



## Review Article

## Lymphangioleiomyomatosis

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## ABSTRACT

Lymphangioleiomyomatosis (LAM) is a rare disease that mainly affects women, particularly those of fertile age. Its presentation is sporadic or associated with tuberous sclerosis complex. It is characterized by an abnormal proliferation of immature smooth muscle cells (LAM cells), which grow aberrantly in the airway, parenchyma, lymph nodes and pulmonary blood vessels and can gradually lead to respiratory failure. It affects several systems, affecting the lymphatic ganglia and causing abdominal tumors. Given its very low prevalence, difficulty in establishing early diagnosis, absence of curative treatment and the difficulty in obtaining information, LAM is placed under the heading of the so-called Rare Diseases. There is a growing interest in the study of this disease which has led to the creation of patient registers and an exponential growth in LAM research, both at a clinical and cellular level.

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## Linfangioleiomiomatosis

## RESUMEN

La linfangioleiomiomatosis (LAM) es una enfermedad rara que afecta predominantemente a la mujer, sobre todo en edad fértil. Se presenta de forma esporádica o bien asociada al complejo de esclerosis tuberosa. Se caracteriza por una proliferación anormal de células musculares lisas inmaduras (células LAM), que crecen de manera aberrante en la vía aérea, parénquima, linfáticos y vasos sanguíneos pulmonares, lo que determina una evolución progresiva hacia la insuficiencia respiratoria. Tiene carácter multisistémico, afectando a ganglios linfáticos y produciendo tumores abdominales. Dadas su escasa prevalencia, la dificultad de establecer un diagnóstico precoz, la ausencia de un tratamiento curativo y la dificultad de obtener información encuadran a la LAM dentro del capítulo de las denominadas Enfermedades Raras. Existe un creciente interés en el estudio de esta enfermedad, lo que ha determinado el establecimiento de registros de pacientes y un crecimiento exponencial en la investigación de la LAM, tanto a nivel clínico como celular.

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## Introduction

Lymphangioleiomyomatosis (LAM) is a rare disease that predominantly affects women, especially those of fertile age. It is characterized by an abnormal proliferation of immature smooth muscle cells (LAM cells), that grow atypically in the airway,

parenchyma, lymph nodes and pulmonary blood vessels, leading to the appearance of pulmonary cystic lesions. The disease has no treatment, with a progressive and unrelenting evolution towards respiratory insufficiency that leads to patient death. The mechanism producing this distortion of the lung architecture is unknown. It is multifocal in character, affecting the axillary, abdominal and retroperitoneal lymph nodes; the appearance of lymphangiomas and angiomyolipomas is not infrequent, especially at the renal level.<sup>1,2</sup>

Due to its low prevalence, the difficulty of establishing an early diagnosis and the absence of curative treatment, LAM is grouped

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under the heading of the so-called Rare Diseases. Within this context, interest in LAM has led to the creation of patient registers<sup>3-5</sup> and exponential growth in its research at the clinical level<sup>4</sup> as well as at the level of its basic cellular pathology.<sup>6-8</sup>

There are two types of presentation, a sporadic form (S-LAM) and another linked to tuberous sclerosis complex (TSC-LAM), which occurs in up to 40% of women with TSC.<sup>9,10</sup> TSC, a dominant autosomal neurocutaneous syndrome, is associated with the formation of hamartomas in the central nervous system, skin, eyes, abdominal organs (especially kidneys) and lungs. Patients with S-LAM may present extrapulmonary manifestations observed in TSC, such as angiomyolipomas, axillary adenopathies and abdominal lymphangiomas, but no cutaneous, ocular or central nervous system manifestations, which are required to establish the diagnosis of TSC based on the criteria by Gómez.<sup>11,12</sup> Both disorders have their origin in mutations of the genes of tuberous sclerosis,<sup>13,14</sup> genes that are involved in the regulation of cell signs critical for energy control and cell nutrition processes.<sup>15</sup>

## Pathogenesis

### The LAM Cell

The LAM cell, the central axis of this disease, is a unique type of mesenchymal cell that expresses specific smooth muscle markers, proliferates continuously and invades the lung parenchyma, forming nodular growths as well as small disperse intraparenchymatous accumulations.<sup>8</sup> It possesses an immature smooth muscle phenotype, thus expressing smooth muscle  $\alpha$ -Actin as well as other muscle differentiation markers.<sup>16</sup> Given its immature character, it occasionally shares striated muscle differentiation markers (myogenin, MyoD1). It is, however, the positivity for HMB-45 (monoclonal antigen against gp100 glycoprotein of the premelanosomes) which distinguishes it from other cell types.<sup>17-19</sup> Thus, it is an anomalous cell element with a dual phenotypic expressivity with simultaneous features of smooth muscle and melanocytic antigen expression, data that suggest its origin in the neural ridge.<sup>20</sup>

There are two LAM cell subtypes<sup>21</sup>: one is fusiform, with a myofibroblastic morphological appearance, and another of larger, polygonal cells with epithelioid appearance. The expression of the premelanosome HMB-45 marker is variable and correlates inversely with the expression of proliferating cell nuclear antigen (PCNA)<sup>22</sup> or with the index of proliferation established by the analysis of the Ki67 antigen (MIB-1). In addition, the expression of gp100 and the proliferation markers (PCNA, Ki67) delimit the two LAM cell subtypes. While the fusocellular subtype shows a low expression of gp100 and a high percentage of cells immunoreactive to PCNA/Ki67, the epithelioid cell subtype exhibits an inverse pattern. Although currently the functional differences of both subtypes are unknown, Finlay<sup>6</sup> has suggested that the fusocellular subtype could represent the proliferative component of LAM lesions. Another hypothesis is that these two cell types correspond to the different phases in the differentiation or maturation of the LAM cell or that they are actually members of two different phenotypes.<sup>7</sup>

It has been recently demonstrated that LAM cells also show positivity, among others, against CD63 and PNL2, both markers for melanocytic differentiation. Thus, Zhe et al.<sup>20</sup> have established three cell subtypes depending on the expression of these markers: those cells that show CD63 immunoreactivity, those that also react to PNL2, and a third group that reacts to HMB-45.

These LAM cell subpopulations differ due to their proliferative capacity, and those with less mitotic activity are positive for PNL2.<sup>20</sup> The subgroup that is positive for HMB-45 shows a proliferative

activity of 6%,<sup>10</sup> similar to that found by Zhe et al.<sup>20</sup> in PNL2-immunoreactive cells.

The incidence of this disease in women, fundamentally those in their reproductive years, with clinical manifestations that worsen during pregnancy and with the administration of estrogen, made researchers consider the existence of estrogen receptors (ER) and/or progesterone receptors (PR) in the LAM cell, which are not present in normally-configured smooth muscle cells.<sup>23</sup> It has been confirmed that the presence of ER/PR can be generally established in LAM cells that are large-sized, with epithelioid habit, negative for HMB-45, and with less expressivity for hormonal receptors in LAM cells with less differentiation and greater proliferative activity.<sup>19</sup>

The etiology of LAM cells is controversial. If initially it was thought that they were derived from the smooth muscle cells of the airway or of the pulmonary vessels, it is currently known that said cells are found throughout the pulmonary territory, with no predominant situation.<sup>8</sup> Although its origin is uncertain, there are clinical and genetic data, and, above all, behavior in the cell cultures suggesting that LAM cells have neoplastic potential. They show an increase in cell motility and in the capacity to invade the collagen matrix, even without any type of environmental stimulation.<sup>24</sup> On the other hand, LAM cells have been detected in blood, urine and chylous liquid,<sup>25</sup> which could explain how primary lesions could disseminate through these pathways and propagate or metastasize at a distance. This also explains the possibility of LAM relapse in patients that undergo lung transplantation (LT).<sup>8</sup>

### LAM Genetics

As we have mentioned, there is a recognized similarity between lesions observed in LAM and in TSC with lung affectation, fuelling the hypothesis that both processes have a common pathogenic mechanism. It is important to point out that approximately one-third of TSC cases are *de novo*, meaning that their origin is in a germline mutation from the parents that affects the descendants but is not present in the rest of the cells of the parents.<sup>26</sup>

In 1993, the TSC2 gene (Tuberous Sclerosis Complex 2) was identified, located in the 16p13 chromosome<sup>27</sup>; in 1997, the TSC1 gene (Tuberous Sclerosis Complex 1) was identified in the 9q34 chromosome.<sup>28</sup> The proteins coded for the cited genes took their names from the phenotypic characteristics of TSC patients: hamartin for the protein produced by TSC1, and tuberin for that produced by TSC2.

The variable and focal nature of the hamartomas in TSC suggest that their development follows the "two-hit" model proposed by Knudson,<sup>29</sup> where two independent mutations are necessary to initiate tumor development. First there is a mutation in one of the copies of the gene and later another mutation would affect the other allele. It would also be possible if one of the copies of the gene is inherited mutated (first step) and later another mutation or deletion affected the remaining copy of the gene.

The genetic-lesion analysis of TSC patients has revealed a loss of heterozygosity (LOH) in TSC1<sup>30</sup> as well as in TSC2,<sup>31</sup> evidence of the tumor suppressor role of both genes. These findings reveal that these tumors develop following the Knudson model mentioned before. The first mutation is expressed in the germinal cells, while a second occurs in the affected tissues, leading to the formation of tumors. Approximately 300 mutations have been reported in both genes, and they are four times more frequent in the case of the TSC2 gene.<sup>32-34</sup> These observations have suggested the possibility that both genes could be implicated in the pathogenesis of S-LAM. Smolarek et al.<sup>13</sup> were able to evaluate LOH in the TSC2 in S-LAM patients and they did not observe any mutation in the TSC1 gene. This same group of

authors have not found evidence of germ-line mutations in S-LAM.<sup>33</sup>

#### *Hamartin and Tuberin*

The proteins coded by the TSC1 and TSC2 gene are called hamartin and tuberin, respectively. These two proteins form a complex involved in the transduction of signals from the receptors of the cell membrane. The main role of the hamartin-tuberin complex is to inhibit mTOR (mammalian target of rapamycin), a central regulatory element of cell growth through protein synthesis.<sup>35</sup> The mechanism by which the hamartin-tuberin complex maintains mTOR inactive involves RHEB (Ras Homolog Enriched in Brain), a GTPase of the family of RAS or small GTPases.<sup>36,37</sup> In order for the mTOR to act, it requires RHEB-GTP; however, the hamartin-tuberin complex maintains RHEB bound to GDP, a form of lesser energy.<sup>38</sup> When the mTOR is activated, it acts as a kinase that phosphorylates and activates S6K (S6 Kinase), an enzyme that stimulates ribosomal protein synthesis from the phosphorylation of the ribosomal protein S6. Although the phosphorylation of S6K is influenced by other multiple signals, such as insulin, amino acids, etc., it has been found that the hamartin-tuberin complex blocks the action of S6K by inhibiting its phosphorylation<sup>32</sup> by maintaining mTOR in a state of deactivation.<sup>39</sup> Moreover, mTOR activates the 4E-BP1 (4E-Binding Protein 1), which binds with the translation initiation factor F4E, freeing it from the state of inhibition and affording the ribosomes the protein synthesis necessary for cell growth and proliferation.<sup>40</sup>

Rapamycin, an immunosuppressive agent able to inhibit S6K phosphorylation, is a drug used to prevent rejection in transplant recipients because of its strong inhibitory effect on lymphocytic activation. This effect is produced by the binding of a protein, FKBP12, establishing a rapamycin-FKBP12 complex that directly inhibits mTOR.<sup>41</sup>

Other kinases involved in transduction, transcription, the cell cycle or apoptosis, likewise seem to play a decisive role in the regulation of the hamartin-tuberin complex. Akt/PKB, a cytosolic kinase linked to membrane receptors like insulin, promotes the dissociation of the hamartin-tuberin complex by phosphorylating tuberin. An overexpression of Akt/PKB leads to a degradation of the complex, therefore extracellular growth factors such as insulin can condition the influence of mTOR, creating conditions for cell growth.<sup>39</sup> Along this line, it is important to highlight the overexpression of extracellular factor receptors in the LAM cell, such as epidermal growth factor,<sup>42</sup> the growth factor derived from the platelets<sup>24</sup> or, more recently, the vascular endothelial growth factor A.<sup>43</sup>

Another recent finding increases our knowledge about these pathways. It seems that an intact and functional hamartin-tuberin complex is necessary for the Akt/PKB to function correctly. According to reports, the excess of phosphorylated S6K, as happens with defective hamartin or tuberin, would act as negative feed-back on Akt/PKB and reduce its expression.<sup>44,45</sup> This mechanism would partly explain the benign nature of the tumors observed in SC and in LAM cells. The loss of hamartin or tuberin produces S6K activation, favoring cell proliferation, but at the same time an inhibition of Akt/PKB is produced, obtaining the opposite effect and therefore halting cell growth.

Other kinases have been described that also regulate the hamartin-tuberin complex, such as MAPK (Mitogen Activated Protein Kinases), ERK2 (Extracellular Signal-regulated Kinase),<sup>46</sup> or AMP kinase.<sup>47</sup> It has also been verified that LAM cells show a deterioration in interferon gamma-JAK-STAT.<sup>48</sup> This finding resulted in treatment assays of interferon gamma associated with rapamycin with promising results at the experimental level.<sup>49</sup>

#### *Role of Estrogen*

Under normal conditions, estrogen binds to an intracellular receptor or ER $\alpha$  (Estrogen Receptor  $\alpha$ ). This union activates a pathway that includes PDGF $\beta$  (Platelet Derived Growth Factor  $\beta$ ) and ERK  $\frac{1}{2}$  (Extracellular signal-Regulated Kinase  $\frac{1}{2}$ ) that leads to cell growth. Tuberin interacts with ER $\alpha$ , which produces its inhibition. This process is influenced by calmodulin, a calcium-binding intracellular protein that can regulate a large number of proteins, modifying the different cell functions. It has been demonstrated to bind with tuberin,<sup>50-52</sup> producing an alteration in its action on ER $\alpha$  which in the end would not be inhibited. Another effect of ER $\alpha$  is its interaction on the Akt/PKB pathway. The result would be permissive, which, as mentioned before, produces inactivation of the hamartin-tuberin complex.<sup>53</sup> On the other hand, estrogen also acts through non-genomic pathways that promote different phosphatases that act on tuberin, dephosphorylating it and leading to its degradation.<sup>54</sup>

#### *The Microenvironment of the LAM Cell*

Hamartin-tuberin complex has a key role in the regulation of Actin cytoskeleton and in cell migration. These actions take place thanks to the interaction with members of the GTPase Rho family.<sup>55</sup> The alteration of the hamartin-tuberin complex finally produces remodeling of the cytoskeleton and anomalous cell motility. These actions are also influenced by mTOR through its binding to different proteins (ricor or raptor), although we do not know its exact mechanism of action.<sup>15</sup>

#### *Role of metalloproteinases*

The extracellular matrix procures structural stability to the lung tissue and participates in the regulation of many critical cell functions. Metalloproteinase (MMP) act as a functional component of the extracellular matrix and play a fundamental role in lymphangiogenesis.<sup>56</sup> It is a well-known fact that the degradation of the extracellular matrix contributes to invasion and metastasis in malignant tumors.<sup>57</sup> Involved in this process are the MMP that act as proteolytic enzymes catalyzing reactions in which zinc participates.

LAM cells, and especially the fusocellular phenotype, overexpress different MMP (MMP-1, MMP-2, MMP-9 and MMP-14, among others),<sup>59,58-60</sup> which would determine the protein degradation of the extracellular matrix, favoring cell migration. In addition, a descent in the tissue inhibitor expressions of the metalloproteinases (TIMP)-3 has been observed in LAM, which could contribute to an imbalance in the MMP/TIMP ratio with an increase in MMP-dependent proteolysis.<sup>61,62</sup> Besides this, MMP can stimulate cell growth as they degrade proteins that bind to the insulin-like growth factor, deactivating it.<sup>6</sup> The expression of MMP is the same in lung LAM cells as it is in angiomyolipomas, which support the theory of a common origin, according to which one of the lesions would come from the other, constituting a "benign metastasis".<sup>14,60,63</sup>

Doxycycline, an MMP inhibitor, affects the growth and migration of the neoplastic cells, angiogenesis and lymphangiogenesis, as well as muscle cell growth,<sup>64</sup> thus its use could be effective in the treatment of LAM.<sup>65</sup>

#### *Pathogenesis of Cystic Lung Disease in LAM*

LAM cells proliferate in the lymphatic vessels of the lung and in the mediastinal, retroperitoneal and pelvic lymph nodes. As they grow, new lymphatic channels are created, which is true lymphangiogenesis. The cells that line these channels express various

lymphatic endothelial markers, such as VEGF-C (Vascular Endothelial Growth Factor)<sup>66</sup> or VEGF-D.<sup>67</sup> High levels of the latter have been detected in the serum of patients with the disease. These new lymphatic vessels cross the LAM cell accumulations, dividing them and even dislodging some.<sup>66</sup> The action would explain a mechanism of metastasis at a distance.

The groups of LAM cells are in the proximity of the airway and the blood vessels, and can block them. The difficult blood flow can produce hemorrhages and hemoptysis. The affection of the airway produces air trapping, which finally translates into cystic changes in the pulmonary parenchyma. The formation of cysts at the pulmonary level would also be favored by the alteration of the extracellular matrix produced by the MMP. The obstruction of the lymphatic vessels explains another of the typical signs of LAM: chylothorax.<sup>68</sup>

### Pathological Anatomy

Architecturally, as verified in the macroscopic study of the lesion, LAM is manifested as a disease with characteristic features, which include the existence of bilateral cystic lung structures, generally between 0.5 and 2 cm in diameter, although they can reach up to 10 cm, associated with large lungs and with alveolar emphysema.<sup>69</sup>

In its morphopathology, LAM is characterized by an abnormal proliferation of oval or elongated cells with muscular habit, known as LAM cells, whose abnormal growth is associated with the cystic destruction of the pulmonary interstitium.<sup>8</sup> LAM cells can affect any lung structure, including the pleura, bronchial walls, pulmonary arteries, venules, small airway, as well as lymph, mediastinal, hilar, mesenteric and retroperitoneal nodes.<sup>70,71</sup> In this way, the LAM cell proliferates, forming nodules around the bronchi and blood vessels while lining the walls of the cysts. The LAM cell nodules are centrally composed of fusiform cells that express smooth muscle markers, such as smooth-muscle specific  $\alpha$ -Actin ( $\alpha$ -AMS), desmin (DM) and vimentin (VT). They are surrounded by a subtype of epithelioid cells that are immunoreactive for HMB-45.<sup>8</sup> Both lesions, cysts and proliferation nodules of the LAM cells, are found together and in varying proportions and their presence may be quite scarce and difficult to recognize in the initial stages of the disease.<sup>2</sup>

With regards to its architectural characteristics, Matsui et al.<sup>72</sup> have proposed a score, called the LAM Histologic Score (LHS), with three degrees of prognostic severity (grades 1, 2 and 3) based on the percentage of lung tissue affected by the cystic and proliferative component. These authors observed that the proportion of affected parenchyma is related to survival, likewise observing a correlation between this gradation and the amount of intra-alveolar hemosiderinic pigment (positive Perls or blue Berlin stain), due to the obstruction (destruction) of septal capillaries. The amounts of hemosiderin and iron pigment are greater in the higher Matsui grades.

Besides the lesions described, there are also other associated lesion findings in the lungs, such as multifocal micronodular pneumocyte hyperplasia, pulmonary clear cell tumor (sugar tumor) and non-necrotizing granulomas.<sup>71,73,74</sup> In addition, extrapulmonary associated presentations in LAM patients include abdominal lymphangiomas and angiomyolipomas.

### Prevalence

The prevalence and incidence of LAM are unknown and have probably been underestimated due to its clinical latency and to the lack of specific laboratory tests.<sup>75</sup> S-LAM is estimated at one case per million inhabitants,<sup>9,10</sup> which would be somewhat more frequent for

TSC-LAM, as up to 40% of women with TSC could have LAM lesions.<sup>9,76</sup>

Derived from the genetic alterations described before, TSC-LAM should be between 5 and 10 times more frequent than S-LAM. This data is not confirmed in the different registers and could be due to the fact that the TSC-associated form is milder or that other associated comorbidities are more important than the lung affection.<sup>1,14</sup> In Spain, prevalence is estimated between one and two patients per million inhabitants.<sup>77</sup>

The disease usually presents in women of fertile age with an average age at presentation of 35. Nevertheless, cases have also been described in post-menopausal women, although some were receiving substitutive hormonal treatment.<sup>78</sup> Other cases have been diagnosed in adolescents and in men with TSC, but these are exceptional.<sup>79-81</sup>

Although there have been reported cases of LAM in men affected with TSC79, S-LAM is found exclusively in women. One controversy is the possibility of the existence of LAM in a male without TSC, as published by Schiavina.<sup>82-84</sup>

### Clinical Manifestations

#### Respiratory Manifestations

In S-LAM, the symptoms derived from the pulmonary affection are the main manifestations, The three most frequent findings are dyspnea on effort, pneumothorax and cough.<sup>1,4,85,86</sup>

Dyspnea on exertion is referred by most patients and is the result of the airflow obstruction and the substitution of the lung parenchyma by cysts. Their establishment is slow and progressive, and from the onset of dyspnea until the diagnosis, 5 or 6 years usually pass.<sup>87</sup>

Pleural pathology is a common complication in LAM and contributes substantially to the morbidity associated with this disease.<sup>88</sup> The first pneumothorax is the sentinel event that directs the diagnosis in a great proportion of patients. Its incidence varies between 39 and 81%, according to several authors,<sup>3,89-93</sup> and pneumothorax recurrence is characteristic of LAM, with an incidence of between 61 and 81%.<sup>3,89,94</sup> With regards to treatment, a conservative attitude such as rest or endothoracic drain has a 66% recurrence rate; thus an interventionist attitude is recommended, although pleurodesis with talc or pleurectomy carry recurrence rates of 27 and 32%, respectively.<sup>95</sup> Why these results are poor compared to those obtained in pneumothorax secondary to other lung pathologies is unknown. It is possible that the profusion of blebs on the pulmonary surface limits the apposition of the visceral and parietal pleura, making the pleural fusion incomplete.<sup>88</sup>

Chylothorax presents an incidence that varies between 7 and 31%.<sup>4,78,89-92</sup> Clinically, it presents as progressive dyspnea accompanied by chest pain and unproductive cough. It is produced by various mechanisms, the most important being the obstruction or rupture of the thoracic duct. Another is a leak from the pleural lymph nodes or transdiaphragmatic flow of chylous ascites.<sup>88</sup> Its management is difficult because it usually recurs if the treatment only consisted of endothoracic drain.<sup>4,86</sup> In addition, it produces a nutritional deficit and a certain degree of immunosuppression, and it is therefore necessary to monitor patient weight, hemogram, electrolytes, serum prealbumin and albumin and total protein.<sup>96</sup> The measures aimed at reducing the production of chyle consist of following a diet of short-chain fatty acids<sup>97</sup> or treatment with octreotide.<sup>98</sup> However, recurrence is the norm, therefore the treatment should include pleurodesis, or pleurectomy, reserving thoracic duct ligation for the most serious cases.<sup>86</sup>

Other respiratory symptoms like hemoptysis and chyloptysis occur in a small number of patients. They are due to the obstruction by the LAM cells of the lymphatic and blood capillaries.

## Extrapulmonary Manifestations

The most frequent extrapulmonary manifestations are lymphadenopathies, lymphangioliomyomas, chylous abdominal collections and the appearance of angiomyolipomas. Although it is exceptional, LAM can start with abdominal pain, and even with severe abdominal symptoms.<sup>99,100</sup>

The increase in size of the abdominal lymph nodes usually affects the retroperitoneal, retrocrural or pelvic area. It is diagnosed with CT in almost one-third of patients, but causes no symptoms itself.<sup>101</sup>

Lymphangioliomyomas are large cystic masses that are a result of the obstruction of the lymph vessels. They are most frequently situated in the abdomen, retroperitoneum and pelvis, although they have also been described in the mediastinum and neck. The symptoms produced include nausea, abdominal distension, edema in legs and urinary alterations. Normally, these manifestations worsen during the day, which has been related with an increase in the size of the lymphangiomas as a result of the accumulation of lymphatic liquid in the lower areas of the body due to standing.<sup>102</sup>

Chylous ascites is present in 10% of patients and occurs in advanced stages of the disease.<sup>101</sup> It is related with the obstruction of abdominal lymph nodes and with the presence of chylothorax.<sup>78</sup>

The most frequent abdominal alteration is angiomyolipomas, predominantly in renal situations. They affect 40% of S-LAM patients and up to 80% in TSC-LAM4. These benign tumors are composed of dysplastic blood vessels, smooth muscle and adipose tissue. Diagnosis is based on CT, as the majority are asymptomatic.<sup>101,103,104</sup> In any event, they can cause flank pain, hydronephrosis, hematuria and loss of renal function. In patients with S-LAM, angiomyolipomas are usually unilateral, small, solitary and restricted to the kidneys, while in those with TSC-LAM they are bigger, bilateral, multiple, multi-organic (affecting spleen or liver) and with a greater tendency towards hemorrhage.<sup>105</sup> This latter complication seems related with the profusion of aneurisms in the tumor mass and with size, so that those that are greater than 4 cm seem to have a greater predisposition for bleeding, and active treatment is therefore recommended.<sup>106</sup>

Other much less frequent alterations have also been reported, such as chyluria or chylopericardium. Also, the incidence of meningiomas seems higher in patients with LAM, regardless of its association with TSC, compared with the general population.<sup>107</sup>

Pregnancy can aggravate LAM symptoms above all because it seems to favor the appearance of pneumothorax and chylothorax, as has been observed in various clinical series.<sup>3,78</sup> Furthermore, it can worsen the angiomyolipomas, producing greater growth and increasing the possibilities for bleeding.<sup>108</sup> In contrast, other patients' pregnancies do not exacerbate their symptoms. This data, along with such little useful information on the true effect of pregnancy on LAM, suggest that final decisions be made individually in each case.<sup>1,86</sup>

## Diagnosis

The mean age of patients at the time of diagnosis is around 35, according to many series consulted.<sup>4,89-93</sup> LAM diagnosis requires pulmonary high-resolution CT (HRCT) showing the presence of thin-walled cysts and, in addition, a positive biopsy including immunohistochemical study with HMB-45 or a compatible clinical context, such as the presence of clinically-confirmed TSC, angioliomatosis or chylothorax.<sup>1,85,86</sup>

Physical examination is usually normal, only detecting some abnormal wheezing-like chest sounds. Unlike other lung interstitial diseases, clubbing does not usually appear.

Blood work-up and chest x-ray do not usually show pathological findings in the initial stages. Later, a reticulonodular pattern and

bullas or cysts can appear, as well as signs of pulmonary hyperinflation. In more advanced phases, there can be a honeycomb pattern similar to other pulmonary interstitial diseases.<sup>109</sup>

HRCT is one of the most important diagnostic tests. It shows multiple thin-walled cysts disseminated homogeneously throughout the lung fields together with areas of conserved parenchyma. The cysts are small in size, usually less than 1 cm.<sup>110,111</sup> Cyst size has been related to the possibility for pneumothorax, and sizes greater than 5 mm are associated with higher probability.<sup>112</sup> Another frequent finding is the existence of non-calcified pulmonary nodules that range from 2 to 10 mm. Their distribution is random throughout the lung fields. In addition, the HRCT study can diagnose other processes such as chylothorax, lymphangioliomyomas or angiomyolipomas. The latter have a characteristic appearance, showing mixed densities with areas of hypoattenuation derived from their adipose component.<sup>101</sup>

There have been a series of differences established between S-LAM and TSC-LAM. In the former, lymphatic affection is more frequent: dilatation of the thoracic duct, chylous pleural effusion, ascites and presence of lymphangioliomyomas. The presence of non-calcified lung nodules and angiomyolipomas, renal as well as hepatic, are more prevalent in TSC-LAM.<sup>105</sup>

Gasometry can be compatible with respiratory insufficiency in approximately 5% of patients, although most show parameters close to normal.

Spirometry usually shows an obstructive pattern in more than half of patients, 25% of which have positive bronchodilator test and impaired diffusing capacity.<sup>4,113,114</sup> The rest may have a mixed pattern or minimal alterations. Total lung capacity, residual volume and the relationship between both are usually higher as an expression of lung hyperinflation and air trapping, respectively. Patients with pleural effusion or those that have been subjected to pleurodesis can present a restrictive pattern.<sup>114</sup> A lower carbon monoxide diffusing capacity (DLCO) is another of the most frequent factors. In a recent study, the mean value obtained was  $67.6 \pm 1.61$  ml/min/mmHg.<sup>4</sup> Likewise, the average reduction is estimated in 0.69 ml/min/mmHg per year.<sup>87</sup>

The tolerance to exercise measured in the effort tests is lower. This is due to reduced oxygen consumption, a lower anaerobic threshold, excessive and abnormal ventilatory response with high respiratory rate, excessive minute ventilation and reduction in respiratory reserve. The dead space volumes at rest and during effort, which are larger than normal, also contribute to this state.<sup>115</sup>

The pressure in the pulmonary artery at rest is within the limits of normal, but it increases with small levels of effort.<sup>116</sup> This establishes the need for specific tests to determine the possible existence of pulmonary hypertension in all these patients.

Another cornerstone in the diagnosis is biopsy and later anatomopathologic study. This can be obtained from the lung parenchyma or from a lymph node. The finding of LAM cells would confirm diagnosis. It is not uncommon for a biopsied retroperitoneal mass suspected of lymphoma or ovarian cancer to show the presence of LAM cells.

It is necessary to establish the differential diagnosis with pulmonary emphysema and Langerhans cell histiocytosis (LCH). In doing so, the history of tobacco habit and the shape of the cysts are very useful.<sup>117</sup> Other diseases that can simulate LAM are: Sjögren's syndrome,<sup>118</sup> follicular bronchiolitis, lymphocytic interstitial pneumonitis,<sup>119</sup> hypersensitivity pneumonitis,<sup>120</sup> amyloidosis, light chain deposition disease,<sup>121</sup> bronchopulmonary dysplasia, metastatic endometrial cell sarcoma,<sup>122</sup> low-grade leiomyosarcoma, Birt-Hogg-Dubé syndrome<sup>123</sup> and lymphangiomatosis.<sup>124</sup>

## Prognosis

The course of the disease varies widely and there are no known relevant prognostic factors.<sup>1,4,85,86</sup>

An example of this variability is the 10-year mortality after the onset of symptoms, which ranges from 10 to 90% according to the series consulted.<sup>3,125</sup> Cases diagnosed in patients in their eighties have also been reported<sup>126</sup> as have disease courses of longer than 30 years.<sup>65</sup>

The deterioration in gas exchange is the most important factor that aggravates prognosis. The average loss of FEV1 is 120 ml per year, although it varies depending on the series consulted.<sup>87,127,128</sup> Patients with positive bronchodilator tests present a faster descent in FEV1, although the cause is unknown.<sup>113</sup> The decrease in DLCO also has a good correlation with the histological severity grade, one of the predictors for transplantation time and death.<sup>114</sup> The deterioration is greater in the presence of pneumothorax or chylothorax.

It seems that the initial presentation with pneumothorax occurs in younger women and it is associated with better prognosis and a 10-year survival of 89%. In contrast, when the initial symptom is dyspnea, survival is lower, around 47%.<sup>129</sup> These data have not been confirmed in other studies, in which survival is independent of pneumothorax.<sup>122</sup>

Quantified HRCT measures the quantity of parenchyma affected by the cysts and is significantly related with functional deterioration.<sup>130</sup>

Histological factors such as the predominance of cystic lesions<sup>90</sup> or the percentage of lung tissue affected by the proliferative cystic component<sup>72</sup> have been previously commented.

Large part of this variability over the course of the disease could be explained by the polymorphism of the genes involved and by the identification of such polymorphisms.<sup>86</sup>

## Treatment

There is currently no curative treatment for this pathology. Progesterone treatment, currently widely-used, and other alternatives including the use of gonadotropin-releasing agonists<sup>131</sup> or tamoxifen<sup>132</sup> have been questioned in recent years. On the one hand, it is criticized that they are based on small case series, fundamentally retrospective and even in one single clinical case, and that randomized clinical assays were not done.<sup>1</sup> Moreover, side effects related with these medications (water retention, swelling, nausea) are not infrequent, and may even lead to suspension of the treatment.<sup>86</sup> There has been a recent report of a possible relationship between progesterone treatment and a greater incidence of meningiomas in these patients.<sup>107</sup> On the other hand, in a retrospective analysis, it was found that progesterone treatment did not stop the fall in FEV1 and in fact it seemed to accelerate the fall in DLCO when compared with untreated patients.<sup>127</sup> Other patients have not confirmed this tendency.<sup>87</sup> There also is no evidence that oophorectomy halts the progression of LAM; therefore, this more aggressive therapeutic approach is much less indicated than it was some years ago.<sup>89</sup>

The results with interferon alpha have not been as expected given the absence of noted benefits and the associated side effects.<sup>133</sup>

Recently, and derived from the knowledge of the pathogenesis in which mTOR is a fundamental piece, inhibiting agents of this compound are being assayed in the treatment of LAM and TSC.<sup>134-136</sup> There are various clinical assays, in phase I as well as II, which include sirolimus and everolimus. The preliminary results are hopeful but further experience is necessary.<sup>137</sup>

Other drugs currently being studied that have therapeutic potential are RHEB inhibitors, selective estrogen antagonists, tyrosine

kinase inhibitors, MMO inhibitors, angiogenesis inhibitors and lymphangiogenesis inhibitors.<sup>1</sup>

Although bronchodilator treatment is recommended in patients with positive bronchodilator tests, the effect obtained has been modest,<sup>1</sup> as in the use of inhaled or systemic corticosteroids.<sup>85</sup>

## LAM and Lung Transplantation (LT)

The utility of LT in LAM has been controversial due to the low incidence of this disease, its systemic character, and the impact that the disease itself could have on perioperative morbidity and mortality. Currently, LT can be a good alternative for these patients when they meet a series of internationally-accepted LT criteria.<sup>138</sup>

According to the registry of the International Society for Heart and Lung Transplantation, 1% of LTs performed annually are done so in patients diagnosed with LAM.<sup>139</sup> Due to this fact, it has taken a long time to establish study series that are more or less extensive in order to evaluate the utility of LT.

Some recurrences of the disease have been reported in lung transplantation cases,<sup>140,141</sup> and although the existence of pleural adherence due to the disease itself or previous pleural procedures can condition intra- or post-operative hemorrhages, especially if it is necessary to use by-pass circulation during LT95. Nevertheless, recent publications,<sup>142,143</sup> some of which report experiences in the USA<sup>144</sup> or Europe,<sup>145</sup> give a survival of 80, 75 and 65% at one, three and five years, respectively. They conclude that, although the perioperative complications in LAM are frequent, LT could be considered a valid treatment in these patients. The results are comparable with those obtained in other lung pathologies, not only for survival,<sup>139</sup> but also for quality of life.<sup>146</sup>

## Conflict of Interest

The authors declare having no conflict of interest.

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