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CASE REPORT

Pulmonary alveolar proteinosis – a rare pulmonary toxicity of sirolimus

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Summary

The aim of our paper is to describe an unusual pulmonary toxicity of sirolimus (SRL) in a kidney transplant recipient. We present a 34-year-old woman with a second renal transplantation, complicated with steroid-resistant acute rejection and chronic allograft dysfunction. Two years after initiating SRL, she presented complaints of progressive dyspnoea, nonproductive cough, chest pain and low-grade fever of 1 month duration. She had chronic allograft nephropathy and slight elevation of lactic dehydrogenase levels. After exclusion of common reasons of this condition, a computed tomography (CT) of the thorax and bronchoscopy was performed, revealing ground-glass opacification with polygonal shapes on CT and an opaque appearance with numerous macrophages on bronchoalveolar lavage. The alveolar macrophages stained positive by Periodic acid-Schiff. Diagnosis of pulmonary alveolar proteinosis (PAP) was made and drug-induced toxicity was suspected. SRL was withdrawn with marked improvement in the patients' clinical and radiological status. PAP resolved within 3 months without further therapy. PAP is a very rare complication of SRL therapy with only a few cases described. Withdrawal of SRL with conversion to another immunosuppressant seems to be an appropriate procedure in this condition.

Case report

We report a 34-year-old white woman, with end-stage renal failure of unknown aetiology since June 1994. She underwent a first cadaveric renal transplantation in September 1994. Initial immunosuppression included ATG, cyclosporine A (CsA) and steroids. This transplantation was complicated by an early episode of severe steroid-resistant acute rejection (AR) which was treated with OKT3. The graft was lost at 2 years because of chronic allograft nephropathy (CAN).

In January 1998, a second living-related renal transplantation (from her mother) was performed (two HLA mismatch; PRA max/actual: 56/10%; donor and receptor cytomegalovirus positive). Initial immunosuppression consisted of CsA, mycophenolate mofetil (MMF) and steroids, which were withdrawn in 2000 because of cosmetic problems. In 2001, she presented CAN and CsA was converted to tacrolimus (FK). In August 2001, there was an episode of steroid sensitive AR. Maintenance immunosuppression was then changed to MMF, FK and steroids. In 2003, she developed severe alopecia and she was then changed from FK to sirolimus (SRL). The patient was also taking furosemide, losartan and atenolol for hypertension and simvastatine for dyslipidaemia. Trough blood levels of SRL ranged from 5 to 20 ng/ml over the next 24 months.

Two years after initiating SRL, she was admitted with complaints of progressive dyspnoea, nonproductive cough, profound fatigue with minimal exertion, chest

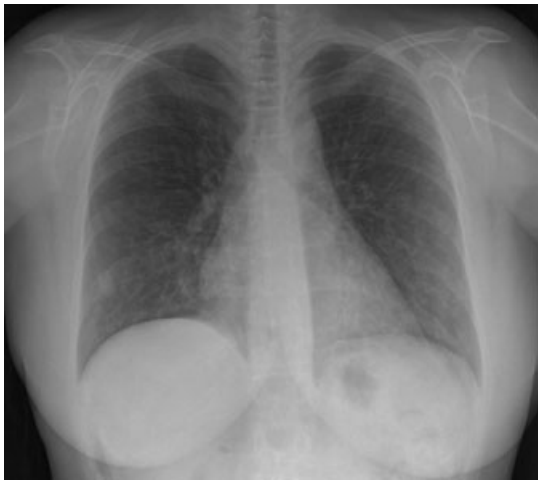

Figure 1 Chest X-ray showing bilateral symmetric alveolar infiltrates, and a nodular pattern in the lower right lung.

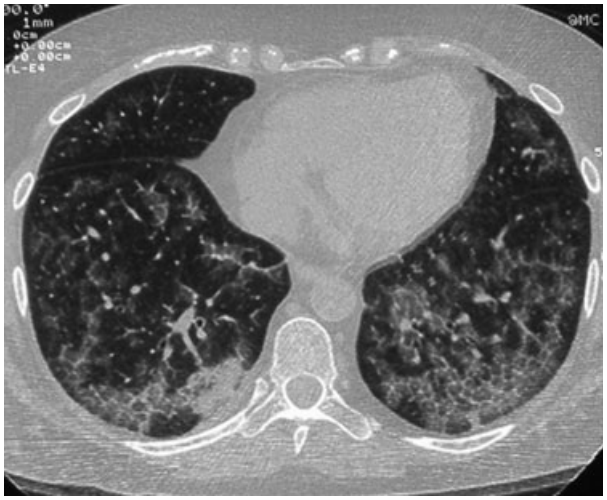

Figure 2 Computed tomography of the thorax showing ground-glass opacification, with polygonal shapes a pattern commonly referred as 'crazy paving' typically seen in pulmonary alveolar proteinosis.

pain and low-grade fever of 1 month duration. Physical examination revealed inspiratory crackles bilaterally and no acute distress syndrome. Blood pressure was 108/69 mmHg. The remaining physical exam was unremarkable. Laboratory examination revealed anaemia (haemoglobin: 10.3 g/dl), with no new abnormalities in blood chemistry, stable renal function with a creatinine of 2.9 mg/dl and elevation of the serum level of lactate dehydrogenase (465 U/l; normal value: 135–214 U/l). Reactive C-protein was normal. SRL trough blood level was 9.1 ng/ml. Arterial-blood gas measurements revealed hypoxemia reversible with O_2 therapy and widened alveolar–arteriolar diffusion gradient (29 mmHg). Arterial blood gases with Fi O_2 of 35% were: pH: 7.39, pCO_2: 29 mmHg, pO_2: 94 mmHg and HCO_3: 19.2 mmol/l. The patient had normal serum complement concentrations. Tests for antinuclear, antineutrophil cytoplasmic and antiglomerular-basement-membrane antibodies were negative. Chest X-ray revealed bilateral symmetric alveolar infiltrates, and ill-defined nodular pattern in the lower right lung (Fig. 1). Because of suspected respiratory infection, the patient received levofloxacin. CT of the thorax showed patchy ground-glass opacifications with polygonal shapes (Fig. 2). Bronchoscopy with bronchoalveolar lavage (BAL) was performed twice revealing an opaque appearance (Fig. 3). BAL showed no evidence of bacterial, mycobacterial, fungal, viral or parasitic infection, or intra-alveolar haemorrhage but did show numerous macrophages (total cell count of 7×10^6 with 9% neutrophils, 7% lymphocytes, 1% eosinophils and 83% macrophages). Periodic acid-Schiff (PAS) stained positive the alveolar macrophages and amorphous lipoproteinaceous material (Fig. 4). Blood, urine and sputum cultures, CMV serology and all other

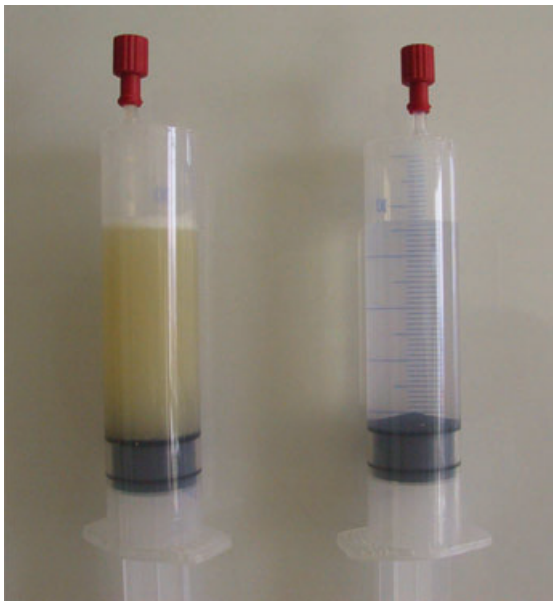

Figure 3 Bronchoalveolar lavage: revealing an opaque appearance in left compared with saline solution in the right.

investigations for virus, opportunistic fungi, mycobacteria and parasites were negative. All antibiotics were discontinued. Pulmonary function tests (PFT) showed severe restrictive ventilatory defect with a total lung capacity of 2.52 l (47% of predicted), a vital capacity of 1.69 l (45.4% of predicted) and a residual volume of 0.84 l (52.3% of predicted), with a disproportionate reduction in the total carbon monoxide diffusion capacity: 2.13 mmol/min/kPa (22.6% of predicted).

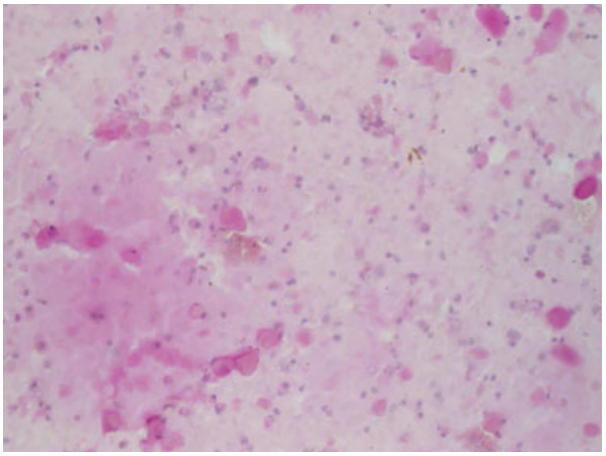

Figure 4 Periodic acid-Schiff stains positive alveolar macrophages and amorphous lipoproteinaceous material (20X).

Diagnosis of pulmonary alveolar proteinosis (PAP) was made and secondary, drug-induced toxicity was suspected. SRL was the last immunosuppressor introduced, so it was decided to withdraw SRL and converted back immunosuppression to FK, maintaining MMF and steroids. No other treatment was given and the other drugs remained unchanged. Discontinuation of SRL was followed by marked improvement in the patient clinical and radiological status and lactate dehydrogenase normalization. She gradually weaned from oxygen (arterial blood gases with FiO_2 of 21%: pH 7.37, pCO_2: 34 mmHg, pO_2: 96 mmHg, alveolar–arteriolar diffusion gradient: 13 mmHg). PAP resolved within 3 months, with improvement in the PFT (total lung capacity of 4.62 l – 86.1% of predicted, a vital capacity of 3.62 l – 98.3% of predicted, and a residual volume of 1.14 l – 70.2% of predicted, with total carbon monoxide diffusion capacity: 6.7 mmol/min/kPa – 71.6% of predicted) and in the lung appearance on CT of the thorax (Fig. 5).

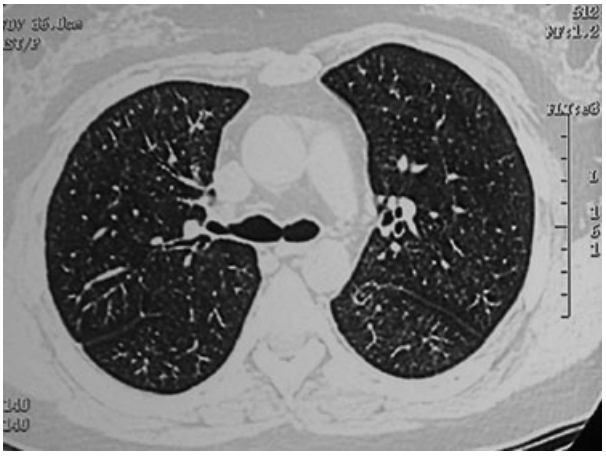

Figure 5 Computed tomography of the thorax showing marked improvement of the lung appearance.

Discussion

Sirolimus is a highly potent immunosuppressant used with increasing frequency in transplant patients as a calcineurin inhibitor sparing agent with minimal nephrotoxicity [1,2]. However, SRL also has side-effects as dose-dependent hyperlipidaemia, anaemia, thrombocytopenia, hepatotoxicity, as well as delayed wound healing and increased incidence of lymphoceles [2–5]. SRL-induced pulmonary toxicity is a rare but serious entity that should be considered in the differential diagnosis of transplant recipients presenting with respiratory compromise. Recently, interstitial pneumonitis, bronchiolitis obliterans with organizing pneumonia, alveolar haemorrhage and noncardiogenic pulmonary oedema have been described in rare transplant patients treated with SRL [6–9]. Infrequent is the description of PAP associated with this drug. SRL-associated PAP was only described in the literature in two lung transplant recipients, which presented complete recovery after discontinuing SRL [9–10]. As far as we know, there are no cases of SRL-associated PAP reported in kidney transplant recipients treated with SRL, MMF and steroids.

Since January 2000, the Food and Drug Administration has been advised of 64 organ transplant recipients who develop pulmonary complications that were temporally associated with SRL use. These consisted of 52 kidney, four lung, three liver, three heart, one heart-lung and one islet cell transplants. Most of these cases resolved with dose-adjustment or cessation of the drug; however, 4.8% of the patients died. All of the deaths occurred among heart recipients [9]. However, two cases of reversible SRL-associated pneumonitis after heart transplantation were recently reported [11,12]. The case of Chau *et al.* [11] refers that SRL-induced pneumonitis can occur even when low dose of SRL is used and outlines the importance of recognize that rapid development of respiratory distress may be a possible adverse effect of the drug, which is reversible on withdrawal of SRL [11]. Time interval between initiation of therapy with SRL and the onset of pulmonary symptoms varied, with some patients initiating the symptoms more than a year after starting the drug [7].

Morelon *et al.* [13] proposed four criteria for the definitive diagnosis of SRL-induced lung toxicity: (i) exposure to SRL preceding the onset of pulmonary symptoms; (ii) exclusion of infection or alternative pulmonary disease; (iii) exclusion of other possible offending agents; and (iv) resolution of symptoms after withdrawal or dose-reduction of SRL.

Although risk factors for pulmonary toxicity were not clearly established, high dose, late exposure to the drug (e.g. switch regimen) and male gender have been suggested [9]. As in our patient, in most reported cases, SRL was added later in the course of transplantation, usually trying to attenuate nephrotoxic effects of a previous immunosuppressive regimen including calcineurin inhibitors. Almost all patients (95%) resolved their clinical and radiological findings with withdrawal or dose-reduction of SRL [9]. Most of the authors describe rapid improvement of clinical and radiological status of the patient within a few weeks of SRL withdrawal and resolution approximately at 2–6 months [6,7,13,14].

Pulmonary alveolar proteinosis is a rare disorder characterized by the accumulation of amorphous, PAS-positive lipoproteinaceous material within alveoli, with little or no lung inflammation and preservation of lung architecture. PAP occurs in three clinical forms: congenital, secondary and acquired or idiopathic. Secondary PAP may be associated with some haematological cancers, pharmacologic immunosuppression, inhalation of inorganic dust (e.g. silica) or toxic fumes and certain infections. These conditions may be responsible for functional impairment or reduced numbers of alveolar macrophages, with consequent impaired production or degradation of surfactant. It has a variable clinical course ranging from respiratory failure to spontaneous resolution that occurs in only 8% of the idiopathic forms. Typical age at presentation is 30–50 years [15]. Long-term outcome of PAP has been described in a retrospective study of 343 cases with 5 years survival rate of 75% [16]. In this study, the deaths were directly attributed to respiratory failure from PAP in 72% of cases and 20% attributed to PAP with uncontrolled infection. The risk of death declined, significantly beyond 12 months from diagnosis. Patients with opportunistic infection seem to have a shorter duration of symptoms before PAP diagnosis, perhaps because the presentation was precipitated by the development of infection itself. These patients were more likely to be diagnosed at autopsy (21% vs. 9%, $P = 0.01$) [16].

Secondary PAP has been described, although rarely, as a complication of underlying immunodeficiency disorders (e.g. AIDS, severe combined immunodeficiency disorder, or immunoglobulin A deficiency) or related to malignant haematological diseases as leukaemia, myelodysplasia and following bone marrow transplantation [16,17]. One report of secondary PAP in patients with haematological malignancies emphasizes that PAP is potentially reversible, especially if complete remission of the disease, and so of immunosuppression induced by the haematological disease, is achieved [17]. Secondary PAP has also been described, rarely, as a result of various immunosuppressive medications after transplantation [15,16]. A recent report [18] described one case of PAP associated with immunosuppression with leflunomide and corticosteroids in a patient with rheumatoid arthritis. Leflunomide is a newly approved disease-modifying antirheumatioid arthritis drug also used in France in psoriatic arthritis. The active metabolite of leflunomide has multiple actions that are dose-related, but the major action at the doses given for arthritis seems to result of the inhibition of *de novo* pyrimidine synthesis at the level of the enzyme dihydroorotate dehydrogenase. In this case, PAP recurred after reintroduction of the leflunomide witch make this drug the most probable medication responsible for the development of PAP [18]. All these situations of immunosuppression, as immunodeficiency disorders, haematological malignancy and treatment with immunosuppressive drugs raise the suspicion of some common pathway that modify alveolar macrophages homeostasis and induce secondary PAP.

Other diseases that can demonstrate, rarely, histopathological findings similar to PAP include infections such as CMV, *Nocardia*, mycobacterial, fungal infections, cryptococcosis and *Pneumocystis carinii* pneumonia either preceding the development of PAP, but often seen complicating the disease [16]. On the other hand, an important feature of PAP is the increased susceptibility to pulmonary superinfection. Such infections include common respiratory pathogens, but also opportunistic organisms such as *Nocardia*, mycobacteria, and various endemic or opportunistic fungi caused by impaired macrophage phagocytic function [15]. Interestingly, infections in PAP not only involve the lung but also occur outside the lung, suggesting systemic alterations in the patient defence [15,16]. In the transplant patients, these opportunistic infections should be intensively searched, as these patients are immunosuppressed presenting more risks for these kinds of infections.

In our immunosuppressed patient, as in other cases described in the literature, the first suspicion was an infectious disease, so antibiotics were initiated. In the absence of improvement, a CT of the thorax and bronchoscopy with BAL was performed. These exams help not only in obtaining material for bacteria, virus, fungi and mycobacteria cultures, but also the CT and BAL characteristics can help to differentiate from another pulmonary diseases. SRL-induced pulmonary disease must be kept in mind as changing immunosuppression can be life saving. The SRL-associated interstitial pneumonitis is the most common described pulmonary toxicity of SRL [9]. Interstitial pneumonitis usually has a typical radiographic pattern with bilateral interstitial infiltrates usually asymmetric. The BAL typically shows an alveolar inflammatory picture with lymphocytic predominance, particularly of CD4 T-cells. The alveolar haemorrhage, also described as SRL-induced toxicity, usually shows a frankly

bloody or blood-tinged BAL fluid. The differential diagnosis of drug-induced toxicity in these immunocompromised patients includes a large number of pulmonary diseases. Among them are interstitial pneumonitis, alveolar haemorrhage, bronchiolitis obliterans with organizing pneumonia, or diffuse alveolar damage of known cause (e.g. sepsis, shock or infection) [6–9].

Pathophysiology of PAP associated with SRL is not well understood. SRL blocks the transduction of signals from the IL-2 receptor to the nucleus and the receptors for other cytokines and growth factors and unlike calcineurin inhibitors it does not inhibit T-cell cytokine gene expression. The overall effect of SRL is to halt cell cycle progression from the G1 to the S phase in lymphocytes as well as other cell types including fibroblast and endothelial cells [5,9]. One might hypothesize that SRL may interfere with cytokines and transforming growth factors that may play a role in pulmonary macrophage dysfunction, leading to decrease clearance of surfactant. The exact mechanisms leading to PAP in this case are unknown.

Treatment of PAP depends on the underlying cause. In the acquired form, whole-lung lavage remains the standard of care. Therapy with subcutaneous granulocyte macrophage colony stimulating factor (GM-CSF) has a poor response rate. Therapy for secondary PAP generally involves treatment of the underlying condition as treatment of an associated haematological cancer or discontinuation of an offending drug [15].

In our case, the patient was on SRL prior the onset of pulmonary symptoms and SRL was the last drug introduced. The absence of pathogens on numerous surveillance cultures and serologies and the absence of improvement with antimicrobial therapy argue in favour of a noninfectious cause. The investigation revealed the typical findings of PAP. Although rare, SRL-induced lung toxicity may be manifested by PAP. It is unlikely that, in our case, PAP was caused by other drugs as no other change in treatment was made. Another observation in favour to SRL-related toxicity inducing PAP was biochemical normalization of lactic dehydrogenase after SRL withdrawal. If even PAP was a consequence of combined administration of SRL with other drug, SRL withdrawal solved the problem. The prompt resolution of clinical and radiological abnormalities on withdrawal of SRL, and the absence of other confounding changes in treatment strongly implicate the drug as the cause of PAP.

In conclusion, replacement of SRL for another immunosuppressant seems appropriate in SRL-associated PAP. Although extensive effort should be made to rule out infections before lung disease can be attributed to SRL, early recognition of this association may avoid invasive investigations such as lung biopsy. Discontinuation of SRL may lead to complete resolution of PAP. Whether dose-reduction will prevent this complication and whether retreatment at lower doses is safe remains to be determined. Lung toxicity of SRL, including PAP, should be considered in the differential diagnosis of pulmonary disease in transplanted patients who are taking the drug.

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