LYMPHANGIOLEIOMYOMATOSIS

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ABSTRACT

Lymphangioleiomyomatosis (LAM) is a slowly progressive, low grade, metastasizing neoplasm, associated with cellular invasion and cystic destruction of the pulmonary parenchyma. Although the source of LAM cells that infiltrate the lung is unknown, available evidence indicates that the disease spreads primarily through lymphatic channels, often involving abdominal, axial, and retroperitoneal nodes, suggestive of an origin in the pelvis. LAM cells harbor mutations in tuberous sclerosis genes and produce lymphangiogenic growth factors, which facilitate access to and movement through the lymphatic system and likely play an important role in destructive tissue remodeling in the lung. Lymphatic manifestations of LAM include thoracic duct wall invasion, lymphangiomyoma formation, chylous fluid collections in the peritoneal, pleural, and pericardial spaces, chyloptysis, chylocolporrhrea/chylometorrhea, chyle leak from the umbilicus, chylous pulmonary congestion, and lower extremity lymphedema. LAM lesions express lymphangiogenic growth factors VEGF-C and VEGF-D; growth factor receptors, VEGFR-2 and VEGFR-3; and markers LYVE-1 and podoplanin, and are laced with chaotic lymphatic channels. Serum VEGF-D is elevated in 70% of patients with LAM and is a clinically useful diagnostic and prognostic biomarker. Molecular targeted therapy with sirolimus stabilizes lung function, is anti-lymphangiogenic, and is highly effective for the lymphatic and chylous complications of LAM. Future trials in patients with LAM who have lymphatic manifestations or elevated serum VEGF-D will likely focus on the VEGF-C/VEGF-D/VEGFR-3 axis.

Keywords: lymphangioleiomyomatosis (LAM), sporadic LAM, tuberous sclerosis LAM, vascular endothelial growth factors (VEGF), angiomyolipoma (AML), sirolimus (Rapamycin)

Lymphangioleiomyomatosis is an uncommon systemic neoplasm targeting women that typically presents in the third decade of life with cystic destruction of the lung associated with dyspnea on exertion and pneumothorax (1). Respiratory limitations in LAM are most commonly caused by airflow limitation and destruction of the pulmonary capillary bed leading to a reduction in diffusing capacity (2). Less common initial pulmonary symptoms include hemoptysis, cough, and chest pain. Patients with LAM may have renal, hepatic or splenic angiomyolipomas (fat containing benign tumors with aneurysmal vessels that are
prone to bleeding). Lymphatic manifestations are present in a substantial fraction of patients with LAM, including chylous pleural effusions in 30%, lymphangioleiomyomas in the abdomen, pelvis, or mediastinum in 29%, chylous ascites in 10%, and lower extremity lymphedema in 4%. Lymphatic fistulas can lead to loss of chyle into other potential spaces, hollow viscera, or the environment, producing manifestations of chylous pericardial effusion, chyloptysis, plastic bronchitis, chylometrorrhoea (chylometrorrhoea), chyluria, and leakage of chyle from the umbilicus. Communication between the intestinal tract and lymphatic system can lead to protein losing enteropathy or lymphatic sepsis and death. Prolonged external drainage of chyle or repeated taps of chylous accumulations in the chest and abdomen can result in nutritional and trace element deficiencies. Serum VEGF-D is elevated in about 70% of LAM patients, especially those who have known lymphatic involvement (3).

Molecular Genetics and Epidemiology of LAM

LAM is caused by mutations in tuberous sclerosis complex (TSC) genes, TSC1, or TSC2 (4). TSC is an autosomal dominant tumor suppressor syndrome with variable penetrance that usually presents in childhood with seizures, skin lesions, and benign hamartomatous tumors of brain, heart, and kidney (5). LAM can occur in patients with TSC (TSC-LAM), and also sporadically in patients who do not have any inherited genetic disease (sporadic LAM or S-LAM). Patients with TSC-LAM have germ line mutations in tuberous sclerosis genes, most often (66% of the time) acquired during embryogenesis rather than inherited from a parent, and develop tumors in locations where second somatic mutations or ‘hits’ occur (6). Sporadic LAM, in contrast, is thought to be due to the occurrence of two somatic hits in TSC2 (4).

Women with TSC develop pulmonary cystic changes in an age-dependent manner, affecting about 22% of patients by the age of 20 years, and up to 80% by the age of 40 years (7). Only a fraction of women with TSC who have cystic changes on HRCT develop pulmonary symptoms, however, perhaps as low as 5-10%. Although up to 10-15% of men with TSC may have cystic changes on HRCT, symptomatic LAM in men is extremely rare (8). It should be noted that not all cystic changes in patients with TSC are due to LAM; biopsies in some cases have shown atypical HMB-45 positive lesions or no evidence of smooth muscle cell infiltration that is characteristic of LAM (9), so studies of LAM prevalence in patients with TSC that rely solely on radiographic changes may overestimate the numbers of patients affected. The global prevalence of TSC is about 1 million people, about half of whom are women. A conservative estimate is that 40-50% of adult females with TSC have cysts consistent with TSC-LAM, suggesting a worldwide prevalence of about 200,000 to 250,000 affected or about 1 per 20,000 persons. S-LAM, in contrast, is thought to affect about 1 in 200,000 persons (10,11).

Molecular Pathogenesis of LAM

LAM can be caused by mutations in either of the tuberous sclerosis genes, TSC1 or TSC2, which encode the proteins hamartin and tuberin, respectively. These proteins form a complex that negatively regulates mTOR activity through an intermediate called Ras Homologue Enriched in Brain (Rheb) (12,13). Defects or deficiencies of TSC1 or TSC2 result in constitutive mTOR activation and dysregulated protein translation and cellular proliferation, autophagy and survival (Figs. 1 and 2) (14-16). Estradiol promotes the proliferation of TSC2 deficient cells in rat models of TSC-LAM (17), perhaps by enhancing the expression of Fra1 (18).

The cells that infiltrate and destroy the lung in LAM arise from an unknown source. They migrate to the lung and form nodular and cystic lesions in the interstitial spaces,
Fig. 1. Signaling networks in LAM cells. TSC1 and TSC2 form a complex that integrates input from upstream signaling cascades, such as those emanating from membrane tyrosine kinase receptors. Rheb, which is normally suppressed by TSC1/TSC2, becomes activated when TSC1 or TSC2 are defective or deficient. Rheb activates mTOR leading to multiple cellular functions that confer a cancer-like phenotype on the LAM cell. Sirolimus binds to FKBP12 and stearically inhibits mTOR actions.

Fig. 2. Role of TSC mutations and lymphatic processes in the pathogenesis of LAM.
enveloping and sometimes invading lymphatics, airways, and blood vessels. LAM lesions are composed of chaotically arranged epithelioid and spindle shaped smooth muscle cells that stain with antibodies against smooth muscle actin, estrogen receptors, progesterone receptors and gp-100 (HMB-45), and other melanocytic proteins (19) (Fig. 3). Lymphangiogenic growth factors, VEGF-C, VEGF-D, are also expressed in the LAM lesion (20), most likely through hypoxia inducible factor (HIF) or HIF-related pathways.

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Fig. 3. (a-h) LAM histology and immunohistochemical staining. a) Involvement of a lymph node by LAM. About 70% of this lymph node was replaced by bundles of immature smooth muscle cells (H&E stain, x1). Higher magnifications (a) revealed two LAM cell clusters in a lymphatic vessel within the lymph node (b; H&E stain, x40) and LAM cell proliferation in the wall of a lymphatic vessel (c; H&E stain, x10. d; H&E stain, x40 of selected area). Immunohistochemical staining of the LAM lesion with anti-alpha-smooth muscle actin (e) highlighted bundles of LAM cells and a LAM cell cluster within the lymphatic vessel (magnification x60). Immunohistochemical staining of a LAM lesion with HMB-45 (f) stained the cytoplasm of LAM cells (magnification x60) while staining of the LAM lesion with anti-progesterone receptor (g) and -estrogen receptor (h) antibodies were positive in a nuclear pattern (magnification for both x60).
VEGF-C and -D are ligands for VEGFR-3, a receptor that exhibits a highly restricted expression pattern in lymphatic endothelial cells and fenestrated blood vessels found in endocrine organs such as pancreas, thyroid, and adrenal glands (21-24). Other lymphatic markers that are present in the LAM lesion are podoplanin and LYVE-1 (20). Cleft like spaces that are lined with VEGFR-3 expressing lymphatic endothelial cells are often found within both pulmonary and extrapulmonary LAM lesions (20). Cyst formation in the lung may be the result of indiscriminate expression of matrix degrading enzymes known to be expressed in LAM lesions including MMP-2, MMP-9, and Cathepsin K, or may be a form of ‘frustrated lymphangiogenesis,’ a term coined to describe chaotic remodeling process occurring in response to lymphangiogenic signals that were appropriate during development but ‘confusing’ to a mature organ (Fig. 2). Cysts are often but not always bordered by crescentic, non-circumferential collections of smooth muscle cells, often with a partial interior lining of hypertrophic alveolar epithelial cells.

Candidates for the primary tumor site in LAM include the bone marrow, angio-myolipoma, lymphatic tree, or the uterus (25,26). Some believe the cell of origin may be the pericyte, whereas others believe that like other PEComas, there is no anatomically normal counterpart for LAM cells; i.e., that their unique phenotype is driven by dysregulated cellular signaling. Circulating LAM cells are present in the blood (27) and lymphatic fluids (28-30). Lymph node involvement is typically restricted to the axial distribution, most often in an ascending gradient pattern from the pelvis and lower abdomen. Mediastinal and hilar lymph nodes may also be involved, but only a few case reports of peripheral lymph node involvement have appeared in the literature. Lymphangioliomyomas are dilated, fluid filled lymphatic neoplasms that occur in the abdomen and pelvis of patients with LAM (31,32). Because of their hypodense centers, they can be easily confused with lymphomas, or partially necrotic genitourinary malignancies such as ovarian or uterine cancer.

Clinical Presentation

LAM typically presents with progressive dyspnea on exertion or recurrent pneumothorax, or can be discovered incidentally on CT scans obtained for another purpose. The presentation mimics asthma and chronic obstructive pulmonary disease and the diagnosis is often delayed. The average number of pneumothoraces prior to diagnosis of LAM in the USA is 2.2 (33,34). Angiomyolipomas (AMLs) are present in about 30% of patients with S-LAM and about 80% of patients with TSC-LAM. Chylous pleural effusions (Fig. 4) or chylous pulmonary congestion (Figs. 4,5) are also seen in about 30% of S-LAM patients, and less frequently in patients with TSC-LAM.

High resolution computed tomography of the chest is the most sensitive diagnostic modality for LAM. Characteristic findings include thin-walled cysts with discrete borders, ranging from a few mm to a few cm in diameter, diffusely distributed throughout the lung. The spaces between cysts are often comprised of radiographically normal appearing pulmonary parenchyma, although recent textural analyses suggest that paracystic lung tissue in LAM may not be truly normal (35).

Pulmonary function tests are often normal in the early stages of LAM, when few cysts are present. Approximately 34% of patients enrolled in the NHLBI registry had normal spirometry (36). The earliest changes in LAM are often a reduction in diffusing capacity and an increase in residual volume. Over time, most patients with LAM develop progressive obstructive ventilatory abnormalities, including a reduction in FEV1 and the FEV1/FVC ratio. Lung volumes can also become abnormal; in the NHLBI registry 11% of patients had a restrictive defect and 6% of patients had hyperinflation (36).
Fig. 4. Chest radiograph (a) and HRCT (b) images of a 37 year old female with a right-sided chylous pleural effusion and cystic changes due to LAM. CT of the abdomen (c) revealed a retroperitoneal mass with hypodense center that was associated with a protein losing enteropathy, suggesting communication with the gut. Needle aspiration showed HMB-45 positive cells consistent with LAM. 1 year of Sirolimus therapy resulted in resolution of retroperitoneal mass (d).

Fig. 5: Pulmonary lymphatic congestion (A) due to chylous reflux is a cause of worsening dyspnea and hypoxemia in LAM. Note the diffuse reticular and ground glass densities. Treatment with Sirolimus has been demonstrated to improve the congestion (B). Reprinted with permission of the American Thoracic Society from Moua et al (2012): Resolution of Chylous Pulmonary Congestion and Respiratory Failure in Lymphangioleiomyomatosis with Sirolimus Therapy. Am. J. of Resp. Crit. Care Med. 186(4): page 390.
Diagnosis

The average age at the time of diagnosis of LAM is 35 years, although it has been described in the young and the old, including a 12 year old girl (37) and in an 86 year old woman (38). A clinical diagnosis of LAM can be based on the European Respiratory Society Guidelines (39), without the need for tissue confirmation in some cases. In a patient with typical cystic changes on HRCT, the presence of any one of the following additional features are diagnostic by ERS Criteria; tuberous sclerosis, chylothorax, lymphangiomyoma or an angiomyolipoma. We maintain that a serum VEGF-D >800 pg/ml is also diagnostic when the CT is characteristic (40) (Fig. 6). When tissue is required, transbronchial biopsy has a yield of >50% and appears to be safe in several small series (41,42). Video-assisted thoracoscopic biopsy remains the gold standard, but is required for diagnosis in only about 15-20% of cases when all of the above modalities are employed, and should be reserved for cases where less invasive approaches fail. Cystic lung diseases that are often considered in the differential of LAM include emphysema, alpha-1 antitrypsin deficiency, pulmonary Langerhans cell histiocytosis, Birt-Hogg-Dubé syndrome, or cystic lung disease due to follicular bronchiolitis, lymphocytic interstitial pneumonitis (as occurs in Sjögren’s syndrome), hypersensitivity pneumonitis or desquamative interstitial pneumonitis. Serum VEGF-D testing can be useful for making the diagnosis of LAM when positive (>800 pg/ml) (Fig. 6), but is not informative when negative. It is often helpful to obtain an alpha 1-antitrypsin protein level, SS-A, SS-B, ANA, RA, and WESR to help with differential diagnosis. Cytological analysis of cells obtained from pleural effusion or ascitic fluid can be diagnostic (29). Pathological examination of tissue obtained by transbronchial or VATSs biopsy includes staining for HMB-45,
an antibody that recognizes the gp-100 protein in the melanogenesis pathway. HMB-45 staining is specific for LAM but it can be sparse and may be even absent. Other markers that stain positive in LAM tissue include alpha-smooth muscle actin, desmin, vimentin, hormone receptors (ER, PR), VEGF-R3, podoplanin, among many others (19).

Involvement of Lymphatics

Approximately 30% of patients with LAM have axial abdominal or thoracic lymphadenopathy, compared to about 9% of patients TSC-LAM. LAM can occasionally be restricted to the retroperitoneum, abdomen, or pelvis, with a normal HRCT or only scattered rare lung cysts, consistent with regional spread from a pelvic or low abdominal source. More often, abdominal LAM manifestations occur in patients who have diffuse cystic change on CT.

Clusters of LAM cells in the chylous pleural fluid of patients with LAM were first described by Valensi in 1973 (43). Later, Itami and coworkers demonstrated that the clusters were also present within the dilated lymphatic circulation and were composed of alpha smooth muscle actin-positive spindle cells enveloped by a single layer of endothelial cells (44). They suggested that LAM cell clusters could be used diagnostically to obviate the need for biopsy in patients with chylous manifestations of LAM. More recent data from Japan provided additional evidence that a likely source and mechanism of spread of LAM may be through the lymphatic circulation. In a small autopsy series, Kumasaka and colleagues described the infiltration of the thoracic duct wall and surrounding fat by LAM cells (30). They also noted the presence of LAM cell clusters enveloped by lymphatic endothelial cells budding from the walls of lymphatic vessels and in the lumen of lymphatic channels and the thoracic duct. LAM cell clusters were found in chylous pleural and peritoneal effusions and within lymphatic vessels.

Lymphangioleiomyomas in LAM most commonly affect the retroperitoneal and pelvic regions (30,36,45-48). On CT screening, lymphangioleiomyomas appear as well circumscribed, lobulated, low density cystic to solid masses of various sizes (31). Diurnal variation in size and echotexture of lymphangioleiomyomas occurs, suggesting gravitational and dietary influences on the retention of lymphatic fluid in these lesions (31), with increase in size between morning and afternoon.

Chyle leakage into body cavities occurs in a subset of LAM patients, due to direct invasion or proximal obstruction of the lymphatic system, particularly the thoracic duct and its tributaries (30,49). Chylothorax (36,50), chylous ascites, chyle leak in pericardial space (51), chyluria (52,53), chyle loss in intestinal lumen (54-55) and also chyle loss in vagina has been described in various studies and case reports (33,49). Chyloptysis can occur with development of a bronchopleural fistula (56). Chylous bronchial casts have been described in case reports (57). About 10-15% of LAM patients have chylothorax at presentation, eventually affecting 20-40% of patients at some point in the disease course (45,48,58,59). In most cases, fluid accumulation is likely the result of chylous reflux, increased pressures in the lymphatic vessels of the lung and retrograde weeping of chyle from visceral and diaphragmatic pleural surfaces (60). Transdiaphragmatic flow of chylous ascites through porous defects in the diaphragm may also result in chylothorax, most commonly on the right. Chyle loss in intestine or protein-losing enteropathy may occur (55) due to retroperitoneal involvement by LAM and associated intestinal lymphangiectasia. Patients may present with diarrhea, peripheral edema, and hypoalbuminemia (34,59). Lymphedema with chyluria was first described in 1968 (61,62). Lymphedema has been described in a case report with LAM without evidence of pulmonary involvement (63).
Lymphatic Biomarkers in LAM

Lymphatic growth factors have shown to have diagnostic, prognostic and predictive utility in patients with LAM. Serum VEGF-D, but not VEGF-C or VEGF-A, is elevated in serum of patients with LAM. There is a negative correlation between serum VEGF-D and markers of disease severity such as FEV1/FVC and diffusion capacity of lung for carbon monoxide (DLCO) (3). A statistically significant correlation between greater lymphatic involvement and higher VEGF-C expression by immunohistochemistry has been demonstrated, and both VEGF-C and -D (by IHC) were associated with worse prognosis and more rapid progression, based on the LAM histology score (LHS) and time to death or transplantation (20). Young et al demonstrated that VEGF-D levels are significantly elevated in patients with LAM compared to those with other cystic lung diseases, such as those due to PLCH, Sjögren’s cystic lung disease, and emphysema, and can obviate the need for lung biopsy in patients with typical cystic change on HRCT (40,64,65). Furthermore, VEGF-D levels were much higher in women with TSC and LAM than in women with TSC and normal HRCT (40,65). Glasgow et al also showed that VEGF-D levels appear to reflect lymphatic involvement. Patients with LAM and lymphatic involvement have significantly decreased pulmonary function (3). VEGF-D has also recently been shown to correlate with disease progression and treatment response, in that patients with higher levels are more likely to progress and more likely to respond to therapy with sirolimus (66).

Treatment

Sirolimus, also called rapamycin, blocks mTOR activation (Fig. 1) and partially restores homeostasis in cells with defective TSC gene function (67). The double blind, randomized Multicenter International LAM Efficacy of Sirolimus (MILES) trial, demonstrated that treatment with sirolimus for one year stabilized FEV1, reduced serum VEGF-D, and improved FVC, quality of life and functional performance. Sirolimus therapy has also been shown to be highly effective for the treatment of chylous effusions and lymphangioleiomyomas (68) (Figs. 4d,5B).

The management of chylous complications in patients with LAM is often challenging. Thoracentesis or paracentesis is indicated for relief of shortness of breath, but repeated chyle drainage can result in malnutrition and immunodeficiencies (69,70). Institution of a fat restricted diet enriched in medium chain triglycerides has been met with variable success in small studies (71). Peritoneovenous shunts have been used for management of refractory chylous ascites (69). Mechanical abrasion and chemical pleurodesis are generally effective therapies for chylothorax (50), but can result in diversion of flow and appearance of chylous fluid collection or drainage in other sites. Octreotide and other somatostatin analogues reduce lymphatic flow and have shown promise for the treatment of chylous effusions in other conditions (72-75) and use in LAM has been trialed (ClinicalTrials.gov Identifier: NCT00005906) and reported (76). Older studies of hormonal treatment with the progesterones or gonadotropin-releasing hormone (GnRH) suggested a salutary effect on chylous effusions in LAM (58). However, many conflicting reports regarding the effects of antiestrogen therapies in LAM have been published. Lymphedema can be managed with leg elevation, compressive hose, physiotherapy, and/or exercise (77).

Future Prospects

Anti-lymphangiogenic strategies are promising in LAM. mTOR inhibitors such as sirolimus and everolimus are anti-angiogenic and anti-lymphangiogenic, and effective in the treatment of chylous complications in LAM. Other candidates include tyrosine
kinase inhibitors such as axitinib, and pazopanib, anti-VEGF-D and anti-VEGF-C antibodies, and anti-VEGFR3 and soluble VEGFR3 have all been mentioned in the context of future trials. Stratification by VEGF-D status and menopausal state may greatly reduce the number of patients required for trials.

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