

## SUCCESSFUL TREATMENT OF PULMONARY AND LYMPHATIC MANIFESTATIONS OF LYMPHANGIOLEIOMYOMATOSIS WITH SIROLIMUS

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### ABSTRACT

*Lymphangioleiomyomatosis (LAM) is a rare, progressive, diffuse cystic lung disease predominantly affecting women of child bearing age. Recently treatment with sirolimus was shown to stabilize lung function decline and improve quality of life in patients with LAM. We treated three premenopausal women suffering from LAM manifesting as diffuse cystic lung disease, chylous effusions, and lymphangioleiomyomas with sirolimus (1-3 mg a day; sirolimus trough levels 2.9-8.5 ng/ml). All three patients had a remarkable response to sirolimus, with resolution of effusions, improvement in lung function and shrinking of abdominal lymphangioleiomyomas. Our case series further complements the literature in that sirolimus is a safe and effective treatment for LAM and its lymphatic manifestations.*

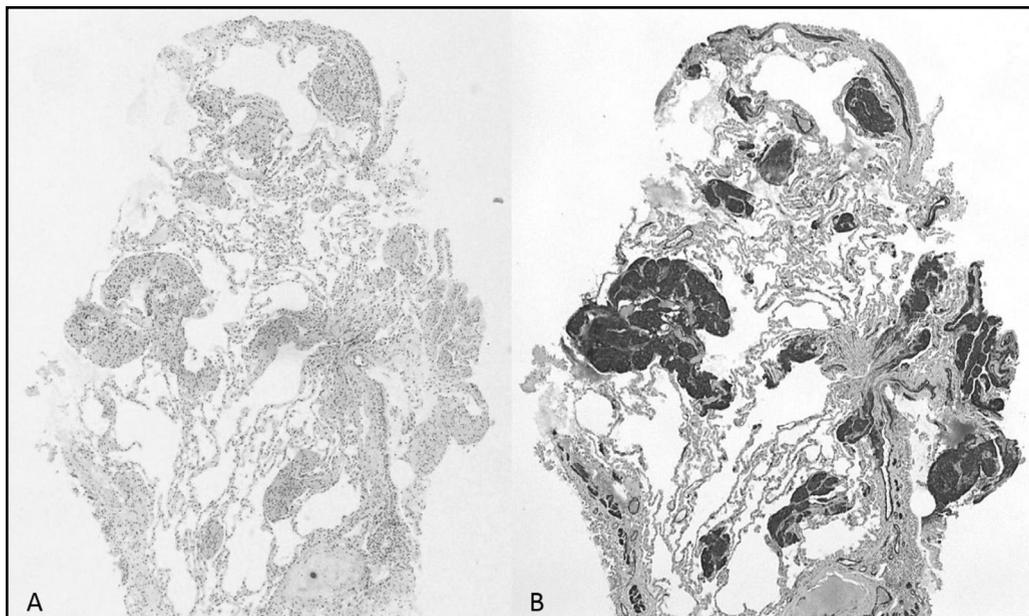
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Lymphangioleiomyomatosis (LAM) is a rare disease with a worldwide prevalence of 3.4-7.8 per million women (1). LAM

predominantly affects women of child bearing age and only a few cases in men have been described (2,3). LAM can be seen either in association with tuberous sclerosis complex (TSC-LAM) or occur alone, also known as Sporadic LAM (S-LAM) (3).

LAM is a progressive, diffuse cystic lung disease and is best characterized as a low-grade, destructive, metastasizing neoplasm (4). TSC-LAM is caused by mutations in either one of the two known TSC genes, TSC1 and TSC2, where as only TSC2 mutations have been reported in cases of S-LAM. The mutations in TSC-LAM are germline (present in all cells), while TSC mutations in cases of S-LAM are somatic (confined to lungs, kidneys, and lymph nodes) (5,6).

Patients with LAM typically present with manifestations due to pulmonary involvement, with dyspnea on exertion or recurrent pneumothorax being the most common modes of presentation (7). High resolution computed tomography (HRCT) of the chest is the most sensitive non-invasive modality to diagnose LAM. Characteristic HRCT findings of LAM include multiple smooth, round, thin-walled cysts ranging in size from a few millimeters to a few centimeters and distributed in a diffuse



*Fig. 1. Transbronchial lung biopsy demonstrating distorted architecture due to proliferation of LAM cells, causing loss of alveolar spaces and small cysts formation (Fig 1A). LAM cells showed strong positive reaction with smooth muscle actin antibody (Fig 1B). (A: hematoxylin eosin stain, objective x4; B: immunohistochemistry, Smooth muscle actin antibody, objective X4)*

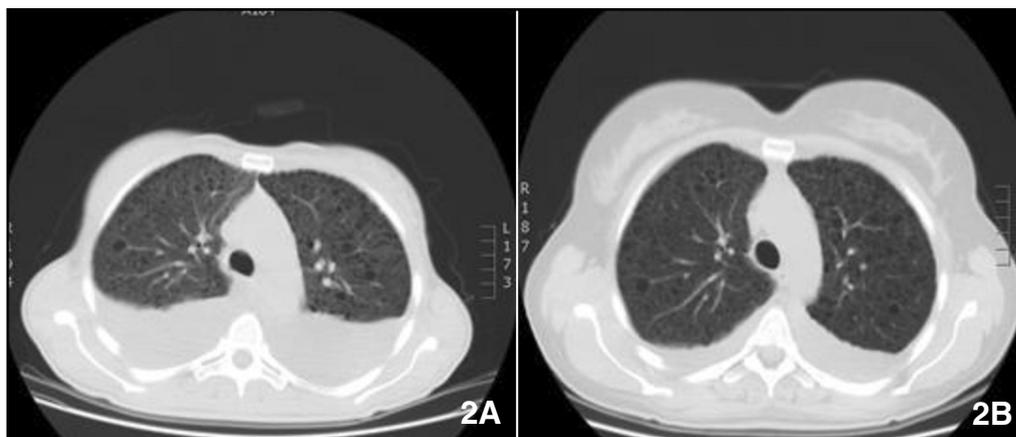
manner. The intervening lung parenchyma appears normal on HRCT in most patients with LAM (8). The primary histopathological abnormality in LAM is the proliferation of abnormal smooth muscle cells (LAM cells) in the lung parenchyma. The LAM cells stain positive for smooth muscle actin and desmin and for HMB-45 (monoclonal antibody against the premelanosomal enzyme glycoprotein-100), which helps confirm the diagnosis on histopathology (9).

In addition to the pulmonary cysts, patients with LAM can have a myriad of extra-pulmonary manifestations, most commonly secondary to lymphatic involvement. Our case series describes three non-smoking women of reproductive age, who had a significant burden of both pulmonary and lymphatic complications from LAM.

#### *CASE REPORT*

##### *Case 1*

Our first patient is a 44-year old female who was healthy until 2010 when she was hospitalized for the first time because of progressively worsening dyspnea on exertion. Imaging performed at that time showed bilateral, diffuse thin-walled cysts in the lungs, bilateral pleural effusions, and abdominal retroperitoneal tumors. Thoracentesis was performed to further evaluate the pleural effusion, which revealed a triglyceride level of 796 mg/dl, consistent with a chylous effusion. She underwent a needle biopsy of the abdominal tumor and a transbronchial lung biopsy. Histopathology from the abdominal tumor was consistent with lymphangioliomyoma and the lung biopsy revealed characteristic changes of LAM (Fig. 1). Proliferation of smooth muscle-like cells (LAM cells) within pulmonary lymphatics was found in both lungs and abdominal tumor. Immunohistochemistry showed positivity for smooth muscle actin and progesterone receptors. The patient's



**Fig. 2.** Chest CT findings before and after treatment in Patient 1. Fig 2A: Chest CT showing bilateral pleural effusions. Fig 2B: Chest CT 10 months after treatment with sirolimus showing significant decrease in the pleural effusions. Also, note the bilateral, diffuse, thin-walled cysts on chest CT consistent with LAM.

spirometry values were within normal limits: forced expiratory volume in 1 second (FEV1) 2.56 liters (84.7% predicted), forced vital capacity (FVC) 3.10 liters (88.8% predicted), but diffusion capacity for carbon monoxide (DLCO) was reduced (DLCO 50% predicted) and she was hypoxemic on room air with a  $pO_2$  of 69 mmHg. During the next three years, there was further deterioration of her lung function and she now required supplemental oxygen at a rate of 4 liters per minute at all times. In March 2013, her DLCO had decreased further to 20% predicted. The FEV1 and FVC had also declined considerably and were as follows: FVC 1.68 L (49.1%), FEV1 1.16L (39.4%), amounting to a 500 ml/year decline in FEV1.

Two months later, in May 2013, she was admitted to the Intensive Care Unit (ICU) because of worsening hypoxemic respiratory failure. Chest X ray showed bilateral pleural effusions. CT abdomen was performed and it showed an increasing size of her existing retroperitoneal lymphangioliomyoma, which now measured 7 cm in diameter as opposed to 5 cm three years ago. Treatment with sirolimus was started in June 2013 alternating between 2 and 3 mg daily (sirolimus trough levels 5.8-7.7 ng/ml). After a few weeks, she had a remarkable clinical

improvement with almost complete resolution of the pleural effusions (Fig. 2) and by the time of hospital discharge, no longer required supplemental oxygen.

After 10 months of treatment, abdominal CT scan was performed and it showed significant reduction in the size of her retroperitoneal lymphangioliomyoma (Fig. 3), which now measured 1.9 x 1.2 cm in diameter. Her pulmonary function tests (PFTs) showed significant improvement and she now had a DLCO of 32% predicted, FVC 2.52 L (76.8%), and FEV1 1.84L (65.5%). The patient continues to be on sirolimus 1 mg daily and has stayed stable with no signs of clinical deterioration.

#### Case 2

A 33 year-old female was admitted to our hospital in September 2013 with complaints of progressively worsening dyspnea on exertion. Three years prior, a large retroperitoneal mass was found on regular gynecology exam. Based on a needle biopsy, the mass was termed as liposarcoma. The patient declined chemotherapy at that time. We re-evaluated the pathology slides and the mass revealed trabeculae of cytologically uniform eosinophilic spindle cells distributed

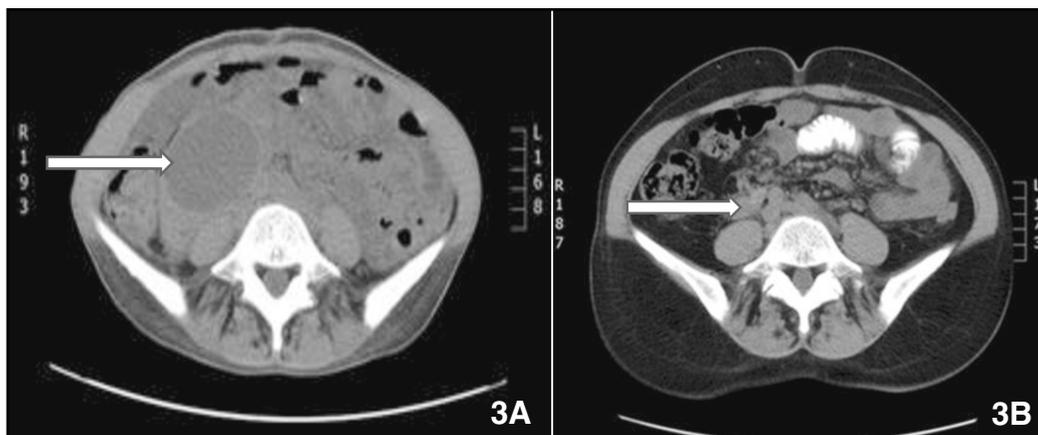


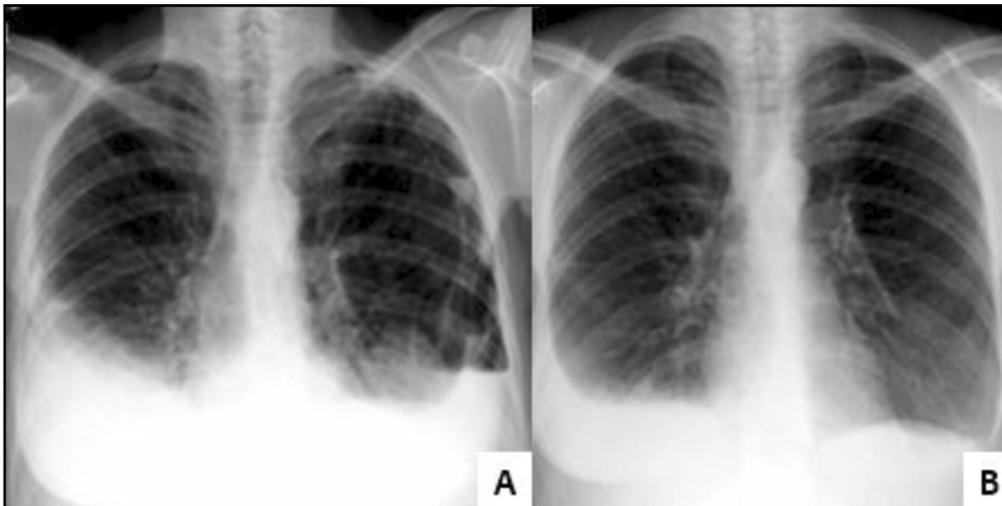
Fig. 3. Abdominal CT scan before (Fig 3A) and after 10 months of treatment with sirolimus (Fig 3B) showing a marked reduction in the size of lymphangioliomyoma (Arrow).

around numerous thin-walled lymphatic vascular channels, highlighted by immunopositivity for smooth muscle actin, desmin, microphthalmia transmission factor (MIFT) and focally for Melan-A. Based on these findings the diagnosis was changed to lymphangioliomyoma and given the characteristic findings, the pathologist decided not to use staining for HMB-45. Further work-up for her dyspnea was pursued with PFTs and chest CT scan. Her PFTs revealed: FVC 2.59 liters (59.8% predicted), FEV1 1.86 liters (49.1% predicted); FEV1/FVC 0.72, and DLCO of 35.6% predicted. Multiple pulmonary parenchymal cysts were found on chest CT scan together with bilateral partial loculated pneumothoraces and pleural effusions. Abdominal CT scan revealed multiple cystic masses containing low attenuation fluid in the retroperitoneum and pelvis, with varying sizes, the largest being 10 cm in diameter. We started treatment with sirolimus 1 mg daily (sirolimus trough levels 2.9-3.2 ng/ml). Three months post treatment, chest X-ray showed almost complete resolution of pleural effusions (Fig. 4). Nine months into the treatment course, the patient's retroperitoneal lymphangioliomyoma had shown a significant reduction in size, and now measured 2 cm in diameter. The lung function tests

were markedly improved with her DLCO now being 68% predicted, FVC 3.60 liters (83.1% predicted), and FEV1 2.68 liters (70.8% predicted).

### Case 3

A 34 year-old female, with no prior medical history, presented to the hospital with complaints of abdominal pain and distension in 2011. An 8 cm retroperitoneal mass was discovered on abdominal imaging. The tumor was extirpated and histopathological analysis confirmed a diagnosis of leiomyoma. Subsequently, the patient was lost to follow up. The patient reported a few mild episodes of hemoptysis to her primary care provider over the next 3 years, but due to the self-limiting nature of the episodes, no further diagnostic work up was pursued. Three years later, in March 2014, the patient delivered a healthy baby. Three months after her delivery, the patient presented with worsening ascites. At the time of admission, she did not have any respiratory symptoms. Diagnostic paracentesis performed on the ascitic fluid revealed elevated triglycerides (1991 mg/dl) in the fluid consistent with chylous ascites. Due to the prior history of hemoptysis, CT scan of her chest was done which showed diffuse involvement of the



*Fig. 4. Chest x-ray findings in Patient 2. Fig 4A: Chest x-ray pre-treatment showing a moderate sized right pleural effusion. Fig 4B: Chest x-ray 3 months after treatment with sirolimus showing significant reduction in the size of right pleural effusion.*

lung parenchyma with multiple, thin-walled cysts. Lung function tests showed a reduced DLCO of 52.3% predicted, while the FEV1 and FVC were within normal limits [FVC 3.48 L (82.0%), FEV1 3.13 L (84.7%)]. The previously resected leiomyoma was re-evaluated and it stained positively for HMB-45. Based on the characteristic HRCT findings, presence of chylous ascites and leiomyoma, a diagnosis of LAM was established. The patient was started on oral sirolimus at a dose of 2 mg daily with resultant serum trough levels of 3.7-4.4 ng/ml. Three months after starting treatment, the patient has had significant reduction in her ascites and improved quality of life. Her PFTs have shown improvement with the current DLCO being 65.8% predicted with stable spirometric values [FVC 3.59 L (84.7%), FEV1 3.18 L (86.0%)].

## DISCUSSION

Lymphatic complications are seen in a substantial fraction of patients with LAM. The most common lymphatic manifestations of LAM include formation of lymphangiolei-

omyomas and collection of chylous fluid in the pleural and peritoneal cavities (10).

Lymphangioleiomyomas are well-circumscribed lobulated masses filled with chylous material and are typically seen in the mediastinum, retroperitoneum or pelvis on CT scans. Lymphangioleiomyomas in LAM are a consequence of the dilation and obstruction of lymphatic channels, lymph nodes, or the thoracic duct due to proliferation of LAM cells (10). Up to 29% of patients with LAM have lymphangioleiomyomas as one of the disease manifestation (10). In about one third of patients, chylous pleural effusions are present and occur as a result of obstruction of thoracic duct or pleural lymphatics by proliferating LAM cells (1,4). The third patient in our case series also had chylous ascites, which is present in about 10-15% of patients (1).

In 2011, results of the Multicenter International LAM Efficacy of Sirolimus (MILES) trial which involved 89 LAM patients were published, and it showed that sirolimus stabilized lung function, reduced serum VEGF-D, and improved quality of life in patients with LAM (11). However, patients with pleural effusions were excluded from the

MILES trial. Taveira-DaSilva et al treated a cohort of LAM patients with lymphatic involvement with sirolimus (12). In their study, there were 12 patients with chylous effusions (pleural effusions as well as chylous ascites), and 11 patients with lymphangioleiomyomas. Treatment with sirolimus resulted in almost complete resolution of these conditions and in 2 patients, enabled discontinuation of pleural fluid drainage (12).

The standard dose and duration of therapy with sirolimus is not well established. With the rate of decline of lung function in treatment group mirroring the placebo arm in MILES trial during the observation phase, it is likely that sirolimus will need to be used long term, even lifelong, to be effective. Recent evidence suggests that low dose sirolimus (trough level less than 5 ng/ml, as opposed to a trough level between 5-15 ng/ml as employed in the MILES trial) is equivalent in terms of efficacy as compared to the dose used in MILES trial (13). In addition, low dose sirolimus was equally effective for treatment of chylous effusions (13).

Our results are comparable to those published before concerning influence of sirolimus treatment on lung function, chylous pleural effusions, and lymphangioleiomyoma. All three patients had improvement in their pulmonary function tests, resolution of chylous effusions, and reduction in size of lymphangioleiomyomas while on sirolimus and none experienced any adverse effects from sirolimus therapy. The three patients had serum sirolimus trough levels of 2.9-7.7 ng/ml. Taken together, our case series adds further proof to the efficacy of sirolimus in treatment of pulmonary as well as lymphatic manifestations of LAM.

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