

# The Natural History of Lymphangioleiomyomatosis: Markers of Severity, Rate of Progression and Prognosis

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## Abstract

Lymphangioleiomyomatosis (LAM) is a multisystem disease of women, characterized by proliferation of abnormal smooth muscle-like cells (LAM cells) that can metastasize, leading to the formation of lung cysts, fluid-filled cystic structures in the axial lymphatics (e.g., lymphangioleiomyomas), and angiomyolipomas, benign tumors usually involving the kidneys, comprising LAM cells and adipocytes, intermixed with incompletely developed vascular structures. LAM occurs sporadically or in association with tuberous sclerosis complex, an autosomal dominant syndrome characterized by hamartoma-like tumor growths.

LAM may present with progressive dyspnea, recurrent pneumothorax, chylothorax, or abdominal hemorrhage. Computed tomography scans show thin-walled cysts scattered throughout the lungs, abdominal angiomyolipomas, and lymphangioleiomyomas. Pulmonary function tests show reduced flow rates (FEV<sub>1</sub>) and diffusion capacity (DL<sub>CO</sub>). Exercise testing may reveal gas exchange abnormalities, ventilatory limitation, and hypoxemia, which can occur with near-normal lung function. Methods used to grade the severity of disease are the LAM histology score, semiquantitative and quantitative computer tomography, pulmonary function testing, and cardiopulmonary exercise testing. Currently, progression of disease is best assessed by serial measurements of FEV<sub>1</sub>, DL<sub>CO</sub>, and exercise performance. New quantitative radiographic techniques that may offer advantages over physiologic testing are now available.

Several potential biomarkers, such as LAM cells in peripheral blood, urine, and chyle and chemokines, vascular endothelial growth factors, and matrix metalloproteinases, may be useful as diagnostic tools or markers of organ involvement, disease severity, and progression.

## Introduction

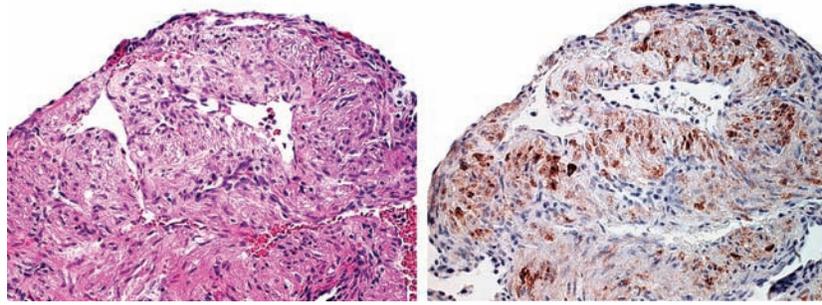
**L**YMPHANGIOLEIOMYOMATOSIS (LAM), A MULTISYSTEM DISORDER primarily affecting women, is characterized by cystic lung destruction, axial lymphatic abnormalities, and abdominal angiomyolipomas.<sup>1–10</sup> A characteristic pathologic feature of LAM is the proliferation of abnormal smooth muscle-like cells (LAM cells), associated with thin-walled cysts in the lungs, fluid-filled lymphatic cystic structures (i.e., lymphangioleiomyomas) in the axial lymphatics and angiomyolipomas (AMLs), mostly involving the kidneys, which are composed of LAM cells and adipocytes, intermixed with incompletely developed vascular structures.<sup>1–10</sup>

LAM occurs sporadically in patients with no evidence of genetic abnormality and in about one-third of women with tuberous sclerosis complex (TSC),<sup>11–13</sup> which is an autosomal dominant syndrome characterized by hamartoma-like tumor growths in various organs, cerebral calcifications, seizures, and mental retardation, that occurs in one of 5,800 live

births.<sup>14,15</sup> Sporadic LAM is a relatively uncommon disease, with a prevalence estimated at 2–5/million.<sup>5,16</sup>

Histologically, LAM lung is characterized by clusters of both small spindle-shaped and larger epithelioid cells with abundant cytoplasm adjacent to cystic lesions and along pulmonary blood vessels, lymphatics, and bronchioles.<sup>7</sup> Both types of LAM cells react with antibodies against smooth muscle cell antigens (e.g., smooth muscle  $\alpha$ -actin, vimentin, desmin).<sup>7</sup> The epithelioid cells react with HMB-45, a monoclonal antibody that recognizes gp100, a premelanosomal protein product of alternatively spliced *Pmel17*<sup>7</sup> (Fig. 1). The spindle-shaped cells react with PCNA, consistent with the conclusion that these cells are more proliferative.<sup>7</sup> Receptors for estrogen,<sup>17</sup> progesterone,<sup>18</sup> insulin-like growth factors,<sup>19</sup> angiotensin II,<sup>20</sup> hyaluronic acid (CD44),<sup>21</sup> and chemokines<sup>22</sup> have been identified in LAM cells.

Sporadic LAM is thought to be caused by a neoplastic cell in which one of the tuberous sclerosis complex genes 1 (*TSC1*) or 2 (*TSC2*) is mutated, more often *TSC2* than *TSC1*.<sup>23,24</sup> Loss



**FIG. 1.** *Left panel* shows a close-up of LAM nodule (hematoxylin-eosin). The *right panel* shows that the same nodule reacts with the monoclonal antibody HMB 45 (original magnification  $\times 200$ ).

of heterozygosity of *TSC2* has been reported in LAM lesions from lung and kidney,<sup>23</sup> consistent with Knudson's "two-hit" hypothesis of tumor development.<sup>25</sup> LAM cells are capable of metastasizing, as identical *TSC2* mutations were found in lung lesions and AMLs from the same patient with sporadic LAM, and recurrent LAM cells of recipient origin were detected in the donor lung of a transplanted patient.<sup>26–28</sup> LAM cells can be detected in body fluids (blood, urine, expectorated chyle, pleural or abdominal chylous fluids) of some LAM patients.<sup>29</sup> The "primary" source of LAM cells in the lungs is unknown. Potential sources include AMLs and the lymphatic system,<sup>30</sup> although 68% of sporadic LAM patients do not have demonstrable AMLs.<sup>31</sup>

LAM presents with symptoms of dyspnea, recurrent pneumothorax, pleural effusion, or extrapulmonary manifestations such as intra-abdominal hemorrhage or an abdominal mass.<sup>4–9</sup> Dyspnea occurs in over 70% of LAM patients;<sup>9</sup> 50% have a history of pneumothorax.<sup>5,32</sup> Chylothorax, with or without ascites,<sup>8,33</sup> and thoraco-abdominal lymphadenopathy and lymphangioliomyomas,<sup>33,34</sup> suggestive of a malignant lymphoproliferative disease,<sup>35,36</sup> are other presentations of LAM (Fig. 2). Abdomino-pelvic lymphangioliomyomas may present with abdominal pain or as an acute abdomen.<sup>37,38</sup> These lymphatic tumors have a distinctive radiologic appearance, and diurnal variation in size of the tumor masses has been demonstrated<sup>34,39,40</sup> (Fig. 2).

Angiomyolipomas are benign tumors usually localized in the kidneys that are found in approximately 80% of patients with LAM and TSC, and in about 40% of those with sporadic LAM.<sup>9</sup> These tumors vary in size from one millimeter to more than 20 centimeters in diameter.<sup>41,42</sup> Radiologically, the tumors comprise areas of fatty density, intermixed with more dense areas and normal-appearing renal parenchyma (Fig. 2).<sup>41,42</sup> The tumors are highly vascular, with the blood supply ordinarily originating from the renal arteries, and may completely disrupt normal kidney architecture. The principal complication of AMLs, especially those greater than 4 centimeters in diameter, is bleeding, which may be abrupt and require blood transfusions, arterial embolization, or surgical resection.<sup>42,43</sup>

The average time from the onset of symptoms related to LAM to the definitive diagnosis of LAM ranges from 3 to 6 years.<sup>44</sup> The physical examination of LAM patients is surprisingly normal, which may account for the frequency of misdiagnosis. Signs consistent with TSC, such as neurofibromas, periungual fibromas, nail ridges, and shagreen patches, are seen in patients with LAM and TSC.<sup>15</sup>

### Natural history of LAM

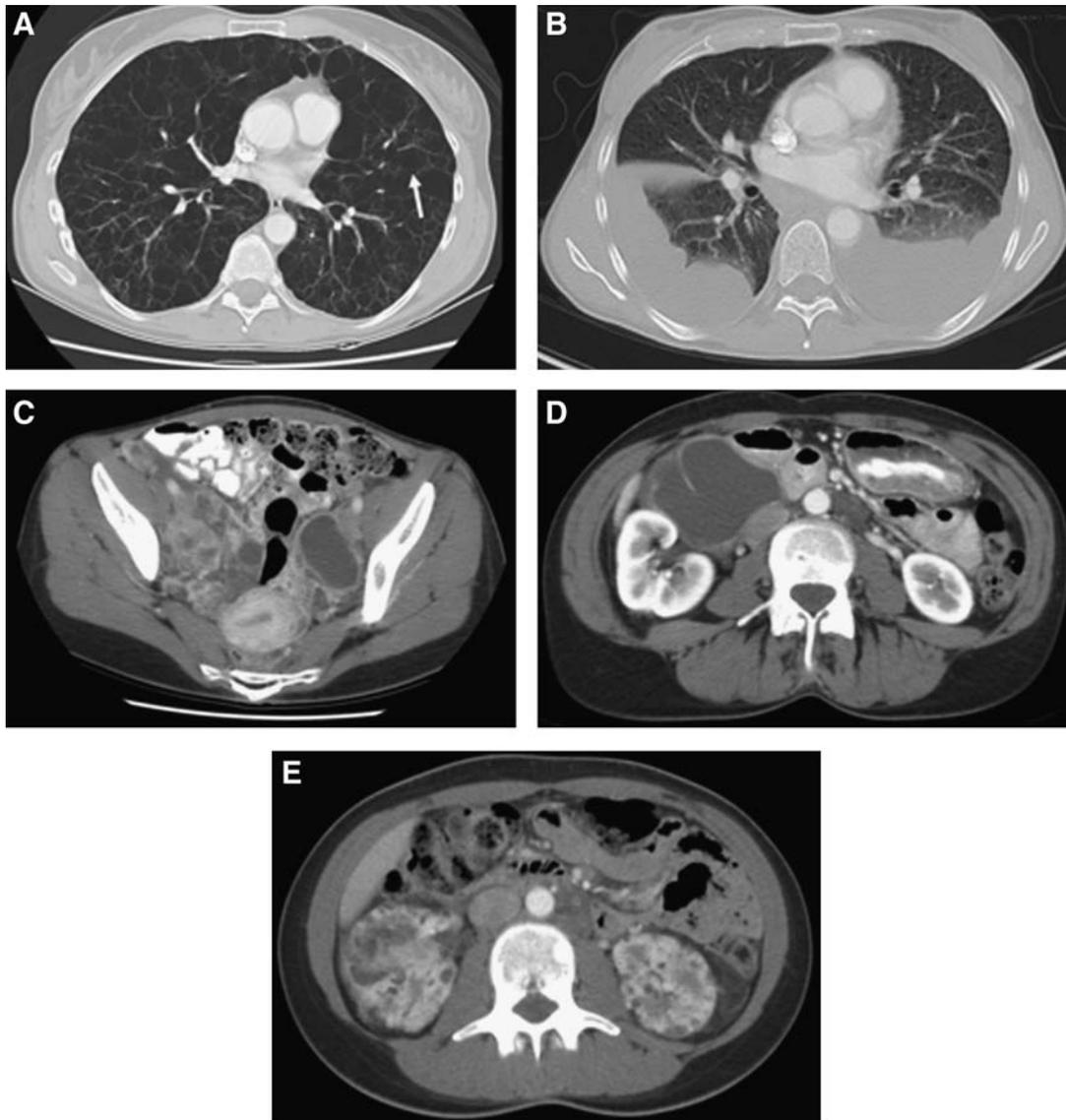
Pathologic, radiologic, and clinical descriptions of LAM first appeared in the literature of the late 1960s and 1970s.<sup>45–50</sup> The first study that established the physiologic abnormalities observed in LAM is that of Carrington et al.<sup>48</sup> Subsequent reports<sup>1,49</sup> added more clinical and physiologic data, but because of the relatively small number of cases, left large gaps in our knowledge of the natural history of LAM. The spectrum of the disease and its prognosis at that time were largely unknown. Indeed, lymphangioliomyomatosis was defined as a fatal disease of women of child-bearing age, characterized by recurrent pneumothorax, chylothorax, hemoptysis, and progressive dyspnea.<sup>49</sup> Patients were offered few therapeutic options besides oophorectomy, progesterone therapy, or lung transplantation.

During the last decade, the establishment of an NHLBI LAM registry, and data from studies done at the NIH, in addition to research outside the USA, especially in the UK, Japan, and France, revealed quite diverse clinical phenotypes.<sup>1,5,6,9</sup> It was found that LAM occurs in postmenopausal women as well as those of childbearing age and is best defined as a chronic disease with a life expectancy spanning decades. Cohen et al.<sup>51</sup> found that LAM was becoming more and more a condition of women aged 45–60 years, as more older women were being diagnosed with LAM. LAM should now be defined as a disease of women of childbearing age and women in their middle years. At the same time, the role of anti-estrogen therapy in LAM is, at best, questionable, given the potential for adverse events in a chronic illness, and oophorectomy is no longer universally recommended.

A retrospective analysis of 505 patients seen at NIH between 1995 and 2009 showed that the mean age at the time of the first visit was  $43 \pm 0.4$  years, and the diagnosis had been established  $2.4 \pm 0.1$  years before that visit. Symptoms attributable to LAM had been present for  $7.4 \pm 0.4$  years prior to the visit. The mean time between symptoms and diagnosis was  $4.8 \pm 0.4$  years. Of the 505 patients, 59 (12%) had died and 64 had undergone lung transplantation with 21 deaths. The total number of deaths between 1995 and 2009 was 80.

### Measures of Disease Severity

Multiple laboratory tests may assist in quantifying disease progression.  $DL_{CO}$  and  $FEV_1$ , in particular correlated with disease severity assessed by computed tomography scans, LAM histology score, and exercise testing.<sup>44,52–59</sup>



**FIG. 2.** Pulmonary and extrapulmonary findings on computed tomography scans of patients with LAM. (A) Multiple thin-walled cysts scattered throughout the lungs, completely replacing the lung parenchyma. *Arrow:* cyst wall. (B) Bilateral chylous pleural effusions. (C) Complex pelvic cystic lymphangiomyomas. (D) Large lymphangiomyoma compressing the inferior vena cava. (E) Multiple bilateral angiomyolipomas.

### LAM Histology Score

The severity of lung involvement in LAM may be assessed in patients who have undergone lung biopsy using the LAM Histology Score (LHS), which grades extent of replacement of normal lung tissue by both cystic lesions and LAM cell infiltrates.<sup>54</sup> The amount of tissue involvement by these two pathological patterns can be estimated semiquantitatively. Grading is based on percent of lung tissue involved: LHS-1, <25%; LHS-2, 25% to 50%; and LHS-3, >50% lung tissue. Significant differences in survival and time to transplantation for patients with LHS-1, -2, and -3 scores (Fig. 3) have been reported.<sup>54</sup> Ten-year survival was near 100% for LHS 1, 74.4% for LHS 2, and 52.3 % for LHS 3. The presence of hemosiderin-laden macrophages is also a marker of severity, as it was associated with higher LHS and worse prognosis.<sup>54</sup> As reported by Kitaichi et al.,<sup>1</sup> patients with more cystic disease, in

general, have worse prognosis than those with a more muscular, solid variety of LAM. These patients are likely to have higher cyst volume, lower  $DL_{CO}$ , and more exercise-induced hypoxemia.<sup>1</sup> Significant variability is observed among LAM patients, however, and this should be taken into account when considering prognosis.

### Computed Tomography

Severity of lung disease in LAM can be graded semiquantitatively by CT scans.<sup>55,56</sup> This method assesses the extent of involvement of each of three equal pulmonary zones and grades them according to the percentage of lung volume judged abnormal using the following scale: 0, absent; 1, less than 30% abnormal; 2, 30%–60% abnormal; 3, more than 60% abnormal. Quantification of CT scans in this way, has yielded good correlations with results of lung function tests<sup>55,56</sup> (Fig. 4).

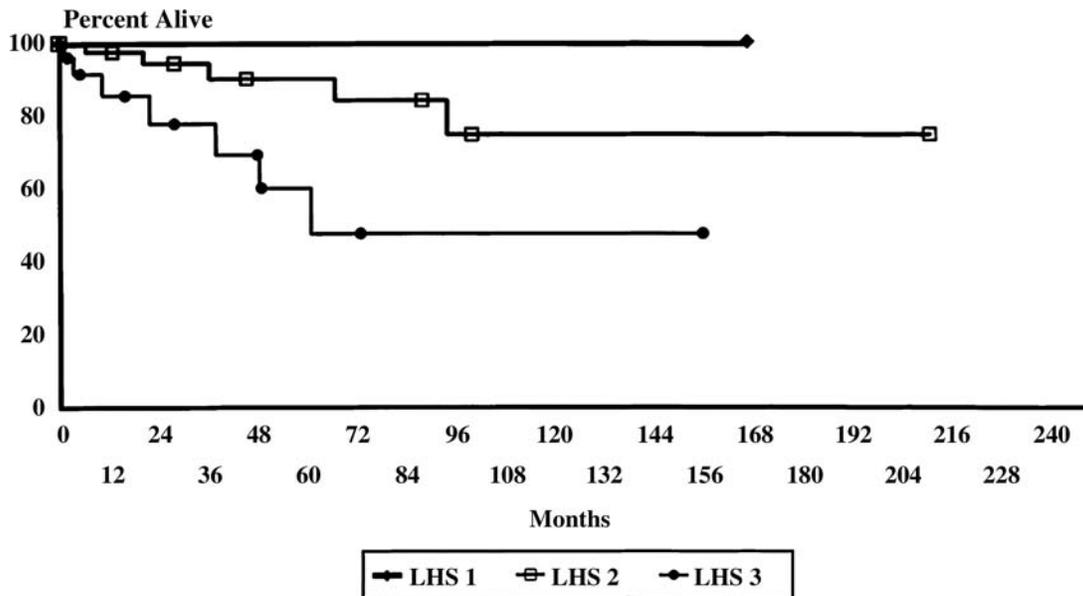


FIG. 3. Kaplan-Meier survival curves of LAM patients according to pulmonary lymphangioleiomyomatosis histologic score (LHS). Patients with LHS-1 have nearly 100% survival at 168 months. Patients with LHS-3 have a 52.3% survival at 156 months, and patients with LHS 2 have a 74.4% survival at 216 months ( $p < 0.002$ ) (From Reference 54).

Quantitative CT scan grading has advantages over semi-quantitative methods, as it correlated well with tests of lung function, gas exchange, and exercise performance.<sup>57,58</sup> Recent advances in assessing the extent and severity of lung disease by computed tomography make it possible to quantify the amount of lung parenchyma affected by cysts and to evaluate the texture of lung areas not apparently involved,<sup>60</sup> (Yao J, unpublished observations). Using these methods, percent of cyst volume correlated with FEV<sub>1</sub>, re-

sidual volume, and DL<sub>CO</sub>.<sup>58,60</sup> With these advances, lung imaging can assist in grading the severity of lung disease, in addition to serving as a useful diagnostic test. Unlike pulmonary function testing, these procedures require minimal patient cooperation.

#### Pulmonary Function Tests

The simplest and most practical method of assessing severity of lung disease in LAM is pulmonary function testing. The most frequent pulmonary function abnormalities in LAM are airflow obstruction and decreased lung diffusion capacity.<sup>9,52,53</sup> Evidence of airflow limitation was found in over 60% of patients. Reduced diffusing capacity was seen at least as frequently as a decreased FEV<sub>1</sub>.<sup>9,52,53</sup> About one-third of the patients had normal tests. In another series, the most frequent abnormality in pulmonary function was a decline in the DL<sub>CO</sub>, which was seen in 80% of patients.<sup>61</sup> Although most patients had both airflow obstruction and impaired gas exchange, a significant number had normal flows or only mild airflow obstruction, along with marked decrease in diffusion capacity. In these patients, the severity of disease should be graded by tests of gas exchange such as DL<sub>CO</sub>, arterial blood gases, alveolar-arterial oxygen (A-a/O<sub>2</sub>) gradient, or with cardiopulmonary exercise testing or 6-minute walk test, to elicit hypoxemia.

#### Reversible Airflow Obstruction

Reversible airflow obstruction has been reported in 25% to 30% of LAM patients.<sup>52,62</sup> Indeed, many LAM patients are treated with inhaled bronchodilators and steroids by their physicians.<sup>52,62</sup> Patients who responded to bronchodilators appeared to have greater predominance of proliferative lung lesions (Fig. 5).<sup>52</sup> Further, the yearly rate of FEV<sub>1</sub> decline was greater in patients with predominantly proliferative,

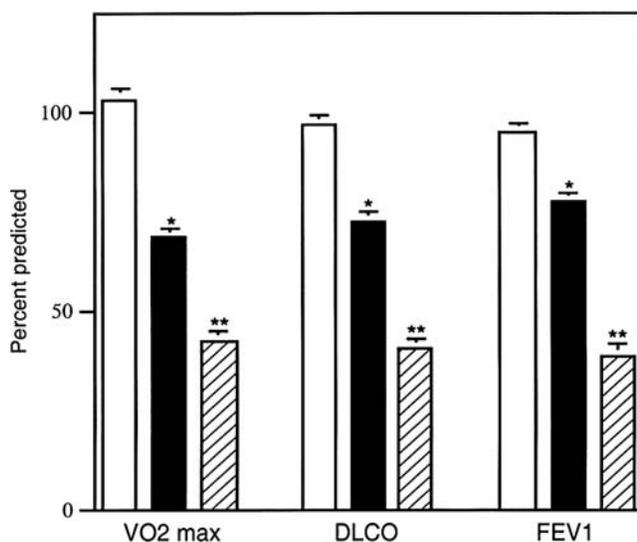


FIG. 4. Relationship between VO<sub>2</sub> max, FEV<sub>1</sub>, and DL<sub>CO</sub> and CT grade of disease severity. Grade I, white bars; grade II, black bars; grade III, cross-hatched bars. \* $p < 0.05$ , versus grade I and III. \*\* $p < 0.01$ , versus grade I and II. (From Reference 53).

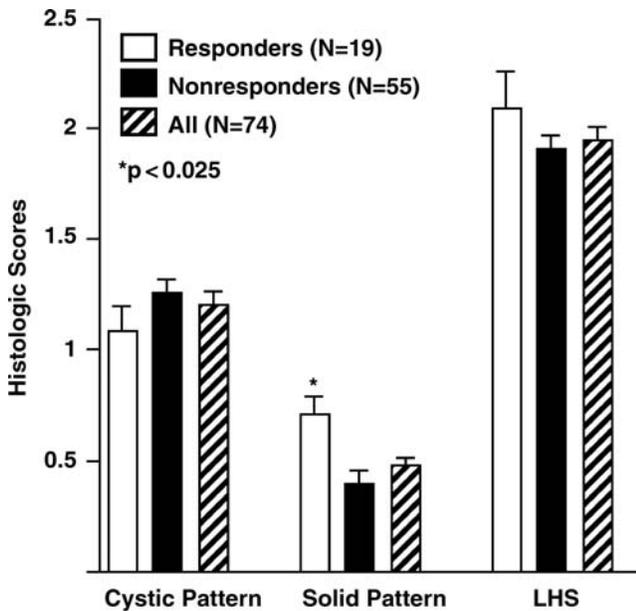


FIG. 5. Scores for predominantly cystic lesions (0–3), predominantly solid lesions (0–1), and LHS (1–3) in 19 patients who had a positive response to bronchodilators (white bars), 55 patients who did not respond to bronchodilators (black bars), and all patients combined (cross-hatched bars). The solid lesion score is significantly greater in patients who responded to bronchodilators than in patients who did not respond (From Reference 52).

“solid’ lesions’.<sup>52</sup> Patients who responded to bronchodilators tended to have greater rates of decline in FEV<sub>1</sub><sup>52,62</sup> and DL<sub>CO</sub>,<sup>63</sup> suggesting that the positive response to bronchodilators may be a marker of disease severity and rate of progression.

**Exercise Testing**

Cardiopulmonary exercise tests (CPET) in LAM are frequently abnormal,<sup>53,59</sup> which may be caused by skeletal muscle fatigue, impaired gas exchange, ventilatory limitation, and cardiovascular dysfunction. Although in general, DL<sub>CO</sub> and FEV<sub>1</sub> can reasonably predict peak oxygen uptake (VO<sub>2</sub> max), a decline in VO<sub>2</sub> max cannot be fully explained by lung function abnormalities. Specifically, exercise-induced hypoxemia may occur in the presence of near-normal DL<sub>CO</sub> and FEV<sub>1</sub>.<sup>53</sup> Exercise-induced pulmonary hypertension may also contribute to exercise-induced hypoxemia (Fig. 6).<sup>63</sup> The best functional predictor of exercise-induced hypoxemia is DL<sub>CO</sub><sup>53</sup> (Fig. 7). This may reflect a predominance of cystic lesions that are likely to impair gas exchange more than air flow.

A correlation was observed between low VO<sub>2</sub> max and worse LHS scores in LAM patients (Fig. 8).<sup>53</sup> Since LHS is a predictor of death and time to transplantation, VO<sub>2</sub>max may also be useful as a predictor of survival of LAM patients. Good correlation between a computed tomography severity index, assessed by a quantitative method, and A-a/O<sub>2</sub> gradient, dead space/tidal volume ratio, and VO<sub>2</sub>max, has also been reported.<sup>57</sup> Similarly, other investigators found good correlation between quantitative scores of cystic lesions obtained from high resolution lung computed tomography scans and

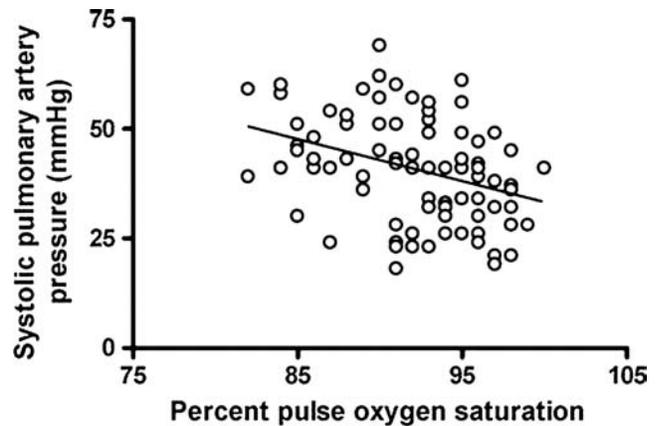


FIG. 6. Relationship between systolic pulmonary artery pressure (PAP) and pulse oxygen saturation (SaO<sub>2</sub>) at peak exercise in 95 patients with LAM (From Reference 63).

the 6-minute walk test (6MWT).<sup>58</sup> Because of the occurrence of exercise-induced hypoxemia in patients with mildly impaired lung function, CPET or other exercise tests such as the 6MWT may be important measures of LAM lung disease severity.<sup>53,58</sup> The 6MWT is a simple, low-cost test and its validity as a measure of functional capacity has been well demonstrated in patients with interstitial lung diseases.<sup>64,65</sup>

**Factors Associated with Severity of Disease. Mortality, Morbidity, and Progression of Disease**

*Age and menopause*

Most LAM patients are diagnosed in the fourth decade of life.<sup>9</sup> Since, however, the average time between the onset of LAM-related symptoms and the definitive diagnosis is 3–6 years,<sup>9,44,52</sup> it is likely that some patients develop the disease during the second or third decade of life, perhaps even during adolescence.<sup>66</sup> Based on a history of pneumothorax or angiomyolipoma preceding the LAM diagnosis, it is also

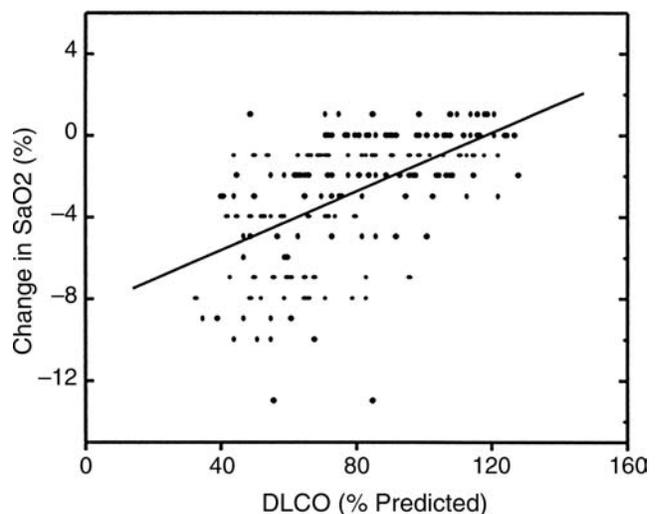
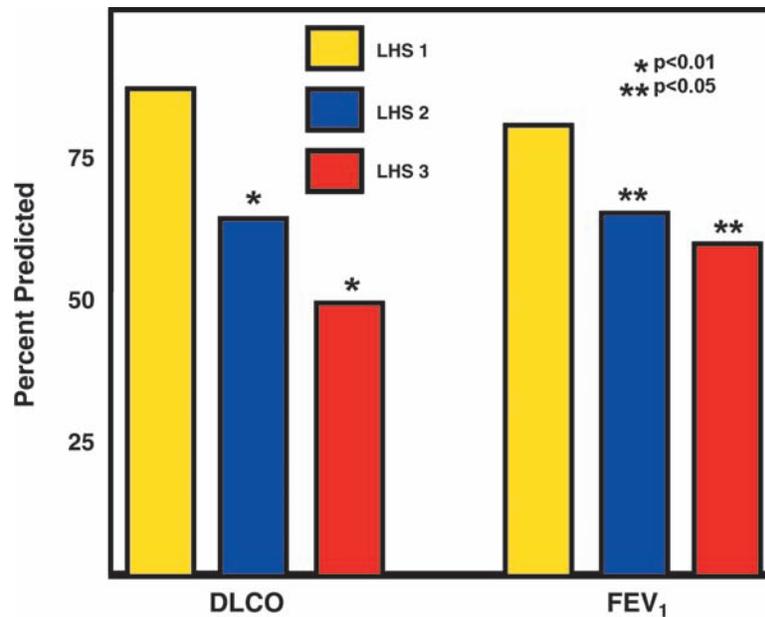


FIG. 7. Correlation ( $n = 217, r = 0.674, p < 0.0001$ ) between percent-predicted lung diffusion capacity (DL<sub>CO</sub>) and change in percent arterial oxygen saturation at peak exercise (From Reference 53).



**FIG. 8.** Relationship between LHS and lung function at the time of biopsy. DL<sub>CO</sub> was lower for patients with LHS2 (blue) or LHS3 (red) than those with LHS1 (yellow). Patients with LHS2 (blue) or LHS3 (red) also have lower FEV<sub>1</sub> than patients with an LHS1. \* $p < 0.01$ , by ANOVA, \*\* $p < 0.05$ , by ANOVA (From Reference 52).

possible that patients diagnosed in the fifth and sixth decades may have had slowly progressing disease for several decades. Indeed, there is some evidence that older age and/or menopause are associated with milder disease and slower progression.<sup>52</sup> In a retrospective study, it was found that older patients tended to have lower initial DL<sub>CO</sub> and lower rates of FEV<sub>1</sub> decline. Whether this could be attributed to age, menopause, or a longer duration of disease, could not be ascertained.<sup>52</sup>

#### Race and ethnicity

There is no evidence that LAM is more frequent or widespread among any race or ethnic group. LAM has been reported from most countries in Western Europe, Southeast Asia, Japan, and China.<sup>1,5,6,9,16,37,38,58,61</sup> Further, there is no evidence that the clinical course of the disease in those countries differs from that in North America.

#### Tuberous sclerosis complex

Overall, lung disease tends to be milder in patients with TSC than is seen in the sporadic LAM population, often only comprising a few cysts scattered throughout the lungs.<sup>9,11-13,31</sup> These patients may have subclinical disease and are only diagnosed because they have TSC. Patients who present with symptoms of LAM and are later shown to have TSC, may have more severe lung disease. In TSC, however, it is possible to determine the potential at risk population. In sporadic LAM, it is more difficult to estimate the prevalence of subclinical LAM.

#### Clinical presentation

LAM most frequently presents with dyspnea or the occurrence of a pneumothorax, chylous pleural effusion, or intra-abdominal hemorrhage.<sup>9</sup> Patients who present with

breathlessness and hemoptysis, in general, have more severe disease than those with a history of recurrent pneumothorax.<sup>51,67-69</sup> This could be due to a delay in diagnosis due to a more insidious course in patients who present with dyspnea. Alternatively, it has been proposed that those who present with dyspnea and those with pneumothorax are two clinically distinct populations. Indeed, patients who present with dyspnea, have a lower FEV<sub>1</sub> and DL<sub>CO</sub>, and higher mortality than those presenting with pneumothorax.<sup>51,61</sup>

#### Lymphangioliomyomas

Lymphangioliomyomas occur more frequently in patients with sporadic LAM (29%) than in those with LAM/TSC (9%).<sup>31</sup> Patients who present with chylothorax, with or without ascites, thoraco-abdominal lymphadenopathy, and lymphangioliomyomas may have a more severe form of disease.<sup>70</sup> There was a correlation between the extent of lymphangiogenesis, expression of VEGF-C, and LHS.<sup>71</sup> LAM tissue with greater reactivity with anti-VEGF-C antibodies also contained more VEGFR-3. Both immunoreactive VEGF-C and VEGFR-3 were associated with disease severity, assessed by LHS.<sup>71</sup> These data are consistent with a lymphangiogenic process associated with LAM via VEGF-C production. Serum VEGF-D levels correlated negatively with FEV<sub>1</sub>/FVC ratio and lung diffusion.<sup>72,73</sup> They were associated with more severe disease by CT scan, suggesting that lymphatic involvement in LAM is also associated with more severe disease.<sup>74</sup>

#### Pneumothorax

Although 57% of patients with LAM have had at least one pneumothorax, some have never had any.<sup>32,75,76</sup> Patients with pneumothorax tend to have larger cysts, and lower FEV<sub>1</sub>, perhaps because of changes in lung volumes caused by therapeutic pleurodesis.<sup>75,76</sup> In one study, patients with mild

disease by CT scan and a history of pneumothorax had greater rates of FEV<sub>1</sub> decline.<sup>75</sup> In this population, no difference in 15-year survival was observed between those with a history of pneumothorax (91.3%) and those without (92%).<sup>75</sup> In agreement with a potential role for extracellular matrix in disease, specific polymorphisms in genes for type I and III collagen and MMP-1 were associated with pneumothorax.<sup>75</sup>

### Angiomyolipomas

Neither the presence nor size of angiomyolipomas was correlated with severity of lung disease. In TSC, angiomyolipomas are frequent among both men and women, whereas women are far more likely to have pulmonary disease. Further, the prevalence of angiomyolipomas is greater in TSC-LAM than sporadic LAM, but pulmonary disease is less severe, suggesting that lung disease in LAM is not due exclusively to metastasis of LAM cells originating from those tumors.<sup>11–13,31</sup>

### Bronchodilator response

Patients with a positive response to bronchodilators tend to have more severe disease and faster declines in pulmonary function, although a cause-effect relationship has not been established.<sup>44,62</sup> It is possible that these patients have more aggressive disease due to greater cell proliferation, leading to a larger LAM cell load.

### Rate of functional decline

The rate at which lung function declines in LAM may tell us how aggressive the disease is and, in a given patient, may have prognostic significance. The yearly rate of functional decline in four LAM cohorts has been reported.<sup>52,61,77,78</sup> In one study of 36 patients, the rate of decline of FEV<sub>1</sub> was 118 ± 21 ml/year and of DL<sub>CO</sub>, 0.905 ± 0.26 ml/min/mm Hg/year.<sup>77</sup> Lazar et al.<sup>78</sup> reported an average decline in FEV<sub>1</sub> of 106 ± 35 ml/year (3.4 ± 0.8 % predicted) in 31 patients followed for approximately 5 years. In a larger study involving 275 patients followed for approximately 4 years,<sup>52</sup> the average yearly rates of decline in FEV<sub>1</sub> and DL<sub>CO</sub> were 75 ± 9 ml (1.7 ± 0.4% predicted), and 0.69 ± 0.07 ml/min/mm Hg (2.4 ± 0.4% predicted), respectively. These data show great variability in the rates of progression of disease. In some patients, LAM can be very aggressive with declines of FEV<sub>1</sub> or DL<sub>CO</sub> ranging from 5%–10% predicted per year. Hayashida et al.<sup>61</sup> found that among patients with FEV<sub>1</sub> above one liter and DL<sub>CO</sub> above 40% predicted, those presenting with dyspnea had a median change in FEV<sub>1</sub> of –285 ml (range –582.9 to –71.3 ml) per year and a median change in percent predicted DL<sub>CO</sub> of –11.9% (range –20.7% to –3.2%). In patients who presented with pneumothorax (*n* = 74), loss of function was slower. The median annual decline in FEV<sub>1</sub> was 16.7 ml (range –157.1 to +46.8) and 1.6% predicted (range –7.2% to +6.4%).

When expressed in percent-predicted values, the yearly rates of decline are adjusted for age. Consequently, any decline in percent-predicted FEV<sub>1</sub> and DL<sub>CO</sub> may be considered abnormal. However, even when rates of decline in FEV<sub>1</sub> and DL<sub>CO</sub> are expressed in absolute values, they are much greater in LAM than in normal subjects. The yearly rate of decline in FEV<sub>1</sub> in normal subjects is <30 ml/year.<sup>79</sup> The mean yearly rate of decline in DL<sub>CO</sub> in a nonsmoking female population is

0.28 to 0.29 ml/min/mm Hg.<sup>80</sup> For comparison, average rates of decline in FEV<sub>1</sub> and DL<sub>CO</sub> in patients with  $\alpha$ 1-antitrypsin deficiency were reported as 67 ± 14 ml/year and 1.07 ± 0.21 ml/min/mm Hg/year, respectively.<sup>81</sup>

Evidently, in some patients, LAM can be a very aggressive disease with declines in FEV<sub>1</sub> or DL<sub>CO</sub> ranging from 5%–10% predicted per year. However, in other patients, loss of function is slow, taking decades to reach a level sufficient to interfere with activities of daily living or require oxygen therapy. For lack of well-characterized markers of disease severity, frequent monitoring of FEV<sub>1</sub>, DL<sub>CO</sub> and 6-minute walk test are currently the best methods to assess the severity and progression of lung disease in LAM.

### Biomarkers of LAM

#### *Vascular endothelial growth factors*

Serum levels of VEGF-D, a lymphangiogenic growth factor, are higher in LAM patients than normal volunteers and are especially elevated in LAM patients with lymphatic abnormalities.<sup>72–74</sup> Further, there may be an association between the clinical phenotype characterized by metastatic lesions, circulating LAM cells, lymphatic involvement, elevated serum VEGF-D levels, and the severity of disease.<sup>72,74</sup> Measurement of VEGF-D in the serum may be of value in establishing a diagnosis of LAM in those patients with no evidence of extrapulmonary involvement or TSC, and to exclude patients with other cystic lung diseases, in which VEGF-D levels are not increased.<sup>73</sup>

#### *Matrix metalloproteinases*

Matrix metalloproteinases (MMPs) are functional components of the extracellular matrix that play an important role in lung remodeling and lymphangiogenesis.<sup>82</sup> MMPs have been also implicated in mechanisms of human cancer and metastasis<sup>83,84</sup> and are associated with LAM lesions.<sup>85,86</sup> LAM nodules contain MMP2, MMP9, MMP1, MMP activators (MT1-MMP), and inhibitors (TIMPs).<sup>87,88</sup> In particular, immunoreactivity with anti-MMP-2 antibodies is strong in LAM cells. Similarly, increased amounts of MMP-1, MMP-9, and MMP-13 have been observed in TSC skin tumors.<sup>89</sup> Levels of tissue inhibitor of metalloproteinase (TIMP)-3, which inhibits some MMPs, were low in LAM lesions, which may contribute to MMP-catalyzed proteolysis.<sup>87,89</sup> Further, serum levels of MMP-9 were higher in patients with LAM than in normal subjects,<sup>90</sup> all suggesting that excessive production of matrix metalloproteinases by LAM cells may contribute to lung destruction.<sup>84–86</sup>

Monitoring of urinary MMPs in LAM could be of value in assessing disease severity and response to therapeutic agents. Indeed, treatment of a patient with severe LAM with doxycycline, an inhibitor of MMP,<sup>91–93</sup> increased FEV<sub>1</sub> and improved gas exchange. Within 3 months of beginning therapy, urinary levels of MMPs became undetectable.<sup>94</sup> A potential role of doxycycline in the therapy of LAM is now being examined in a placebo-controlled trial in Nottingham, U.K. (Dr. Simon Johnson, personal communication).

#### *CD44v6 as a molecular determinant of metastasis*

CD44, a transmembrane glycoprotein that binds hyaluronic acid, osteopontin, and metalloproteinases, is present

in LAM lung cells and nodules.<sup>21</sup> CD44v6, which has been found in various cancers,<sup>95</sup> is localized on the LAM cell surface.<sup>21</sup> In addition, cells expressing CD44v6 had loss of heterozygosity for TSC2. Levels of osteopontin, a regulator of CD44 gene expression, alternative splicing, and function, were higher in the serum of LAM patients than healthy volunteers, although there was no correlation between plasma levels of osteopontin, or CD44v6, and severity of lung disease.<sup>21</sup> Nevertheless, CD44v6 could play a role in the pathogenesis of LAM and confer metastatic potential to the LAM cells by enabling them to adhere to the extracellular matrix, which means that this surface protein could be targeted to block a metastatic spread.<sup>21,96</sup>

### Chemokines

Metastasis of cells in specific organs may depend in part on cytokines<sup>97</sup> and the interplay of soluble factors produced in the microenvironment with receptors on the metastatic cell. Several cytokines (CCL2, CCL19, CXCL1, CXCL11, CXCL12, and CXCL16) were differentially expressed in the bronchoalveolar lavage fluid of LAM patients.<sup>22</sup> Among the LAM cell chemokine receptors, those most commonly seen microscopically in lung sections were CCR2, 7, 10, and CXCR2, 1 and 4. Of the potential chemokine receptor ligands, CCL2/MCP-1 immunoreactivity was found in LAM nodules in 70% of 30 LAM patients.<sup>22</sup> This evidence supports the hypothesis that chemokine receptors and their ligands participate in the pathogenesis of LAM.

TSC2 regulates CCL2/ MCP-1 production<sup>98</sup> and CCL2/MCP-1 selectively attracts LAM cells. MCP-1, a chemokine that stimulates angiogenesis, fibrosis, and recruitment of monocytes, was overexpressed in TSC skin angiofibroma and periungual cells.<sup>96</sup> Overexpression of MCP-1 in *Tsc2*<sup>-/-</sup> cells was mTOR-dependent and caused by loss of tuberlin function.<sup>95</sup> This effect was reduced by transfection of *Tsc2*<sup>-/-</sup> cells with human tuberlin or treatment with rapamycin. Thus, MCP-1 may be a paracrine factor contributing to TSC tumorigenesis.<sup>95</sup> These data suggest also that TSC2 downregulates CCL2/MCP-1 production.<sup>98</sup> Interestingly, CCL2 gene polymorphisms were found to be more frequent in LAM than in healthy volunteers and correlated with the rate of decline in FEV<sub>1</sub>.<sup>22</sup> Altogether, these data suggest that CCL2 may be involved in LAM cell metastasis by a paracrine feedback loop.

### Disseminated LAM cells

It has been shown that LAM cells are able to metastasize to lungs but the mechanism for their spread is not completely understood. Among the factors contributing to metastasis, it appears that estrogens and chemokines could play an important role.<sup>22,99</sup> The metastatic potential of LAM cells was recognized largely based on observations made in donor male lungs that were transplanted to female LAM patients and later were found to have been invaded by the recipient LAM cells. Consistent with a metastatic model, LAM cells were identified in blood, chylous effusions, and urine.<sup>29</sup> Thus, circulating LAM cells could be used as diagnostic biomarkers.

Since LAM cells had been defined as possessing alterations in TSC complex genes, loss of heterozygosity was investigated in cells isolated from blood by a density gradient followed by cell-based negative staining with CD45 and CD235a anti-

bodies. The cell fraction separated from blood by density gradient centrifugation from blood had TSC2 LOH in 55% of LAM patients.<sup>28</sup> In one series, 81% of LAM patients examined were informative, and, of these, greater than 90% had LOH to TSC2 (Xiong Cai and Joel Moss, personal communication). Cells in urine (79%) and chylous fluid (33%) also had TSC2 LOH. LAM cells in these fluids were not quantified, but the findings may justify the use of disseminated cells to assist in LAM diagnosis and the search for new membrane-associated markers that could facilitate identification of LAM cells.

### Renin-angiotensin system

A system comprising angiotensinogen, angiotensin II, renin, angiotensin-converting enzyme, and angiotensin I and II receptors (ATR1 and ATR2) appears to be active in LAM cells *in vivo* in their microenvironment.<sup>20</sup> Indeed, all these components were identified in LAM nodules and localized in the proliferative spindle-shaped LAM cells.<sup>20</sup> Angiotensin II could contribute to LAM cell proliferation and migration through its potential role in signaling, whereas activation of angiotensin receptors ATR1 and ATR2 could also upregulate VEGF production, leading to activation of STAT1 and STAT3 and stimulation of LAM cell growth.<sup>20</sup>

### Summary and Conclusions

LAM is an uncommon multisystem disease of women that is caused by proliferation of a neoplastic smooth muscle-like cell that exhibits metastatic properties. LAM occurs in about one-third of women with TSC and, much less commonly, in a sporadic form. It has been established that, in both cases, LAM is caused by mutations of the *TSC1* or *TSC2* genes, two tumor suppressor genes that encode, respectively, hamartin and tuberlin, resulting in dysfunction of these two major regulatory proteins, leading to unchecked proliferation of LAM cells.

The natural history and clinical phenotypes of LAM have been well described. Research is now focusing on uncovering biomarkers with potential diagnostic and prognostic value and unraveling the mechanisms regulating LAM cell proliferation and metastasis. As in the case of the inhibitor of mammalian target of rapamycin inhibitors, this research may lead to development of effective specific targeted therapies.

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