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Sirolimus Therapy for Patients With Lymphangioleiomyomatosis Leads to Loss of Chylous Ascites and Circulating LAM Cells

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A young woman received a diagnosis of abdominal, sporadic lymphangioleiomyomatosis (LAM) and multiple abdominal lymphangioleiomyomas and was referred for recurrent chylous ascites responding only to a fat-free diet. On admission, pulmonary function test (PFT) results showed a moderate reduction in the transfer factor for carbon monoxide with normal exercise performance. The serum vascular endothelial growth factor D (VEGF-D) level was 2,209 pg/mL. DNA sequences, amplified at loci kg8, D16S3395, D16S3024, D16S521, and D16S291 on chromosome 16p13.3, showed a loss of heterozygosity (LOH) only for kg8. Fat-free total parenteral nutrition in association with sirolimus (2 mg po daily) was initiated. Serum sirolimus levels were maintained at concentrations between 5 and 15 ng/mL. After 1 month, reintroduction of a low-fat oral feeding was achieved without recurrence of ascites. PFT results were stable. Interestingly, clinical improvement was associated with a reduction in the VEGF-D serum level (1,558 pg/mL). LOH at the kg8 biomarker in blood LAM cells was no longer detected.

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Case Report

A 39-year-old woman received a diagnosis of multiple retroperitoneal, retrorenal, and retropancreatic masses seen on an abdominal CT scan performed for a subfascial abscess following caesarean section. A biopsy specimen from the biggest mass was consistent with a lymphangioleiomyoma. A subsequent chest CT scan showed diffuse thin-walled lung cysts suggestive of lymphangioleiomyomatosis (LAM).

Six months later, ascites was detected by abdominal ultrasound and 6 L of chylous fluid was drained. The patient was given fat-free, total parenteral nutrition (TPN) with complete resolution of the abdominal effusion, but a relapse occurred in the attempt to shift from parenteral to enteral nutrition. The patient was referred to our center.

On admission, the patient was in good health, with an indwelling drainage catheter in place.

Pulmonary function test results showed a moderate reduction in the transfer factor for carbon monoxide (FEV_1: 2.17 L, 72% of the

ABBREVIATIONS: LAM = lymphangioleiomyomatosis; LOH = loss of heterozygosity; TPN = total parenteral nutrition; TSC = tuberous sclerosis complex; VEGF-D = vascular endothelial growth factor D

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predicted value; FVC: 2.21 L, 72% of the predicted value; TLC: 3.98 L, 86% of the predicted value; transfer factor for carbon monoxide: 12.7 mL/min/mm Hg, 52% of the predicted value) and a normal 6-minute walking distance, without clinically significant desaturation. Blood test results were within normal limits except for a high serum level of vascular endothelial growth factor D (VEGF-D) (2,209 pg/mL). VEGF-D is a lymphangiogenic growth factor, which plays a key role in tumor metastasis. It is also a useful biomarker in LAM, used for diagnosis in patients with cystic lung disease and no extrapulmonary manifestations. It directly correlates with disease severity and response to treatment.[1]

LAM cells were isolated from the patient's blood by fluorescence-activated cell sorting as previously described.[2] DNA sequences were amplified at loci kg8, D16S3395, D16S3024, D16S521, and D16S291 on chromosome 16p13.3, near the tuberous sclerosis complex 2 (*TSC2*) gene[2] (Fig 1A). Quantitative loss of heterozygosity (Q^{LOH}) was calculated and evaluated as described.[3] LOH was detected only for kg8.

The initial attempt to feed the patient with a low-fat enteral diet caused a relapse of abdominal pain, with large ascites and multiple abdominal masses on CT scan (Fig 1B). Fat-free TPN in association with sirolimus (2 mg po daily) was started. After several days we added a low dose of fat in the parenteral nutrition. After discharge, the patient continued this parenteral nutrition with a monthly check of liver function test results and serum sirolimus levels to maintain a concentration between 5 and 15 ng/mL. After 1 month, the reintroduction of low-fat oral feeding was achieved without recurrence of ascites.

After 3 months, MRI showed that the abdominal masses were smaller when compared with the previous abdominal CT scan (Fig 1C), with no signs of ascites. Pulmonary function test results were stable and the serum VEGF-D level was 1,558 pg/mL. Interestingly,

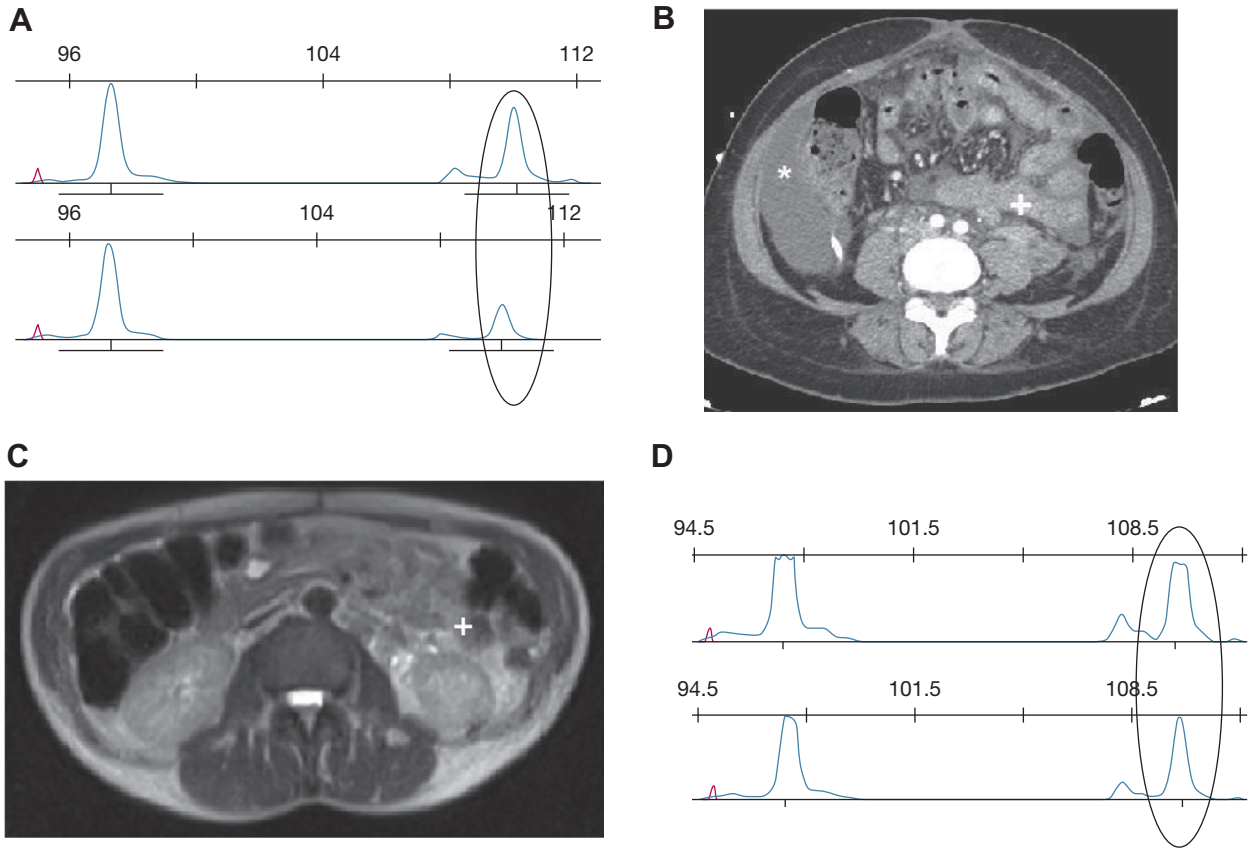

Figure 1 – *A-D, A 29-year-old woman affected by lymphangioleiomyomatosis presented on CT scan with ascites (*) and a retroperitoneal lymphangioleiomyoma appearing as a mass with enhancement and low-density intralesional area (+) (B). DNA sequences of lymphangioleiomyomatosis cells were amplified at loci kg8, D16S3395, D16S3024, D16S521, and D16S291 on chromosome 16p13.3. Before sirolimus therapy was begun, loss of heterozygosity (LOH) was detected only for kg8 (A). LOH was no longer detectable after 1 month of treatment (D). This finding was associated with complete resolution of abdominal ascites and a reduction in size of the lymphangioleiomyoma (C).*

LAM cells in blood having *TSC2* LOH at the kg8 biomarker were no longer detected (Fig 1D). The patient continued the same sirolimus treatment regimen and was stable at follow-up visits at 6, 12, and 18 months with a normal diet regimen. Recurrent ascites was not observed by abdominal ultrasound.

Discussion

LAM, a rare multisystem disease affecting primarily women, is characterized by the dissemination of abnormal smooth muscle-like LAM cells, leading to cystic lung destruction, development of thoracic and abdominal lymphatic masses, and abdominal angiomyolipomas. LAM may occur sporadically or in association with tuberous sclerosis complex (TSC).[4,5] The metastatic dissemination of LAM cells, bearing inactivating mutations or LOH of the tumor suppressor gene *TSC1* or *TSC2*, is hypothesized to be the cause of the disease.[2,6] Sirolimus, approved by the US Food and Drug Administration for the treatment of LAM, is an inhibitor of mechanistic target of rapamycin complex 1 (mTORC1) and an immunosuppressant, which was shown to decrease the size of angiomyolipomas in patients with TSC-related and sporadic LAM.[7] The effect of sirolimus on lung function in LAM was studied in a multicenter, double-blinded trial (MILES [Multicenter International Lymphangioleiomyomatosis Efficacy of Sirolimus] trial) of 46 patients treated with sirolimus and 43 with placebo. The sirolimus group, when compared with patients taking placebo, showed significant improvements in FVC and functional performance status, with a stabilization of FEV_1.[8] The effect of sirolimus on circulating LAM cells was evaluated in 23 patients with LAM. After 2 years of treatment, on average, detection of LAM cells was significantly decreased in blood (from 100% to 25%) and urine (from 75% to 8%).[9]

Sirolimus was found to be effective in patients with thoracic chylous effusions. In fact, Taveira-DaSilva et al[10] described 12 patients with LAM with chylous abdominal and thoracic effusions and in whom complete resolution was observed in nine of 11 patients with pleural effusions and in all eight patients affected by ascites after 410 $\pm$ 111 days of sirolimus therapy.

Before the availability of sirolimus for the treatment of patients with LAM, the management of chylous ascites was difficult. Diuretics, paracentesis, and a low-fat diet, with mainly medium-chain triglycerides, were used to reduce the production of chylous effusions. In other cases, a surgical approach was considered.[11]

We report the case of a woman with sporadic LAM, who had mild/moderate respiratory disease, multiple abdominal lymphangioleiomyomas, and recurrent chylous ascites. After 3 months of therapy with sirolimus, complete resolution of the chylous ascites was observed, with disappearance from the blood of circulating LAM cells bearing the *TSC2* LOH. The simultaneous restarting of TPN and introduction of sirolimus may be a potential confounder, as it has been reported that TPN alone decreases the volume of lymphangioleiomyomas and alleviates obstruction in the abdomen. In our case, when the patient was treated with TPN in another hospital, although a resolution of chylous ascites was observed, abdominal masses were not reduced in size, and at the time of admission to our center, VEFG-D values were high. Thus, TPN alone was not effective in reducing the size of the lymphangioleiomyomas.

Although resolution of chylous ascites after treatment with sirolimus has been described in a previous article,[9] as has the effect of treatment on the disappearance of circulating blood LAM cells,[10] this case describes for the first time, to our knowledge, a direct temporal relationship between clinical response to sirolimus and disappearance of blood circulating LAM cells in a patient with chylous ascites.

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