Secondary pulmonary alveolar proteinosis after lung transplantation: a single-centre series

To the Editor:

Pulmonary alveolar proteinosis (PAP) is a rare disease characterized by accumulation of surfactant in alveolar space related to alveolar macrophage dysfunction. PAP occurs in three clinically distinct forms: autoimmune PAP (90% of cases), secondary PAP and genetic PAP [1]. Secondary PAP may be related to immunosuppressive disorders, with few cases associated with solid-organ transplant [1]. Indeed, to our knowledge, only five cases of symptomatic PAP secondary to lung transplantation have been reported [2–4].

Here, we report a series of four new cases of PAP in lung transplantation recipients, with death associated with PAP-onset in all cases. One patient showed impaired granulocyte-macrophage colony-stimulating factor (GM-CSF) signalling in the absence of auto-antibodies, which argues for similar mechanisms as in autoimmune PAP. A specific enhanced risk associated with lung transplantation was also suggested by the lack of native lung involvement in single-lung transplantation recipients.

Patient one

A 66-year-old man with idiopathic pulmonary fibrosis (IPF) underwent left single-lung transplantation from a 34-year-old male smoker. The immediate post-operative course was uneventful, with primary graft dysfunction determined to be grade 0. On post-operative day 72, acute rejection (grade A2B0) [5] was diagnosed without complement component 4d (C4d) staining. Donor-specific antibodies were detected by Luminex (human leukocyte antigen DQ7 (HLA-DQ7); mean fluorescent intensity (MFI)=4030) and steroid boluses were administered followed, on post-operative day 101, by anti-thymocyte globulins due to the persistence of histologically proven acute rejection (grade A2B0) [5]. Subsequent transbronchial biopsies (post-operative day 134) showed complete resolution of the acute rejection episode (grade A0B0). Furthermore, bronchoalveolar lavage fluid (BALF) analysis did not show parasitic *(Pneumocystis jirovecii*), mycological, viral (using multiplex PCR assay), or bacterial infection (including *Nocardia*), and cardiac ultrasonography revealed normal left-ventricular function. Nevertheless, in parallel with histological resolution of the steroid-resistant acute rejection episode, repeat computed tomography (CT) scans showed progressively worsening lung opacities from post-operative day 129, with a "crazy-paving" pattern located exclusively in the graft (figure 1a). Re-examination of transbronchial biopsies from post-operative day 134 (figure 1b) and a new BALF analysis stained with periodic acid–Schiff (PAS) reagent (figure 1c) led to a diagnosis of PAP. Anti-(GM-CSF) antibodies were absent and the possibility of impaired GM-CSF signalling was investigated using a recently described test based on the ability of GM-CSF to rapidly increase cell-surface CD11b levels on neutrophils in flow cytometry (reflected by a CD11b stimulation index) [6]. The GM-CSF stimulation index in patient one was very low, with a value of seven as compared to values of 101, 55 and 102 in three stable lung-transplantation recipients used as positive controls (mean GM-CSF stimulation index of stable lung transplantation=86±26). Tacrolimus trough levels were tapered to 5–7 $ng \cdot mL^{-1}$. The patient remained dependent on oxygen, with persistent and progressively worsening PAP lesions (as evidenced by CT-scan) in the following months. He ultimately died from sepsis associated with catheter-related bacterial infection and pneumonia on post-operative day 516. Telomerase reverse transcriptase (*TERT*)/ telomerase RNA component (*TERC*) gene mutation, which has been shown to be associated with PAP [7], was absent in post-mortem investigations.

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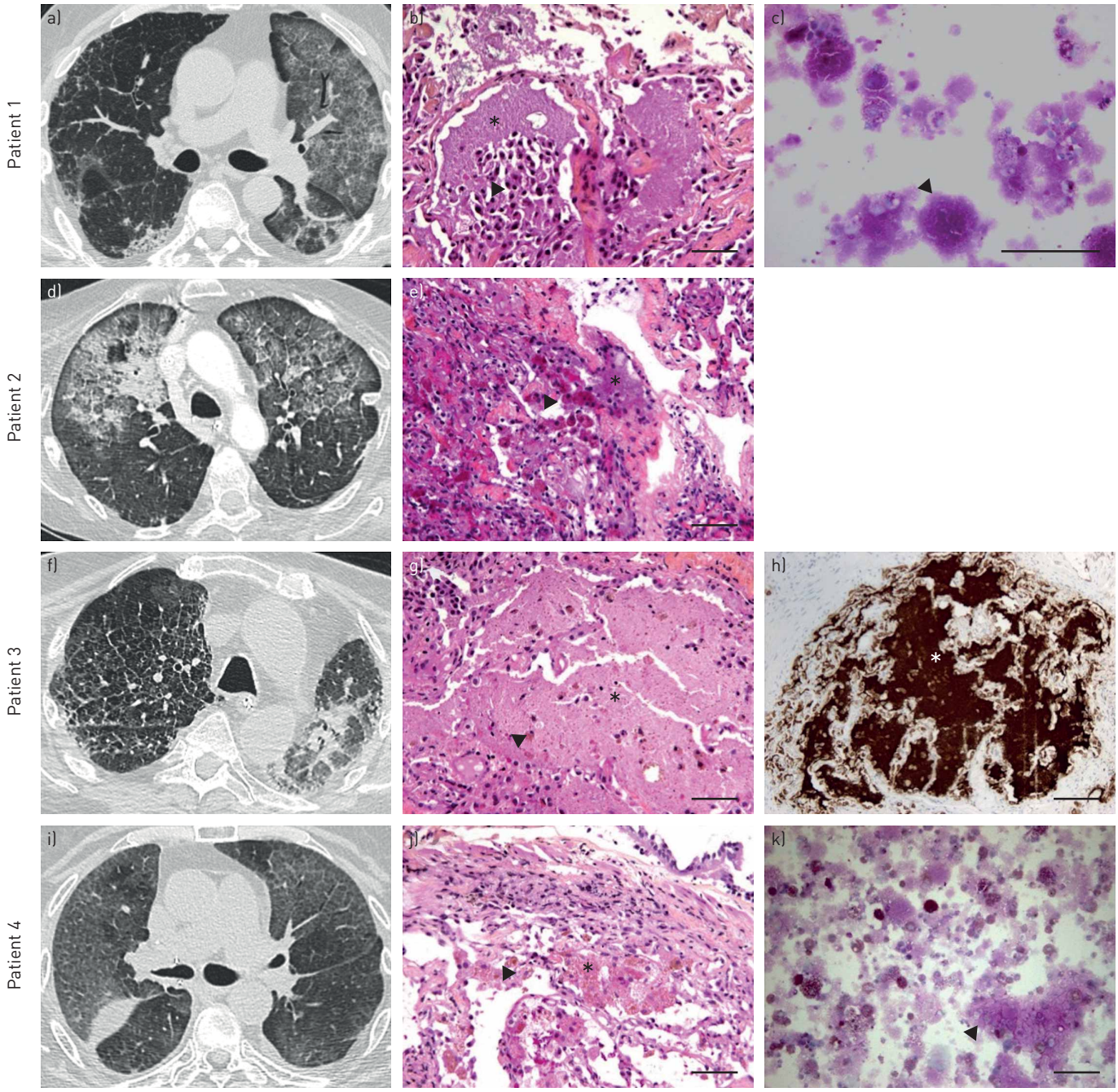

FIGURE 1 Images obtained for four patients exhibiting pulmonary alveolar proteinosis (PAP) after lung transplantation. Images (a), (d), (f) and (i) show axial transverse chest CT (computed tomography) results for patients 1, 2, 3 and 4, respectively. Reticulations are superimposed on ground-glass opacities forming a "crazy-paving" pattern with a geographic distribution (a juxtaposition of healthy and sick zones). Images (b), (e), (g) and (j) are transbronchial lung biopsies of patients 1, 2, 3 and 4, respectively, showing intra-alveolar accumulation of amorphous granular eosinophilic material (*) and numerous alveolar macrophages with finely granular brownish cytoplasm (arrowhead). Images (c) and (k) are stains of bronchoalveolar lavage fluid (BALF) from patients 1 and 4, respectively, showing periodic acid–Schiff (PAS) positive extracellular material (arrowhead). Image (h) is a transbronchial lung biopsy of patient 3 and is an example of immunohistochemical staining with a surfactant antibody [anti-(surfactant protein A), anti-(SP-A), $32^{E}12$, from Abcam Plc, Cambridge, UK], with a marked intensity of intra-alveolar amorphous material (*). Positive anti-(SP-A) staining was observed when performed for both patients 3 and 4. Scale bars=100 µm.

Patient two

A 61-year-old woman with emphysema underwent a double-lung transplantation from a 44-year-old male smoker. The post-operative course was uneventful with primary graft dysfunction determined to be grade 1. On post-operative day 33, a thoracic CT-scan showed discrete alveolar opacities considered to be nonspecific. A transbronchial biopsy showed no acute rejection (grade A0B0) [5], and donor specific

antibodies were absent. Repeated BALF analysis showed *Pseudomonas aeruginosa* bronchial colonization without parasitic (*Pneumocystis jirovecii*), mycological, viral, or bacterial (*Nocardia*) infection. From post-operative day 75, respiratory status deteriorated with the appearance of new lung opacities. On post-operative day 91, invasive aspergillosis was diagnosed with cavitary lung lesions seen on the lung CT-scan (figure 1d). The CT-scan also showed a coexisting "crazy-paving" pattern associated with aspergillosis lesions (figure 1d). The patient died from sepsis related to *P. aeruginosa* pneumonia on post-operative day 96. After death, re-examination of transbronchial biopsies performed on post-operative day 33 ultimately led to a diagnosis of PAP (figure 1e).

Patient three

A 63-year-old man with IPF underwent right-hand side, single-lung transplantation from a 40-year-old female smoker. The post-operative primary graft dysfunction was determined to be grade 3, with delayed ventilator weaning on post-operative day 20. Transbronchial biopsies performed on post-operative day 32 were normal (grade A0B0) and donor-specific antibodies were absent. On post-operative day 42, new-onset lung opacities were identified on the CT-scan (exclusively in the lung graft and with a "crazy-paving" pattern) (figure 1f). Analysis of BALF showed features of PAP lesions, with bronchial colonization by *Serratia marcescens* but without viral, parasitic (*Pneumocystis jirovecii*), bacterial (*Nocardia*), or mycological infection. Nevertheless, an acute rejection episode without histological proof was not definitively ruled out and steroid boluses were administered without clinical improvement. In the absence of a definite diagnosis, a retrospective analysis of the findings of previous transbronchial biopsies, BALF and CT-scans then led to a diagnosis of PAP (figures 1f–1h). Anti-(GM-CSF) antibodies were absent and GM-CSF therapy was started (250 mg·day^{-1}) with conversion from tacrolimus to cyclosporine. Nevertheless, the patient's condition progressively worsened, resulting in respiratory failure, and he ultimately died from sepsis at post-operative day 80.

Patient four

A 65-year-old man with IPF underwent double-lung transplantation from a 27-year-old female non-smoker. The primary graft dysfunction was determined to be grade 3 and subsequent *Enterobacter cloacae* pneumonia delayed weaning from the ventilator until post-operative day 16. From post-operative day 90, the patient's respiratory condition progressively deteriorated and CT-scans displayed a "crazy-paving" pattern. Transbronchial biopsies revealed no cellular acute rejection (grade A0B0) while BALF displayed *Staphylococcus epidermidis* colonization without viral, parasitic (*Pneumocystis jirovecii*), bacterial (*Nocardia*), or mycological infection. Due to the detection of donor-specific antibodies (HLA-DQ9; MFI=7598), and although C4d staining was negative [8], antibody-mediated rejection was considered and the patient underwent plasma exchange (n=5) starting from post-operative day 101. This allowed a decrease in donor-specific antibody MFI (HLA-DQ9; MFI=947) albeit without respiratory improvement. Administration of steroid boluses on post-operative day 108 also led to no improvement and, on post-operative day 114, retrospective re-examination of CT-scans and transbronchial biopsies (from post-operative day 97), together with new BALF findings, led to a diagnosis of PAP (figures 1i–1k). Anti-(GM-CSF) antibodies were absent and sequential therapeutic bronchoalveolar lavages (BALs; n=4), GM-CSF administration and withdrawal of mycophenolate mofetil led to transient improvement. From post-operative day 142, PAP lesions increased in number (as revealed by CT-scan), while progressive respiratory failure occurred. The patient died from bacterial pneumonia on post-operative day 153.

Summary

The occurrence of secondary PAP in our single cohort of lung-transplant recipients within a 4-year period suggests a possible under-recognition of this complication in this population. This situation could result from the difficulty in diagnosing PAP using post-transplantation BALF, which often contains microorganisms, inflammatory cells and mucus, or from the absence of routine systematic PAS staining of BALF after lung transplantation. In this setting, the positivity of PAS staining can also allow exclusion of other causes of intra-alveolar material accumulation, such as oedema or fibrin, and causes of vacuolated macrophages as seen with amiodarone toxicity. In our series, histological diagnosis of PAP was additionally supported (when performed) by positive staining with an anti-surfactant antibody (figure 1h). Exclusion of silicoproteinosis was systematically performed by polarized light examination with no birefringent silica particles. In all patients, PAP developed early after lung transplantation (before month 3, post-transplantation) with a fatal outcome being associated with this onset. Diagnosis was delayed in the four case studies and was established only after specific re-examination of the transbronchial biopsies/BALF. In the autoimmune and genetic forms of PAP [6, 9], the alveolar macrophage dysfunction is known to be driven by impaired GM-CSF signalling. A similar impaired GM-CSF pathway was found in one patient by using a recently described test based on the ability of GM-CSF to rapidly increase cell-surface CD11b levels on neutrophils in flow cytometry [6, 9]. A coincidental primary form of PAP was ruled out

by the lack of anti-(GM-CSF) antibodies (n=3). The striking lack of involvement of the native lung in recipients of single-lung transplantation may indicate an alveolar macrophage injury linked to a pathogenic process located specifically in the graft; however, this remains to be determined. In addition, the promoting role of immunosuppressive therapy has previously been evoked in cases of symptomatic PAP reported to develop in lung- or kidney-transplant recipients [2–4, 10], including almost all well-described cases related to mammalian target of rapamycin inhibitor (mTOR inh) use [4, 10].

In the lung-transplantation setting, it is important to be able to distinguish between PAP and an acute rejection episode, especially as inter- and intra-lobular septal thickening are included in the features of acute rejection [11, 12]. In our patients, the initial misdiagnosis of PAP was associated with a suspected or proven acute rejection episode, which was treated with increased immunosuppression that possibly worsened the alveolar macrophage dysfunction. Finally, our cases often showed subsequent pulmonary infection, which is consistent with the less-efficient alveolar macrophage reported in PAP for anti-infection defence [13, 14]. Due to these diagnostic challenges, physicians should be aware of this rare, life-threatening, post-transplantation complication in an attempt to provide specific treatment.

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