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Early View

Original research article

Clinical, tomographic, and functional comparison of sporadic and associated tuberous sclerosis complex forms of LAM: a retrospective cohort study

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Title Page

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Take home message: Patients with sporadic lymphangioleiomyomatosis present higher annual rates of functional decline and lung cysts extent than those with tuberous sclerosis complex form of the disease, who, however, suffer a greater impact on vitality and emotional health.

Clinical, tomographic, and functional comparison of sporadic and associated tuberous sclerosis complex forms of LAM: a retrospective cohort study

Abstract

Background: Lymphangioleiomyomatosis (LAM) is a rare disease that can occur sporadically (S-LAM) or associated with the tuberous sclerosis complex (TSC-LAM). The natural history of LAM is not completely understood, including whether there is a difference between the clinical courses of the two forms. This study aimed to compare the clinical, functional, and tomographic features between S-LAM and TSC-LAM, and evaluate the annual rates of change in lung function.

Methods: This retrospective cohort study included patients with LAM followed up between 1994 and 2019. Clinical, functional and imaging variables were evaluated, and the lung cysts were automatically quantified. Quality of life and predictors of lung function impairment were accessed, and the annual rate of lung function decline was compared between S-LAM and TSC-LAM.

Results: Of the 107 patients included, 77 had S-LAM and 30 had TSC-LAM. Although patients with TSC-LAM had a higher prevalence of renal angiomyolipomas and neurological and dermatological manifestations, pulmonary function tests were similar. Patients with S-LAM had a greater rate of FEV_1 decline and a higher extent of cysts. Pneumothorax, desaturation in the six-minute walking test and a higher extent of lung cysts were predictors of functional impairment. A greater impact on vitality and emotional health was observed in the TSC-LAM.

Conclusion: Greater functional decline and a higher cystic extension were found in patients with S-LAM. Our study provides a broad clinical, functional, and tomographic characterisation of patients with LAM, adding valuable information to the existing evidence to better understand the two forms of the disease.

Keywords: Computed tomography; follow-up; lymphangioleiomyomatosis; pulmonary function tests; quality of life; tuberous sclerosis.

Introduction

Lymphangioleiomyomatosis (LAM) is a rare neoplastic disease that predominantly affects women of reproductive age and is caused by mutations in the tuberous sclerosis complex (TSC) genes TSC1 and TSC2, culminating in the hyperactivation of the mechanistic target of rapamycin (mTOR) signaling pathway.[1] LAM causes inappropriate cell growth, proliferation, invasion, and metastatic spread of abnormal smooth muscle-like cells (LAM cells), resulting in cystic destruction of the lung parenchyma, and tumour lesions such as lymphangioleiomyomas and renal angiomyolipomas (AML).[2-5]

LAM may occur sporadically (S-LAM) or associated with TSC (TSC-LAM), a genetic autosomal dominant disorder characterised by hamartomas in different organs.[6-8] Recent findings have shown that lung cysts suggestive of LAM can complicate TSC in up to 80% of subjects aged > 40 years.[9-12]

Dyspnoea and spontaneous pneumothorax are the most common clinical manifestations.[13, 14] The clinical course of LAM is heterogenous and may vary from asymptomatic to progressive disease culminating in death or lung transplantation. Previous studies reported that the estimated annual rate of decline in forced expiratory volume in the first second (FEV_1) is 47–134 mL/year.[17]

TSC-LAM is usually considered milder and less progressive than S-LAM. Patients with TSC-LAM often show higher FEV_1 and diffusing capacity for carbon monoxide (DLCO), and a higher proportion of asymptomatic disease.[15, 16] Nonetheless, recent cohorts found no difference between the rate of lung function decline between these two groups, including one involving only patients with incidental diagnosis and asymptomatic disease aiming to eliminate possible selection bias in TSC-LAM group due to preconised screening.[15, 17, 18]

The natural history of LAM is mainly derived from retrospective cohort studies and has not yet been completely elucidated, especially the differences between the clinical courses of TSC-LAM and S-LAM.[19] Therefore, we decided to further analyse our cohort of patients with LAM to better understand and compare both groups, with a special emphasis on functional decline. The purpose of this study was to compare the

main clinical, functional, and radiological features of S-LAM and TSC-LAM, and to investigate the annual rate of change in lung function in our cohort.

Methods

Design and population

This retrospective cohort study included patients with LAM who were followed up at a tertiary centre from 1994 to 2019. The included patients were at least 13 years old and had a definitive LAM diagnosis according to international guidelines.[20] TSC was diagnosed based on previous recommendations.(21) All variables were accessed at inclusion, which occurred in 2019. The study protocol was approved by the local research ethics committee (79217317.5.0000.0068), and signed informed consent was obtained from all patients.

Clinical and demographic data

Data on the age, number of patients with TSC, time from diagnosis, symptoms, and pulmonary and extrapulmonary manifestations at inclusion and during the course of the disease and treatment were collected. All patients were referred for a dermatological evaluation to investigate the presence of cutaneous manifestations associated with TSC. Quality of life was accessed using the Short Form 36 Health Survey (SF-36) questionnaire, which has been validated in the Brazilian population.[22, 23]

Pulmonary function tests

Spirometry was performed using a calibrated pneumotachograph, and lung volumes and DLCO values were obtained using a body plethysmograph. The following variables were obtained: forced vital capacity (FVC), FEV_1, FEV_1/FVC ratio, total lung capacity (TLC), residual volume (RV), RV/TLC ratio, and DLCO. Predicted values were derived from *Global Lung Function Initiative*.[24-26]

The prevalence of obstructive, restrictive, mixed, and nonspecific patterns; positive response to bronchodilators (BD); air trapping; pulmonary hyperinflation; and reduced DLCO were determined as recommended.[25] Pulmonary function tests (PFTs) performed within six months before the clinical evaluation were considered.

Patients with two or more PFTs available during follow-up were included in the analysis to determine the annual rate of change in FEV_1. The results of all available spirometry tests performed since diagnosis of each patient were collected.

Predictors of lung function impairment defined by FEV_1 below the lower limit of normality (LLN) were identified.

Six-minute walk test

The six-minute walk test (6MWT) was performed according to recommended standards.[27, 28] Peripheral oxygen saturation (SpO_2), heart rate (HR), the 6-minute walking distance (6MWD) and breathlessness were recorded. SpO_2 was measured using pulse oximetry (Onyx, model 9500; Nonin, Plymouth, MN, USA) at rest and at the end of exercise. Breathlessness was evaluated using the modified Borg scale before and after exercise.[29] The 6MWD was expressed as a percentage of reference values for the Brazilian population.[30]

Imaging tests

The patients underwent chest computed tomography (CT) in the supine position without intravenous contrast injection. Quantification of the volume of cysts was obtained automatically by densitovolumetry using a computer program (Advantage Workstation Thoracic VCAR software; GE Medical Systems, Milwaukee, WS, USA) and by selecting pixels between −1000 and −950 HU on soft tissue filter images. The total lung volume, volume occupied by cysts, and ratio of abnormal cyst volume to total lung volume were calculated automatically. Image analysis and manual correction were performed by a thoracic radiologist (M.W). All CTs scans were performed in a stable clinical setting within one year before clinical evaluation.

Two chest radiologists (M.W. and R.C.C) with 8 and 20 years of experience, respectively, performed a qualitative analysis of CT scans to determine the prevalence of other thoracic findings. A consensus was established in cases of divergent opinions.

Other imaging tests performed at inclusion or within a year prior, including cranial CT or MRI, abdominal CT and transthoracic echocardiography, were reviewed to access the presence of TSC neurological manifestations, angiomyolipomas, lymphangioleiomyomas, and cardiac rhabdomyomas.

Statistical analysis

Data are reported as numbers and percentiles, as mean ± SD for variables with a normal distribution, and as median (25^{th}–75^{th} percentiles) for variables with a non-normal distribution The Shapiro-Wilk test was used for normality. Continuous variables were compared using the unpaired t-test or Mann-Whitney U test, whereas categorical variables were compared using Fisher's exact or chi-square tests.

Univariate logistic regression analysis was performed to select the variables associated with FEV_1 below LLN. Variables that resulted in $p \leq 0.1$ were included in multivariate analysis using the Stepwise forward Likelihood Ratio logistic regression model to predict factors that were related to lung function impairment. The level of statistical significance was set as $p \leq 0.05$ for variables to be included in the final model. Odds ratios (OR) and 95% confidence intervals were determined.

The annual rate of change (slope) of FEV_1 was calculated using linear regression. An adjusted analysis was performed using mixed-effects models with a random intercept and random slope to estimate the decline in FEV_1 over time. These data were compared between TSC-LAM and S-LAM groups and are reported as mean (standard error).

All statistical analyses were performed using SPSS software (version 21.0; IBM Inc., Chicago, IL, USA), and statistical significance was set at $p \leq 0.05$.

Results

Clinical and demographic features, and treatment description

Among the 116 patients regularly followed-up at our centre, two refused to participate in the study and seven were excluded due to a previous pulmonary transplant. Finally, our study included 107 women with a definitive diagnosis of LAM and a mean age of 43 ± 11 years. Of the 107 patients, 72% had S-LAM and 28% had TSC-LAM. Patients with TSC-LAM were younger at the time of inclusion and diagnosis. Diagnosis was confirmed in all patients with TSC-LAM using a combination of clinical and tomographic findings. However, lung biopsy was necessary to confirm the diagnosis in 42% of the patients with S-LAM. The most frequent clinical manifestations were dyspnoea (57%) and spontaneous pneumothorax (50%), with no intergroup differences. Table 1 summarises the patients' clinical and demographic characteristics.

At the time of inclusion, 35% of all LAM patients were taking mTOR inhibitors, with no significant difference between the two groups. Renal angiomyolipoma (23%) and lung function decline (18%) were the main reasons for the use of mTOR inhibitors in patients with TSC-LAM and S-LAM, respectively. Continuous oxygen supplementation was prescribed to 9% of the patients (Table 1).

Extra-thoracic manifestations

Extra-thoracic manifestations are presented in Table 2. More than half the patients had renal angiomyolipomas, which were significantly more prevalent in patients with TSC-LAM. There was no difference in the prevalence of lymphangioleiomyomas between the two groups. Imaging findings suggestive of neurological impairment were observed in 73% of the patients with TSC-LAM. The most frequent cutaneous manifestation was facial angiofibroma (28%), with a higher prevalence in the TSC-LAM group.

Pulmonary function tests

The functional data are presented in Table 3. Approximately 50% of all LAM patients had normal PFTs. The most frequent abnormalities observed were reduced DLCO (46%), air trapping (41%), and obstructive impairment (40%), with no significant intergroup differences. Functional variables were similar between the two groups, except for FEV_1/FVC and TLC, which were lower in the S-LAM and TSC-LAM groups, respectively.

Previous pneumothorax, mMRC dyspnoea score, the distance walked and desaturation ≥4% in the 6MWT, the extent of pulmonary cysts and lymphatic involvement were identified as predictors of FEV_1 below LLN in the univariate logistic regression. Previous pneumothorax (OR 3.206, p=0.050), desaturation ≥4% in the 6MWT (OR 7.026, p=0.004), and higher extent of lung cysts (OR 1.150, p=0.005) persisted as independent factors for FEV_1 below LLN in the multivariable logistic regression (Table S1).

Eighty-two patients (63 with S-LAM and 19 with TSC-LAM) were included in the analysis of the annual rate of change in FEV_1. The median follow-up interval (years) and the number of PFTs were 6 (3 - 6) and 5 (3 - 8) in S-LAM patients, and 4 (2 - 7) and 4 (2 - 6) in those with underlying TSC, with no significant differences observed between groups (p = 0.079 and p = 0.089, respectively). The mean (SE) FEV_1 decline for all LAM patients was 51.7 (4.7) mL/year and patients with S-LAM presented a significantly higher annual decline compared to those with TSC-LAM (55.8 (5.08) mL *vs.* 31.7 (10.6) mL, p=0.044) (Figure 1). An adjusted analysis was conducted, incorporating initial FEV_1, the use of mTOR inhibitor, and age as covariates in the model. The patients with S-LAM persisted with higher rate of FEV_1 decline.

The same analysis performed on patients not treated with mTOR inhibitors (47 S-LAM and 7 TSC-LAM) yielded higher annual rate of FEV_1 decline in S-LAM (51.3 (5) mL *vs.* 7.4 (12.3) mL, p=0.002) (Figure S1). Additionally, we compared the slopes between the S-LAM and TSC-LAM groups, including patients who died or underwent lung transplantation. There was a tendency to higher annual rate of FEV_1 decline in S-LAM (59.5 (5.3) mL *vs.* 35.7 (11.6) mL, p=0.067) (Figure S1).

Six-minute walk test and SF-36 questionnaire

6MWT and SF-36 datasets are shown in Table 4. The median distance walked was 495 m (435–559 m), which corresponded to 86% (72–96%) of the predicted distance, with no difference observed between the two groups. The S-LAM group showed lower SpO_2 at the end of the 6MWT [93% (88–95) *vs.* 96% (88–97), p=0.044].

The SF-36 questionnaire showed the lowest scores for role limitations owing to emotional health and vitality in patients with TSC-LAM.

Chest High-Resolution Computed Tomography

Chest CT was available for automatic cystic quantification in 86% of the patients. Patients with S-LAM presented with a higher extent of lung cysts than those with TSC-LAM [5.1% (1.2–13.6) *vs.* 1.2% (0.2–7.8), p=0.027]. CT scans with different extents of cysts are shown in Figure 2.

The most prevalent thoracic findings were sclerotic bone lesions (22%) and ground-glass opacities (16%). A higher prevalence of nodules suggestive of multifocal micronodular pneumocyte hyperplasia (MMPH), ground-glass opacities, and sclerotic bone lesions was found in the TSC-LAM group (Table 5 and Figure 3).

Discussion

The natural history and clinical course of S-LAM and TSC-LAM are not completely understood, particularly whether the latter represents a less severe phenotype. Our study provides valuable information by comparing S-LAM and TSC-LAM in a unique Latin American cohort, with an emphasis on the annual rate of change in lung function. The main findings of our study were as follows: 1; 1) Patients with S-LAM presented higher annual rates of lung function decline, which did not appear to be related to age, baseline severity or use of mTOR inhibitors, according to our findings. 2) Functional features were similar between the two groups; 3) There were no differences in thoracic and extra-thoracic clinical manifestations between the two groups, except for a higher prevalence of lung ground-glass opacities, nodules suggestive of MMPH, sclerotic bone lesions, renal angiomyolipoma, neurological and dermatologic features in TSC-LAM; 4) The main indication for treatment with mTOR inhibitors was lung function decline in S-LAM and renal angiomyolipoma in TSC-LAM; 5) Lower lung cyst extensions on CT scan was observed in TSC-LAM; 6) Patients with TSC-LAM seem to suffer a greater impact on vitality and emotional health.

In accordance with previous studies, reduced DLCO, air trapping, and obstructive patterns were the most common functional abnormalities identified in our study, with no significant differences between S-LAM and TSC-LAM.[14, 31] The S-LAM group presented with a lower FEV_1/FVC ratio and higher TLC, suggesting higher obstruction and hyperinflation in this population. Previous studies have demonstrated lower

functional impairment in TSC-LAM, with higher levels of FEV_1 and DLCO compared to S-LAM, which was not confirmed in our study.[14, 15]

Patients with S-LAM presented a higher annual rate of FEV_1 decline than patients with TSC-LAM, which was not related to age, baseline FEV_1 or the use of mTOR inhibitors. As patients with TSC-LAM presented a higher proportion of mTOR inhibitor use than those with S-LAM (63% *vs.* 25%, p=0.002), we performed an additional analysis accessing only those not treated with sirolimus, and obtained similar results. A few studies have compared the FEV_1 slope between S-LAM and TSC-LAM, and observed no differences.[12, 15, 17, 19] The NHLBI group demonstrated that, although there was no difference in FEV_1 decline between the S-LAM and TSC-LAM groups matched for age and PFTs, a greater proportion of patients with S-LAM presented higher rates of FEV_1 decline.[15] Additionally, the annual rates of functional decline in our population were lower than those observed in previous studies, possibly because of the higher proportion of patients treated with mTOR inhibitors. Although the estimated number of patients with TSC-LAM exceeds those with S-LAM, the latter usually constitute the majority in reference centres, which is similar to our findings, and commonly require medical interventions.[6, 16, 32, 33] This finding may explain the difference observed in the functional decline in our study. Moreover, we cannot rule out a lead time bias due to preconized screening of LAM in patients with TSC.

Other studies have demonstrated various predictors of greater functional decline and disease severity, such as lower baseline FEV_1, reduced DLCO, dyspnoea, desaturation during the 6MWT, and cystic extension.[13, 17, 34] In our study, the occurrence of pneumothorax, desaturation during the 6MWT, and extent of lung cysts were the predictors of functional impairment.

Evaluation of lung cyst extension using semi-quantitative or quantitative methods has been used to assess disease severity with a good correlation with PFTs.[13, 35] Some authors analysed and compared the extent of pulmonary cysts between the S-LAM and TSC-LAM, and reported varying results. Avila et al. demonstrated less severe lung disease in the TSC-LAM group[32], whereas other authors found no significant differences between the two groups.[15, 36] Our study showed mild cystic involvement and a higher extent of lung cysts in patients with S-LAM. We observed a higher prevalence of nodules suggestive of MMPH in the TSC-LAM group. Moreover, in contrast

to other reports, patients with TSC-LAM presented a higher prevalence of ground-glass opacities, which may represent alveolar haemorrhage or lymphatic congestion.[36] Contrary to previous studies[32], we demonstrated a similar prevalence of lymphatic abnormalities between the two groups, including pleural effusion, and thoracic lymphadenopathy.

Renal angiomyolipomas were the most common extra-thoracic findings in our study, with a higher prevalence in TSC-LAM, similar to previous reports.[6, 14] Additionally, previous nephrectomies were more frequent in this group. Angiomyolipomas may increase in size, causing pain and haemorrhage, which may contribute to morbidity and a higher risk of death.[4, 37] Previous studies have demonstrated that LAM is associated with reduced quality of life.[14, 38, 39] Lower scores were found in all domains of the SF-36 questionnaire in patients with LAM compared to healthy Brazilian women paired for age, with worse scores in the domains of general health perception, vitality and mental health.[40] In our study, patients with TSC-LAM demonstrated lower emotional health and vitality scores than those with S-LAM, which may be associated with a higher prevalence of extrapulmonary manifestations. This finding differs from the results of the NHLBI LAM registry, which showed no difference in SF-36 scores between these groups.[14] Our findings suggest that multidisciplinary care and holistic management plans should be provided to patients with LAM.

This study has several limitations. First, the retrospective design was an expected limitation, considering the rarity of the disease. Second, data were obtained from routine clinical follow-ups of the patients, which may explain the occurrence of missing data. Third, some patients were diagnosed close to the time of data collection and not all patients were included in the analysis of the rate of FEV_1 decline. Even with these missing data, we could demonstrate the difference between the slopes of the S-LAM and TSC-LAM. Finally, we cannot exclude the possibility that the higher prevalence of mTOR inhibitors in patients with TSC-LAM affected the differences in the rates of FEV_1 decline observed between the two groups. However, this hypothesis is less likely because we performed an analysis only including patients not treated with mTOR inhibitors, and obtained similar results. Although this was a single-centre study and biases associated with institutional practices should be considered when extrapolating

these findings to other LAM populations, our cohort is representative of the whole country, as patients from different regions are referred to our centre.

To the best of our knowledge, this is the first study to describe a faster rate of functional decline in patients with S-LAM than in those with TSC-LAM. Higher obstruction and hyperinflation as well as a greater extent of lung cystic destruction were demonstrated in patients with S-LAM. The quality of life in patients with TSC-LAM showed greater impairment due to lower emotional health and vitality scores, which may be related to the extrapulmonary manifestations of the disease. As the largest LAM cohort in Latin America, our study provides a valuable and broad clinical, functional, and tomographic characterization of patients with LAM, contributing to a better understanding of the differences between the two forms of the disease.

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Conflict of Interest Disclosure: The authors declare to have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Tables

Table 1 – Clinical and demographic data, and treatment description of the patients included in the study

	LAM (n=107)	**Sporadic LAM (n = 77)**	**TSC-LAM (n = 30)**	**p**
Female sex	107 (100%)	77 (100%)	30 (100%)	
Age at diagnosis, y	38 ± 10	39 ± 10	33 (26 - 42)	0.033
Age at inclusion, y	43 ± 11	45 ± 11	39 ± 11	0.012
Time from diagnosis to inclusion, y	4 (1 - 8)	5 (2 - 9)	2.5 (1 - 6)	0.079
BMI [1]	24 (22 - 28)	25 (23 - 28)	24 (22 - 28)	0.568
Obesity[1]	16 (15%)	13 (17%)	3 (10%)	0.548
Current/former smokers	24 (22%)	20 (26%)	4 (13%)	0.159
Diagnosis confirmation				
Clinical-tomographic	67 (63%)	37 (48%)	30 (100%)	<0.001
Lung biopsy	32 (30%)	32 (42%)	0	<0.001
Angiomyolipoma exeresis	6 (5%)	6 (8%)	0	0.182
Lymphangioleiomyoma exeresis	2 (2%)	2 (2%)	0	1
Clinical features				
Asymptomatic	34 (32%)	27 (35%)	7 (23%)	0.242
Dyspnoea	61 (57%)	42 (54%)	19 (63%)	0.409
-mMRC	1 (0 - 1)	1 (0 - 1)	1 (1 - 1)	0.201
-Mahler[2]	11 (8 - 12)	11 (8 - 12)	10 (8 - 12)	0.756
Cough	24 (22%)	17 (22%)	7 (23%)	0.889
Wheezing	7 (6%)	6 (8%)	1 (3%)	0.670
Haemoptysis	3 (3%)	3 (4%)	0	0.558
History of pneumothorax	54 (50%)	39 (51%)	15 (50%)	0.952
- Number of episodes	0.5 (0 - 3)	0.5 (0 - 2)	0.5 (0 - 3)	0.763
- Pleurodesis	37 (34%)	29 (37%)	8 (27%)	0.311
- Pleurectomy	7 (6%)	6 (8%)	1 (4%)	0.670
History of chylothorax	16 (15%)	11 (14%)	5 (17%)	0.768
Current treatment				
Doxycycline	2 (2%)	2 (2%)	0	1
Goserelin	17 (16%)	12 (15%)	5 (17%)	1
Progesterone	2 (2%)	1 (1%)	1 (3%)	0.484
mTOR inhibitor[*]	38 (35%)	25 (32%)	13 (43%)	0.291
Long-acting bronchodilator[3]	30 (29%)	24 (32%)	6 (20%)	0.219

Indications for the use of mTOR inhibitor				
Lung function decline	17 (16%)	14 (18%)	3 (10%)	0.386
Renal angiomyolipoma	9 (8%)	2 (2%)	7 (23%)	0.001
Lymphatic involvement	5 (5%)	5 (6%)	0	0.320
Lung function decline and renal angiomyolipoma	5 (5%)	2 (2%)	3 (10%)	0.125
Lung function decline and lymphatic involvement	2 (2%)	2 (2%)	0	1
Supplemental oxygen therapy	10 (9%)	8 (10%)	2 (7%)	0.722

Values expressed are mean ± SD, median (25th-75th percentil) or n (%).
BMI: body mass index; LAM: lymphangioleiomyomatosis, mMRC: modified Medical Research Council; mTOR: mechanistic target of rapamycin; TSC: tuberous sclerosis complex;
[*]All patients using sirolimus.
[1]n expressed in each column, respectively: 106 (total), 76 (S-LAM) and 30 (TSC-LAM)
[2]n expressed in each column, respectively: 106 (total), 77 (S-LAM) and 29 (TSC-LAM)
[3]n expressed in each column, respectively: 105 (total), 75 (S-LAM) and 30 (TSC-LAM)

Table 2 – Extrathoracic manifestations of the patients included in the study

	LAM (n = 107)	Sporadic LAM (n = 77)	TSC-LAM (n = 30)	p
Renal				
Angiomyolipoma	59 (55%)	30 (39%)	29 (97%)	<0.001
- Right	13 (12%)	10 (13%)	3 (10%)	1
- Left	20 (19%)	13 (17%)	7 (23%)	0.442
- Bilateral	26 (24%)	7 (9%)	19 (63%)	<0.001
Cysts	21 (20%)	12 (16%)	9 (30%)	0.092
Previous partial/total nephrectomy	36 (34%)	17 (22%)	19 (63%)	<0.001
Neurological[1]				
TSC suggestive findings	22 (21%)	0	22 (73%)	<0.001
Cortical tubers	14 (14%)	0	14 (47%)	<0.001
Subependymal nodules	20 (19%)	0	20 (67%)	<0.001
Astrocytoma	3 (3%)	0	3 (10%)	0.023
Dermatologic and dental[2]				
Hypomelanotic macules	14 (15%)	0	14 (52%)	<0.001
“Confetti”skin lesions	17 (18%)	0	17 (63%)	<0.001
Facial angiofibromas	27 (28%)	4 (6%)	23 (85%)	<0.001
Ungueal fibromas	17 (18%)	1 (1%)	16 (59%)	<0.001
Gengival fibromas	15 (16%)	1 (1%)	14 (52%)	<0.001
Dental enamel pits	11 (12%)	1 (1%)	10 (37%)	<0.001
Shagreen patch	11 (12%)	0	11 (41%)	<0.001
Fibrous cephalic plaque	15 (16%)	0	15 (56%)	<0.001
Other findings				
Lymphangioleiomyoma	11 (10%)	9 (12%)	2 (7%)	0.724
Chylous ascytes	5 (5%)	5 (6%)	0	0.319
Previous/current pecoma	2 (2%)	2 (3%)	0	1
Previous/current uterine leiomyoma	29 (27%)	22 (29%)	7 (27%)	0.815
Cardiac rhabdomyoma[3]	2 (2%)	1 (1%)	1 (3%)	0.492

Values expressed are n (%).

LAM: lymphangioleiomyomatosis; TSC: tuberous sclerosis complex

[1]n expressed in each column, respectively: 103 (total), 73 (S-LAM) and 30 (TSC-LAM)

[2]n expressed in each column, respectively: 95 (total), 68 (S-LAM) and 27 (TSC-LAM)

[3]n expressed in each column, respectively: 105 (total), 75 (S-LAM) and 30 (TSC-LAM)

Table 3 – Pulmonary function data obtained at the study inclusion

	LAM (n = 103)*	**Sporadic LAM (n = 75)**	**TSC-LAM (n = 28)**	**p**
Lung function patterns				
Normal	49 (48%)	36 (48%)	13 (46%)	0.887
Obstructive	41 (40%)	33 (44%)	8 (29%)	0.155
Non-specific	6 (6%)	2 (3%)	4 (14%)	0.045
Restrictive	6 (6%)	3 (4%)	3 (11%)	0.341
Mixed	1 (1%)	1 (1%)	0	1
Positive BD[1] response	9 (14%)	8 (16%)	1 (7%)	0.670
Air trapping[2]	41 (41%)	33 (46%)	8 (29%)	0.115
Hyperinflation[2]	8 (8%)	8 (11%)	0	0.102
Reduced DLCO[3]	46 (46%)	36 (52%)	10 (40%)	0.297
Lung function parameters				
FEV_1(L)	2.07 ± 0,69	2.05 ± 0.68	2.13 ± 0.74	0.635
FEV_1 % predicted	79 (57 – 93)	73.60 ± 23.25	80.50 (61 – 91.25)	0.654
FVC (L)	2.90 (2.56 – 3.31)	2.99 ± 0.72	2.85 ± 0.58	0.348
FVC % predicted	86.4 ± 17.10	88.23 ± 17.33	81.44 ± 15.70	0.078
FEV_1 /FVC	0.73 (0.61 – 0.81)	0.71 (0.60 – 0.79)	0.81 (0.69 – 0.85)	0.019
RV(L)[2]	2.02 (1.46 – 2.49)	2.16 ± 0.78	1.76 (1.37 – 2.24)	0.076
RV % predicted[2]	137 (114.5 – 177)	150.61 ± 52.96	131.5 (110.25 - 149.75)	0.213
TLC (L)[2]	5.03 (4.58 – 5.52)	5.22 (4.67 -5.60)	4.63 (4.28 – 5.16)	0.003
TLC % predicted[2]	105 ± 16.59	107.87 ± 17.12	97.82 ± 12.83	0.006
RV/TLC[2]	0.39 (0.33 – 0.48)	0.41 ± 0.12	0.39 (0.30 – 0.42)	0.407
DLCO (mL/min/mmHg)[3]	17.25 ± 6.64	17.14 ± 6.31	17.58 ± 7.64	0.776
DLCO % predicted[3]	66.71 ± 25.37	66.58 ± 24.28	67.08 ± 28.58	0.933

Values expressed are mean ± SD, median (25^{th}-75^{th} percentil) or n (%).

BD: Bronchodilator; D_{LCO}: diffusing capacity of the lung for carbon monoxide; FEV_1: forced expiratory volume in the first second; FVC: forced vital capacity; LAM: lymphangioleiomyomatosis; RV: residual volume; TLC: total lung capacity; TSC: tuberous sclerosis complex;

*4 patients from the initial sample could not perform lung function tests due to the following reasons: cognitive impairment (3) and refusal to perform the test (1).

[1]n expressed in each column, respectively: 65 (total), 51 (S-LAM), 14 (TSC-LAM)

[2]n expressed in each column, respectively: 101 (total), 73 (S-LAM), 28 (TSC-LAM)

[3]n expressed in each column, respectively: 94 (total), 69 (S-LAM), 25 (TSC-LAM)

Table 4 – Six-minute walk test (6MWT) variables and Quality of life data (SF-36) obtained at the study inclusion

	LAM (n = 103)*	Sporadic LAM (n = 76)	TSC-LAM (n = 27)	p
6MWT[1]				
Distance, m	495 (435 - 559)	492 (440 - 562)	482 ± 109	0.994
Distance, % pred	86 (72 - 96)	86 (78 - 96)	81 ± 19	0.472
Initial SpO_2	96 (95 – 98)	96 (95 – 97)	98 (94 – 98)	0.071
Final SpO_2	93 (87 – 96)	93 (88 – 95)	96 (88 – 97)	0.044
Change in SpO_2 , %	-3 (-7 - -1)	-3 (-7- -1)	-2 (-6 - -1)	0.229
Initial HR[2]	84 ± 12	84 ± 12	84 ± 11	0.915
Peak HR	115 ± 17	118 ± 17	111 ± 18	0.068
Initial Borg dyspnea score	0 (0 – 0)	0 (0 – 0)	0 (0 – 0)	0.406
Peak Borg dyspnea score	3 (0 – 5)	2 (0 – 5)	3 (0 – 5)	0.936
Initial Borg leg discomfort score[3]	0 (0 – 0)	0 (0 – 0)	0 (0 – 0)	0.556
Peak Borg leg discomfort score[3]	2 (0 – 4)	1 (0 – 3)	2 (0 – 5)	0.264
SF-36				
Physical functioning	75 (50 - 90)	75 (49 - 90)	75 (50 - 85)	0.319
Role limitations due to physical health	100 (50 - 100)	100 (50 - 100)	75 (12 - 100)	0.097
Role limitations due to emotional health	100 (33 - 100)	100 (58 - 100)	33 (0 - 100)	0.017
Vitality	65 (50 - 75)	70 (50 - 80)	55 ± 19	0.046
Mental health	72 (52 - 84)	76 (52 - 84)	61 ± 19	0.058
Social functioning	75 (62 - 100)	75 (62 - 100)	62 (50 - 87)	0.098
Bodily pain	70 (45 - 90)	77 (45 - 100)	62 ± 25	0.223
General health	58 ± 22	58 ± 23	58 ± 19	0.810

Values expressed are mean ± SD, or median (25th-75th percentil).

HR: heart rate; LAM: lymphangioleiomyomatosis; SpO_2: peripheral oxygen saturation; TSC: tuberous sclerosis complex;

*Four patients from the initial sample did not perform the SF-36 questionnaire due to the following reasons: cognitive impairment (2) and refusal to answer (2).

[1]n expressed in each column, respectively: 99 (total), 72 (S-LAM) and 27 (TSC-LAM); Eight patients from the initial sample could not perform the 6MWT due to the following reasons: osteoarticular limitation (2), cognitive impairment (3), pregnancy (1), loss of follow up (1) and refusal to perform the test (1).

[2]n expressed in each column, respectively: 98 (total), 71 (S - LAM) and 27 (TSC - LAM);

[3]n expressed in each column, respectively: 97 (total), 70 (S – LAM) and 27 (TSC – LAM).

Table 5 – Chest CT variables obtained at the study inclusion

	LAM (n = 97)	**Sporadic LAM (n = 69)**	**TSC-LAM (n = 28)**	**p**
Extension of cysts %[1]	3.8 (0.9 – 13.4)	5.1 (1.2 – 13.6)	1.2 (0.2 – 7.8)	0.027
Other thoracic findings				
- Nodules suggestive of MMPH	12 (12%)	1 (1%)	11 (39%)	<0.001
- Ground-glass opacities	16 (16%)	5 (7%)	11 (39%)	<0.001
- Pleural effusion	9 (9%)	7 (10%)	2 (7%)	1
- Bronchovascular bundles thickening	6 (6%)	5 (7%)	1 (4%)	0.669
- Interlobular septal thickening	7 (7%)	6 (9%)	1 (4%)	0.669
- Hilar lymphadenopathy	2 (2%)	1 (1%)	1 (4%)	0.496
- Mediastinal lymphadenopathy	8 (8%)	6 (9%)	2 (7%)	1
- Supraclavicular/ axillary lymphadenopathy	6 (6%)	5 (7%)	1 (4%)	0.669
- Thoracic duct dilatation	4 (4%)	4 (6%)	0	0.321
- Lymphangioleiomyomas	14 (14%)	12 (17%)	2 (7%)	0.338
- Sclerotic bone lesions	21 (22%)	1 (1%)	20 (70%)	<0.001

Values expressed are median (25th-75th percentile) or n (%).

LAM: lymphangioleiomyomatosis; MMPH: multifocal micronodular pneumocyte hyperplasia; TSC: tuberous sclerosis complex;

[1]n expressed in each column, respectively: 92 (total), 66 (S-LAM) and 26 (TSC-LAM); Fifteen patients from the initial sample were not included in the lung cysts quantification due to technical limitations on importing and/or processing the images by the software (7) or unavailability of the chest CT images (8).

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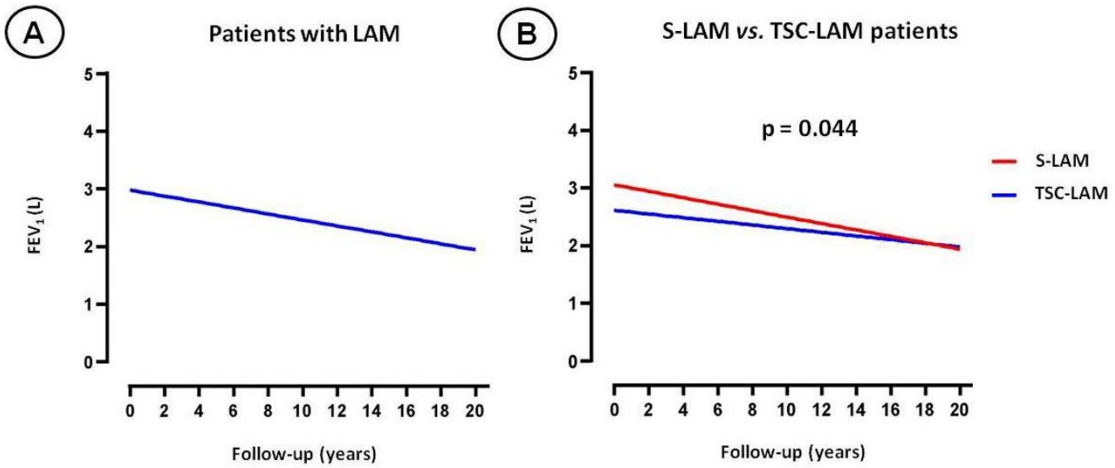
A
Patients with LAM
FEV1 (L)
0
1
2
3
4
5
0 2 4 6 8 10 12 14 16 18 20
Follow-up (years)
B
S-LAM vs. TSC-LAM patients
p = 0.044
S-LAM
TSC-LAM
FEV1 (L)
0
1
2
3
4
5
0 2 4 6 8 10 12 14 16 18 20
Follow-up (years)

Figure 1

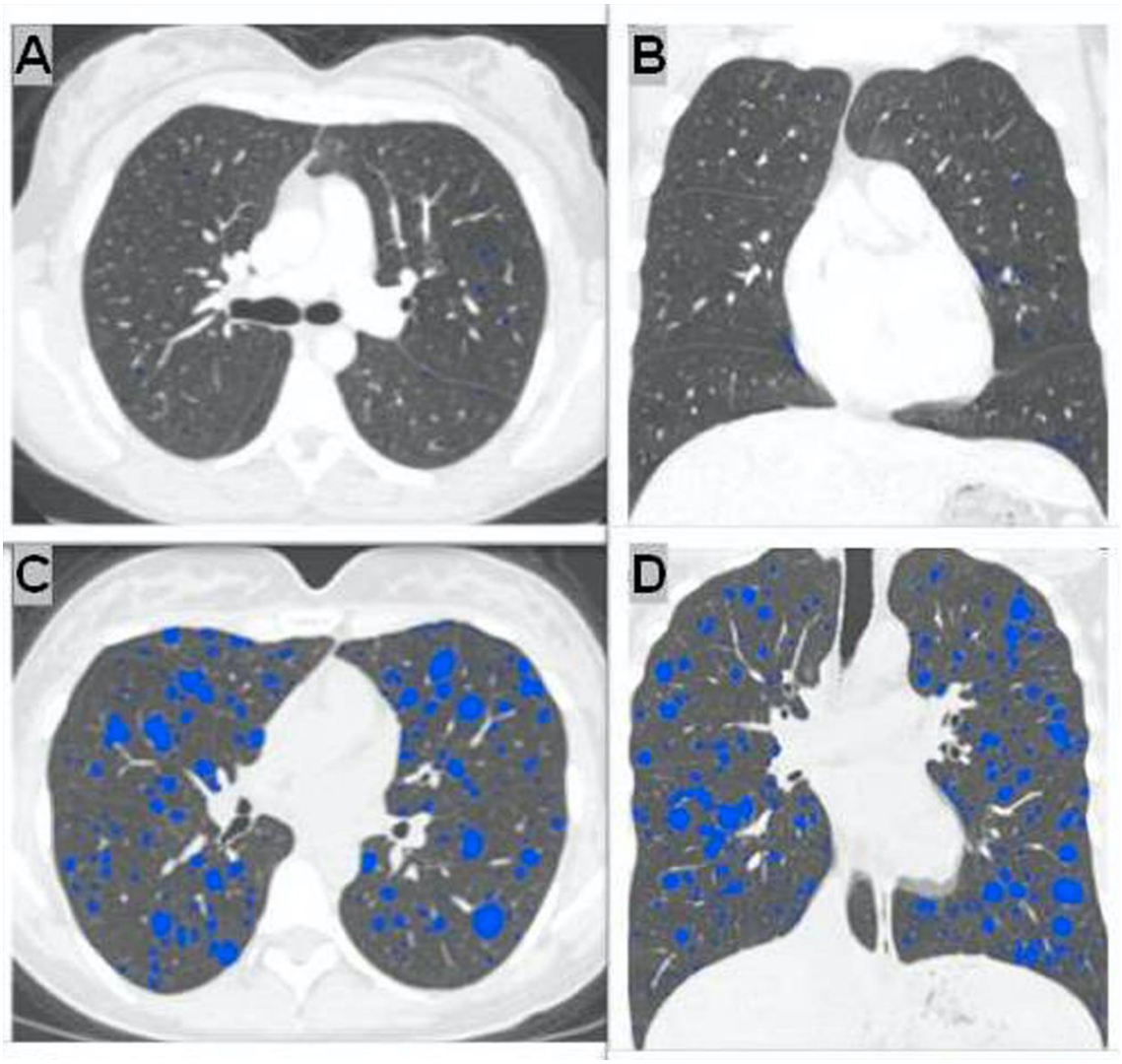

Figure 2

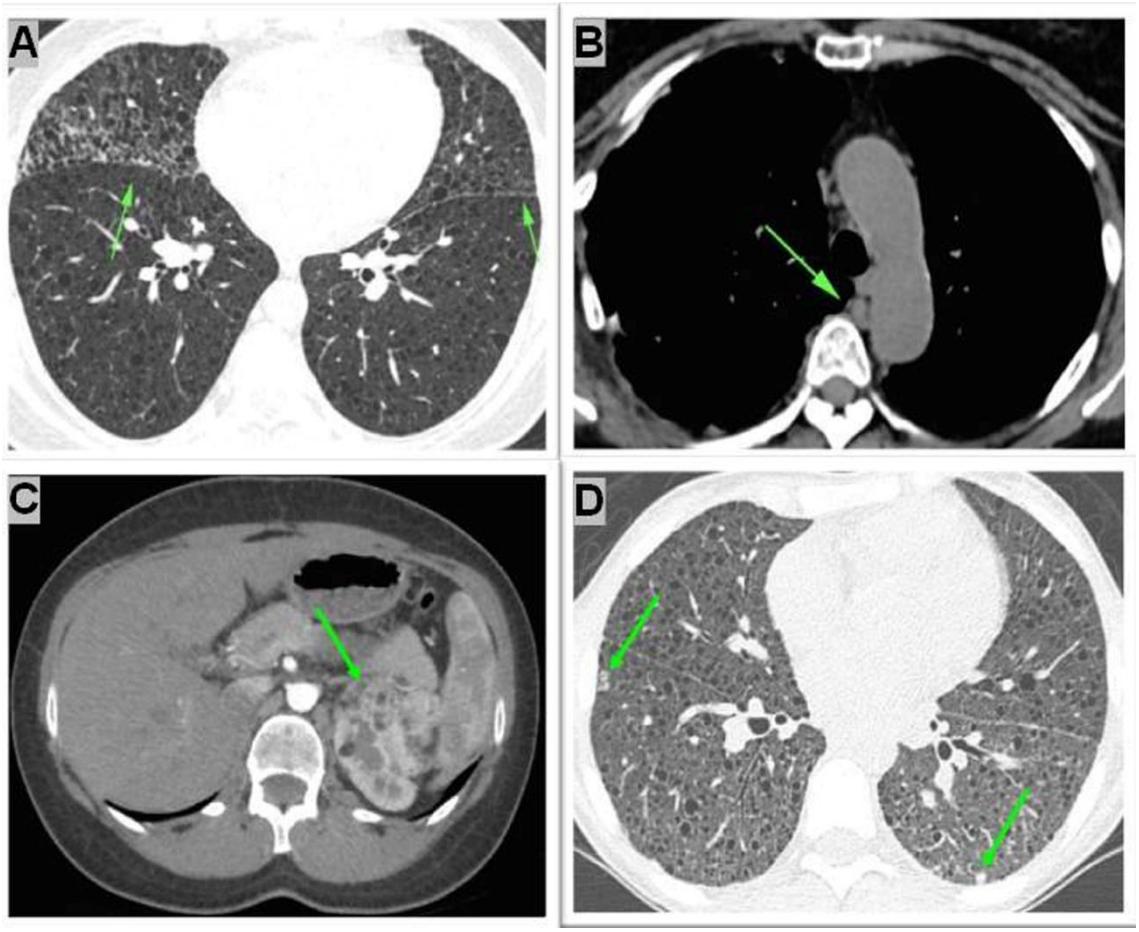

Figure 3

Supplementary data

Clinical, tomographic, and functional comparison of sporadic and associated tuberous sclerosis complex forms of LAM: a retrospective cohort study

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Supplementary Table 1 – Predictors of lung function impairment (FEV_1 < LLN)

Variable	OR	IC (95%)	p
Univariate analysis			
LAM group (S-LAM *vs.* TSC-LAM)	1.083	0.455 – 2.581	0.857
Time from diagnosis to inclusion, y	1.023	0.949 – 1.103	0.546
mMRC	2.313	1.276 – 4.195	0.006
Pneumothorax (yes *vs.* no)	2.933	1.316 – 6.536	0.008
BD response (yes *vs.* no)	0.545	0.130 – 2.283	0.406
Distance walked, % predicted	0.958	0.932 – 0.985	0.003
Change in SpO_2 ≥ 4% in 6MWT (yes *vs.* no)	12.495	4.246 – 36.770	<0.001
Lung cysts extension, %	1.187	1.093 – 1.288	<0.001
Lymphatic involvement* (yes *vs.* no)	1.919	0.817 – 4.509	0.135
Renal angiomyolipoma (yes *vs.* no)	1.029	0.474 – 2.236	0.942
Multivariate analysis			
Pneumothorax (yes *vs.* no)	3.206	1.001 – 10.270	0.050
Change in SpO_2 ≥ 4% in 6MWT (yes *vs.* no)	7.026	1.895 – 26.046	0.004
Lung cysts extension, %	1.150	1.042 – 1.268	0.005

* chylous ascites, chylothorax and/or lymphangioleiomyomas

BD: bronchodilator; LAM: lymphangioleiomyomatosis; mMRC: modified Medical Research Council; 6MWT: six-minute walk test; SpO_2: peripheral oxygen saturation; TSC: tuberous sclerosis complex

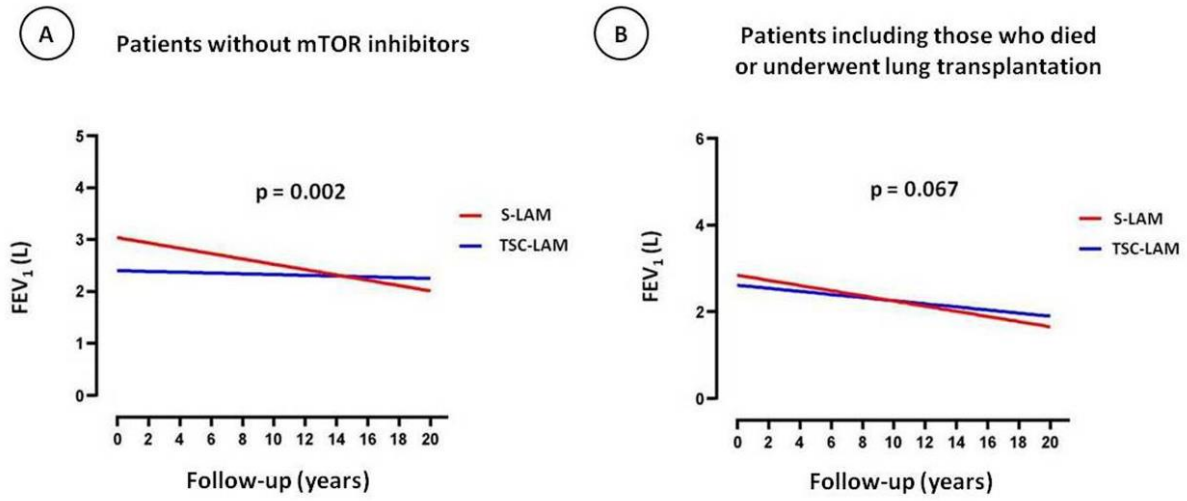

Figure S1. A) FEV_1 annual rates of change in patients with S-LAM (n = 47) *vs.* patients with TSC-LAM (n = 7) without mTOR inhibitors at inclusion in the study. Data expressed as mean (SE): -51.3 (5) mL/year *vs.* -7.4 (12) mL/year, p = 0.002; B) FEV_1 annual rates of change in S-LAM (n = 75) *vs.* TSC-LAM (n = 20) including patients who underwent lung transplantation or died during the cohort assessment. Data expressed as mean (SE): -59.5 (5) mL/year *vs.* -35.7 (11) mL/year, p = 0.067.

Abbreviations: FEV_1: forced expiratory volume in the first second; LAM: lymphangioleiomyomatosis; mTOR: mechanistic target of rapamycin; S-LAM: sporadic LAM (red straight); TSC-LAM: LAM associated with tuberous sclerosis complex (blue straight).