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TITOLO TESI

EFFECT OF ESTROGEN SUPPRESSION ON LUNG FUNCTION IN PULMONARY LYMPHANGIOLEIOMYOMATOSIS

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Pulmonary lymphangioleiomyomatosis (LAM) is a rare interstitial disease of uncertain etiology that almost exclusively occurs in women, most often of childbearing age. LAM is characterized by proliferation in the bronchioli, pulmonary veins and lymphatic vessels of cysts lined by atypical smooth muscle cells (LAM cells) [1][2]. Progressive occlusion of these structures most often leads to serious clinical manifestations including worsening dyspnea, often accompanied by cough, chest pain, hemoptysis, pneumothorax and chylothorax. Pneumothorax results from cystic rupture, chylothorax results from obstruction of pulmonary lymphatics and hilar lymph nodes by the slowly proliferating LAM cells. Pulmonary function abnormalities in LAM consist of gas exchange abnormalities, characterized by a decreased diffusion capacity of the lung for carbon monoxide (DLCO) and airflow obstruction. In many patients, progressive lung damage occurs and functional impairment is severe, leading to incapacitation and complete disability, requiring long-term oxygen therapy and lung transplantation, or causing death. The rate of progression of disease, however, is variable, and some patients have a long- term course lasting >20 years [3][4].

Prevalence of LAM

LAM occurs sporadically in patients with no evidence of genetic disease and in about one third of women with Tuberous Sclerosis complex (TSC) [5][6][7], an autosomal dominant syndrome characterized by hamartoma-like tumor growths in various organs, cerebral calcifications, seizures, and mental retardation that occurs in 1 of 5,800 live births [8].

Based on the prevalence of TSC, it is estimated that there may be as many as 7,500 TSC patients with LAM in the United States.

Sporadic LAM, however, which is not associated with a TSC phenotype, is a relatively uncommon disease with a prevalence that has been estimated at 2 to 6 per million women [9].

Our group of patients represents one of the largest series with sporadic LAM ever reported in Italy (75 patients at writing). We described the first case of definite diagnosis of LAM in a karyotypically normal man without TSC [10].

Diagnosis of LAM

The diagnosis of LAM should be considered in a woman of any age who presents with recurrent pneumothorax, chylous pleural effusions, and/or ascites, or an unexplained decrease in exercise tolerance. The single most important diagnostic test is a CT scan of the thorax, with high resolution views to facilitate visualization of the cysts. In patients with TSC, the identification of lung

cysts on the CT scan strongly suggests the diagnosis of LAM. The coexistence of angiomyolipomas (AMLs) and lung cysts is also virtually diagnostic of LAM. On the other side patients presenting with apparent sporadic LAM may have TSC. As TSC has a highly variable phenotype and two thirds of cases arise as spontaneous mutations the diagnosis can be overlooked. Patients should undergo a full history and physical examination to exclude TSC. Physical examination should include the skin, retina and nervous system by a physician familiar with the manifestations of TSC [11]. (Fig.1) As TSC comprises manifestations in multiple organ systems, full evaluation for TSC may require more than one specialist. Table 1-2

Major feature	**Minor features**
Facial angiofibromas or forehead plaque	Multiple randomly distributed pits in dental enamel
Non-traumatic ungual or periungual fibroma	Hamartomatous rectal polyps[c]
Hypomelanotic macules (more than three)	Bone cysts[d]
Shagreen patch (connective tissue nevus)	Cerebral white matter migration lines [a,d,e]
Multiple retinal nodular hamartomas	Gingival fibromas
Cortical tuber[a]	Non-renal hamartoma[c]
Subependymal nodule	Retinal achromic patch
Subependymal giant cell astrocytoma	"Confetti" skin lesions
Cardiac rhabdomyoma, single or multiple	Multiple renal cysts[c]
Pulmonary lymphangioleiomyomatosis or renal angiomyolipoma[b]	

Table 1. Revised Diagnostic Criteria for Tuberous Sclerosis Complex [11]

Definite TSC: either 2 major features or 1 major feature and 2 minor features.
Probable TSC: one major feature and one minor feature.
Possible TSC: either 1 major feature or 2 or more minor features.
(a) Co-existent cerebral cortical dysplasia and cerebral white matter migration tracts count as one feature. (b) Co-existent LAM and renal angiomyolipomas count as one feature. (c) Histologic confirmation suggested. (d) Radiographic diagnosis sufficient.

Assessment	**Timing / repeat testing**
Neurodevelopmental testing	At diagnosis / as indicated
Ophthalmic examination	At diagnosis / as indicated
Electroencephalography	If seizures occur / as indicated
Echocardiography	If symptoms occur / as indicated
Renal ultrasonography	At diagnosis / every 1-3 years
Cranial CT / MRI	At diagnosis / children adolescents 1-3 years or as indicated

Table 2. Recommended assessment of new patients with TSC.
TSC Consensus Conference 1998.

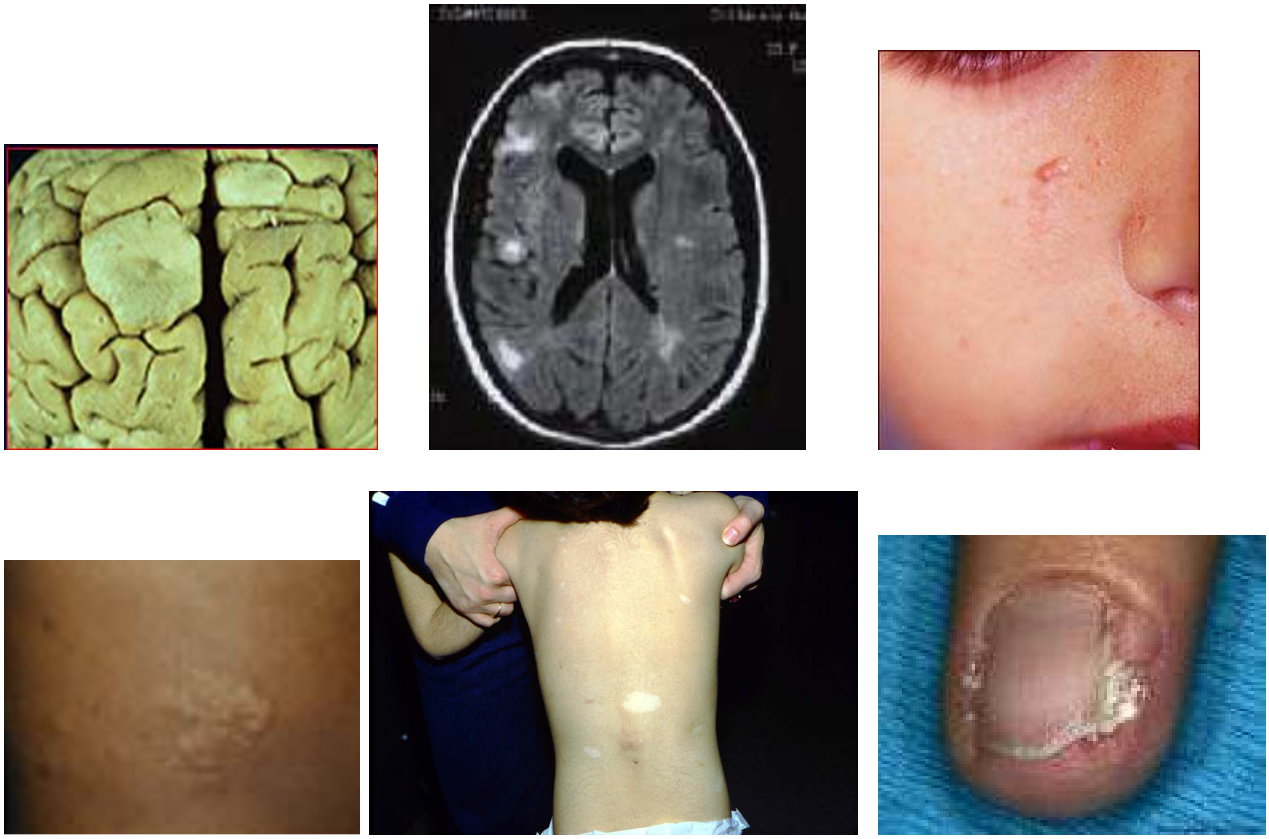


Fig.1 Neurological and cutaneous manifestation of TSC

Lung cysts are the hallmark lesion in LAM and are present in all patients [12][13]. Their appearance, size and contour vary considerably typically ranging from 2-5mm in diameter but occasionally as large as 30mm [14]. Cysts are usually round, distributed evenly throughout the lungs with normal lung parenchyma. Cysts wall thickness ranges from barely perceptible to 2mm in most series but has been described as measuring up to 4mm [14]. (Fig 2 a-b)

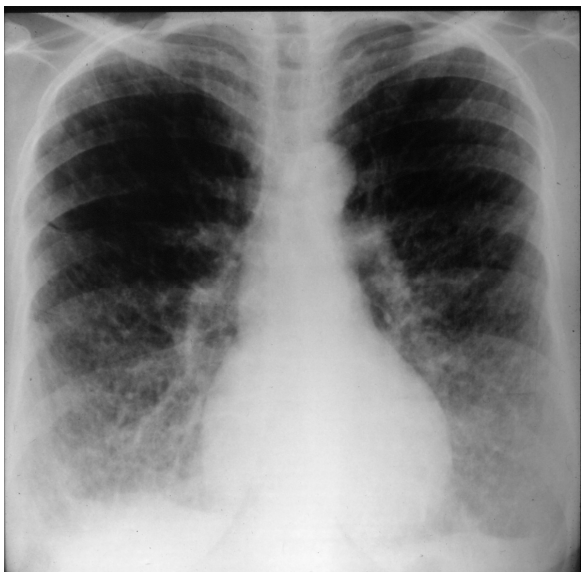


Fig.2a Chest radiography of a patient with LAM

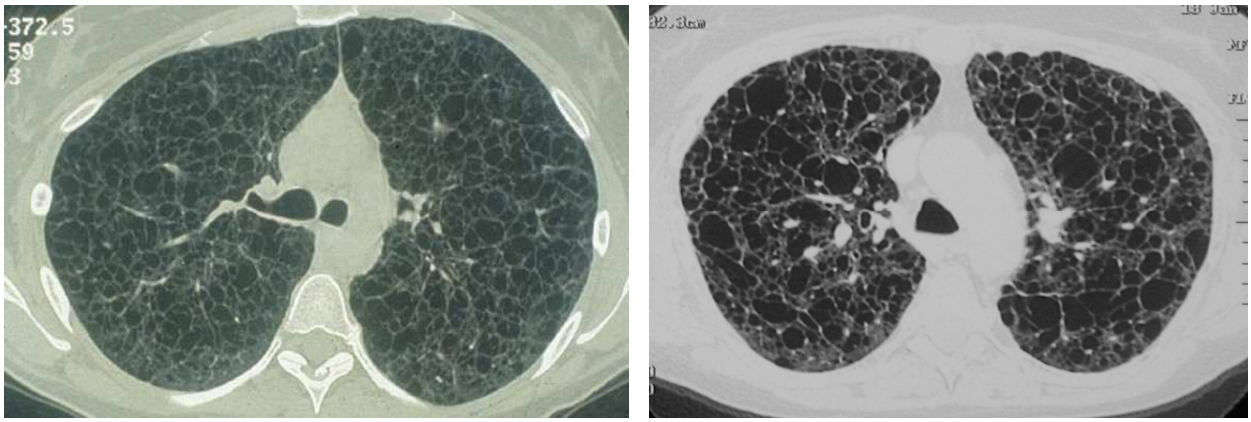


Fig.2b High resolution axial CT image of the chest of a patient with LAM. Note diffuse involvement of the lung parenchyma, with numerous thin-walled cysts.

The presence of lung cysts on a CT scan with no evidence of TSC, AMLs, lymphangioleiomyoma or chylothorax is not diagnostic of LAM. Other uncommon diseases presenting with lung cysts, such as Birt-Hogg-Dubé syndrome, Langerhans cell histiocytosis, and Sjögren's syndrome, should be considered. Under these circumstances, a lung biopsy is recommended [15]. Abdominal CT scanning can be used to detect angiomyolipomas, lymphangioleiomyomas or lymphadenopathy to support the diagnosis, to plan the management of angiomyolipomas, and to follow their evolution. Abnormal abdominopelvic imaging findings in patients with LAM are found in up to two thirds of patients [16]. CT is more sensitive and specific than ultrasound and can detect tumour <1cm in diameter [16]. Magnetic resonance imaging (MRI) with and without fat suppression techniques may be adequate for the diagnosis of fat-containing tumours when iodinated contrast is contraindicated.

Gross specimens from open lung biopsies or autopsies contain cysts throughout the lung parenchyma that are 0,5 to 2,0 cm in size [17]. (Fig.3)

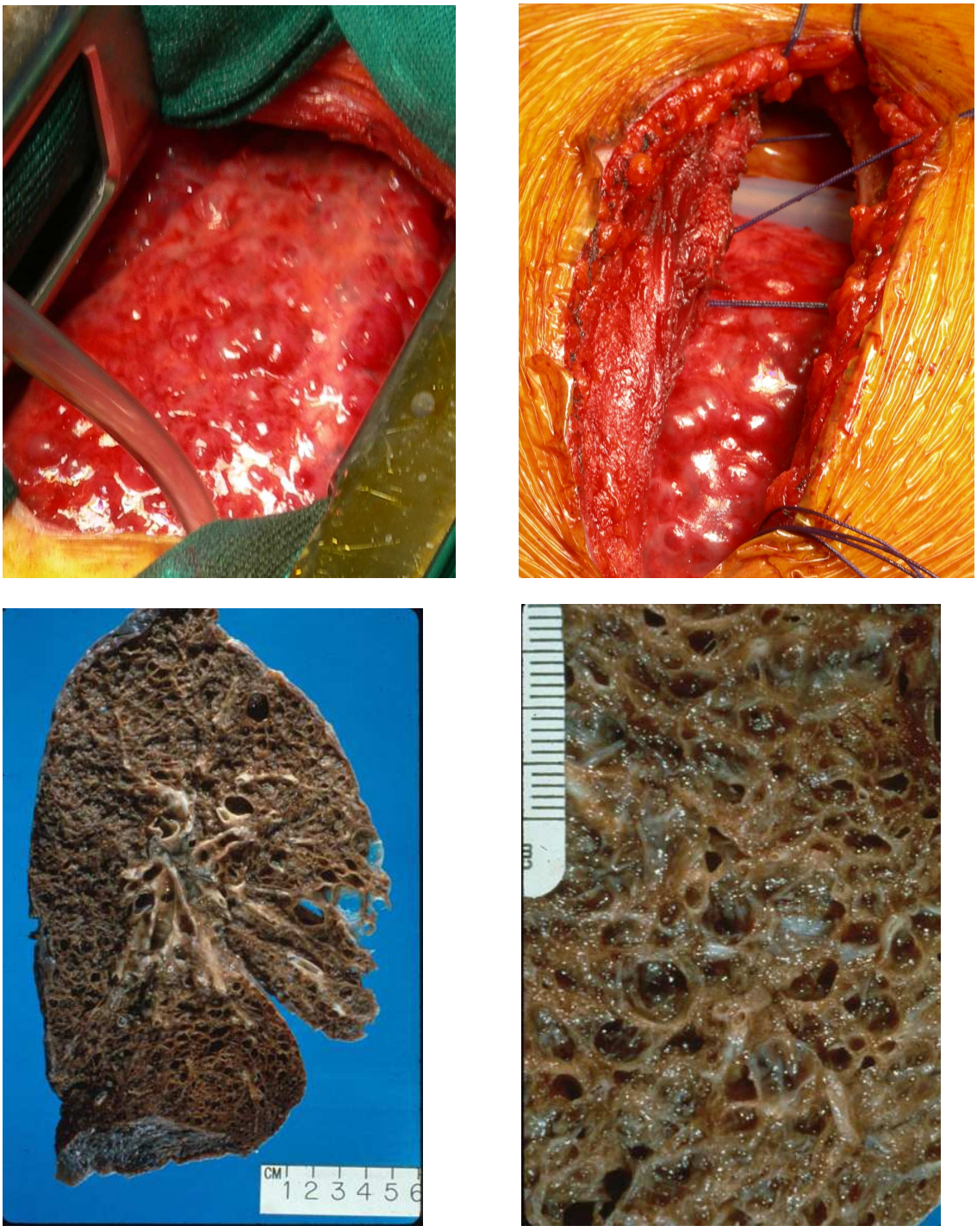


Fig.3 Gross pathology of lung in a patient with severe LAM.

On microscopic examination, discrete foci of abnormal smooth muscle cells abut cystic structures lined with hyperplastic type II pneumocytes. The foci contain centrally located, small, spindle-shaped cells with larger epithelioid cells at the periphery, all of which are arranged in haphazard fashion. In addition to LAM cell foci, patients with TSC may also exhibit MMPH, a feature characterized by ill-defined nodules consisting of alveoli with hyperplastic type II pneumocytes.

Consistent with their classification as smooth muscle cells, LAM cells react with several smooth muscle-specific antibodies and with HMB-45, a monoclonal antibody that reacts with gp100, a melanocyte antigen found in premelanosomes. In support of a role for sex hormones in the pathogenesis of LAM, epithelioid LAM cells reacted strongly with antibodies to estrogen and progesterone receptors [17]. (Fig.4)

Herein, special immunofluorescent stains for smooth muscle α-actin, vimentin, desmin, and HMB45 should be performed especially where morfologic features do not allow a secure diagnosis to be made. The estrogen and progesterone receptor may be an adjunct to diagnosis [11].

Like the radiological pattern, the histological one is also characteristic in LAM, so that transbronchial or surgical lung biopsy and histological studies, integrated with immunohistochemistry, have to be used to confirm the diagnosis. The use of immunohistochemical staining with HMB45, the hallmark of

diagnosis, has improved the usefulness of transbronchial biopsy in the diagnosis of LAM. (Fig.5)

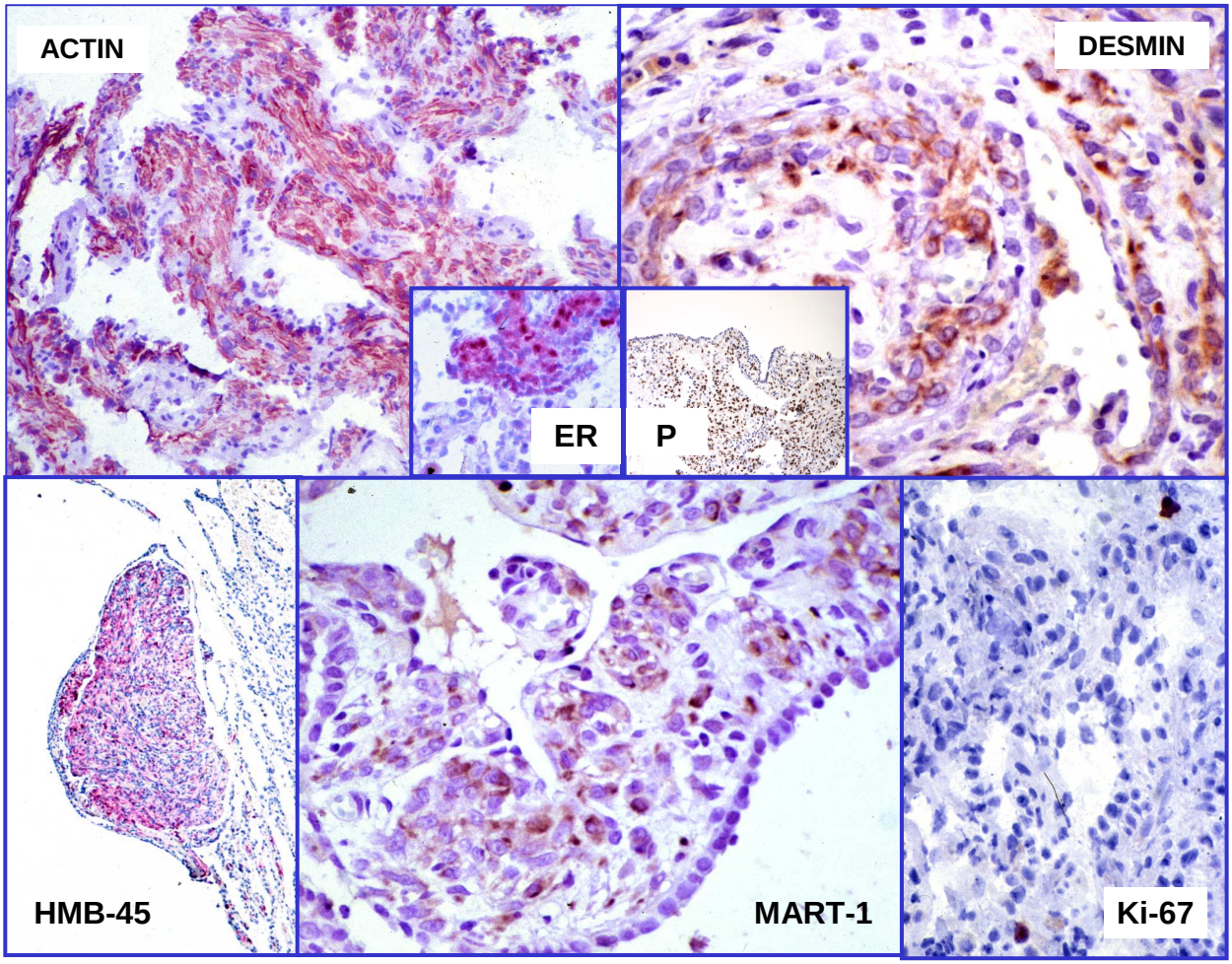
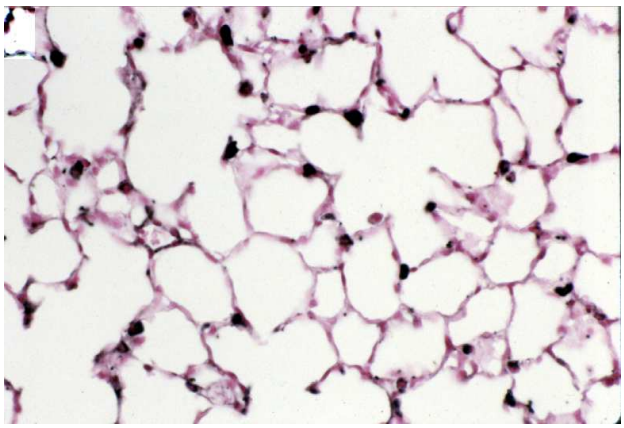


Fig.4 Histological pattern of LAM. From reference 41



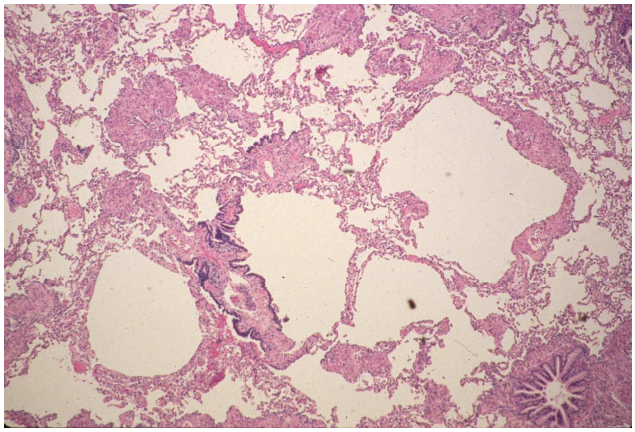
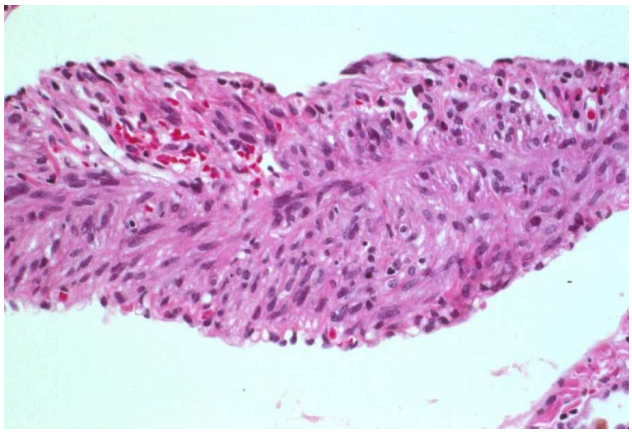
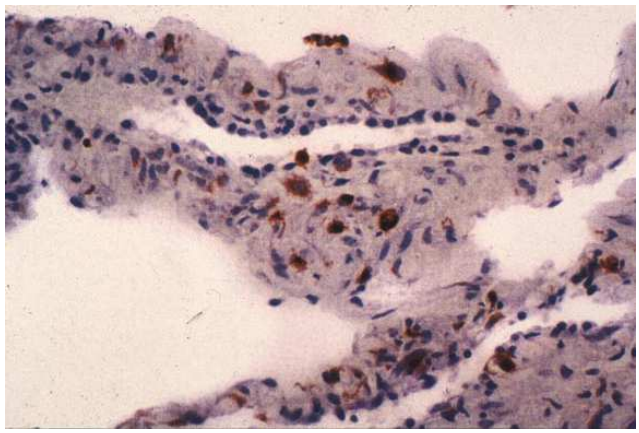


Fig.5 a)Normal lung, low power b)LAM, low power





c)LAM, high power d)LAM, HMB-45 stain

Although most AMLs occur in the absence of LAM (Fig.6), the accidental finding of a kidney mass by abdominal sonogram or CT scan suggesting the presence of an AML mandates a CT scan of the lungs to look for lung cysts[15].

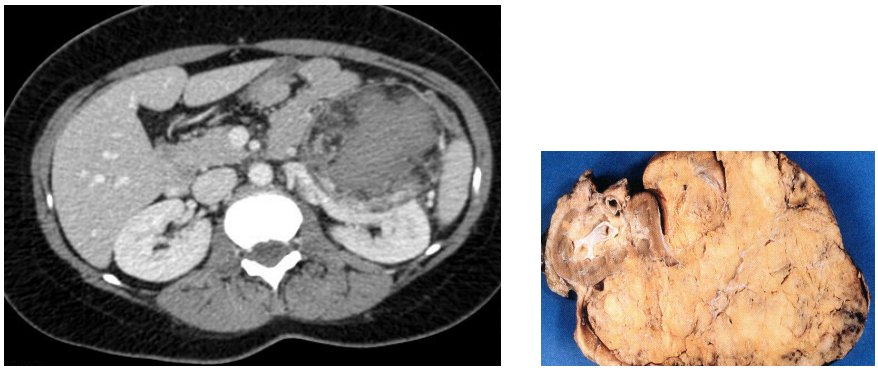


Fig.6 Renal angiomyolipomas in LAM patients

Extrapulmonary LAM can be observed in 3 major locations: the posterior mediastinum, the upper retroperitoneal areas close to the abdominal aorta, and the pelvic cavity. These predominant locations of LAM are related to the anatomic distribution of lymphatic vessels; the morphologic and immunohistochemical heterogeneity of LAMcells in extrapulmonary LAM is similar to that in pulmonary LAM [18].

Matsui described the lesions of lymphangioleiomyomatatosis in patients with masses occurred in the mediastinum, in the retroperitoneum and in the pelvis;

sometimes the diagnosis of pulmonary LAM was established after that of extrapulmonary LAM.

We recently described a young woman presenting with chyluria secondary to the presence of a large retroperitoneal lymphangioleiomyoma [19]. (Fig.7)

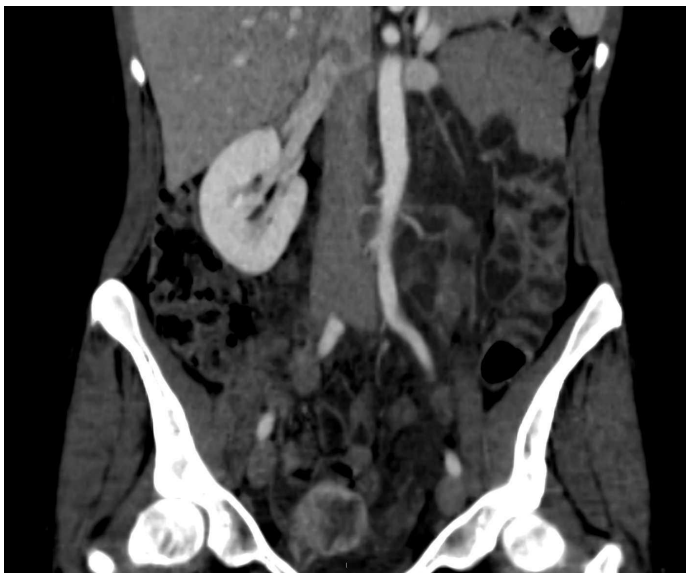


Fig.7 Multi-slice spiral CT, coronal reformatted image showing a retroperitoneal mass located in close proximity with the thoracic duct.

From reference 19

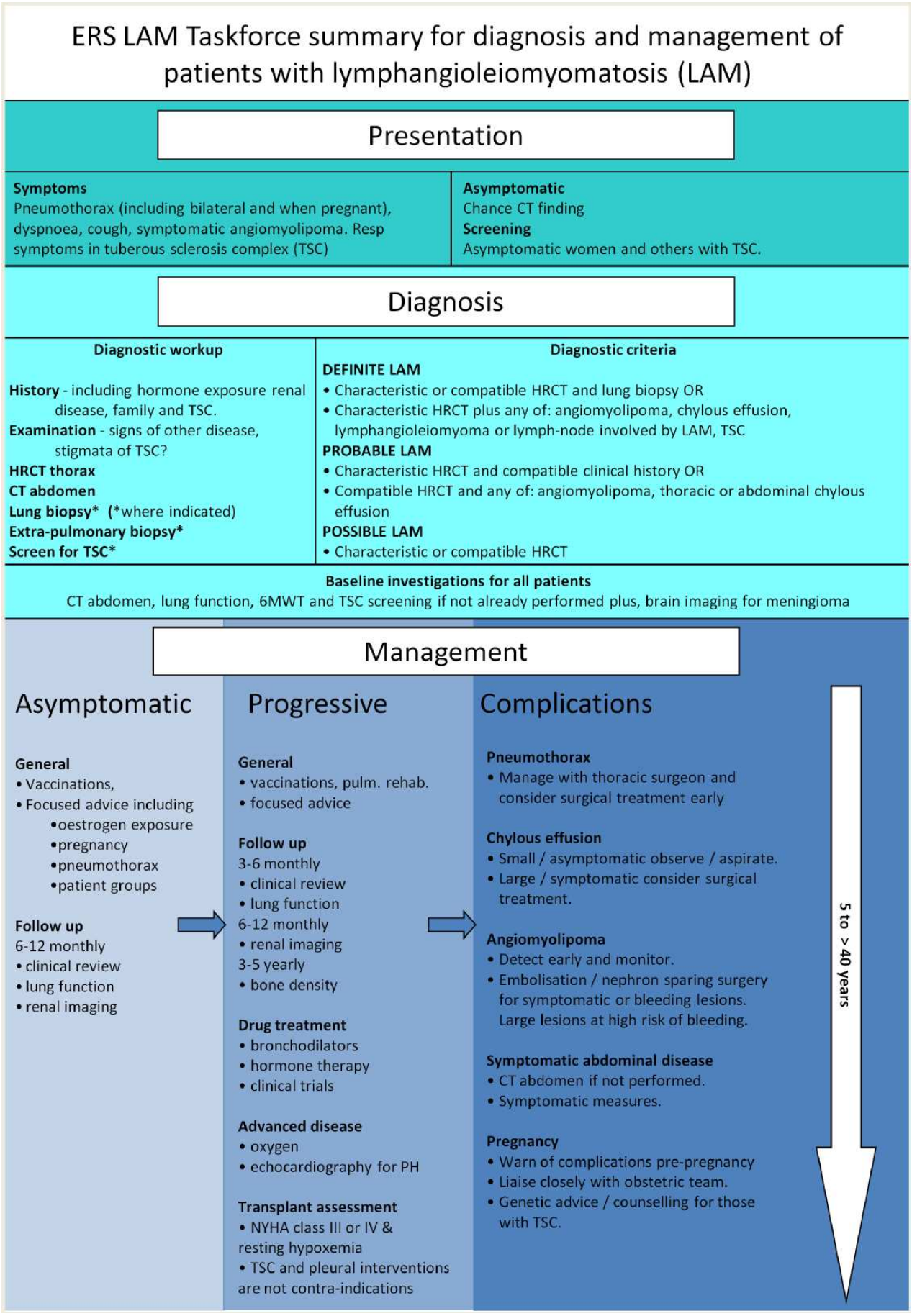


Fig.8 From reference 11

Treatment

As soon as the diagnosis is established, therapy should be initiated, because of its progressive nature. LAM is a rare disease and it explains the lack of data from controlled trials focusing on treatment. Estrogens have been implicated in the pathogenesis of LAM based on a number of convincing evidence, including the identification of ERs in the context of LAM cells [20][21][22], and the occurrence of the disease primarily in women of childbearing age.

There are numerous reports of patients being treated with oophorectomy [23][24][25][26], progesterone [24][25], tamoxifen or other antiestrogen agents[23][24][27], gonadodropin-releasing hormone analogue[28][29][30], or radioablation of the ovaries. In many reports, various combinations of these treatments have been attempted, and responses to such treatments have been variable. The choice between the different approaches is largely dictated by the specific experience of the different Authors.

More recently, patients with end-stage lung disease related to LAM have been treated with lung transplantation [31][32][33]; some reports document LAM recurrence after single-lung transplantation [34][35][36], raising the possibility of some type of circulating mitogen in the pathogenesis of the disease.

Lung transplant shouldn’t be regarded as the first choice treatment of LAM, but the extreme measure only in patients in end-stage disease.

The use of tamoxifen as antiestrogenic therapy was first described in 1982 [37] and since then it has been used, with varying results, in different studies. In some cases it led to a worsening of the symptoms [38]. The weak partial estrogen-agonist activity that tamoxifen is known to have might have caused the stimulation of estrogens receptors in those cases. Moreover, in some models tamoxifen acts as an estrogens antagonist, but may not produce the expected results because acting on atypical receptors of LAM muscular tissue. Because of these contradicting data tamoxifen is now not recommended in the treatment of LAM.

Medroxyprogesterone acetate is used because of its effect as an estrogens antagonist; however, a higher incidence of meningiomas in patients treated with this drug compared to the general population has been recently reported [39], and this observation has raised some concerns about the use of this medication. A case of symptomatic meningioma requiring surgical excision also occurred in one of our patients [40].

As an alternative to medroxyprogesterone it is possible to use letrozole, a pharmacological inhibitor of the enzyme aromatase, in order to inhibit the estrogenic activity in LAM patients.

We suggest a therapeutic opportunity through sex hormonal manipulation: discontinuation of preparations containing estrogens, avoiding pregnancy, eradication of estrogenic activity and induction of menopause. Our data by women with LAM, collected over a period of more than 20 years, led us to the

assumption that hormonal factors play an important role in the pathogenesis of the disease [41]. In fact the timely administration of estrogen suppression allows a dramatic improvement in the prognosis of these patients, leading to higher survival rates than studies appeared before hormonal treatment was introduced. These clinical findings are supported by *in vitro* and *in vivo* data about LAM pathogenesis [22][42].

However, other studies showed that hormonal modulation (using a number of different approaches) failed to provide consistent, reproducible proofs of efficacy [37][43][44][45]. In this regard, a recent report suggested limited efficacy of triptoreline therapy [46]. However, in this study on 11 patients (9 of which had pathologic confirmation of their disease) no data were reported about the expression of estrogen receptors and HMB-45.

LAM is associated with inappropriate activation of mammalian target of rapamicin (mTOR) signaling, which regulates cellular growth and lymphangiogenesis. Sirolimus (also called rapamicin) inhibits mTOR. (Fig.9)

A report including both patients with TSC and sporadic LAM showed that sirolimus monotherapy reduced angiomyolipoma volume of nearly 50%. Conversely, improvements in airflow and gas trapping were limited to patients with LAM [47]. However, the rate of adverse effects was high, and only a small number of patients had renal and pulmonary benefits that persisted after the drug was stopped. Another recent report [48] shows that treatment with sirolimus for 1 year has beneficial effects in patients with LAM, including

the stabilization of FEV1 and improvement in FVC, quality of life, and some functional performance measures. But sirolimus therapy positively affected lung function only during the treatment period and it was associated with a large number of different adverse events.

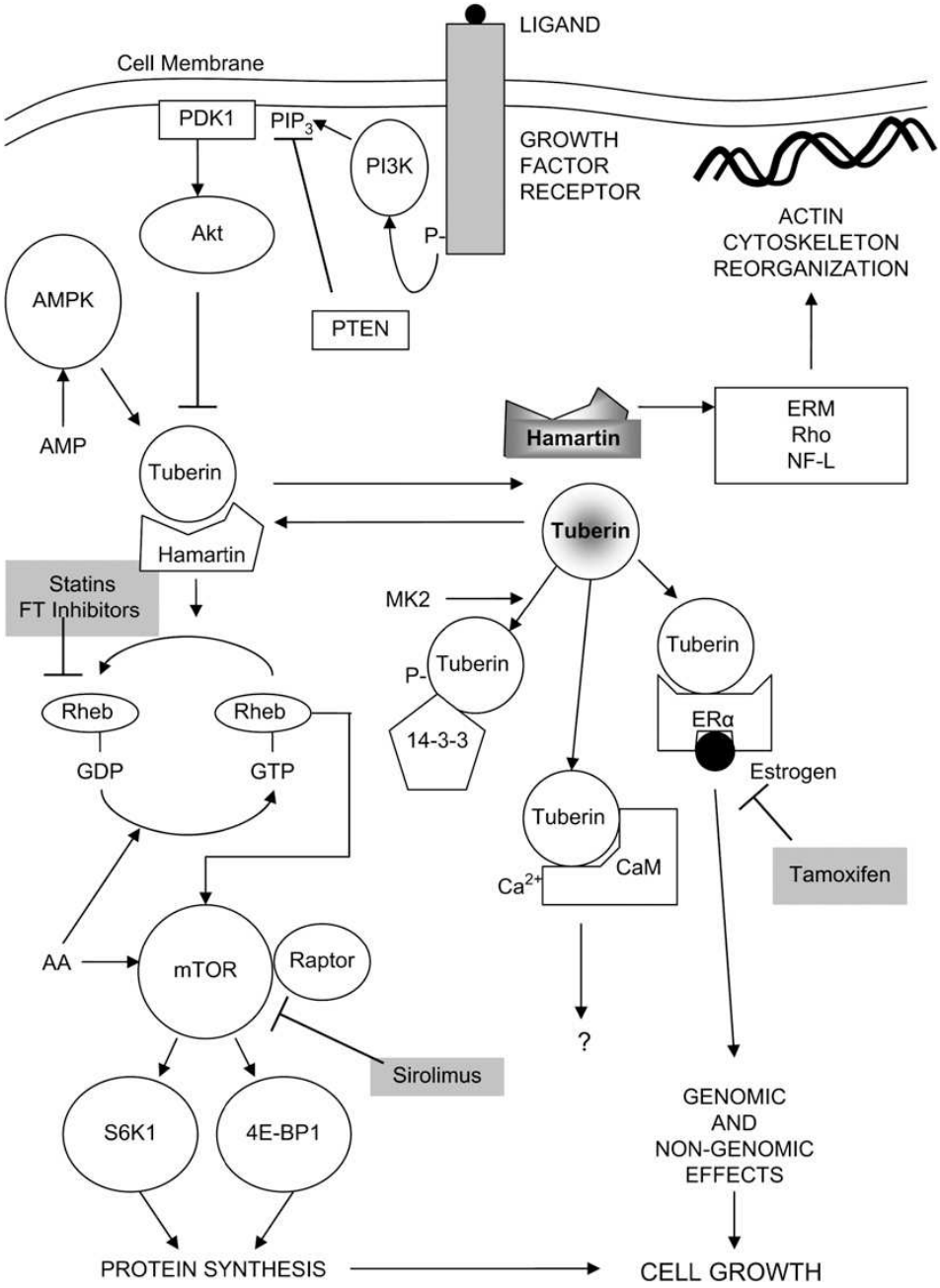


Fig.9 The mTOR pathway is constitutively activated in LAM.

From reference 47

Recently the European Respiratory Society (ERS) guidelines for the diagnosis and management of LAM recommended that hormone treatments should not be used in patients with LAM (Grade: Inconclusive, Quality: low, Benefit: conflicting, Consensus: very good) [11]. On the other side, ERS guidelines also recommended that sirolimus should not be prescribed routinely outside clinical trials and that in renal angiomyolipoma mTOR inhibitors should not be used as first-line therapy (Grade: C, Quality: low, Benefit: small/weak, Consensus: very good) [11].

As LAM is rare, there have been no controlled trials of its management. Therefore, despite recent somewhat promising results, effective treatment for patients with LAM is still lacking and ERS guidelines for LAM don't produce conclusive indications about effective therapeutic strategies.

Management

In common with other pulmonary diseases, patients with LAM should be encouraged to maintain a normal weight and refrain from smoking. The diagnosis of an orphan disease and its consequences often leave patients with a feeling of isolation. Patient groups can help with these issues and other practical matters. Patients with both sporadic and TSC-LAM including those with no or minimal symptoms must be warned of the risk of pneumothorax and

told to seek urgent medical attention in the event of symptoms of pneumothorax.

Indeed LAM is associated with an increased risk of pneumothorax which occurs in ~40% of patients at presentation and 66% of patients during the course of the disease. The estimated rate of recurrence after the first episode in LAM is ~75% [11].

It is likely that pregnancy in LAM is associated with an increased risk of pneumothorax and chylothorax. It is suspected that pregnancy may accelerate the decline in lung function. It is likely that patients with poor baseline lung function are less likely to tolerate a pneumothorax or chylous effusion during pregnancy. There may be an increased risk of bleeding from angiomyolipoma during pregnancy [11].

Anyway to become pregnant is the patients' decision. However all patients, including those with few or no symptoms, should be informed that there is a greater risk of pneumothorax and chylous effusion during pregnancy. Those with recurrent pneumothorax or effusion outside pregnancy and those with poor baseline lung function are at greater risk during pregnancy. It may be appropriate to discourage patients with severe disease from becoming pregnant.

Exogenous oestrogens may promote the progression of pulmonary LAM in at least some cases [11] therefore females with LAM should avoid oestrogen

containing treatments including the combined oral contraceptive pill and hormone replacement therapy.

Reports of pneumothorax occurring in flight have resulted in females with LAM being advised not to travel by air. Patients with sporadic and TSC-LAM with well preserved lung function do not need to take specific precautions or avoid air travel. Those with advanced disease should be evaluated for the need for oxygen during flight to prevent hypoxaemia and as they are less likely to tolerate pneumothorax.

Patients with a known untreated pneumothorax, or a pneumothorax treated within the previous month, should not travel by air.

LAM is associated with reduced bone mineral density in a significant proportion of patients. Patients with LAM, especially those post-menopause, should undergo periodic evaluation of bone mineral density. Those with osteoporosis should be treated the same as other patients with osteoporosis. In view of the rapid deterioration in bone mineral density observed after lung transplantation, aggressive therapy for osteoporosis should be initiated early in LAM patients with severe lung disease and osteopenia at any bone site. In addition to pharmacological therapy, weight-bearing exercise and strength training should be encouraged [11].

One quarter of patients respond to inhaled bronchodilators according to standard objective criteria and more may obtain some clinical benefit. Patients

who respond to bronchodilators tend to have airflow obstruction and have a greater rate of decline in FEV_1. Although bronchiolar inflammation is seen in some patients the efficacy of inhaled corticosteroids in LAM has not been assessed.

Complications and co-morbidities

Pneumothorax occurs in the majority of patients, results in significant hospital stays, and is frequently recurrent. Conservative treatments are associated with higher rates of recurrence than pleurodesis *via* chest tube or appropriate surgical interventions. Lung transplantation in patients with previous thoracic surgery or pleural procedures may be associated with increased technical difficulty and an increased risk or perioperative bleeding.

Chylothorax in LAM may be almost asymptomatic or cause marked dyspnoea. The interventions used for the management of chylothorax in LAM should be appropriate for the size and clinical impact of the effusion, comorbid factors and local expertise. A fat-free diet (with or without oral supplementation of medium-sized triglycerides) or fat-free total parenteral nutrition has been used occasionally to minimise the volume of chyle formation. For small effusions, observation or thoracocentesis may be sufficient [11].

Finally patients should be advised to seek urgent medical attention in the presence of symptoms of bleeding angiomyolipoma.

Although experience is based on data from case series, both embolisation and nephron sparing surgery have been performed safely without compromising renal function both in elective cases and acute renal haemorrhage, including during pregnancy. Embolisation can be performed for active bleeding, is less invasive and does not require general anaesthesia but may need to be repeated. Embolisation may be favoured over surgery in patients with bleeding angiomyolipoma, although no trials have compared the two strategies. The intervention used depends upon technical factors associated with the tumour and local expertise. When bleeding is not present, nephron sparing surgery may be preferred when a malignant lesion is suspected. Surgery with intra-operative frozen section biopsy may be considered with the option to perform conservative or radical surgery; the risk of a false diagnosis of carcinoma must be borne in mind [11]. Decisions are best made electively after screening for angiomyolipoma or in response to symptoms rather than in the setting of an acute haemorrhage, early detection of angiomyolipoma is therefore important. The risk of bleeding is linked to angiomyolipoma size and is clinically appreciable in tumours ≥4 cm in diameter and where aneurysms are ≥5 mm. Therefore asymptomatic renal angiomyolipoma <4 cm should not be treated, but should be followed by yearly ultrasound unless symptoms occur. Renal angiomyolipomas >4 cm or with renal aneurysms >5 mm in diameter are at an increased risk of bleeding, and should be followed by ultrasound

imaging twice yearly to evaluate growth. Treatment by embolisation or nephron sparing surgery should be considered.

LAM accounts for 1.1% of lung transplant recipients [49]. LAM compares favourably to patients transplanted for other indications [31]. In a recent survey the actuarial survival of lung transplantation for LAM was 86% at 1 yr, 76% at 3 yrs, and 65% at 5 yrs [50].

Due to the small number of patients treated and variable rates of decrease in lung function, firm recommendations are difficult to make. In a recent survey of patients undergoing transplantation for LAM, most had severe airway obstruction and were transplanted with a mean FEV_1 of ~25% and $D_{L,CO}$ of 27% predicted [50]. Patients should be considered for lung transplantation when they reach New York Heart Association (NYHA) functional class III or IV with severe impairment in lung function and exercise capacity ($V'_{O2,max}$ <50% pred, hypoxaemia at rest). Transplantation in patients >65 yrs of age may only be considered exceptionally.

Both single, and more commonly, bilateral lung transplantations have been performed for LAM. Although a bilateral lung transplant is associated with better post-transplant lung function and a reduction in LAM-related complications there is no difference in survival between the two procedures[50]. Therefore the choice of a single, or bilateral lung transplant in LAM should be determined by surgical technical factors and organ availability.

Patients with TSC have received successful lung transplantation for severe LAM [51][52]. Although no TSC specific problems have been identified, patients with TSC-LAM are likely to have more comorbidities than those with sporadic LAM. The impact of these processes and their treatment require careful pre-transplant evaluation.

Prognosis and Natural history

Predicting the prognosis of individual patients is difficult. Histological extent of disease and some lung function variables have been found to be predictive at diagnosis, of either survival or more rapid deterioration of lung function. LAM is less severe in TSC-LAM than sporadic LAM although this may reflect ascertainment bias. None of these predictors have been validated prospectively and moreover, some of these variables may only reflect more advanced disease at the time of diagnosis resulting in shorter survival. DLCO and FEV1 are likely to be the best current indicators of disease progression and survival. Patients with TSC-LAM may have a more indolent course than those with sporadic LAM.

Forced expiratory volume in 1s (FEV1) and transfer factor of the lung for carbone monoxide (DLCO) correlate with CT and histological abnormalities in LAM and change over time as the disease progresses. DLCO, abnormal in more patients than FEV1, may be a more sensitive indicator of early disease.

Spirometry, bronchodilator testing and DLCO should be performed in the initial evaluation of patients with LAM (including TSC-LAM); then FEV 1 and DLCO should be performed to assess disease progression and response to treatment. Lung function tests should be repeated every 3-6 months in patient with progressive disease and every 6-12 months in those with more stable disease, as determined by a period of observation of 1year [11].

The natural history of the disease has been dramatically changed by hormonal treatment. Up to the 80s the survival rate was 20% at 10 years from the time of the onset, while in more recent studies the survival has risen to 70% at 10 years and 71% at 15 years [49]]53][54].

In our group of patients the survival was 97% at 5 years, 90% at 10 years and 71% at 25 years [41].

STUDY

INTRODUCTION

Lymphangioleiomyomatosis (LAM) is a rare progressive cystic lung disease of uncertain etiology that affects young women. The most common clinical manifestation is the insidious onset of exertional dyspnoea; patients may also experience a non productive cough. Other common features include spontaneous pneumothorax, which results from cystic rupture, and chylothorax, which results from obstruction of pulmonary lymphatics and hilar lymph nodes by the slowly proliferating LAM cells. Less frequently, haemoptysis or chyloptysis may occur [55]. Death usually occurs from respiratory failure and it is a common opinion that there is no proven treatment.

LAM is a destructive, eventually fatal lung disease, which affects women of childbearing age at the time of diagnosis [56][57]. Pregnancy and use of oral contraception are associated with a higher frequency of exacerbations and a more aggressive disease course [58][59][60]. LAM can also be found in post menopausal women taking hormone replacement therapy. In support of a role of sex hormones in the pathogenesis of LAM biochemical and immunohistochemical techniques revealed expression of estrogen receptors (ER) and progesterone receptors in cells of LAM lesions [61]. Based on these clinical and biochemical findings, estrogens were hypothesized to invoke or, at least, contribute in a principal manner to the progression of LAM.

Treatments for LAM have consisted mostly of oophorectomy and hormonal manipulations with progesterone or other agents. The efficacy of gonadotrophin-releasing hormone (Gn-RH) agonists and analogues in the treatment of LAM has also been the subject of several reports with conflicting results [41] [55]. Harari et al, in a prospective analysis of lung function decline in 11 patients with LAM, found that treatment with triptorelin did not affect disease progression [61].

We analysed the lung function in 21 patients with LAM treated with estrogen suppression obtained with oophorectomy alone, triptorelin alone, or triptorelin followed by oophorectomy.

METHODS

We reported the analysis of the response to estrogen suppression in 21 LAM patients.

To quantify the lung function, we selected FEV1, FVC and DLCO, because these tests correlate well with disease severity defined by CT-scans and LAM histology scores; they also reflects the airway and alveolar involvement by Lymphangioleiomyomatosis. We also examined gas exchange at rest.

We evaluated hormonal assays (FSH, LH, 17 β estradiol), pulmonary function tests and arterial blood gases at baseline and after 12, 24 and 36 months after initiating hormonal manipulation. Flow rates and single-breath DLCO were

measured according to the American Thoracic Society recommendations [62][63].

All spirometric data reported in this work are from pre-bronchodilator studies. Room air, resting arterial blood samples were drawn from the radial artery and tests were run immediately.

The yearly rate of decline in lung function was calculated from a linear regression using FEV1 and DLCO as the response variables and the time of each test as the independent variable, considering the first test as time zero. The Student's t-test was employed to compare data sets. All reported p-values are two-sided. All data are shown as means ± SD.

RESULTS

Of the 21 patients, sixteen had a histological proven diagnosis of LAM (13 surgical biopsy and 3 transbronchial biopsy) and five patients exhibited clinical and radiographic features consistent with LAM. Three patients were ex-smokers, two current smokers; the remaining patients were non-smokers.

One patient had LAM in association with Tuberous Sclerosis Complex. Of the 21 patients, one was a male [10].

Estrogens suppression was obtained with oophorectomy (13 patients), triptorelin (6 patients), or triptorelin followed by oophorectomy (2 patients). Gonadal suppression was achieved in all patients.

Twelve patients experienced a decline in FEV1, nine patients showed improvement in FEV1. The mean rate of decline in FEV1 was 103mL±549mL after 3yrs treatment (Figure A). Indeed, we observed that the decline in FEV1 was lower in ten of the twenty-one patients who had milder disease (initial FEV1>80%) than in the remaining patients (initial FEV1 <80%): 52mL vs 158mL. It is possible that the milder disease subgroup had a more benign course.

Ten patients experienced a decline in FVC, eleven patients showed improvement in FVC. The mean rate of improvement in FVC was 45mL±648mL after 3yrs treatment (Figure B). Changes in FEV1 and FVC are not statistically significant.

DLCO test was available only for thirteen of twenty-one patients; nine of them had a decline in function, four showed improvement. The mean rate of decline in DLCO was 2mL/min/mmHg and this value is statistically relevant (p=0,01). (Figure C)

Eight patients had an improvement of PaO2 after three years of treatment; nine patients showed a decline in PaO2; four patients had gas exchange stability. We found that only the mean rate of improvement in PaO2 at the first year was statistically significant (p= 0.04).

DISCUSSION

The goal of this study was to evaluate the effect of hormonal manipulation on lung function in patients with LAM (Table A). The mean yearly rate of decline in FEV1 and DLCO was lower than those observed by Johnson and Tatters-field [64], Taveira-Da Silva et al. [65] and Harari et al. [61] (Table B).

The PaO2 value on the third year of the study was higher than at baseline, but it was significantly higher only on the first year of the treatment (p=0,04). Harari et al. [61] instead found a significant decline in PaO2 on the third year such as at the first year of the study.

It should be noted that the patients of the current report had a higher initial lung function than the subjects of historical controls. In our group we observed a lower decline in FEV1 and DLCO, and an improvement of FVC and PaO2.

It is possible that subjects with a mild disease, because of a diagnosis in an early stage, can have a more benign course when rapidly treated with estrogen suppression.

The current study shows that the suppression of ovarian function may affect disease progression and it is especially true for LAM patients with a lightly–moderate disease. So we can conclude that it’s extremely important to obtain an early diagnosis of LAM and to start the appropriate treatment as soon as possible. However because this was a retrospective study and the population was heterogeneous (including patients of all ages and grades of disease), our

findings have to be interpreted with caution. Of note, the encouraging results of this analysis highlight the role of the hormonal manipulation as a main stay in the treatment of LAM.

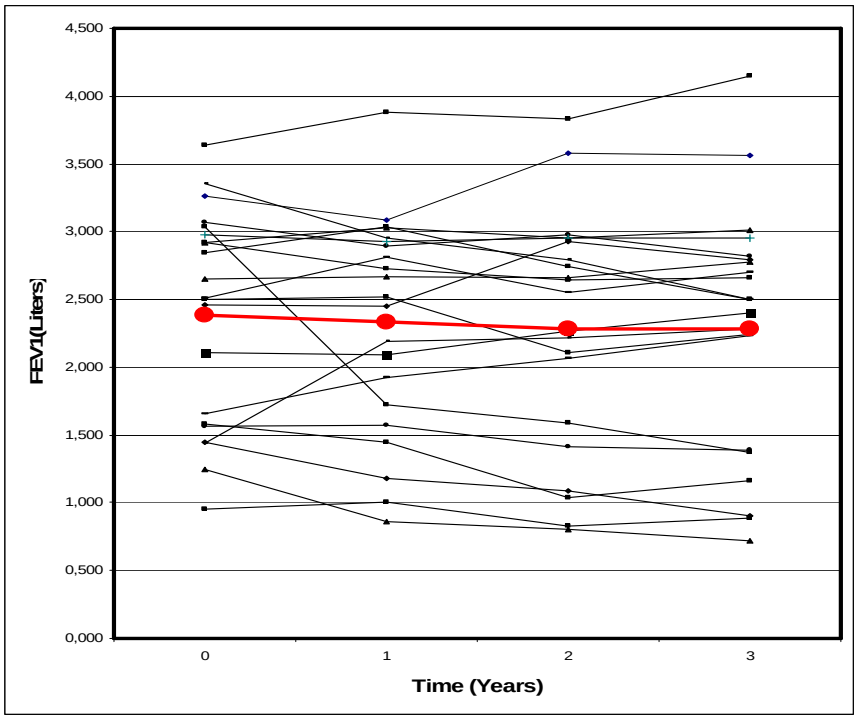


Fig.A Changes in FEV1 in twenty-one patients with LAM who were treated with hormonal manipulation.

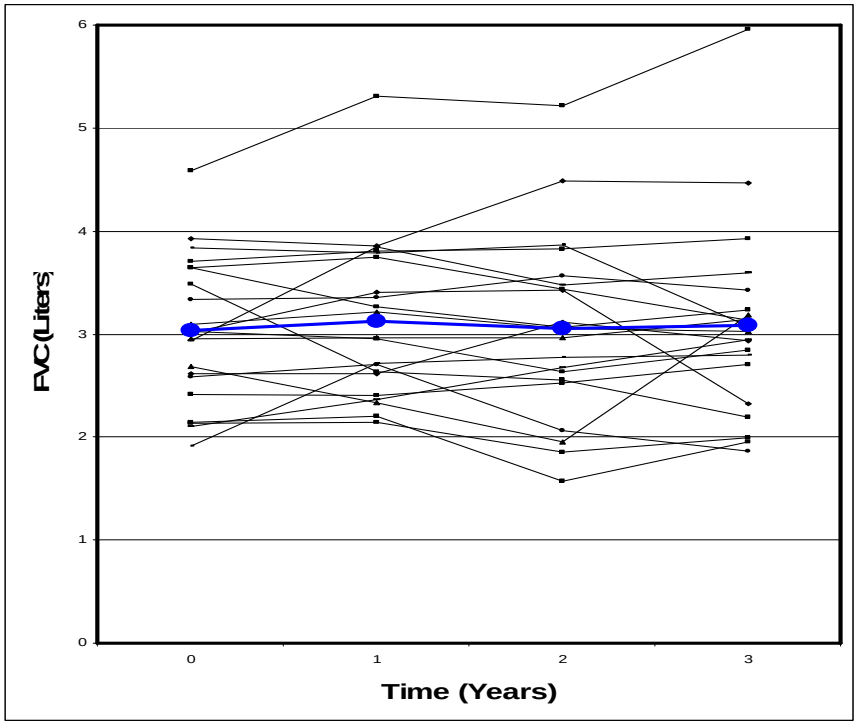


Fig.B Changes in FVC in twenty-one patients with LAM who were treated with hormonal manipulation.

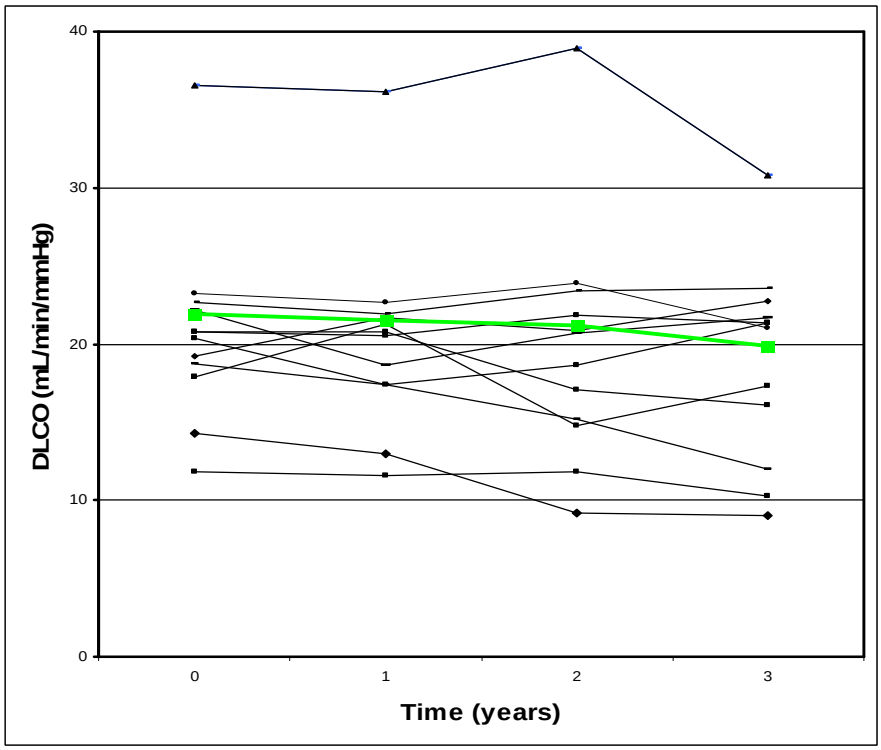


Fig.C Changes in DLCO in thirteen patients with LAM who were treated with hormonal manipulation.

Table A. Pulmonary function before and during estrogen suppression in twenty-one patients with LAM.

		INITIAL	1YEAR	2YEAR	3YEAR
FEV1	(liters)	2.38 ±0.7	2.33 ±0.8	2.28±0.87	2.28±0.9
FVC	(liters)	3.03±0.7	3.12±0.7	3.05±0.8	3.08±0.9
DLCO	(mL/min/mmHg)	21.94±7.2	21.48±7.3	21.18±9.0	19.84±6.85
PaO2	(mmHg)	84.9±14	89.0±15.5	88.7±15.6	85.2±13.9

Table B. Comparison between rates of lung function decline in study subjects and historical control subjects

Number of patients	Change FEV1/year mL	Change DLCO/year mL/min/mmHg	Years
32 †	-108±101	-0.905±1.54	3-3.5
275 ‡	-75±17	-0.69±1.2	4
10 §	-156±184	-1.3±0.4	1-3
21 ⊾	-34±183	-0.69± 1.08	3

† From reference 64; ‡ From reference 65; § From reference 61; ⊾ From the current study

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