe is verified by flexible bronchoscopy. One of the probe channels is used to ventilate the patient (protective ventilation, FiO2 = 100%, continuous capnography (EtCO2)) and the other channel is used for lavage. The supine patient is sedated and curarized and 1 to 2L of saline (without N-acetylcysteine or heparin) are instilled at 37°C. The wash liquid is evacuated by gravity ("siphoning").[5, 18] The first liter of this effluent is routinely analysed for bacteria (standard, *Nocardia, Actinomyces*, mycobacteria) and myco-parasites (*Aspergillus, Cryptococcus*). The procedure (instillation-retraction) is repeated until the effluent liquid is no longer cloudy (Figure 5). PaO2 increases during the lung "filling" phase by increasing the pressure in the airways and shunting blood to the contralateral ventilated lung.[41] The pressure in the airways decreases during the "emptying" phase and blood returns to the unventilated lung, increasing the shunt and thus decreasing PaO2.[41] Invasive hemodynamic monitoring is not required, a standard scope (heart rate, blood pressure and saturation) is sufficient.[42] Each lung is usually washed with 15 to 20 L saline, but the volume can be as high as 40 L.[43, 44] Patients are extubated a few

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hours later and the contralateral lung is washed 1 to 2 days later using the same protocol.[45, 46] WLL can be used together with extracorporeal membrane oxygenation (ECMO) to treat patients with severe respiratory insufficiency.

The WLL technique has not been standardized[2, 44] and many centers have developed adaptations to improve the quality of WLL (increase effluent turbidity).[18] Changes may include concomitant thoracic percussion (manual percussion is more effective than mechanical percussion), washing the contralateral lung during the same anaesthesia, changing positions (alternating procubitus-decubitus) during washing, or a specific type of ventilation (repeated periods of manual balloon ventilation during WLL). Segment washing under local anesthesia and flexible bronchoscopy have also been used successfully to treat patients with less severe forms of PAP. These differences in the WLL techniques in current use could be resolved if an international consensus were to be reached. All those involved agree, however, that these washes should be carried out by a trained team. Perhaps the ideal would be to have one or two national WLL reference centers.

8.1.2 WLL indications

There is no consensus.[2] The indications for WLL are set by expert centers and based on worsening dyspnea, gas exchange (PaO2, desaturation on the 6MWT, A-aDO2), interstitial lesions and PFT parameters. WLL should not be performed if there is any evidence of concurrent active bacterial pneumonia, as it increases the risk of disseminated infection and sepsis.[2]

8.1.3 Complications and effectiveness of WLL

The complications of WLL are minimal when it is performed in expert centers.[44] They include desaturation, pneumothorax, subcutaneous emphysema, headache, fever, cardiac pulmonary

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edema, convulsion, pneumonitis, intubation dislocation (with contralateral leakage) and prolonged intubation.[18, 43]

Seymour's 2002 literature review found that survival at 5 years after WLL was 94 ± 2%.[8] WLL improved symptoms, exercise tolerance, chest imaging, and PaO2 and PFTs parameters.[8, 43] About half of the patients have only needed a single WLL procedure (bilateral), suggesting that the presence of anti-GM-CSF antibodies is not sufficient to maintain the disease.[3] The other patients will need repeated WLL, varying from 1 to 22.[8] WLL is also associated with fewer opportunistic infections.[42]

Smoking seems to be linked to failure of WLL. Bonella et al. reported that active smokers required an average of 5 WLL to achieve remission, while the non-smokers in their cohort required only 2.4.[4]

8.2 GM-CSF Supplementation

8.2.1 Subcutaneous GM-CSF

Several studies have reported that autoimmune PAP patients benefit from subcutaneous injections of GM-CSF. Three prospective studies used daily injections of GM-CSF (5-9 µg/kg/day for 12 weeks), with a gradual increase in the dose to 18 µg/kg/day depending on the clinical response.[47] Treatment was effective in nearly half of the patients: in 43% of patients in the longest study (1 year) and up to 75% in the shortest study (12 weeks).[47] A few patients have been successfully given a maintenance dose for 1 year (20 µg/kg/3 times per week). Adverse reactions were minor: edema, erythema and puncture site pain, fever, chills, nausea, vomiting, malaise, headache, fatigue, arthralgia and dyspnea.[47]

8.2.2 Inhaled GM-CSF

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Inhaled GM-CSF delivers the drug directly to the lungs with fewer side effects. It also allows doses to be less frequent so reducing the cost of treatment. Inhaled GM-CSF therapy results in better responses by autoimmune PAP patients, greater increases in PaO_2 and $D(A\text{-}a)O_2$ than subcutaneous GM-CSF.[47]

A retrospective analysis of the responses of 12 patients with autoimmune PAP treated with inhaled GM-CSF (sargramostim, at a dose of 250 µg b.i.d. every other week for 12 week) was performed.[47] The dose can be increased to 500 µg b.i.d. every other week if there is no clinical response. Almost all (11/12; 92%) patients responded, with improvements in clinical, radiological, biological and/or PFT parameters.[47]

A prospective study of 39 autoimmune PAP patients treated with inhaled GM-CSF (sargramostim, 250 µg/d, every other week for 12 week (loading dose) and 125 µg/d, every other week for 12 week (maintenance dose) with follow-up for 1 year was also performed.[47] Over half (62%) of the patients responded, 17 patients while on the loading dose, 7 patients during the maintenance phase. This response persisted in 66% of patients even after follow-up for nearly 3 years. Only 12/35 patients required rescue treatment (mostly WLL).[47]

None of these studies found any specific adverse effects. Blood leukocyte counts remained unchanged despite the GM-SCF. No factor predicting the efficacy of inhaled GM-CSF has been identified.

Two major randomized trials, IMPALA[30] and PAGE[29], in which autoimmune PAP patients were treated with inhaled GM-CSF have been completed recently.

The IMPALA trial was a randomised, double-blind, placebo-controlled multicenter clinical trial on 135 autoimmune PAP patients that investigated the efficacy and safety of inhaled molgramostim (rhGM-CSF).[30] The primary objective was to assess the effect of a 24-week treatment on the alveolar-arterial oxygen difference. The secondary objectives were its effects

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on tolerance of exercise, quality of life, time to WLL, pulmonary function, dyspnea and cough, and chest CT-scan score. The number of reported adverse events (AEs), serious AEs, and adverse drug reactions were also monitored. The results should be published soon.

The PAGE trial was a double-blind, placebo-controlled trial of daily inhaled that examined the responses of 64 patients with autoimmune PAP to recombinant human GM-CSF (sargramostim).[29] The change in the alveolar–arterial oxygen gradient was significantly better in the GM-CSF group (33 patients) than in the placebo group (30 patients), with a mean change from baseline (−4.50±9.03 mm Hg) to 0.17±10.50 mm Hg (P = 0.02). The secondary endpoints of dyspnea and CT-scan score were also better in the GM-CSF group.

8.3 Rituximab

Rituximab is a monoclonal antibody directed against the CD20 antigen of B lymphocytes. Rituximab reduces the number of B lymphocytes in patients suffering from autoimmune diseases, which in turn decreases the secretion of antibodies including anti-GM-CSF. Borie et al. first described the effects of rituximab on an autoimmune PAP patient who refused WLL.[48] Kavuru et al. then treated 10 patients with autoimmune PAP using the same protocol (1000 mg IV infused on days 1 and 14) and found improvements in PaO2, PFT parameters and chest CT-scan in 7 of the 9 patients who completed the study.[26] This clinical improvement was also reflected in improved alveolar macrophage lipid homeostasis.[49] The main adverse effects of rituximab are due to reactions occurring during infusion, usually during the first injection: fever, chills, flu-like syndrome. Other rarer symptoms are: nausea, pruritus, angioedema, asthenia, hypotension. They can be minimized by premedication (antipyretic and antihistamine) and slowing the rate of infusion.

However, a real-life setting retrospective study has obtained contrasting results.[50] None of 13 patients with autoimmune PAP treated with rituximab showed any improvement 6 months after

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treatment, while the alveolar-arterial oxygen difference of 4 patients (30%) was significantly smaller 1 year after treatment. No serious adverse event was observed. The authors concluded that their data do not support rituximab as a second line therapy for patients with refractory autoimmune PAP.

8.4 Plasmapheresis

The rationale for using plasmapheresis is based on the proven pathogenicity of plasma anti-GM-CSF antibodies.[16] Plasmapheresis could decrease circulating anti-GM-CSF antibodies enough to improve surfactant clearance by restoring alveolar macrophage catabolic functions. Unfortunately, when plasmapheresis was used to treat five patients in whom WLL had failed, it appeared to be of limited efficacy.[23, 51-55] The protocol included 10 plasmapheresis sessions, over 2 months, in each of which the plasma mass exchanged was 1.5 L. The anti-GM-CSF antibody concentration decreased in 3 patients,[51, 53, 54] no dosage was performed in the two other cases. There is also evidence that plasmapheresis induces immunodepression.

8.5 Lung transplantation

Very few published data are available on lung transplantation and autoimmune PAP in adults. One case of PAP recurring on lungs transplanted for pulmonary fibrosis-complicated PAP within 3 years of surgery has been reported.[56] There have also been a few reports of other cases of PAP occurring as a complication in lungs transplanted to treat other diseases.[57-59] Immunosuppressive treatment, especially sirolimus, has been incriminated.[60]

8.6 Novel therapeutic approaches

As described in the pathophysiology section of this article, targeting lipid homeostasis could be a new way to treat patients with PAP. To date, statin therapy has been described to improve two patients with autoimmune PAP.[10] *In vitro* and *in vivo* studies have shown a potential role for PPARγ-agonist therapy in PAP patients.[11] A pilot phase I/II human clinical trial of oral

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pioglitazone as therapy for autoimmune PAP is reported on the portal Clin.trial.gov (NCT03231033).

Finally, given the recently published positive results of antifibrotics in progressive fibrosis interstitial lung diseases (PF-ILD),[61] PAP patients with PF-ILD phenotype could potentially benefit from these treatments in the future.

9. Future directions and unmet needs in PAP

With the recent large randomized clinical trials on inhaled GM-CSF,[29, 30] the algorithm of treatment of autoimmune PAP patients may change in the near future. In addition, the optimal dose, timing or duration of administration of inhaled GM-CSF have not been clearly defined, so there is still room for improvement in the current response rate.[2] Assessment of drugs targeting lipid homeostasis could also make it possible to increase the therapeutic arsenal against PAP. The combination of several of the available treatments should also be evaluated. At the moment, we are lacking reliable predictive biomarkers that could be used in daily practice. The development of such tools would help to better manage patients with autoimmune PAP.[39] Standardization of WLL is also a necessary goal as mentioned by experts in the field.

10. Conclusion

Recent advances in the treatment, such as inhaled GM-CSF, of the ultra-rare disease autoimmune PAP have led to a better understanding of the processes underlying the disease.

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Tables

Table 1: Differential diagnosis of the « crazy paving » pattern (classical / main causes).[20]

Classical causes	Pulmonary alveolar proteinosis Pulmonary hemorrhage Invasive mucinous adenocarcinoma Pulmonary MALT lymphoma Lipoid pneumonia

MALT: mucosa-associated lymphoid tissue.

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Table 2: Commonly reported opportunistic infection in patients with PAP (Punatar et al.)[31].

Pathogen, n (%)	**Total (n=75)**
Nocardia (n=32)	*N. asteroides*, 19 (59 %) *N. brasiliensis*, 1 (3 %) *N. farcinica*, 1 (3 %) *Nocardia spp*., 11 (34 %)
Mycobacteria (n=28)	*M. tuberculosis*, 21 (75 %) *M. kansaii*, 4 (14 %) *M. avium intracellulare*, 3 (11 %)
Fungi (n=15)	*Aspergillus spp*., 4 (27 %) *Cryptococcus spp*., 5 (33 %) *Histoplasma capsulatum*, 4 (27 %) *Aspergillus spp*. et *Cryptococcus spp*, 1 (7 %) *Zygomyces*, 1 (7 %)

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Figure legends:

Figure 1:
Illustration of pathophysiology of autoimmune PAP adapted from Trapnell et al.[2] The alveolar surfactant homeostasis is represented in normal subjects (A) and in autoimmune PAP patients (B).
In normal subjects, pulmonary surfactant is synthetized and secreted by type II alveolar epithelial cells (AEC2), and forms a thin layer of polar lipids (mostly phospholipids) with low amounts of neutral lipids (mostly cholesterol) as well as surfactant-associated proteins located at the alveolar air–liquid interface. AEC2 cells also participate in the degradation and recycling of surfactant. Alveolar macrophages are also involved in the clearance and catabolism of surfactant by degrading phospholipids and promoting reverse transport of cholesterol to the liver.[2]
In PAP patients, neutralizing polyclonal anti-GM-CSF antibodies block GM-CSF binding to its receptor and subsequent downstream signaling altering multiples genes and mechanisms in alveolar macrophages. This leads in a reduction in cholesterol efflux by alveolar macrophages and impaired surfactant clearance from the alveolar surface. Eventually, lipid droplets containing esterified cholesterol accumulate within macrophages, resulting in the formation of foam cells.
GM-CSF: granulocyte-macrophage colony-stimulating factor; SP-B: surfactant protein B; SP-C: surfactant protein C.

Figure 2:
Auto-immune PAP in a 37-year old man with progressive dyspnea, cough and expectoration. A. Plain chest radiograph showing bilateral central symmetrical lung opacities with relative apical and costophrenic angle sparing ("batwing opacities"). B, C. HRCT showing a typical crazy paving appearance (superimposition of ground glass opacities, interlobular and intralobular septal lines).

Figure 3:
Auto-immune PAP in a 46-year old woman with dyspnea on exertion. A. Plain chest radiograph showing a reticulonodular syndrome with a patchy distribution. B, C, D. HRCT showing geographic or lobular distribution of "crazy paving" (i.e. sharply demarcated from the surrounding normal lung).

Figure 4:
Milky aspect of broncho-alveolar lavage with sedimentation of lipoproteinaceous material from a patient with PAP.

Figure 5:

Liquid collected by whole lung lavage (WLL) (one lung) from a patient with autoimmune PAP. The turbidity decreases from the right (start of the procedure) to left (end). The amount of lipoproteinaceous material deposited at the bottom of the jars decreases during WLL.

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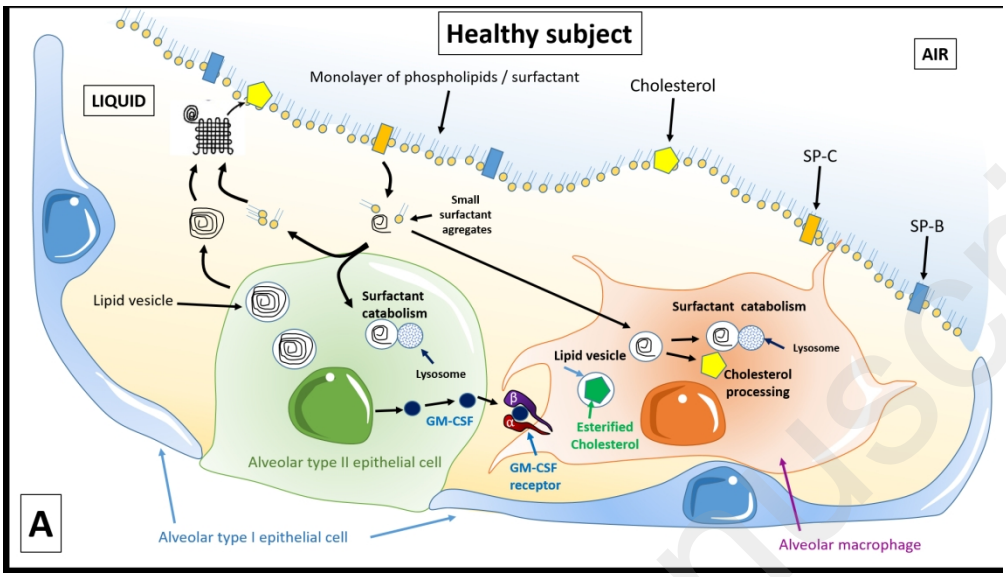

New figure 1A

340x193mm (150 x 150 DPI)

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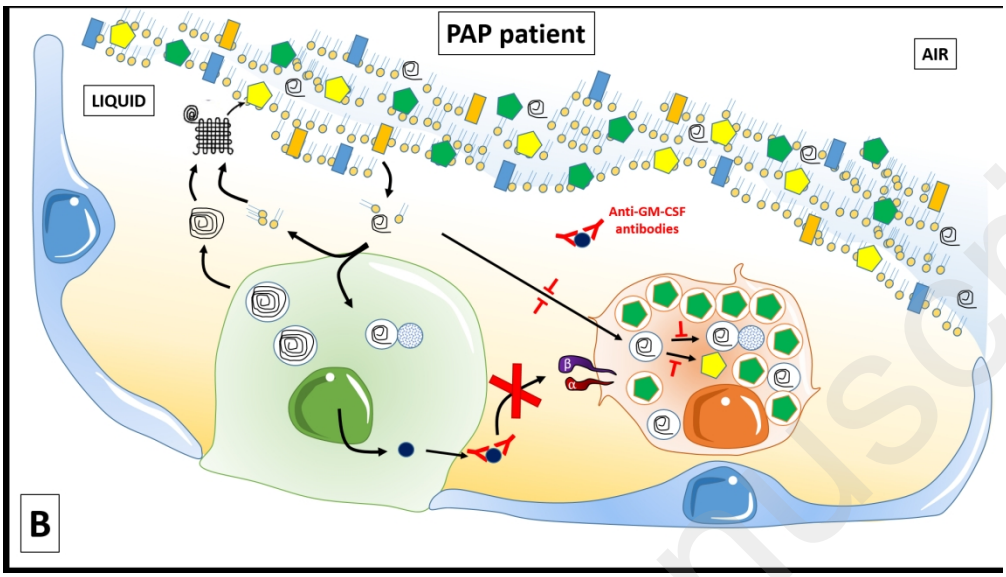

New figure 1B

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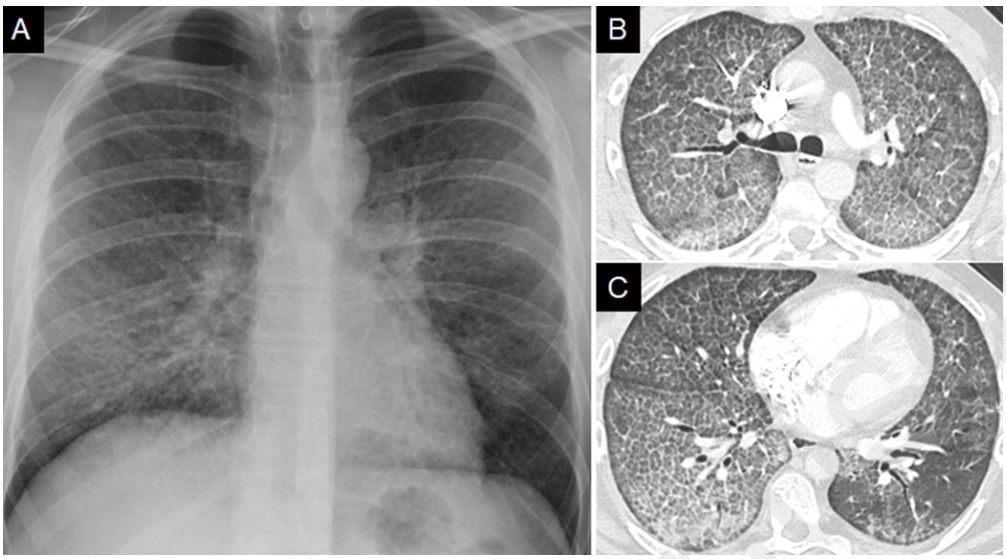

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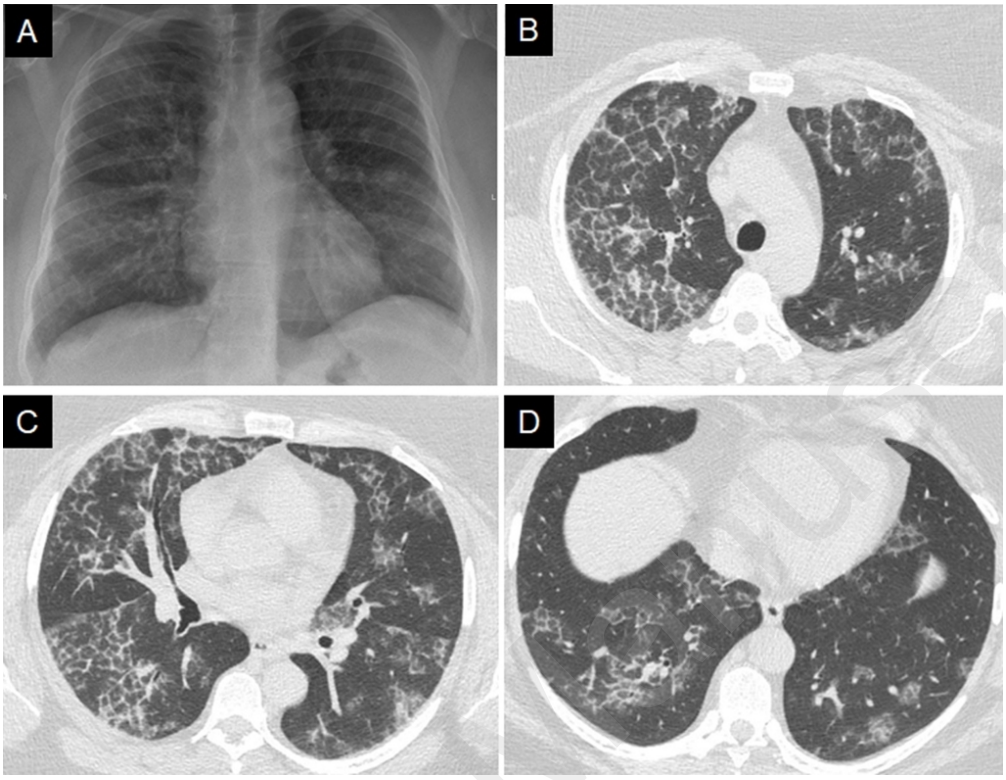

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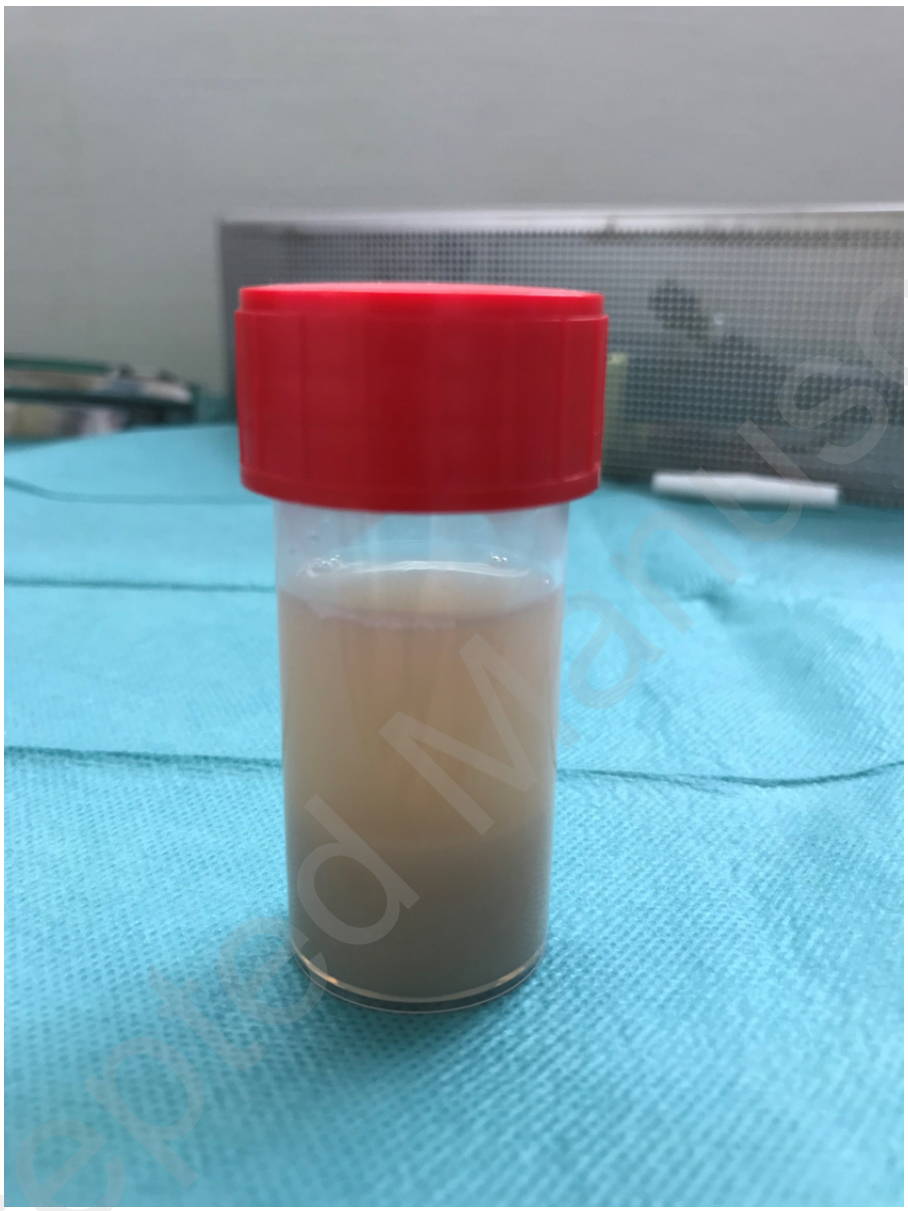

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