Lymphangioleiomyomatosis: New Concepts in Pathogenesis, Diagnosis, and Treatment

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Abstract

Lymphangioleiomyomatosis (LAM) is a slowly progressive lung disease that is associated with mutations in tuberous sclerosis complex genes, infiltration of the pulmonary parenchyma and lymphatics with neoplastic smooth muscle cells, extensive tissue remodeling and architectural distortion of the lung, and tumors of the chest and abdomen, including lymphangiomyomas and angiomyolipomas. LAM occurs in women in the general population and in patients of both genders with tuberous sclerosis. Overt clinical manifestations of LAM occur almost exclusively in females, however, and include progressive dyspnea on exertion, recurrent pneumothorax, and chylous effusions. The molecular basis of LAM has been extensively characterized over the past decade, resulting in the development of a targeted therapy. This article reviews emerging approaches to the diagnosis and treatment of LAM.

Keywords
- lymphangioleiomyomatosis
- lymphangiomyomas
- angiomyolipomas
- tuberous sclerosis

Lymphangioleiomyomatosis (LAM) is a slowly progressive systemic disease characterized by smooth muscle cell infiltration of the lung and lymphatics, and diffuse, cystic remodeling of the pulmonary parenchyma.[1,2] Lymphatic obstruction and infiltration can result in chylous fluid collections and fluid-filled lymphangiomyomas in the chest and abdomen.[3] Angiomyolipomas (AMLs), benign tumors composed of fat and smooth muscle, can occur in the kidney but are also found in liver or spleen. LAM occurs in up to 40% of women with tuberous sclerosis complex (TSC-LAM) and in a nontransmissable sporadic form (S-LAM) that affects ~3 to 5 women per million in the general population.[4,5] Cystic changes consistent with LAM also occur in up to 10 to 15% of men with TSC,[6,7] although there are few biopsy-documented cases, and symptomatic disease in males is rare. Only a single case of sporadic LAM in a male has been reported.[8] The average age at the time of diagnosis of LAM is ~35 years, although the disease has been described in children[9] and in the elderly.[10] LAM typically presents with progressive dyspnea on exertion, pneumothorax, and chylothorax, or as an incidental finding on computed tomographic (CT) scan of the chest or abdomen obtained for another purpose. Less common presentations include hemoptysis, chyloptysis, chylous ascites, or chyluria. Recurrences of pneumothorax are common.[11,12] Chest high-resolution CT (HRCT) scans reveal round, thin walled cysts of diameters varying from 0.2 to 2 cm diffusely distributed throughout both lungs. The diagnosis can be made clinically based on typical HRCT findings and at least one compelling corroborative feature, such as a history of tuberous sclerosis, an AML, a lymphangiomyoma or a chylothorax.[13] In the absence of these findings, a biopsy may be necessary, and video-assisted thoracoscopic surgery is the most common approach. At a microscopic level, nodular foci of smooth muscle cell infiltration are found adjacent to areas of cystic change.[14,15] Smooth muscle cells of two morphologies populate the LAM foci; epithelioid cells and spindled cells. Both cell types have immunohistochemical staining patterns of normal smooth muscle cells including positive staining for alpha smooth muscle actin, desmin, and vimentin. Subpopulations of

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these LAM cells, most prominently the epitheliod cells, also stain for HMB-45, an antibody that recognizes a protein in the melanogenesis pathway. Immunohistochemical evidence of expression of estrogen and/progesterone receptors is also often present.[16,17] Antiestrogen treatment strategies have been commonly employed in the past, including oophorectomy,[18] high-dose progesterone,[19–21] and GnRh agonists,[22–24] but there have been no controlled trials with hormonal agents, and the risks and benefits of these approaches remain incompletely defined. Advances in the understanding of the molecular basis of LAM have identified new targets for therapy, and a recent clinical trial demonstrated that inhibition of the cellular signaling pathway that is dysregulated in LAM with sirolimus results in stabilization of lung function.

There have been several recent reviews of the clinical aspects of LAM, and the reader is referred to those works for a comprehensive perspective on the disease.[2,25,26] This article focuses exclusively on newer concepts in the pathogenesis, diagnosis, management, and treatment of LAM.

Molecular and Cellular Insights

Constitutive Activation of the mTOR Pathway Is the Primary Molecular Defect in LAM

The genetic link between LAM and tuberous sclerosis has greatly accelerated the pace of discovery in the disease. The tuberous sclerosis genes were cloned in 1994[27] and 1997.[28] The domain structure of the encoded proteins, hamartin and tuberin, revealed few initial clues about their function. The only true "domain" was a GTP (guanosine 5′ triphosphate)-ase activating protein (GAP) region, which bore homology to a rap1GAP. Subsequent studies demonstrated that tuberin and hamartin form a complex which inhibits cell growth, but the mechanism of this activity was unknown. About that time, in 1999, a laboratory in San Francisco reported that the *Drosophila* homologue for tuberin controlled cell size in the wings and eyes of the insect.[29] Using genetic techniques, fly biologists in several laboratories were able to position the tuberin/hamartin complex downstream of multiple growth factor receptors and other pathways that control growth, nutrient utilization, and energy state within the cell, including Akt and PI3K in the mTOR signaling pathway.[30–32] A signaling molecule called Ras homologue enriched in brain (Rheb), was found to operate downstream of the tuberin/hamartin complex to drive mTORC1 activation.[33–35] The GAP domain of TSC2 maintains Rheb in an inactive state. When tuberin or hamartin function is defective or deficient, Rheb and, in turn, mTOR, remain constitutively activated. Activated mTOR drives protein translation through effectors S6K and 4EBP1, and also controls aspects of cell migration, survival, angiogenesis, and autophagy. The demonstration by Goncharova et al that LAM tissue expresses high levels of phosphorylated S6 revealed that LAM is, in essence, a disease of inappropriate, constitutive Rheb and mTOR activation.[36]

Sirolimus, which is sold under the trade name Rapamune or Rapamycin (Pfizer, Bedminster, NJ), binds specifically to FKBP12 to form a complex that directly inhibits mTOR. Preclinical studies in tuberous sclerosis rodent models revealed that sirolimus caused reduction in cell size, and apoptosis and regression of tumors in the kidney and the liver.[37–40] The growth of early passage cells isolated from explanted human lungs was suppressed by sirolimus.[36] In an open-label pilot study of sirolimus therapy in 24 patients with AMLs due to either TSC or LAM, tumor volume decreased by half over the course of 1 year on treatment.[41] Surprisingly, in the 11 patients with LAM in the trial, lung function improved by approximately 7% based on change in forced expiratory volume in 1 second (FEV_1). Although most kidney tumor volumes increased to near baseline levels over the year while the drug was held, lung function benefits were more durable, and both FEV_1 and forced vital capacity (FVC) remained significantly above baseline at 24 months. There were several adverse events, including six hospitalizations. Two subsequent AML trials with mTOR inhibitors have shown similar results,[42,43] and everolimus is now U.S. Food and Drug Administration (FDA)-approved for the treatment of AMLs, as well as for subependymal giant cell astrocytomas.[40]

LAM is a Low-Grade Metastatic Neoplasm Due to Mutations in TSC Genes

S-LAM and TSC-LAM were first described in 1918[44] and 1937,[45] respectively, and have historically been classified in pulmonary textbooks and clinically managed as nonneoplastic interstitial lung diseases. Despite accumulating data that LAM exhibits the genetic properties and clinical behavior of a low-grade cancer, the pulmonary field has been slow to adopt this view. The pathology community, however, came to this conclusion several years ago. The World Health Organization classified LAM as "tumor-like" in 1999, and as a "low-grade malignant neoplasm" in 2004.[46,47] The National Cancer Institute Web site lists LAM as a "lung soft tissue neoplasm," in the same category as lung sarcomas, chondromas, and leiomyomas.

Tuberous sclerosis is a classic tumor suppressor syndrome, meaning that the proteins encoded by TSC genes function to regulate cell growth and proliferation. Patients with tuberous sclerosis harbor a germ line mutation (ie, present in every cell in the body) in either TSC1 or TSC2, which in approximately one third of cases is inherited from a parent and in two thirds of cases occurs postconception. For this reason, only a minority of patients with tuberous sclerosis have a history of an affected family member. In a patient with TSC, tumors form in the various organs when a second random mutation occurs in a somatic tissue, through the typical, spontaneous DNA damage/DNA repair process that naturally occurs in all organisms. The tumors and dysplasias that occur in patients with TSC are typically benign but rarely can be malignant, such as in the cases of renal cell carcinomas and malignant perivascular endothelial cell tumors (PEComas).

Although sporadic LAM patients do not have germ line mutations in TSC, the lesions in affected lymph nodes, AMLs, and lung tissue contain classic bi-allelic ("two-hit") mutations in the TSC2 gene. These data support a model in which both the primary and the secondary TSC2 mutations occur in somatic tissues. It is difficult to completely exclude the possibility of low-level somatic mosaicism (in which an initial TSC mutation occurs during embryogenesis, resulting in a

only a fraction of cells being affected) as a mechanism for the "first hit" toward tumor formation in sporadic LAM.

In the small number of patients who have been tested, the mutations found in the kidney and lung tumors of patients with S-LAM are identical. Furthermore, genetic techniques have shown the recurrent LAM lesions in the donor lung of patients who have undergone lung transplant arise from the recipient. Collectively, these results indicate that the cells which populate the lung, kidney, and lymph nodes in LAM arise from a common precursor, and that LAM is metastatic.

The source of LAM cell metastasis remains a mystery. Although some investigators have speculated that AMLs may be a source, not all sporadic LAM patients have a renal AML.[48] CT scans of the abdomen often show evidence of pelvic and retroperitoneal adenopathy, and autopsy studies have shown extensive involvement of the thoracic duct,[49–51] suggesting the lymphatic system may be a possible source in addition to its more obvious role as a key conduit for the spread of the disease. There have been many case reports of LAM discovered in the uterus, and more recently a small but systematic analysis demonstrated LAM lesions in 9/10 uteri from patients with TSC-LAM and S-LAM.[52] The finding that LAM cells in the lung express smooth muscle markers and estrogen/progesterone receptors, and that symptoms in LAM patients often vary during the menstrual cycle, is also circumstantial evidence supporting a uterine origin.

The fact that LAM cells harbor growth-promoting gene mutations and metastasize is prima facie molecular and cellular evidence that it is a malignant neoplasm. The cancer definition also fits from a clinical behavior standpoint, in that LAM cells invade and destroy the lung by inducing or orchestrating matrix degradation and remodeling. Additional evidence has emerged which demonstrates that LAM cells exhibit many other properties of cancer cells. These include mechanisms to enhance cellular survival through inhibition of apoptosis (programmed cell death),[53] suppression of autophagy (a cellular process in which intracellular organelles are cannibalized to provide nutrients and substrate for biosynthetic pathways),[54] overexpression of telomerase (which enhances immortality),[55] and feedback inhibition of PI3K and other upstream kinases (which restrains unchecked cellular proliferation and protein translation, and probably explains why LAM is not more aggressive).[56,57] LAM cells also upregulate the expression of cell cycle mediators cyclin D1, p27,[58] and PLK1[59] to promote cellular proliferation, and the β-catenin[60,61] and MMP-7 mediators that promote cell invasiveness. Finally, LAM cells preferentially utilize glycolysis for energy production, even under aerobic conditions that would support the much more efficient process oxidative phosphorylation. This "Warburg physiology" is an incompletely understood but universal phenomenon in cancer cells, which most likely facilitates biosynthetic programs (such as the glycolysis and the pentose phosphate pathway) that generate substrates for macromolecular synthesis.

Lymphangiogenesis Plays a Central Role in the Pathogenesis of LAM

One of the most exciting developments in LAM science has been the recognition of the central role that lymphangiogenesis plays in the disease. It has been clear for some time that LAM invades and spreads through the axial lymphatics in the abdomen, retroperitoneum, and pelvis, and occasionally in the chest. LAM infiltration of the supraclavicular lymph nodes has been reported on a few occasions, but involvement of peripheral lymph nodes is exceedingly rare. The reason for the remarkably restricted distribution of lymphatic lesions in LAM is not clear. One possible explanation, however, is that target lymph nodes must be conditioned by factors expressed in the primary LAM lesions arising in abdominal or pelvic viscera before metastasis occurs, as has been reported for other neoplasms. Because the blind-ended source of the lymphatic tree is in the periphery and flow occurs in only one direction toward the neck, only lymph nodes and lymphatic structures downstream of the source are vulnerable. In 2004, Kumasaka et al reported that LAM lesions express the lymphangiogenic growth factors, vascular endothelial growth factor C (VEGF-C) and VEGF-D, and contain abundant cleftlike spaces that are lined with lymphatic endothelial cells which express the receptor for these ligands, VEGFR3, as well as the LYVE-1 and podoplanin (D2–40).[50] After examining pathological and autopsy specimens, the authors concluded that lymphangiogenic remodeling demarcates LAM lesions in the uterus, lymphatics, and other locations into endothelial-lined islands, which are liberated into the lymphatic lumen.[51] These LAM cell clusters (LCCs) are spherical collections of LAM cells enveloped by a single layer of lymphatic endothelial cells (►**Fig. 1**). They have been found in lymphatics within LAM lesions in the uterus and lung, in the lumen of the lymphatic duct, and in chylous fluid collections in the chest and abdomen. Lymphatic flow propels the LCCs cephalad in the thoracic duct to the junction of the lymphatic system with the internal jugular vein in the neck. Upon gaining access to the venous circulation, the LCCs are distributed to the pulmonary capillary bed where they presumably become impacted. Long dwell times may facilitate infiltration of the pulmonary interstitium, perhaps by a mechanism that is similar to the "invasion-independent" metastasis that has been reported in renal cancer. The lymphatic channel–centric distribution of LAM cells may suggest that they

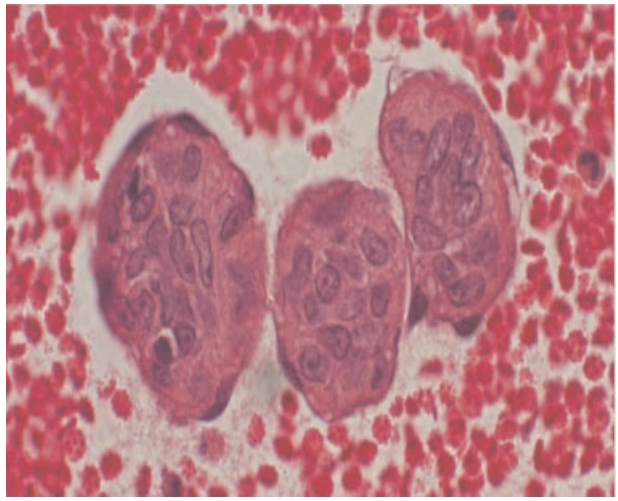

Figure 1 Cytological diagnosis of lymphangioleiomyomatosis (LAM). LAM cell cluster in pleural fluid cell block isolated from the chylous effusion of a patient with LAM.

find their way to spaces where they can remain in close proximity to lymphatic endothelial cells. Perhaps the abundant lymphatic vascular bed and expression of VEGF-D and VEGFR-3 in the lung explain the overall trophism of LAM for the lung. Although the mechanism of cyst formation is not clear, one hypothesis for the extensive remodeling in LAM is that it represents a form of "frustrated lymphangiogenesis" in which VEGF-C and VEGF-D drive a program of inappropriate expression of matrix-degrading proteases that are naturally associated with lymphatic vessel formation during development. Candidates for remodeling proteases with support in the literature include MMP-2, MMP-9,[62–64] and Cathepsin K,[64] among others

Although the molecular signaling pathways involved have not been completely worked out, it is very likely that VEGF-C and VEGF-D expression is driven by activation of HIF pathways, which in turn are driven by constitutive activation of mTOR. The expression of the lymphangiogenic growth factors, or least VEGF-D, is clearly cell-type specific because not all patients with TSC have elevated serum VEGF-D. In fact, in women with TSC, only those with LAM tend to have elevated VEGF-D levels.

There are some missing pieces to the puzzle. No pathologist has described tumor emboli impacted in the pulmonary vascular bed on biopsy specimens or at autopsy. It is not clear why the cysts are diffusely distributed rather than basilar as is the case with most metastatic processes.

Approach to Screening and Diagnosis

Screening

General Population

The diagnosis of LAM is often appropriately considered in young women who present with chylothorax or recurrent pneumothorax. However, exclusively dyspneic presentations are often mistaken for asthma or COPD despite the absence of a history of tobacco use or exacerbations and remissions that are characteristic of these more common obstructive lung diseases. The diagnosis in these patients is typically delayed for 3 to 5 years, and the average age at diagnosis is ~35 years.

Screening of Patients with Pneumothorax

It is unfortunate that the diagnosis of LAM is also often missed in women who present with a first pneumothorax. In the United States, the average number of pneumothoraces prior to diagnosis is 2.2.[11,12] In Japan and other countries where CT scans are routinely used for investigation of a first pneumothorax, LAM is diagnosed at an earlier age.[65] It is likely that the first pneumothorax due to LAM in the United States and United Kingdom is often mistaken for primary spontaneous pneumothorax (PSP), which is a much more frequent cause of pneumothorax. CT scanning is not particularly helpful in the management of PSP, so in US emergency departments it is not typically performed in patients who present with a first pneumothorax. However, in the more narrowly defined demographic of 25- to 54-year-old nonsmoking women,[66] the prevalence of LAM is estimated to be ~5%. Hagaman et al found that CT screening for pneumothorax in this group is likely to be cost-effective insofar as complications of future pneumothoraces could be limited by early pleurodesis when patients are discovered to have LAM instead of PSP.[67] Although not evaluated in that study, the added radiation risk of more aggressive screening in the population could be reduced by employing low-dose CT protocols such as those currently being developed for lung cancer screening. Identifying patients with LAM has assumed enhanced importance in the past few years because a therapy capable of stabilizing the disease has become available.[68]

Screening of Patients with Angiomyolipomas

Ryu et al reported that ~11% of patients with a sporadic AML on abdominal and chest CTs had cystic changes in the lung.[69] Screening with an HRCT scan of the chest should be considered in this population, and for those with bilateral renal AMLs, screening for TSC should also be performed.

Screening of Patients with Incidental Cystic Changes on CT Scans Obtained for Other Purposes

LAM can be discovered incidentally on CT scans of the abdomen or chest obtained for unrelated reasons, such as abdominal pain, suspicion of pulmonary embolism, or evaluation of coronary calcifications. The natural history of LAM discovered at presymptomatic stages in this manner is poorly understood but is likely to be much more favorable than that of LAM ascertained through such symptoms as shortness of breath or exercise limitation.

Screening of Patients with Tuberous Sclerosis

LAM has been known to occur in women with tuberous sclerosis complex. In the early 2000s, three groups reported that cystic changes consistent with LAM were ~10- to 15-fold more common in women with tuberous sclerosis than had been previously appreciated, occurring in up to 34% of women in a cross-sectional screen.[70–72] Recently, evidence has surfaced that cystic change also occurs in up to 10 to 15% of men with TSC.[6,7] The Tuberous Sclerosis Alliance has recommended that HRCT screening for LAM be completed in females at least once after the age of maturity.[73] The European Respiratory Society has recommended that, in addition, a scan be repeated at age 30.[13]

There are several challenges to establishing the diagnosis of LAM in patients with TSC. Biopsy is done infrequently in either gender in these populations, except when completed before the diagnosis of TSC is made, or by physicians who are uninformed about the strong association between LAM and TSC. It is not usually possible, therefore, to definitively exclude the other known disease processes that can be associated with cyst formation, such as emphysema (with or without α one antitrypsin deficiency), or connective tissue disease. It is also possible that TSC can result in cystic change by mechanisms that do not include smooth muscle cell infiltration, as occurs in Birt-Hogg-Dubé (BHD) syndrome.[74] We are aware of at least one case in which a male with TSC underwent biopsy for cystic changes in the lung, and no diagnostic features of LAM were found. There is also no consensus about how many cysts are required to constitute a diagnosis of LAM because a

few cysts are occasionally encountered on CT scans in normal patients. One group recently proposed that four cysts is a reasonable cutoff because up to three cysts can be found on CT as a normal variant in healthy populations.[69] The structural characteristics and distribution of cysts are also important, as will be outlined here.[75] Young et al reported that serum VEGF-D was elevated in patients with TSC and cystic change on CT, but not in women with TSC and a normal chest CT.[76,77] These data suggest that serum VEGF-D may be useful as a screening test in LAM and perhaps reduce the burden of radiation exposure in these patients, but prospective studies are needed to answer this important question definitively.

Differential Diagnosis

In patients referred with multicystic lung disease for suspicion of LAM, the most common diagnoses in the differential are emphysema with or without α one antitrypsin deficiency, follicular bronchiolitis with or without lymphocytic interstitial pneumonitis (which can be idiopathic or due to connective tissue disease such as Sjögrens lupus, or other connective tissue disease), pulmonary Langerhans cell histiocytosis, or BHD syndrome.[25] Less common LAM mimics include cystic change related to prior infection or barotrauma, light chain deposition disease, hyperimmunoglobulin E (hyper-IgE) syndrome, or recurrent papillomatosis.

Diagnosis

The first consideration when one is confronted with a patient with cystic lung disease of unknown etiology is deciding whether a definitive diagnosis is essential. For many of the diagnoses in the LAM differential, there are no known disease-course-altering interventions beyond common sense recommendations, such as discontinuation of smoking or avoidance of environmental irritants, that would justify an aggressive approach. For others with available interventions, such as α-1 antitrypsin deficiency and LAM, therapeutic intervention may not be indicated at early stages of the disease. For instance, one might consider recommending HRCT and pulmonary function follow-up in 12 months rather than a biopsy in patients with only a few cysts on high-resolution CT. At the other end of the spectrum, for patients with extensive, end-stage disease who are approaching lung transplantation, obtaining a definitive diagnosis may be risky and unnecessary. The decision of whether to pursue a definitive diagnosis should be made jointly with the patient, after a thorough discussion of the risks and benefits. In all cases, the diagnostic evaluation should be tailored to obtain the most definitive possible result in the most noninvasive manner possible.

Noninvasive diagnostic measures should include a careful history including tobacco use, sicca complex and connective tissue disease symptoms, physical exam findings to include a search for skin lesions consistent with tuberous sclerosis or BHD syndrome, and serologies including RA (rheumatoid antigen), ANA (anti-nuclear antibody), anti-CCP (cyclic citrullinated peptide), SSA and SSB antibodies and serum level of α-1 antitrypsin to exclude connective tissue disease.

LAM cysts vary from 2 mm to a few centimeters in size and are very thin walled and relatively uniform in appearance.[75] Other abnormalities that can be noted on HRCT of LAM patients and can be a clue to the diagnosis include retrocrural lymphadenopathy (26%), pleural effusion (14%), dilated thoracic duct (11%), pericardial effusion (6%), and pneumothorax (6%). Mediastinal and hilar lymphadenopathy are unusual in LAM. Ground-glass opacities on HRCT are relatively uncommon in LAM and may represent pulmonary hemorrhage or lymphatic congestion. LAM cysts rarely contain internal structure. The dilated airspaces in emphysema contain imperceptible walls, and the cysts in pulmonary Langerhans cell histiocytosis (PLCH) are thicker walled, more bizarrely shaped, and spare the cardiophrenic angles.[78] The cysts in BHD are usually basilar or perivascular, subpleural, and crescent shaped or elliptical.[74,79–82]

HMB-45, an antibody that recognizes an enzyme in the melanogenesis pathway, is quite specific for LAM and is very useful diagnostically. Staining can be sparse and variable, however, and cases of LAM that are HMB-45 negative despite adequate tissue, while rare, do occur. Other markers that frequently stain positive in LAM tissues include those associated with the smooth muscle lineage (smooth muscle actin, desmin, vimentin), hormone receptors [estrogen receptor (ER), progesterone receptor (PR)], and markers of lymphangiogenesis (podoplanin or D2–40,VEGFR-3).[14,15]

There are several approaches to the diagnosis of LAM that can be considered: (1) clinical diagnosis based on European Respiratory consensus criteria, (2) serologic diagnosis using VEGF-D, (3) cytologic diagnosis based on analysis of pleural or ascitic fluid, (4) low tissue volume pathological diagnosis based on needle biopsy of axial lymph node or transbronchial biopsy, and (5) large tissue volume pathological diagnosis based on video-assisted thoracoscopic biopsy.

Based on the recently developed European Respiratory Guidelines, a confident clinical diagnosis of LAM can be made in patients who present with a compatible HRCT pattern and either AMLs, TSC, or chylous effusion.[13] The diagnosis of AML can be made radiographically, based on the presence of fat density, so an abdominal CT (without contrast) or magnetic resonance imaging (MRI) can be helpful in the 30% or so of sporadic LAM patients who have AMLs. It is important to exercise judgment in the application of these criteria because misdiagnoses are possible with the use of clinical grounds alone. For instance, AMLs can occur sporadically, in patients with BHD syndrome[74,83] or MEN (multiple endocrine neoplasia) 2. Cystic pulmonary changes and chylothorax can occur in patients who have lymphoma. Patients with TSC may have other conditions that can result in cystic pulmonary disease, such as emphysema, follicular bronchiolitis or Langerhans cell histiocytosis. Serological, cytological, or pathological confirmation may still be required in cases where doubt remains, especially if therapy with toxic therapies such as mTOR inhibitors is being considered.

Serological Diagnosis

Recent reports suggest that a serological diagnosis can be made using the level of serum VEGF-D. In 2006, Seyama et al reported that serum VEGF-D, but not VEGF-A or VEGF-C, was elevated in the serum of patients with LAM.[84] In the Japanese cohort,

VEGF-D correlated with several markers of disease severity, including diffusing capacity for carbon monoxide (DL_{CO}), and FEV_1:FVC, but not FEV_1, residual volume (RV), or total lung capacity (TLC). Glasgow et al subsequently reported an inverse relationship between DL_{CO} and VEGF-D levels in the subset of patients with lymphatic involvement.[85] In a cross-sectional study using serum samples and data collected from patients referred to the University of Cincinnati and a variety of remote sources, Young et al reported that serum VEGF-D levels are elevated in a majority of women with LAM but normal in women with other causes of cystic lung disease, including PLCH, emphysema, connective tissue disease–associated follicular bronchiolitis or lymphoid interstitial pneumonia, and BHD syndrome.[76,77] A serum level of 800 pg/mL distinguished LAM from other mimics that present with thin-walled cysts on CT, with a sensitivity of 73% and specificity of 100%; at a cutoff of 600 pg/mL, the diagnostic sensitivity and specificity of serum VEGF-D were 83.9% and 97.6%, respectively. No significant association between VEGF-D levels and age, FEV_1, or the use of supplemental oxygen was found in that study. Other groups have also found that serum VEGF-D levels have diagnostic utility.

Cytological Diagnosis

Cytological analysis of pleural effusion fluid or ascites fluid can also lead to the diagnosis of LAM[86–90](►**Fig. 1**). LAM cell clusters are present in the pleural or ascitic fluid of the 15% of patients who present in this manner, or in the 22 to 39% of patients who develop fluid collections over the course of disease. Cytological analysis and immunocytochemical staining of cells isolated by centrifugation reveal spindle-shaped and epithelioid cells that react with antibodies to α smooth muscle actin, and HMB45.[91,92] The diagnosis of LAM can occasionally be made based on the laparoscopic or percutaneous needle biopsy of pelvic mass, or retroperitoneal or axial abdominal lymph nodes. Frequently, the biopsy is initially read as mistargeted and nondiagnostic by operators expecting to find lymphoma or ovarian cancer when the report returns

Pathological Diagnosis

Transbronchial Biopsy

Transbronchial biopsy (TBB) is a potentially useful diagnostic technique in patients with LAM (►**Figs. 2** and **3**). There are 21 reports containing 100 cases of LAM diagnosed by TBB in the literature[18,93–96](►**Table 1**). Small numbers and incomplete reporting of complications make it difficult to draw definitive conclusions about the yield and safety of the procedure in patients with LAM.

However, the theme that emerges from review of the available studies is that the diagnosis can be made in substantial proportion of patients with the assistance of an expert pathologist using this technique (►**Fig. 4**). Naalsund et al reported a case series of eight patients with pulmonary LAM (PLAM).[94] Transbronchial lung biopsy was conclusive in four of five patients who underwent the procedure. In two of the eight cases, review of the TBB slides revealed that the diagnosis was evident. In another series of five patients with LAM, the atypical smooth muscle cells obtained by TBB were initially misinterpreted as fibrocytes. When the biopsies were reviewed by the expert LAM pathologist, three out of four were found to be "diagnostic for LAM" and the fourth one was interpreted as "suggestive of LAM."[18] Torre and Harari

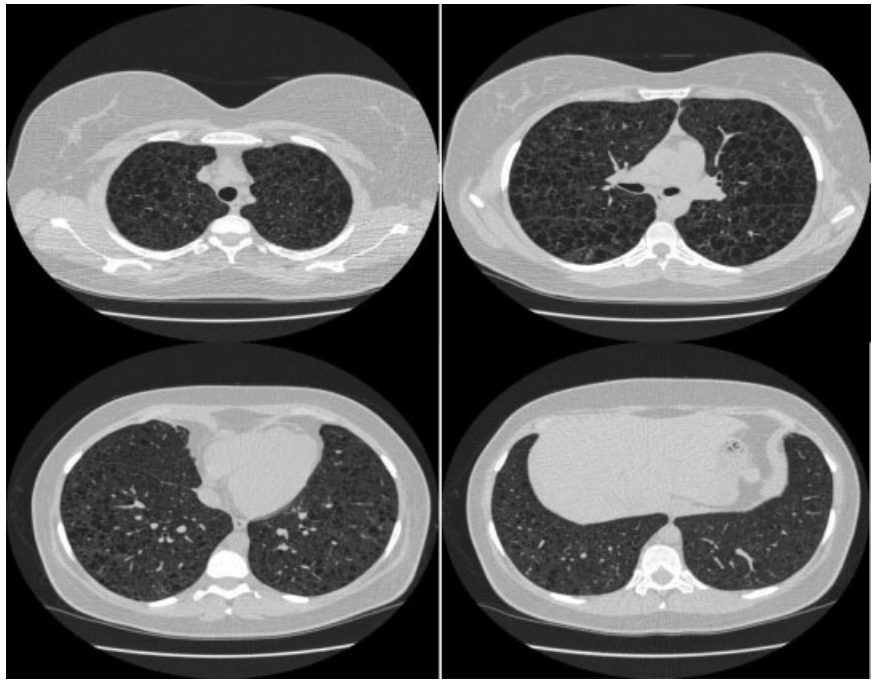

Figure 2 Transbronchial biopsy–based diagnosis of lymphangioleiomyomatosis (LAM) radiology. High-resolution computed tomographic (CT) scan images of a patient with cystic disease who was initially diagnosed with pulmonary Langerhans cell histiocytosis based on CT interpretation by two expert chest CT radiologists. The patient was initially excluded from a clinical trial based on these opinions. Subsequent transbronchial biopsy was diagnostic for LAM, and the patient was enrolled in the trial.

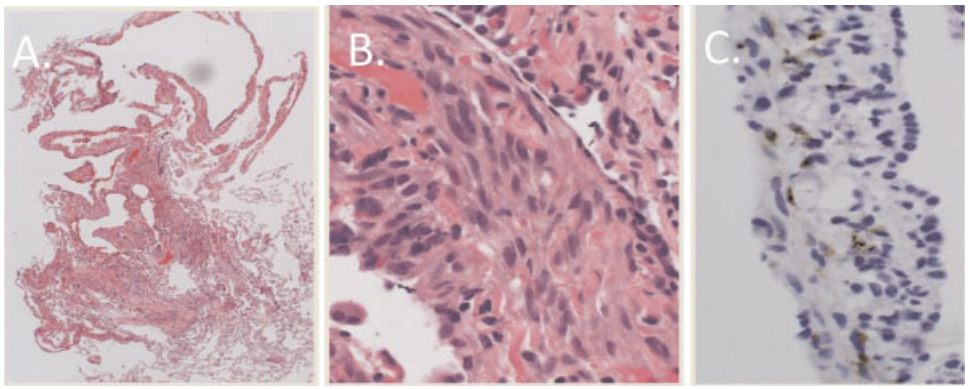

Figure 3 Transbronchial biopsy–based diagnosis of lymphangioleiomyomatosis (LAM) pathology. (A) A low power field of lung tissue obtained by transbronchial biopsy in a patient with cystic lung disease on high-resolution computed tomographic scan compatible with LAM. (B) High power field showing abnormal proliferation of smooth muscle cells or LAM cells. (C) A subpopulation of LAM cells are HMB (human melanoma black) -45 positive by immunohistochemical stain.

recently reported that six out of seven TBB specimens obtained from patients with a high clinical-radiological suspicion for LAM were diagnostic for the disease.[97] There was one pneumothorax in that study. TBB has been reported to be diagnostic in patients with reoccurrence of LAM in transplanted lungs.[98] Ye et al reported a series of 108 patients with LAM, 97 of whom were biopsy proven.[93] In 49 of the biopsied patients, pulmonary samples were obtained by TBB. The yield of TBB was not reported in that paper.

Table 1 Transbronchial Biopsy for LAM

References	No. of Patients Diagnosed with TBB
Kuwabara[114]	1
Chen[98]	1
Guinee[95]	1
Delgrange[96]	1
Yamauchi[88]	1
Naalsund[94]	4/5
Yamamoto[115]	1
Díaz Pedreira[116]	1 suggestive, confirmed by VATS biopsy
Torre[97]	6/7, no major complications
Taylor[18]	3/4 diagnostic. 1 suggestive
Urban[20]	2/6 diagnostic. 1/6 suggestive
Chu[117]	3
Kitaichi[118]	6
Høie[119]	3
Benden[120]	7
Seyama[121]	2
Lim[122]	3
Hayashida[65]	18
Ye[93]	49
Bonetti[123]	3

VATS, video-assisted thoracoscopic biopsy.

A set of sequential surveys about TBB administered to a total of 847 and 1082 LAM patients by the LAM Foundation resulted in a response rate of 25% and 35%, respectively. A total of 35/63 patients reported a positive TBB for a yield of ~55%. Histologic slides from eleven TBB cases were obtained for pathological review by one of the authors (KWB). Of these, six were diagnostic for LAM and five were nondiagnostic, consistent with a yield of 55%.

Transbronchial biopsy in experienced hands is generally considered to be a safe and well-tolerated procedure.[99,100] The two most common complications of TBB are hemorrhage and pneumothorax, each of which have been reported to occur in ~1 to 5% of patients. The overall mortality secondary to TBB ranges from 0.12 to 0.24%.[101,102] The risks of the procedure in patients with LAM and other multicystic lung diseases have not been studied, however.

Based on our literature review and a questionnaire-based survey of patients registered with the LAM Foundation, TBB may be considered as a relatively effective method for establishing the diagnosis of LAM, with a yield in the range of 33 to 87%. Additional studies will be needed to determine the safety of the procedure and the relationship between profusion of cysts on CT and the yield of biopsy. Expert pathological review of the tissue morphology and immunohistochemical staining for smooth muscle actin and HMB-45 are critical components.

Video-Assisted Thoracoscopic Biopsy

Video-assisted thoracoscopic (VATS) biopsy has a higher diagnostic yield than does TBB, but it is associated with greater potential morbidity.[103] VATS requires general anesthesia and double-lumen endotracheal tube intubation and can occasionally be associated with airway injury, prolonged air leak, persistent chest pain, or death. The sensitivity and specificity of pathological examination of tissue obtained by a properly performed VATS with adequate tissue recovery approaches 100% when expert pathological assistance is available. In one series, VATS biopsy was noted to be diagnostic in 26 of 30 LAM patients (87%), but review of the remaining four cases by an expert pathologist revealed LAM in all cases.[18]

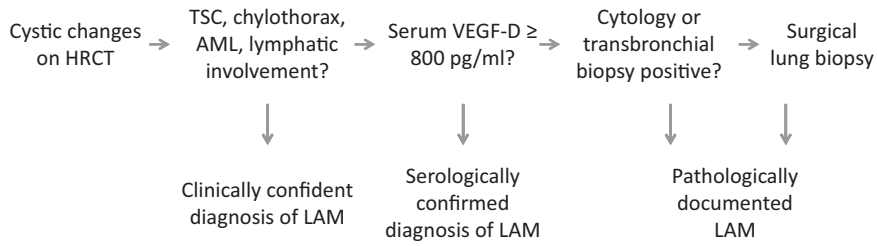

Figure 4 Proposed diagnostic algorithm. In a patient with typical cystic change on high-resolution computed tomographic scan of the lung, a clinical diagnosis of lymphangioleiomyomatosis (LAM) can be based on corroborating features such as a history of tuberous sclerosis complex (TSC), an angiomyolipoma (AML), or a chylous effusion. Most patients with LAM do not present with these clues, however. In the 70% of LAM patients with an elevated serum vascular endothelial growth factor D (VEGF-D), a value of 800 pg/mL can be used to make a serological diagnosis. If chylous fluid or lymph nodes accessible to transcutaneous needle aspiration are available, an attempt at cytological diagnosis can be made. Transbronchial biopsy also has an appreciable yield, perhaps as high as 50% or greater, in patients with diffuse disease. Biopsy obtained by video-assisted thoracoscopy remains the gold standard, with a specificity of almost 100%, albeit at the risk of greater morbidity than other procedures.

Summary

A diagnostic algorithm for the sequential use of serum VEGF-D and TBB is proposed for women in the reproductive age group who present with cystic lung disease on HRCT suggestive of LAM (►**Fig. 4**). Following the strategy, almost 70% of LAM patients can likely be diagnosed by HRCT using European Respiratory Society Guidelines or serum VEGF-D levels. The remaining 30% will require lung biopsy to make a definitive diagnosis of LAM, but perhaps half of those could be achieved by TBB. VATS may be considered as a last resort. The use of TBB to make the diagnosis of LAM in the least invasive manner possible may be especially appealing for those patients with a clinical diagnosis of LAM who are seeking a definitive diagnosis to facilitate decisions regarding long-term therapy with mTOR inhibitors or entry into clinical trials.

Approach to Management

Management of Pneumothorax

Approximately 70% of patients who participated in the NHLBI (National Heart, Lung, and Blood Institute) LAM Registry had a history of prior pneumothorax, and the average number of recurrences was 3.4.[104] This value is higher than the 2.0 recurrences (3.0 ± 2.6 pneumothoraces per patient, total) reported in Japan,[65] for reasons that are unclear. A pleurodesis procedure is recommended with the first pneumothorax, given the high rate of recurrence and associated morbidity.[11] Chemical sclerosis, mechanical abrasion, talc poudrage, and pleurectomy have all been effective in patients with LAM,[105] but the first two options are preferred because the intensity of pleural symphysis with talc or pleurectomy can complicate subsequent pulmonary transplantation. Prior pleural procedures can increase perioperative bleeding in transplant patients but do not affect candidacy or survival.[106] Indeed, in the study by Almoosa et al[11] 56% (45/81) of patients who had undergone transplant had had prior pleurodesis, bilateral in 75% (34/45). The failure rate following pleurodesis is high (on the order of 35%) for reasons that are not understood. Chyle does not generally cause pleural inflammation or fibrosis, and small chylous effusions can often be managed without intervention once the diagnosis of LAM is made. Shortness of breath may mandate drainage, however; and in some cases repeatedly. Pleurodesis may be required to prevent nutritional and lymphocyte deficiencies that can result from repeated taps or persistent drainage.

Differential Diagnosis of Progressive Dyspnea on Exertion in LAM

There are several mechanisms for worsening shortness of breath in LAM, including progressive airflow obstruction, chylous pleural effusions, diaphragmatic impingement by chylous ascites or large angiomyolipoma, tumor emboli, pulmonary hypertension, and lymphatic parenchymal congestion. The latter two etiologies are discussed.

Pulmonary Hypertension

Pulmonary hypertension should be considered in LAM patients who experience unexplained worsening exercise tolerance. Pulmonary vascular infiltration, invasion, and disruption were first reported in patients with LAM over 4 decades ago.[107] Two common presentations of LAM that suggest possible pulmonary hypertension include dyspnea out of proportion to the degree of measured airflow limitation, and reduction in diffusing capacity in the face of well preserved pulmonary mechanics. In a screen of 120 patients with LAM, eight patients were found to have resting pulmonary arterial hypertension with a mean pulmonary artery pressure of 43 ± 3 mm Hg.[108] Of 95 patients who underwent formal exercise testing, 61 (65%) experienced exercise-induced desaturation and 56 had an elevation in systolic pulmonary artery pressure to >40 mm Hg. There was a significant association between the degree of exercise-induced desaturation and the peak systolic pulmonary artery pressure. These data suggest that resting pulmonary arterial hypertension occurs but is uncommon in patients with LAM, and that exercise-induced pulmonary artery pressure elevation is common and may contribute to exercise-induced dyspnea.

A recent case series suggests that targeted treatment of pulmonary hypertension in patients with LAM can be beneficial. Cottin et al reported 20 patients with LAM who were referred from centers throughout France for pulmonary arterial hypertension.[109] The cohort had a mean pulmonary artery pressure (PAP) of 32 ± 6 mm Hg, and mean pulmonary vascular resistance (PVR) of 376 ± 184 dynes.s.cm^{-5}. Mean FEV_1 was 42 ± 25% predicted, and transfer factor was 29 ± 13% of predicted. The PAP and PVR decreased by over

20% in the six patients who received oral pulmonary arterial hypertension therapy, and symptoms improved.

Lymphatic Congestion

One of the lesser-known manifestations of LAM in the lung is lymphatic parenchymal congestion. Patients may present with a history of thoracic or abdominal lymphangiomyomas, chyloptysis, pleural effusion, and subacute or rapid clinical worsening. Often a history of pleurodesis is reported, suggesting that efforts to restrict access of chylous fluid from one space may contribute to development of chylous fluid accumulations elsewhere. Chest radiography may reveal bilateral interstitial infiltrates, pleural effusion, and diffuse cystic changes. Chest CT scan may reveal diffuse cystic disease along with interstitial and interlobular septal thickening, patchy areas of consolidation, and pleural effusions. These patterns are reminiscent of pulmonary edema and can often be confused as such. Although several cases have been reported in the literature,[110,111] Moua et al were the first to note the clinical importance of this mechanism of clinical worsening.[112] In that study, sirolimus resulted in cessation of chyloptysis, radiographic resolution, pulmonary function improvement, and withdrawal from consideration for transplant in patients with chylous manifestations. Patients with a previous history of failure to improve on sirolimus may benefit from another trial of the drug if lymphatic congestion develops.

Approach to Treatment

A recent double-blind, randomized, controlled trial demonstrated that sirolimus stabilizes lung function and improves some measures of quality of life and functional performance in patients with moderately severe LAM.[68] Serum VEGF-D declined in the treatment group and remained stable in the placebo group. There were several adverse events on the drug during the 1 year treatment period, most of them consistent with the known toxicities of mTOR inhibitors, but serious adverse events were no more common in the sirolimus group than in the placebo group. When the drug was held in the second (observation) year, lung function decline resumed at the same rate as the placebo group. These data suggest that sirolimus is an effective treatment for selected patients with LAM, but the benefits of the drug only persist while therapy continues. Additional trials will be required to determine whether the drug benefits patients with milder or more severe lung function impairment, the optimal dosing, and the benefits and risks of sustained treatment.

A recent series of 19 patients who were treated with sirolimus for an average period of 2.5 years demonstrated sirolimus resulted in regression of lymphangiomyomas and resolution of chylous effusions in 11/11 patients with these disease manifestations.[110] A German study of patients referred for lung transplantation showed reversal of rapidly declining lung function in 10 subjects who had a mean improvement in FEV_1 of 345 ± 58 mL after 6 months on therapy.[113] Three patients discontinued use of the drug due to side effects, however.

Summary

Taken together, the data suggest that patients with symptomatic LAM or chylous complications should be considered for therapy. The major side effects of mTOR inhibitor treatment include mucositis, elevation in serum cholesterol, bone marrow suppression, lymphedema, and acneiform rash. More serious potential complications include drug-induced pneumonitis and latent malignancy. Whether the risk/benefit ratio favors treating patients with progressive LAM who have normal lung function or who are asymptomatic is not yet clear.

Future Directions

The discovery of an effective therapy for LAM was the direct result of the remarkable advances in our understanding of the genetic, molecular, and cellular basis of LAM that have occurred over the past decade. Continuing progress in these areas is essential for the design of future trials.

Better biomarkers are also desperately needed. Lung function and exercise tolerance are suboptimal end points because of inter- and intratest variation and time required for response. Serum VEGF-D has proven to be useful for diagnosis but is not elevated in all patients. Serum VEGF-D correlates with multiple markers of disease severity and is also promising as a prognostic biomarker for disease progression and treatment response. Unbiased biomarker screens are required to identify new and better biomarkers that correlate with severity of disease and change rapidly with disease progression or treatment response. Imaging techniques that can reveal the total body burden of LAM and metabolic state of LAM cells are also very important.

The most pressing need in the development of therapies for LAM is to determine if early administration of mTOR inhibitor therapy can prevent progression of disease. Examples of other ideas for therapy that have promise based on biological plausibility or preclinical evidence include statins as inhibitors of Rheb, metformin as an alternative mTOR inhibitor, pazopanib as a lymphangiogenesis inhibitor, and hydroxychloroquine as an inhibitor of autophagy.

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