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Pulmonary hypertension in lymphangioleiomyomatosis: characteristics in 20 patients

Original article

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Abstract (word count: 200)

This retrospective, multicenter study evaluated patients with lymphangioleiomyomatosis (LAM) and precapillary pulmonary hypertension (PH) by right heart catheterisation.

It was conducted in 20 women with a mean age of 49 ±12 years and a mean time interval between LAM and PH diagnoses of 9.2 ±9.8 years. All except 1 were receiving supplemental oxygen. Six-min walk distance was 340 ±84 m. Haemodynamic characteristics were: mean pulmonary arterial pressure (PAP) 32 ±6 mmHg, cardiac index 3.5 ±1.1 $L.min.m^{-2}$ and pulmonary vascular resistance (PVR) 376 ±184 $dyn.s.cm^{-5}$. Mean PAP was >35 mmHg in only 20% of cases. Forced expiratory volume in 1s was 42 ± 25%, carbon monoxide transfer factor was 29 ±13%, and arterial oxygen tension (PaO_2) was 7.4 ±1.3 kPa on room air. Mean PAP and PVR did not correlate with PaO_2. In 6 patients who received oral pulmonary arterial hypertension (PAH) therapy, the mean PAP decreased from 33 ±9 to 24 ±10 mmHg and PVR from 481 ±188 to 280 ±79 $dyn.s.cm^{-5}$. The overall probability of survival was 94% at 2 years.

Precapillary PH of mild haemodynamic severity may occur in patients with LAM, even with mild pulmonary function impairment. PAH therapy might improve haemodynamics in PH associated with LAM.

Keywords: Pulmonary hypertension; lymphangioleiomyomatosis; interstitial lung disease

Introduction

Lymphangioleiomyomatosis (LAM), a disease affecting women mostly young and middle-aged [1-3], is characterised by the proliferation of abnormal smooth muscle-like cells (so-called LAM cells) along lymphatics in the lungs and abdomen. Manifestations of LAM include diffuse cystic lung disease, recurrent pneumothoraces, benign renal tumors (e.g. angiomyolipomas), and lymphatic abnormalities, including pleural and peritoneal chylous effusion as well as abdominal lymphangioleiomyomas. Pulmonary involvement is dominated by the formation and progression of thin-walled cysts, the pathogenesis of which may implicate metalloprotease secretion by LAM cells, leading to airflow obstruction, impairment of carbon monoxide diffusion capacity, and chronic respiratory insufficiency [1-4]. Although sirolimus (a mammalian target of rapamycin (mTOR) inhibitor) has recently been demonstrated to slow the rate of lung function decline [5], lung transplantation is the sole treatment for LAM patients with advanced disease [6-9]. Kaplan-Meier analysis estimated transplantation-free survival to be about 80-90% at 10 years in recent series of LAM patients [10, 11]. However, the rate of disease progression is highly variable among patients [12-15].

Pulmonary hypertension (PH), which may occur in LAM patients [16], is included in the PH group with unclear and/or multifactorial mechanisms in the Dana Point clinical classification of PH (group 5) [17]. Likely multifactorial [18], PH pathogenesis in LAM patients is related, at least in part, to hypoxia and reduced pulmonary vascular capacitance caused by cystic lesions [19]. In addition, mTOR expression is up-regulated in LAM [20], and activation of mTOR complexes 1 and 2 is further enhanced by hypoxia, contributing to smooth muscle cell proliferation [21] and conceivably to PH pathogenesis. Taveira-DaSilva et al. [19] reported 7% PH prevalence in 95 patients with LAM, as defined by estimated systolic pulmonary artery pressure (PAP) >35 mmHg on echocardiography. PH, confirmed by right heart catheterisation (RHC) (mean PAP >25 mmHg), was diagnosed in 9 of 20 patients (45%)

evaluated for lung transplantation [7]. Little information is available regarding the haemodynamic profile of PH in LAM, and there are no data on the effect of pulmonary arterial hypertension (PAH)-specific therapy, such as endothelin receptor antagonists, type 5 phosphodiesterase inhibitors and prostacyclin derivatives.

This study aimed to: (1) evaluate by RHC the haemodynamic characteristics of patients with LAM and PH and not explained otherwise; (2) determine whether haemodynamics may be related to pulmonary function; (3) ascertain the survival of PH patients with LAM; and (4) explore whether PH-specific therapy, given off-label on an individual basis, can bring about significant clinical and/or haemodynamic improvements.

Patients and methods

Study design

This multicenter study was undertaken by the French Reference Center for Rare Pulmonary Diseases (Coordinator: JFC), the French Reference Center for Pulmonary Hypertension (Coordinator: GS), the Center for Rare Pulmonary Diseases in Milan (Coordinator: SH), the Network of French Competence Centers for Rare Pulmonary Diseases and Competence Centers for Pulmonary Hypertension, and the *Groupe d'Etudes et de Recherche sur les Maladies "Orphelines" Pulmonaires* (GERM"O"P), a collaborative group dedicated to research on rare (so-called “orphan”) pulmonary diseases. Participating physicians prospectively reported all cases of LAM to the GERM"O"P registry (Coordinator: JFC). PH was screened by echocardiography at the physicians’ discretion, but most groups in France and Italy perform echocardiography once a year in LAM patients with impaired lung

function. RHC was implemented in case of suspected PH (with estimated systolic PAP of 40 mmHg or greater on echocardiography) or evaluation for lung transplantation.

Data on PH were collected prospectively from the Registry of the French Network of Pulmonary Hypertension that now comprises 24 university pulmonary vascular centers [22]. The registry was opened in 2002 and enrolled all consecutive patients aged 18 years or older with precapillary PH seen at these centers. Additional results on LAM were obtained retrospectively, and data collection ended in December 2010. A control group of patients with LAM (without PH) was obtained from the active file of the centres. This study was compliant with the requirements of the *Commission nationale de l'informatique et des libertés*, the organisation dedicated to privacy, information technology, and civil rights in France. All patients provided informed consent before participation [22]. The study was approved by the Institutional Review Board of the *French Learned Society for Respiratory Medicine* and registered at www.clinicaltrials.gov (NCT00960895).

Inclusion criteria

The following inclusion criteria applied:

1. Definite or probable LAM diagnosed according to European Respiratory Society recommendations [6].

2. Precapillary PH, defined by mean PAP ≥25 mmHg and pulmonary artery wedge pressure ≤15 mmHg at RHC [23].

Patients with PAH, either idiopathic, heritable, or associated with connective tissue diseases related to portal hypertension, congenital heart disease, human immunodeficiency virus infection, or PH due to left heart disease or chronic thromboembolic PH, were excluded. Chronic thromboembolic PH was ruled out by ventilation perfusion scanning and high

resolution computed tomography of the chest. “Out-of-proportion” precapillary PH was defined by mean PAP >35-40 mmHg.

Investigations

RHC was performed as described elsewhere [24] with values obtained at end of expiration. Cardiac output was measured by the standard thermodilution technique. A vasodilator test with inhaled nitric oxide (10 ppm for 5-10 min) was carried out, and positive acute responses were defined as a decrease in mean PAP of >10 mmHg compared with baseline mean PAP, with mean PAP <40 mmHg, and normal or increased cardiac output [24].

Pulmonary function tests followed the joint guidelines of the American Thoracic Society and the European Respiratory Society [25-27]. Lung volume was measured by whole-body plethysmography (Jaeger Masterscreen Body®, Sebbac, Wuerzburg, Germany), and data were expressed as percentages of predicted values [28]. A non-encouraged 6-min walk test was performed according to recommendations [29].

The date of PH diagnosis was defined as the date of RHC, and all data (symptoms including NYHA class, 6-min walk test, pulmonary function, echocardiography) were obtained within two months of RHC. PH treatment was left to the physicians’ discretion, including the management of pleural and other LAM manifestations, oxygen supplementation as needed, oral anticoagulation, diuretics, and possible PAH-specific therapy initiated after RHC.

Pathology

Explanted lungs of transplanted patients were reviewed by a pathologist with particular expertise in pulmonary vascular disease (PD). The presence of LAM / perivascular

epithelioid cells (PEComa cells) was semi-quantified as absent, mild, moderate, or high, along cyst edges, alveolar walls, bronchioles, pulmonary arteries, veins and lymphatics. Cells within the remodeled pulmonary arteries were further characterised by HMB45 immunostaining.

Statistical analysis

The data were analysed by Microsoft Excel 2003 and SPSS 17.0 (SPSS, Inc.). All values were expressed as mean ± standard deviation (SD). Correlations were calculated with Pearson's correlation coefficient. The probability of survival at each time point was estimated according to the Kaplan-Meier method, from the date of the first haemodynamic evaluation demonstrating PH to the endpoints of death or censoring. All-cause mortality was included in survival statistics. For overall survival calculation, transplanted subjects were censored at the time of transplantation. Alive patients were censored at the date of the last visit. Categorical data were compared using the Fisher's exact test. Haemodynamic and pulmonary function variables were compared by the 2-tailed t test. Statistical significance was established at $p<0.05$.

Results

Patient population

Twenty nine LAM patients with suspected PH at echocardiography underwent RHC. The estimated systolic PAP at echocardiography significantly correlated with the systolic PAP ($r=0.66$, $p=0.001$) and with the mean PAP measured at RHC ($r=0.69$, $p=0.0006$). The mean difference between the systolic PAP estimated at echocardiography and the systolic PAP measured at RHC was -5.4 mmHg (95% confidence interval (95% CI): -13 to -2 mmHg). The systolic PAP at echocardiography was overestimated by 10 mmHg or more as

compared to RHC in 4 cases (14%), and was underestimated by 10 mmHg or more in 9 cases (31%). The difference between the estimated systolic PAP at echocardiography and the measured systolic PAP at RHC was greater than 10 mmHg mostly in patients with a mean PAP less than 25 mmHg (Figure 1).

Nine patients had mean PAP between 20 and 24 mmHg and were excluded from the subsequent analysis. The study population thus comprised 20 patients, including 18 with sporadic LAM and 2 with LAM associated with tuberous sclerosis complex; all were female, with a mean age of 49 ± 12 yrs not reported previously [7, 16]. Totally, 270 LAM patients were included in the GERM"O"P registry (n=222) or followed in the Milan referral center (n=48) during the study period; therefore, it was estimated that PH patients represented a minimum of 7% of LAM patients. The reasons for RHC were evaluation for transplantation (n=9) and/or suspicion of PH based on systematic echocardiography (n=11).

The baseline clinical data are reported in Table 1. The diagnosis of LAM was definitive in 19 patients, and probable in 1 non-smoker with characteristic chest imaging, obstructive ventilatory defect, and compatible medical history. A pattern characteristic of LAM [6] was present on high-resolution computed tomography of the chest in all patients. The diagnosis was confirmed by video-assisted thoracoscopic lung biopsy in 13 cases (65%). Fifteen patients were ex-smokers, with a median of 10 pack-years. One patient had a history of splenectomy, and another had taken anorexigens. One patient had undergone unilateral nephrectomy for angiomyolipoma. As compared to LAM patients without PH, patients with LAM and PH had more severe dyspnoea and presented more frequently with right heart failure or haemoptysis.

LAM was treated as follows: inhaled bronchodilators, 75% of patients; progesterone derivatives and/or anti-estrogen therapy, 45%; doxycycline, 30%; sirolimus, 25%.

Clinical and functional evaluation

The mean time interval between LAM diagnosis and the first RHC demonstrating PH was 9.2 ± 9.8 years (0-36 years), and the mean time period between first respiratory symptoms and PH was 10.4 ± 7.5 years (3.0-22.1 years). New York Heart Association functional class was III or IV in 95% of these patients.

Echocardiography revealed dilated right heart cardiac cavities in 7 / 20 patients (35%). The mean value of systolic PAP estimated at echocardiography was 56 ± 18 mmHg (range, 40-108). Mild pericardial effusion was reported in 1 patient. B-type natriuretic peptide (BNP) level was normal in 7 of 7 patients tested. Haemoglobin was higher than 160 g/l in 2 patients (10%).

Six-min walk distance was 340 ± 84 m, with mean desaturation of 10 ± 8%. The median Borg index value of dyspnoea at the end of the 6-min walk test, available in 11 patients, was 4 (range: 3-6). Table 2 presents the pulmonary function test results. Obstructive ventilatory defect, defined by forced expiratory volume in 1 s (FEV_1): forced vital capacity (FVC) <70%, was present in 83% of patients. FEV_1 was <80% of predicted value in 84% of patients, and <50% of predicted value in 63% of patients. Gas exchange was severely impaired, with a mean single breath-diffusing capacity of the lungs for carbon monoxide (DLco) of 29 ± 13% of predicted value. As compared to LAM patients without PH, patients with LAM and PH had more severe airflow obstruction, lower diffusion capacity for carbon monoxide, more severe hypoxaemia and impairment of exercise capacity.

Haemodynamics

Table 3 reports the results of RHC. Mean PAP was 32 ± 6 mmHg, and pulmonary vascular resistance (PVR) was 376 ± 184 dyn.s.cm^{-5}. None of the 8 patients tested was acutely vasoreactive to inhaled nitric oxide. Mean PAP was >35 mmHg in 4 patients with definite

LAM (20%), 2 of whom had normal FEV_1, and one with mean PAP of 40 mmHg and FEV_1 of 52% of predicted had a history of anorexigen intake. Mean PAP was >40 mmHg in 1 patient (5%) who had normal FEV_1

Significant correlations were observed between haemodynamic parameters and pulmonary function (Table E1, Figure 2), especially between PVR and FEV_1 and DLco and the transfer coefficient for carbon monoxide (Kco), but not with arterial oxygen tension (PaO_2) or peripheral oxygen saturation (SpO_2) at the end of the 6-min walk test. Mean PAP correlated with estimated systolic PAP at echocardiography (r=0.583; p=0.063).

Outcome and survival analysis

Ninety-five percent of patients received longterm supplemental nasal oxygen therapy; 30% took diuretics and 25% were given oral anti-coagulant therapy, for PH.

Six of 20 patients (30%) received first-line therapy for PAH with dual endothelin receptor antagonist (bosentan, n=5) or type 5 phosphodiesterase inhibitor (sildenafil, n=1) with no concomitant change in supplemental oxygen therapy. No patient was administered prostacyclin derivatives. In this subgroup of 6 treated patients, no statistically significant difference was observed on functional class (p=0.987), 6-min walking distance (p=0.983), cardiac index (p=0.786), FEV1 (p=0.530), PaO_2 (p=0.179) or SpO_2 (p=0.880) between the evaluation before PAH therapy and the last evaluation on therapy (Figure 3). Right heart cavities were dilated in 3 / 6 patients, with no change upon PH therapy. Mean PAP decreased significantly in the 6 treated patients with PAH therapy from 33 ± 9 to 24 ± 10 mmHg (mean of differences: 9 mmHg [95% confidence interval (95% CI): 5-14]; p=0.003) after a median of 38 months (interquartile range: 14.5-34 months). PVR declined in the 6 treated patients from 481 ± 188 dyn.s.cm^{-5} to 280 ± 79 dyn.s.cm^{-5} (mean of differences: 201 dyn.s.cm^{-5} [95% CI: 18-384]; p=0.037). Overall, an improvement (predefined by NYHA functional class

reduction and/or 20% increase in 6-min walk distance and/or 20% decrease in PVR with 20% diminution of mean PAP) was seen in the 5 patients who received bosentan, but not in the patient given sildenafil.

In addition, one patient was treated with sirolimus (but no PAH treatment) for LAM progression and had follow-up evaluation after 10 months of therapy: NYHA functional class had changed from IV to III, 6-min walk distance from 330 m (SpO_2 of 84%) to 350 m (SpO_2 of 90%), FEV_1 from 27% to 53% of predicted value, FVC from 63 to 102%, mean PAP from 35 to 23 mmHg, PVR from 168 to 178 dyn.s.cm^{-5}, and cardiac index from 5.2 to 3.9 l.min^{-1}.m^{-2}.

Overall, the study subjects were followed for a mean of 2.5 ± 2.1 years from the diagnosis of PH. No patient was lost to follow-up. At the end of follow-up, 1 patient had died from cardiac arrest, and 5 patients had undergone single or double lung transplantation (3 and 2 patients, respectively). The overall probability of survival was 94% at 1 year, 94% at 2 years, and 78% at 3 years (Figure 4). The transplant-free probability of survival was 87% at 1 year, 78% at 2 years, and 56% at 3 years.

Pathology assessment of explanted lungs

Pathologic assessment of explanted lungs in 5 patients demonstrated pronounced vascular remodelling, with involvement of the pulmonary arterial walls by characteristic LAM cells (so-called perivascular epithelioid cells or PEComa cells) (Table 4, Figure 5). Cells within the remodelled pulmonary arteries were further characterised as LAM / PEComa cells by positive HMB45 immunostaining in 3 / 3 cases available. As expected, LAM / PEComa cells were also observed along the edges of the lung cysts, bronchioles, and pulmonary lymphatics.

Discussion

The present study is the first to report the haemodynamic evaluation of LAM patients with precapillary PH confirmed by RHC, the gold standard for PH diagnosis [23].

The main findings were as follows: (1) PH was generally of only mild haemodynamic severity, with mean PAP of 32 ± 6 mmHg and PVR of 376 ± 184 dyn.s.cm^{-5}, and only 20% of patients had mean PAP >35 mmHg (out-of-proportion PH); (2) PH was diagnosed after a mean of 9.2 ± 9.8 years following the LAM diagnosis in patients with mean FEV_1 of 46.4 ± 26% of predicted value, chronic hypoxaemia (mean PaO_2 of 7.4 ± 1.1 kPa), and moderate to severe exercise intolerance as shown by mean 6-min walk distance of 340 ± 84 m with mean SpO_2 of 81.3 ± 9.3% at the end of the test; (3) patients with PH had more severe dyspnoea, airflow obstruction, hypoxaemia, and impairment of exercise capacity than patients with LAM without PH; (4) haemodynamic parameters correlated with pulmonary function and especially with FEV_1, DLco, and Kco; (5) significant remodelling of the pulmonary arteries was observed with involvement by HMB45 positive LAM/PEComa cells; (6) the overall probability of survival was 94% at 2 years, and transplant-free survival was 78% at 2 years; (7) off-label oral PAH therapy in a subgroup of patients was associated with a significant decrease in mean PAP and PVR without a significant difference in exercise capacity or dyspnoea.

Impairment of pulmonary function in our study population was more severe than in most recent series of LAM patients, who had mean FEV_1 of 65% to 75% of predicted value at the time of diagnosis [10, 12, 13, 30, 31]. Patients with PH had more airflow obstruction than LAM patients without PH, indicating that PH generally occurs in subjects with more advanced pulmonary disease. Consistent with this hypothesis, LAM diagnosis preceded PH diagnosis by almost a decade, and 5 patients (25%) underwent lung transplantation within 2 years after PH diagnosis.

However, unexpected correlations were found between lung function and haemodynamic parameters, with greater mean PAP, superior PVR, and lower cardiac index in patients with better preserved FEV_1 and DLco, indicating that PH may progress independently of parenchymal lung disease. In contrast, systolic or mean PAP correlate inversely with DLco in idiopathic pulmonary fibrosis patients, although it correlates poorly with lung volume [32-35]. SpO_2 was significantly decreased during exercise, and most LAM and PH patients required longterm oxygen therapy. However, mean PAP did not correlate with PaO_2 at rest, consistent with a previous observation that PH (during exercise) cannot be predicted from resting pulmonary function testing [19]. Although our small sample size prevents firm conclusions, our study indicates that PH can occur in patients with mild to severe lung function impairment and may reflect intrinsic vasculopathy in an unknown proportion of patients, as suggested by pathological findings in 5 patients and earlier descriptions of the involvement of pulmonary arteries by cell proliferation in LAM [36, 37]. It is conceivable that mTOR activation in LAM may contribute to PH in patients with LAM [21], as in patients with neurofibromatosis type 1 [38].

The mean PAP in our patients (32 ± 6 mmHg) was strikingly similar to that reported by one of us (33 ± 8 mmHg) in 9 patients evaluated for lung transplantation [7], and higher than that observed by Harari et al. (26.0 ± 2.5 mmHg) in 6 patients also evaluated for lung transplantation [16], but not included in the present series. PH in the context of chronic parenchymal lung disease is usually mild or moderate (i.e. with mean PAP <35-40 mmHg). However, subgroups of patients with chronic obstructive pulmonary disease (COPD) [39-41], idiopathic pulmonary fibrosis [42], or combined pulmonary fibrosis and emphysema syndrome [43] have severe "out-of-proportion" precapillary PH, usually defined by mean PAP >35-40 mmHg [17, 39]. These subjects are prone to right heart failure and may share similarities with idiopathic PAH [43, 44]. Only 20% of patients in the present study had mean

PAP >35 mmHg with 5% disclosing mean PAP >40 mmHg, fulfilling this definition. Interestingly, 2 of them had normal FEV_1 with PH and were thus "out-of-proportion". The highest mean PAP recorded was 51 mmHg in our study, and 47 mmHg in that of Reynaud-Gaubert et al. [7]. The mean haemodynamic profile of LAM patients in the present series was much less severe than that of PAH patients in the national French registry (with a higher PVR index of 1,640 dyn.s.cm^{-5}.m^{-2} and cardiac index of 2.5 L.min^{-1}.m^{-2}) [22]. It was also less severe than in patients with precapillary PH and pulmonary Langerhans cell histiocytosis [45, 46], combined pulmonary fibrosis and emphysema [43], or sarcoidosis [47], and somewhat comparable to that of PH associated with idiopathic pulmonary fibrosis [32, 35, 42]. However, although mean PAP was only mildly elevated, some patients with mild lung function impairment had severe and "out-of-proportion" PH.

The clinical relevance of PH in patients with LAM is currently unknown. In a study by Taveira-DaSilva et al. [19], peak exercise PAP correlated negatively with SpO_2, indicating that PH may contribute to exercise-induced hypoxaemia and exercise limitation; increased systolic PAP was observed during low-level exercise corresponding to daily living activities. Predictors of a worse prognosis could not be evaluated in the present series because of the small sample size. However, LAM patients with PH had lower carbon monoxide diffusion capacity, more severe hypoxaemia, and impaired exercise capacity, as compared to LAM patients without PH, suggesting that a low diffusion capacity in LAM should prompt to perform an echocardiography. A significant proportion of our PH and LAM patients died or underwent lung transplantation within 2 years of PH diagnosis. Although no formal survival comparison was made to a group of LAM patients without PH, the present finding questions whether PH might have prognostic significance in LAM, similar to what has been reported in idiopathic pulmonary fibrosis [42, 48], COPD [49], and combined pulmonary fibrosis and emphysema [50, 51]. The 10-year survival of patients with LAM has been reported to 71%,

79%, and 91% from the time of diagnosis in large series [10, 11, 52]. The potential impact of PH on long-term survival in LAM thus deserves further analysis in prospective studies.

The potential benefit of PAH-specific therapy is not known in PH patients with associated pulmonary parenchymal disorders, as these drugs have not been approved in this setting. It is unlikely that the rare occurrence of PH in LAM (an "orphan" disease) will be investigated in a dedicated clinical trial. A minority of patients in the present series were treated off-label on an individual basis, providing some interesting preliminary information on the efficacy and safety of PAH therapy in this condition. PAH treatment with bosentan or sildenafil was well-tolerated, especially since gas exchange was not significantly impaired as observed in some COPD and PH patients [53]. Although involvement of pulmonary veinules was observed pathologically, pulmonary oedema was not observed at initiation of PH therapy. Haemodynamics improved significantly with a 28% decrease in mean PAP and a 42% reduction of PVR, but no significant effect of therapy was apparent on dyspnoea or exercise capacity (although such analysis was underpowered). Sirolimus, which has proven beneficial in lung function and clinically-relevant parameters [5], should be considered in LAM patients with progressive disease. The present study did not allow us to evaluate whether sirolimus may also contribute to improvement in PH patients with LAM. Nasal oxygen supplementation should be considered whenever appropriate [6]. Younger LAM patients should be evaluated early for lung transplantation, which remains the issue in advanced disease [6-9].

This study sheds light on the utility of non-invasive methods screening for PH in LAM patients. No patients tested in the present series had elevated serum BNP levels, and only 35% had dilated right heart cavities on echocardiography. All patients with mean PAP >25 mmHg at RHC had estimated systolic PAP of 40 mmHg or greater based on systolic regurgitation of the tricuspid valve. Conversely, patients who eventually manifested mean

PAP between 20 and 25 mmHg had estimated systolic PAP ≤40 mmHg on echocardiography. Although this study was not designed to evaluate the diagnostic value of echocardiography, we suggest that LAM patients with estimated systolic PAP ≥40 mmHg on echocardiography undergo RHC. It is known that echocardiography lacks specificity and accuracy in patients with advanced lung disease, including emphysema [54] and idiopathic pulmonary fibrosis [55, 56]. No patients in the present study had postcapillary PH. Although the current study was not designed to evaluate the prevalence of PH, a conservative 7% was estimated for PH prevalence in LAM patients, consistent with a previous similar assessment based on echocardiography [19].

Our investigation has some limitations, especially its observational and uncontrolled design owing to the rarity of this condition. However, LAM cases were prospectively included in the GERM"O"P registry, and haemodynamic data were collected prospectively in the setting of the French national PH registry. Haemodynamic and pulmonary functions were not evaluated during exercise. As indications for echocardiography and treatment were left to the physicians' discretion, the data presented here are potentially subject to bias and should not be interpreted as proper evaluation of therapeutic efficacy. However, data on haemodynamic parameters and survival were unlikely to be affected by the study design.

In conclusion, PH may occur in a small subset of LAM patients after a mean of 9 years after diagnosis. It can affect LAM patients with mildly to severely-impaired lung function, most of them requiring longterm oxygen therapy. However, mean PAP and PVR did not correlate with PaO_2, indicating that factors other than hypoxia, and especially LAM/PEComa cell vasculopathy, may contribute to PH. Whether PAH-specific therapy may be beneficial in LAM patients with PH deserves further study.

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Table 1. Characteristics, clinical manifestations, and pulmonary function tests at diagnosis of precapillary pulmonary hypertension in 20 patients with lymphangioleiomyomatosis, as compared to 72 patients with lymphangioleiomyomatosis without pulmonary hypertension.

Variables	*LAM with PH (n=20)*	*Control LAM without PH (n=72)*	*P*
Age, years	49 ± 12 (33-73)	44 ± 19 (33-73)	0.362
Post-menopausal	10 (50%)	30 (71%)	0.612
Smokers, current:ex:never	0:5:15	2:22:48	0.644
LAM diagnosis, definite:probable	19:1	66:6	1
Lung biopsy with LAM	13 (65%)	41 (57%)	0.612
Tuberous sclerosis complex	2 (10%)	12 (17%)	0.726
Renal angiomyolipoma	9 (45%)	32 (44%)	1
History of pneumothorax*	8 (40%)	36 (50%)	0.460
History of chylothorax**	5 (25%)	13 (18%)	0.529
History of chylous ascites	2 (10%)	5 (7%)	0.643
Lymphangioleiomyoma	3 (15%)	20 (28%)	0.382
Lymph node involvement (pathology)	1 (5%)	6 (8%)	1

Dyspnoea	20 (100%)	53 (74%)	0.009
NYHA classes I :II:III:IV	0:1:10:9	11:22:18:4	<0.001
Haemoptysis	2 (10%)	0 (0%)	0.045
Finger clubbing	2 (10%)	N/A	N/A
History of right heart failure or lower limb oedema	4 (20%)	0 (0%)	0.002
Syncope at exercise	0 (0%)	0 (0%)	1
Body mass index, kg/m²	21 ± 3 (14-30)	22 ± (16-37)	0.212

Data are presented as mean ± SD (range) or n (%), unless otherwise stated, for the population of 20 patients. LAM: lymphangioleiomyomatosis; N/A, not available;NYHA: New York Heart Association. *bilateral in 7 of 8 cases; **6 other patients had a history of pleural effusion not otherwise specified.

Table 2. Pulmonary function and 6-min walk test in patients with lymphangioleiomyomatosis, as compared to 72 patients with lymphangioleiomyomatosis without pulmonary hypertension.

		LAM with PH	*Control LAM without PH*	
Variables	Number	Mean ± SD (range)	Mean ± SD (range)	*P*
FVC, % predicted	18	76 ± 28 (27-121)	88 ± 25 (30-145)	0.08
FEV_1 prebronchodilator, % predicted	19	42 ± 24 (13-96)	63 ± 25 (16-129)	0.002
FEV_1 postbronchodilator, % predicted	19	46 ± 26 (13-96)	N/A	N/A
FEV_1/FVC, %	18	47 ± 15 (22-75)	60 ± 16 (24-95)	0.003
TLC, % predicted	20	104 ± 16 (77-143)	109 ± 23 (51-169)	0.404
RV, % predicted	20	162 ± 52 (63-243)	143 ± 58 (65-309)	0.461
DLco, % predicted	18	29 ± 13 (14-57)	50 ± 25 (15-111)	0.002

Kco, % predicted	13	35 ± 14 (19-69)	57 ± 22 (15-93)	0.002
PaO_2 at rest, kPa	17	7.4 ± 1.1 (5.5-9.5)	10.1 ± 1.9 (6.7-14.5)	<0.001
$PaCO_2$ at rest, kPa	18	4.8 ± 0.5 (3.9-5.9)	4.7 ± 0.6 (3.7-6.7)	0.188
6-min walk distance, m	18	340 ± 84 (200-475)	474 ± 144 (110-770)	0.001
SpO_2, %, at end of 6-min walk test	18	81 ± 9 (57-91)	88 ± 8 (62-99)	0.009
SpO_2, %, decrease during 6-min walk test	18	-10 ± 8 (-28-0)	-8 ± 8 (-36;+2)	0.189

FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s; N/A, not available; TLC: total lung capacity; RV: residual volume; DLco: single-breath diffusing capacity of the lungs for carbon monoxide; Kco: single breath transfer factor of the lungs for carbon monoxide; PaO_2: arterial oxygen tension; $PaCO_2$: arterial carbon dioxide tension; SpO_2: peripheral oxygen saturation.

Table 3. Haemodynamic data at the time of pulmonary hypertension diagnosis

Variable	*No*	*Mean ± SD*
Mean PAP, mmHg	20	32 ± 6 (25-51)
Diastolic PAP, mmHg	19	22 ± 5 (12-30)
Systolic PAP, mmHg	19	48 ± 11 (38-84)
Cardiac output, L/min	20	5.4 ± 1.9 (3.1-9.5)
Cardiac index, L/min/m²	20	3.4 ± 1.1 (2.1-5.7)
PVR, dynes.s/cm^{-5}	20	376 ± 184 (118-776)
PVR index, dynes.s/cm^{-5}/m²	20	572 ± 307 (190-1,433)
Right atrial pressure, mmHg	19	7 ± 3 (0-12)
Capillary wedge pressure, mmHg	19	10 ± 3 (4-15)
SvO_2 (%)	12	69 ± 7 (59-80)

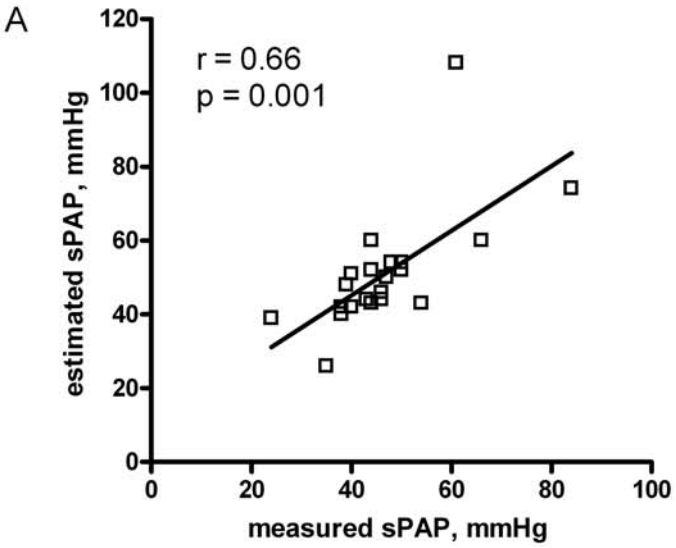
Data are presented as mean ± SD (range). PAP: pulmonary arterial pressure; PVR: pulmonary vascular resistance; SvO_2: venous oxygen saturation.

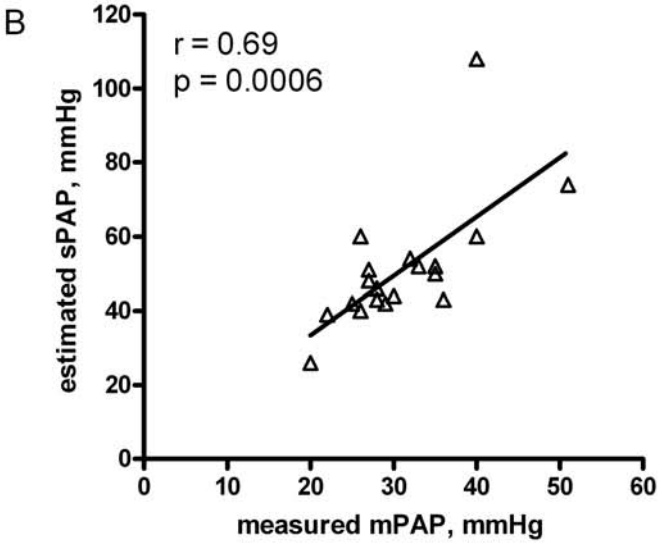
Table 4. Semi-quantitative assessment of LAM/PEComa cells (LC) and their distribution in the diseased lungs of five transplanted patients. HMB-45 staining was available in three patients and unavailable (UA) in two.

Case No	*LC along cyst edges*	*LC along alveolar walls*	*LC along pulmonary arteries / veins*	*LC along lymphatics*	*LC along bronchioles*	*HMB-45+ LC*
#1	+++	++	+	+	+	++
#2	++	++	+	+	+	+
#3	+++	+	++	++	++	++
#4	+++	+	++	++	+	UA
#5	+++	+	+++	+++	+++	UA

Figure legends

Figure 1. Correlation between systolic pulmonary arterial pressure (PAP) estimated at echocardiography and systolic PAP measured at right heart catheterisation (A) or measured mean PAP (B). C: accuracy of estimated systolic PAP compared to systolic PAP as measured by right heart catheterisation in relation to mean PAP.


A
r = 0.66
p = 0.001
estimated sPAP, mmHg
measured sPAP, mmHg
0
20
40
60
80
100
120


B
r = 0.69
p = 0.0006
estimated sPAP, mmHg
measured mPAP, mmHg
0
10
20
30
40
50
60

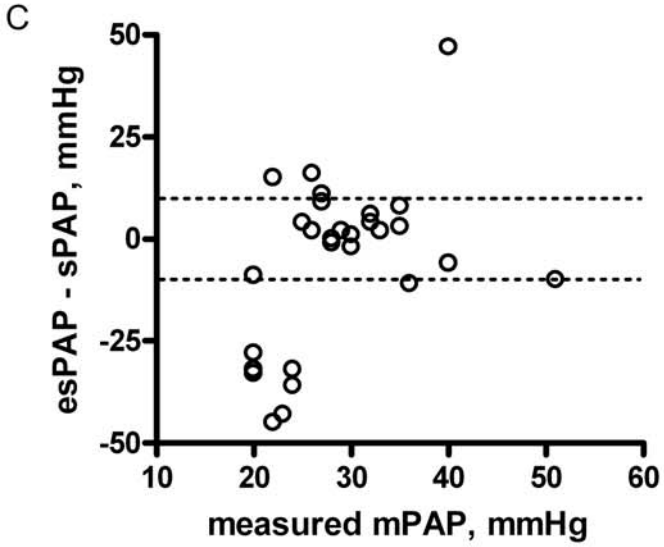

C
esPAP - sPAP, mmHg
measured mPAP, mmHg
50
25
0
-25
-50
10
20
30
40
50
60

Figure 2. Correlation between mean pulmonary arterial pressure (mean PAP) or pulmonary vascular resistance (PVR) and forced expiratory volume in 1 s (FEV_1), single-breath diffusing capacity of the lungs for carbon monoxide (DLco), or single breath transfer factor of the lungs for carbon monoxide (Kco). Regression lines are indicated.

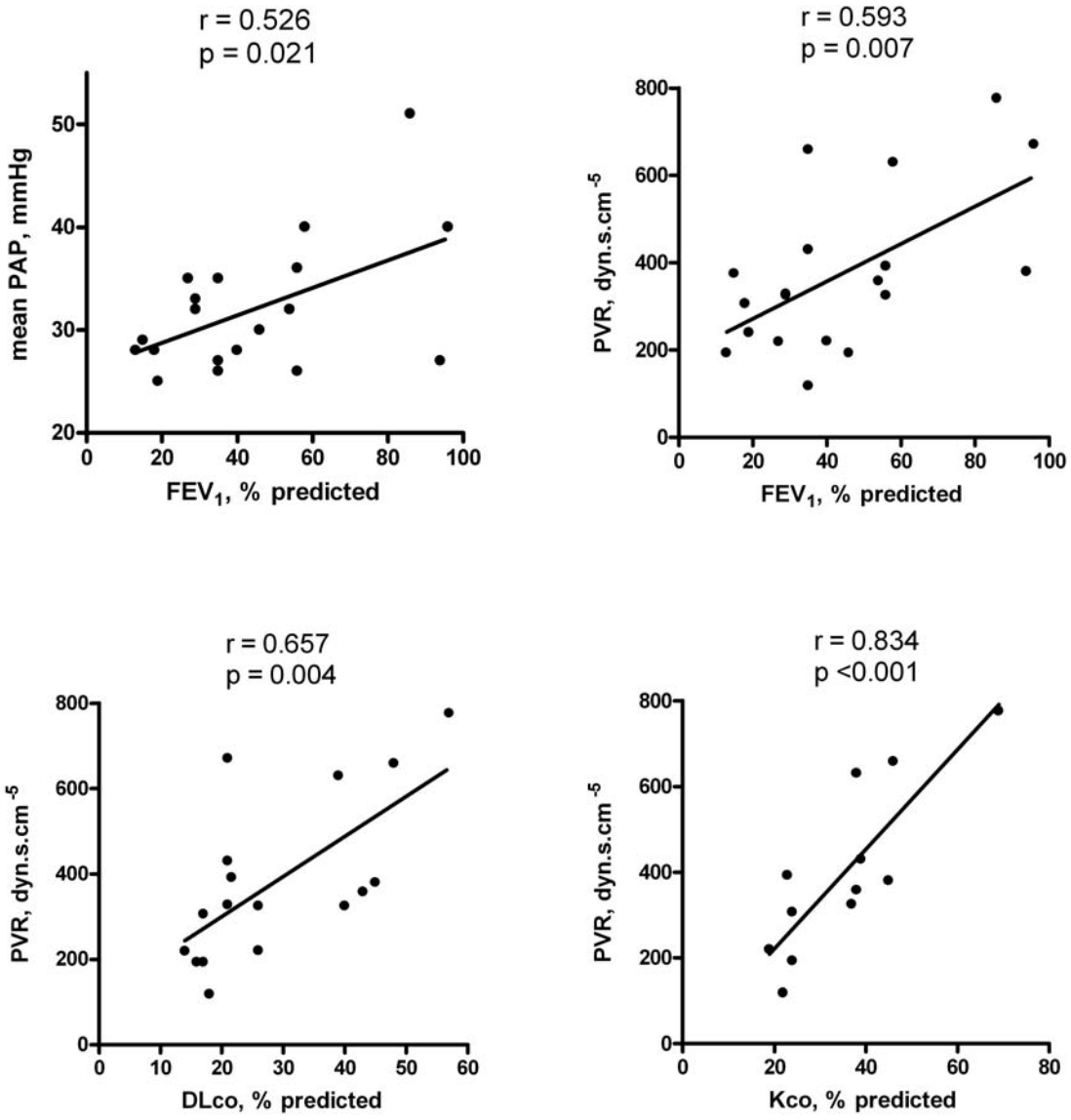


Figure 3. Outcome in 6 patients who received bosentan (open squares) or sildenafil (black squares). PAP: pulmonary arterial pressure; PVR: pulmonary vascular resistance; PaO_2: arterial oxygen tension (on room air); SpO_2: peripheral oxygen saturation (on room air); FEV_1: forced expiratory volume in 1 s; *$p<0.05$ (versus baseline).

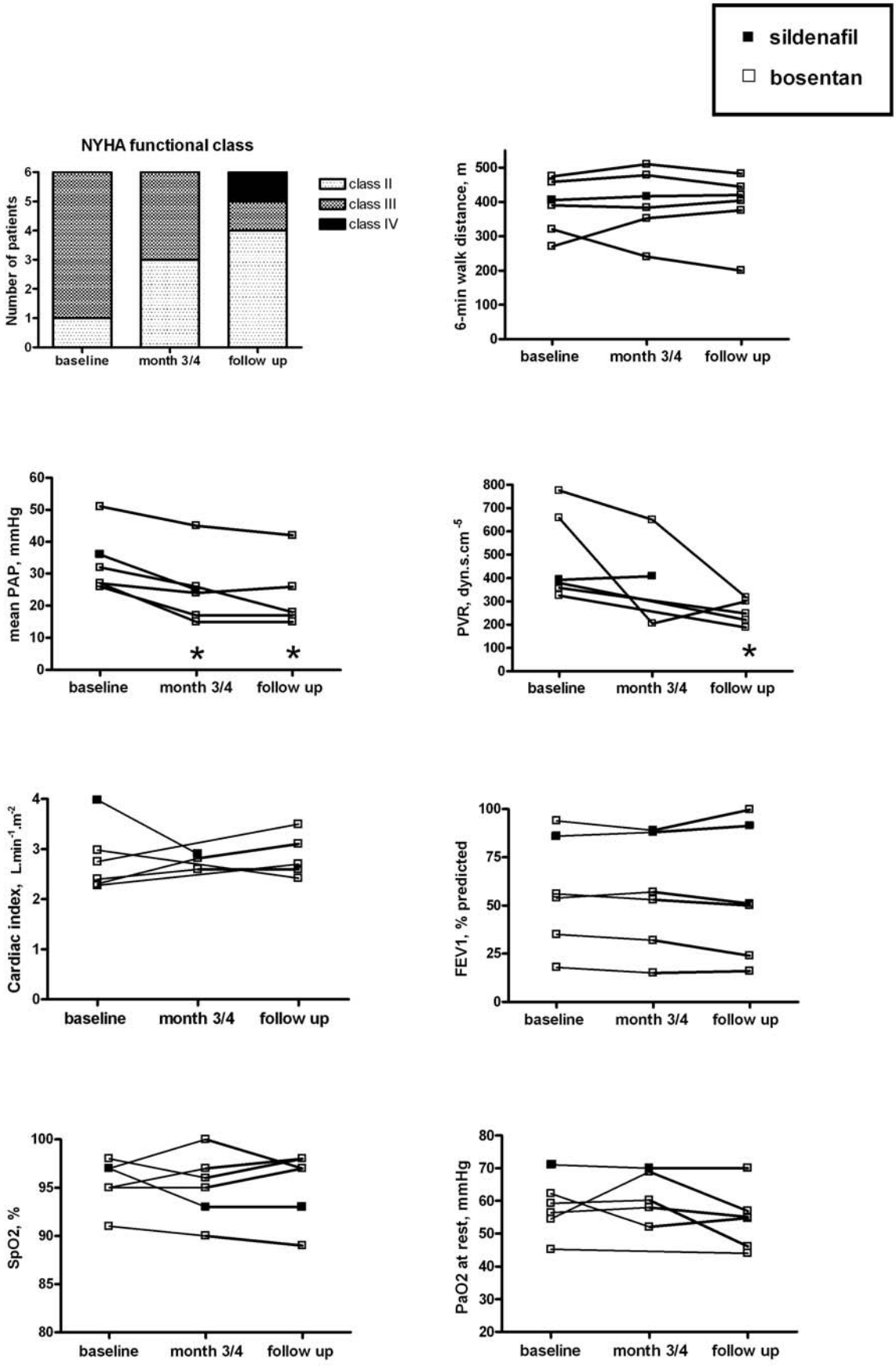
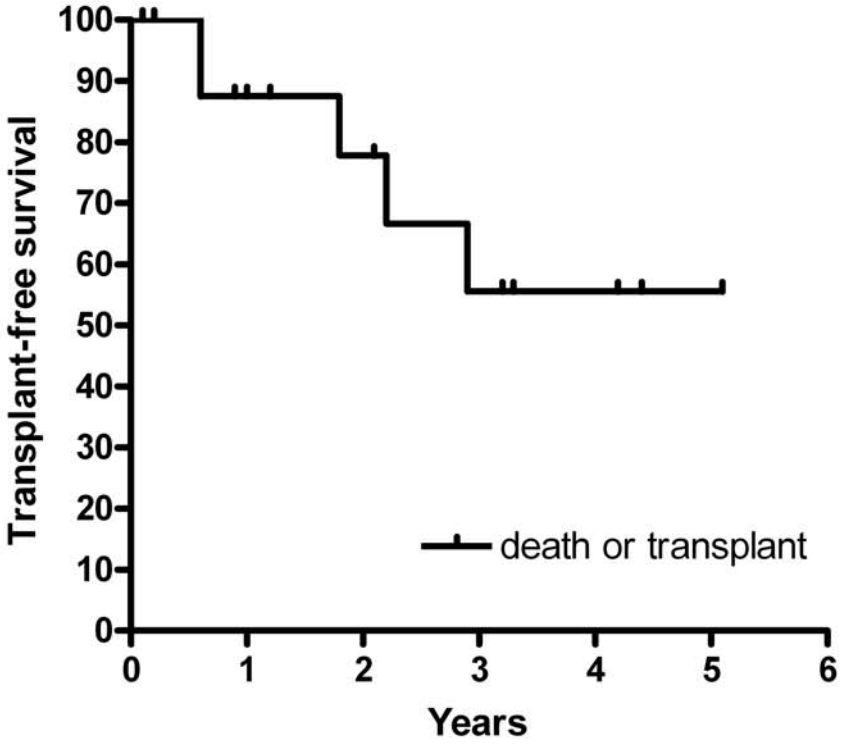
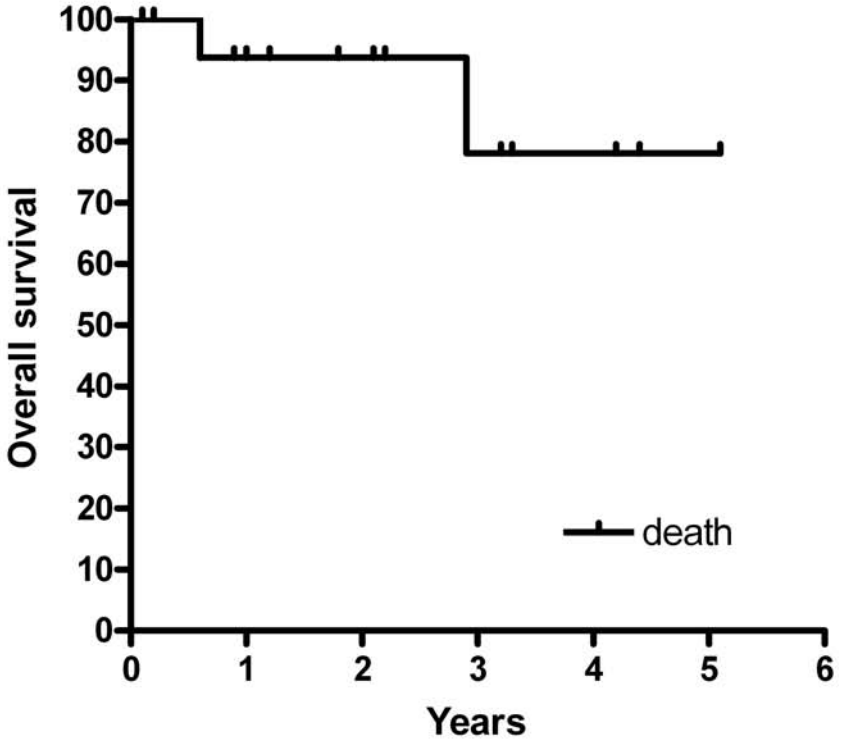

sildenafil
bosentan
NYHA functional class
Number of patients
class II
class III
class IV
baseline
month 3/4
follow up
6-min walk distance, m
mean PAP, mmHg
PVR, dyn.s.cm-5
Cardiac index, L.min-1.m-2
FEV1, % predicted
SpO2, %
PaO2 at rest, mmHg

Figure4. Transplant-free and overall survival in patients with lymphangioleiomyomatosis and pulmonary hypertension.

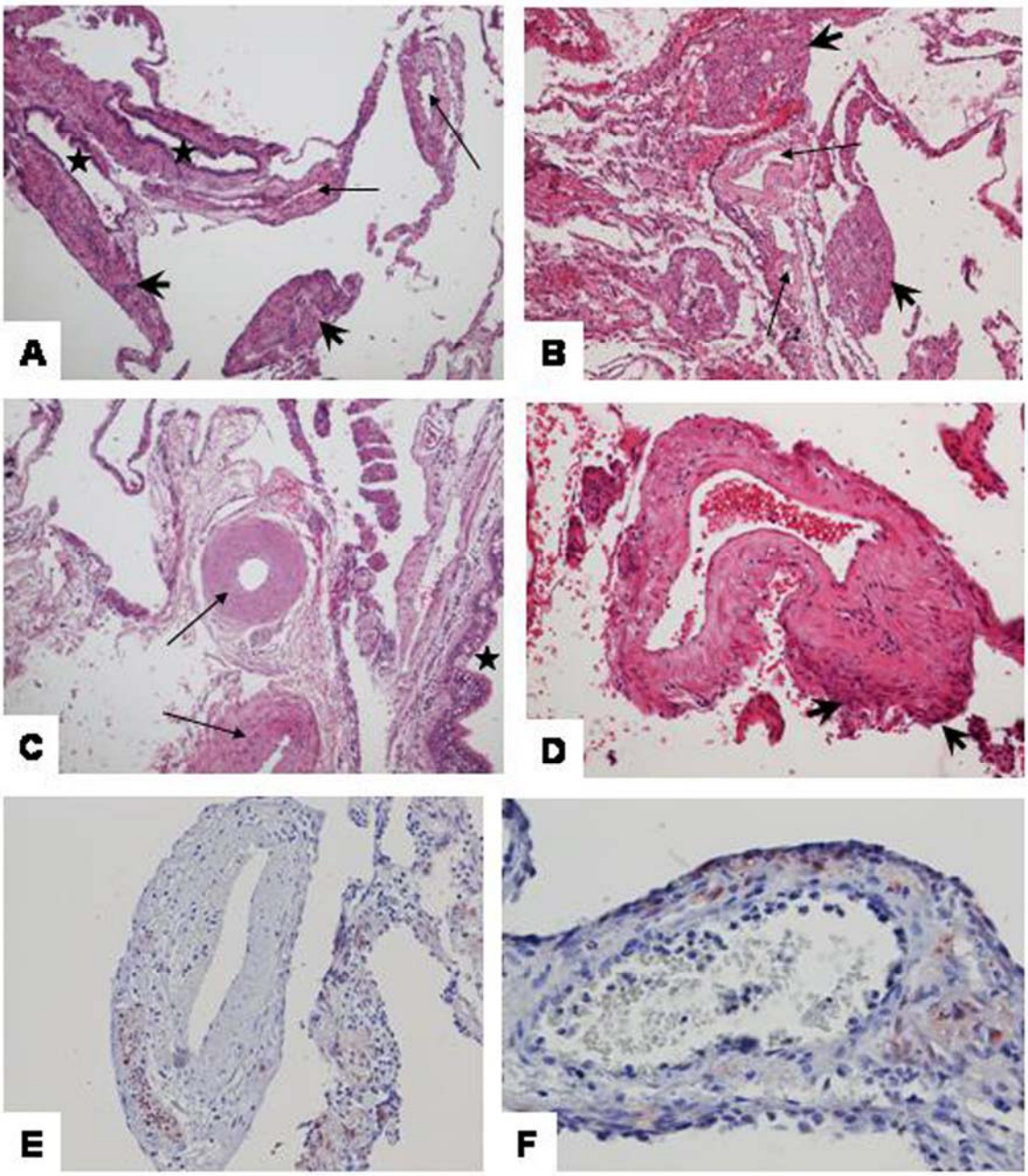


No at risk 20 12 9 6 4 2



No at risk 20 16 9 6 4 2

Figure 5: Pathologic assessment of lungs at the time of transplantation in three patients with pulmonary lymphangioleiomyomatosis. **A-D**: Haematoxylin-eosin staining in one patient. **A:** Two bronchioles (asterisk) and their adjacent pulmonary arteries (arrows). Both structures show remodelling: the bronchioles are directly involved with characteristic LAM/PEComa-cell proliferation (arrowheads), while pulmonary arteries display intimal fibrosis and some minor LAM/PEComa involvement. Magnification x100. **B:** Two pulmonary veins with paucicellular collagen-rich intimal fibrosis (arrows). Note the 2 lymphangioleiomyomatous LAM/PEComa-foci (arrowheads) surrounding the blood vessels. Magnification x100. **C:** Two pulmonary arteries displaying medial hypertrophy and intimal fibrosis (arrows). The adjacent bronchiole is pinpointed with an asterisk. Magnification x100. **D:** A pulmonary artery showing tortuosity and intimal fibrosis. Note involvement of the artery with PEComa cell proliferation at the periphery of the vessel (arrowheads). Magnification x200. **E:** Immunohistochemical staining for HMB45 demonstrating a pulmonary artery with HMB-45+ cells within the remodelled vessel-wall (magnification x200). **F**: Immunohistochemical staining for HMB45 showing a small arteriole or venule presenting muscularisation and involvement of HMB-45+ LAM cells (magnification x400).


A
B
C
D
E
F

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