Lymphangioleiomyomatosis: A Clinical and Pathophysiologic Review

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0. Abstract

Lymphangioleiomyomatosis (LAM) is a systemic, progressive, and rare disease that predominantly affects women during their reproductive years. This disease is characterized by the abnormal proliferation of smooth muscle-like cells, referred to as LAM cells. This leads to cystic destruction of the lung, abnormalities in the lymphatic system, and the emergence of renal tumors. This review aims to elucidate the pathophysiological mechanisms underlying LAM, drawing on current research to highlight the cellular and molecular pathways involved. Approximately 30% of women diagnosed with a genetic disorder known as tuberous sclerosis complex (TSC) also exhibit LAM. TSC is commonly characterized by cardiac rhabdomyomas, facial angiofibromas, and various forms of brain tumors. The clinical manifestations of LAM are diverse, with dyspnea being a common symptom, which is observed in approximately 66% of all cases. This is largely attributed to the obstruction of airflow and the replacement of healthy lung tissue with cysts. Pneumothorax is another common symptom, occurring in approximately 70% of all cases. In such instances, pleurodesis is often recommended to prevent recurrence.

Despite extensive research, no single clinical or serological factor or test has been identified that can reliably predict the prognosis of LAM patients. As such, the disease continues to pose significant challenges in terms of diagnosis and management. Although tests such as a VEGF-D serum level test, high-resolution contrast tomography, and lung cell biopsy have improved specificity in diagnosis, the advent of newer technologies promises to further enhance diagnostic accuracy. Further research is imperative to better understand the pathophysiology of LAM and to develop more effective therapeutic strategies.

1. Epidemiology

LAM is estimated to affect approximately 3-8 per million women[1] , although precise prevalence rates are challenging to determine due to underdiagnosis and misclassification with other respiratory diseases such as asthma, chronic obstructive lung disease, or bronchitis.[2] The disease is characterized by the abnormal proliferation of smooth muscle-like cells, known as LAM cells, which invade the lungs, lymphatics, and other organs.[3] Epidemiologically, LAM occurs in two distinct forms: sporadic LAM (S-LAM) and tuberous sclerosis complex-associated LAM (TSC-LAM). The former is almost exclusively diagnosed in women who typically have no prior history of tuberous sclerosis complex (TSC), whereas the latter is seen in women with TSC, an autosomal dominant genetic disorder. The prevalence of LAM is notably higher in women with TSC, with estimates suggesting that up to 30-40% of women with TSC may develop

LAM.[4] LAM occurs almost exclusively in women, underscoring a potential hormonal influence in its pathogenesis. Although LAM occurs almost exclusively in women, few cases have been reported in males, as confirmed with radiological and/or histological reports. [5] [6] The median age of diagnosis is around 35 years, though the disease can manifest earlier or later. Despite being rare, LAM's incidence may be underreported due to its non-specific clinical presentation, which often mimics other pulmonary conditions such as asthma or chronic obstructive pulmonary disease (COPD).[7] This dysregulation promotes the proliferation and migration of LAM cells, contributing to the progressive lung damage seen in patients. The rarity and gender-specific prevalence of LAM highlight the importance of further epidemiological studies to better understand the disease's risk factors, natural history, and potential environmental or genetic modifiers that may influence its development and progression.

2. Pathophysiology

Lymphangioleiomyomatosis (LAM) involves the proliferation of abnormal smooth muscle-like cells, known as LAM cells, in the lungs, lymphatics, and kidneys. This cell growth results in cyst formation, progressive respiratory failure, and other systemic symptoms. LAM occurs in two forms: sporadic LAM (S-LAM) and tuberous sclerosis complex-associated LAM (TSC-LAM), both of which have similar clinical and histopathological characteristics. The pathogenesis of LAM is tied to mutations in the TSC1 or TSC2 genes, which encode the proteins hamartin and tuberin. These proteins form a complex that inhibits the mammalian target of rapamycin (mTOR) pathway, which is crucial for cell growth and proliferation. In TSC-LAM, germline mutations in TSC1 or TSC2 cause widespread cellular dysfunction, whereas, in S-LAM, somatic mutations are the cause. The loss of hamartin or tuberin function leads to the constant activation of the mTOR pathway, which promotes abnormal cell proliferation, survival, and migration.

Understanding the regulation of the mTOR pathway is essential to grasp LAM pathogenesis. The proteins hamartin and tuberin, encoded by TSC1 and TSC2, form a complex with TBC1D7 that regulates the kinase mTOR via the G protein Rheb.[8] The hamartin-tuberin complex usually keeps Rheb in an inactive GDP-bound state. Akt phosphorylation of tuberin disrupts this complex, increasing Rheb-GTP levels and enabling the formation of the mTORC1 complex with Raptor, mLST8, and PRAS40. [9] This activation leads to the phosphorylation of downstream targets such as mTOR, p70S6K, and 4E-BP1[10,11], which boosts the expression of proteases, vascular endothelial growth factor A (VEGF-A), VEGF-C, VEGF-D, Hypoxia-inducible factor 1-alpha (HIF-1α), Inosine-5'-monophosphate dehydrogenase (IMPDH), while also inhibiting calcineurin inhibitor FKBP38–BCL2 and Unc-51-like kinase 1 (ULK1). [12] Additionally, the formation of the mTORC2 complex with Rictor, mSIN1, and mLST8 activates Akt, ROCK, and RhoA kinase, enhancing glucose metabolism, cell survival,

cytoskeletal rearrangement, cell movement, and prostaglandin metabolism. The hyperactivity of mTORC1 in TSC mutant cells drives anabolic metabolism by increasing protein, lipid, and nucleotide synthesis, leading to cell growth and proliferation. Furthermore, elevated levels of angiogenic factors VEGF-A, VEGF-C, and VEGF-D promote angiogenesis and the development of lymphatic channels, potentially aiding LAM cells in spreading through the circulation. [13] Emerging evidence indicates that LAM cells can evade the immune system through mTOR-dependent expression of checkpoint ligands and modulation of natural killer cell function, as supported with experimental animal models and in glioma [14,15]. This deeper understanding of LAM pathophysiology has identified various therapeutic targets, with mTOR inhibitors like sirolimus showing promise in slowing disease progression by targeting the hyperactive mTOR pathway.

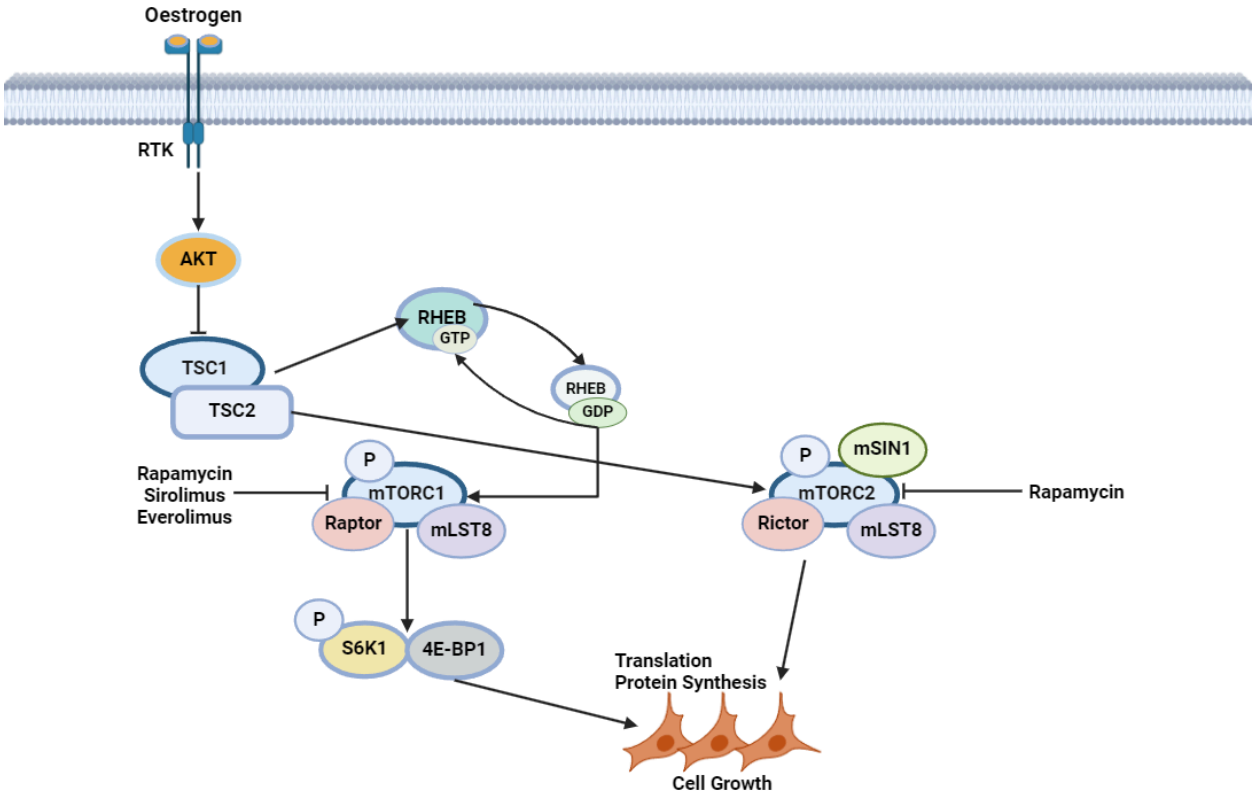

Figure 1: The schematic illustrates the mTOR signaling pathway, highlighting the regulatory role of the TSC1-TSC2 complex in cellular growth and metabolism. Upon estrogen binding to receptor tyrosine kinases (RTKs), AKT is activated, which phosphorylates and inhibits the TSC1-TSC2 complex. This inhibition facilitates the accumulation of GTP-bound RHEB (Ras homolog enriched in brain), which activates mTOR complex 1 (mTORC1). The mTORC1 complex, consisting of mTOR, Raptor, and mLST8, phosphorylates downstream effectors such as S6 kinase 1 (S6K1) and 4E-binding protein 1 (4E-BP1), driving translation, protein synthesis, and cell growth.

mTOR complex 2 (mTORC2), composed of mTOR, Rictor, and mLST8, promotes cell survival and cytoskeletal organization by phosphorylating AKT. Dysregulation of this pathway is central to the pathogenesis of Lymphangioleiomyomatosis (LAM), as mutations in TSC1 or TSC2 lead to constitutive activation of mTORC1, resulting in uncontrolled cell proliferation. Therapeutic agents such as rapamycin and its analogs (sirolimus and everolimus) inhibit mTORC1 activity, providing a targeted approach to modulate disease progression.

2.1 Impact of VEGF-A, VEGF-C, and VEGF-D upon Pathogenesis

The impact of vascular endothelial growth factors VEGF-A, VEGF-C, and VEGF-D on the pathogenesis of lymphangioleiomyomatosis (LAM) is profound, primarily due to their roles in angiogenesis and lymphangiogenesis, which significantly contribute to disease progression. VEGF-A is a potent angiogenic factor that is markedly elevated in LAM patients.[16] This overexpression results in abnormal blood vessel formation within lung tissues, facilitating the growth and survival of LAM cells by supplying essential nutrients and oxygen.[17] This angiogenic process not only supports LAM cell proliferation but also contributes to increased vascular permeability [18,19], leading to recurrent chylous effusions, which complicates the clinical course of 9-30% of LAM patients.[20–22] Furthermore, the abnormal vasculature formed under the influence of VEGF-A creates a microenvironment that protects LAM cells from apoptosis, allowing them to persist and invade surrounding tissues.[23] This contributes to the progressive loss of pulmonary function, with the rate of decline in forced expiratory volume in one second (FEV1) being highly variable and largely unpredictable with the mean annual decline in FEV1 ranging from 60 to 134 mL per year as determined through analysis of several clinical trials. [24,25]

VEGF-C and VEGF-D are integral to the development and remodeling of the lymphatic system, which is severely affected in LAM. These growth factors are markedly upregulated in LAM patients, with serum VEGF-D levels often exceeding 800 pg/mL[26] compared to less than 200 pg/mL[27] in healthy individuals. VEGF-C and VEGF-D drive lymphangiogenesis, resulting in the formation of dysfunctional, dilated lymphatic channels, as supported by the hyperplasia seen in transgenic mice lymphatic vessels[28]. This aberrant lymphangiogenesis is a key factor in the spread of LAM cells through the lymphatic system, contributing to the formation of lymphangioleiomyomas and the accumulation of lymphatic fluid by impairing lymphatic drainage.[29] The disrupted lymphatic architecture also leads to complications such as chylothorax, which occurs in up to 20-30% of LAM patients[19], further aggravating the clinical manifestations of the disease. Elevated VEGF-D levels serve as a diagnostic biomarker for LAM, with a specificity of around 97% and sensitivity of approximately 82%[30], providing a non-invasive means of assessing disease presence and progression.

The interaction between the hyperactive mTOR pathway and elevated VEGF levels presents a complex network of pathogenic mechanisms in LAM. The overactivation of mTORC1 not only promotes LAM cell growth and proliferation through anabolic metabolism[31] but also increases the expression of VEGF-A, VEGF-C, and VEGF-D, thereby enhancing angioenesis and lymphangiogenesis. This multifaceted pathogenic process underscores the potential of targeting VEGF signaling pathways as a therapeutic strategy. mTOR inhibitors like sirolimus have shown efficacy in stabilizing lung function, with clinical trials demonstrating a stabilization or improvement in FEV1 in 70% of treated patients and a reduction in lymphatic complications.[32] By mitigating the effects of the hyperactive mTOR pathway and subsequently decreasing VEGF-mediated angiogenesis and lymphangiogenesis, sirolimus reduces VEGF production, which in turn diminishes the aberrant vascular and lymphatic networks supporting LAM cell survival and dissemination. Continued research into the specific roles of these VEGFs in LAM will likely lead to more refined and effective therapeutic approaches, improving outcomes for patients suffering from this debilitating disease. The development of novel therapies that target both mTOR signaling and VEGF pathways holds promise for more comprehensive management of LAM, potentially halting or reversing disease progression and significantly enhancing patient quality of life.

2.2 Warburg Metabolism and LAM

In lymphangioleiomyomatosis (LAM) and tuberous sclerosis complex (TSC), sustained cell proliferation driven by mTOR activation requires major metabolic adaptations. Under normal conditions, cells generate ATP through two main pathways: aerobic glycolysis in the cytosol, which yields pyruvate and a small amount of ATP (approximately 2 ATP molecules per glucose molecule), and oxidative phosphorylation in the mitochondria, which results in a significantly greater ATP yield (up to 36 ATP molecules per glucose molecule)[33]. In cancer cells, energy production shifts predominantly to aerobic glycolysis via the Warburg effect. This metabolic reprogramming, although less efficient in terms of ATP production, allows for the diversion of glycolytic intermediates into biosynthetic pathways necessary for cell growth and proliferation.[33] This shift is evidenced by increased glucose uptake and lactate production, which can be up to 10 times higher in rapidly proliferating cells compared to normal cells.[34]

Otto Warburg reported that cancer cells preferentially utilize glycolysis rather than oxidative phosphorylation for energy production, even under aerobic conditions, despite the significantly lower ATP yield per glucose molecule. This process is termed the Warburg effect. In TSC-deficient cells, mTOR activation appears to promote the Warburg effect by upregulating HIF-1α which in turn upregulates sterol regulatory element-binding proteins (SREBP1 and SREBP2), which are integral to glycolysis, the oxidative arm of the pentose phosphate pathway, and lipid biosynthesis[35]. For instance, studies have shown that in mice models, HIF-1α levels can increase up to 3-fold in TSC-deficient cells, leading to enhanced expression of glycolytic enzymes such as

hexokinase 2 and lactate dehydrogenase A.[36] This upregulation supports the shift to aerobic glycolysis, ensuring a steady supply of biosynthetic precursors for rapid cell proliferation.

Hypoxia-inducible factor 1α (HIF1α) plays a crucial role in this shift and is upregulated under low-oxygen conditions to promote glycolysis, glucose transport, and angiogenesis, thus supporting tumor survival and growth[36]. In LAM, mTORC1 activation further increases HIF1α transcription and translation even under aerobic conditions, thereby sustaining Warburg metabolism. This finding underscores the pivotal role of mTOR in maintaining the glycolytic pathway and highlights the significance of metabolic adaptation in the growth and survival of neoplastic cells in LAM and TSC. Quantitatively, HIF-1α can enhance the expression of genes involved in glycolysis by up to 4-5 times, promoting an increased glucose uptake that is often detected in imaging studies using fluorodeoxyglucose (FDG)-PET, a common diagnostic tool in cancer metabolism. This metabolic shift not only supports the anabolic needs of proliferating cells but also helps in maintaining redox balance and cell survival under the proliferative stress conditions typical of LAM and TSC.

2.3 Cellular interactions and matrix remodeling

LAM cells secrete matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9[37], which are believed to degrade ECM components and facilitate cell migration and invasion[38]. This matrix remodeling contributes to the cystic destruction of lung tissue and the formation of abnormal airspaces. This has been modeled in vivo, utilizing a novel technique of implanting nanosensors in a preclinical mouse model of LAM. These nanosensors consist of bar-coded peptides with specific proteinase cleavage sequences attached to nanoparticles. When active proteases cleave these peptides, the resulting fragments are excreted in the urine and measured via mass spectrometry.[39] The results indicated that the MMP activity could be effectively monitored, providing valuable insights into the dynamic proteolytic environment in LAM.

The significance of MMPs in LAM pathogenesis underscores the need for targeted therapies to inhibit their activity. Pharmacological inhibitors of MMPs, such as doxycycline, have shown promise in preclinical models by reducing lung destruction and improving respiratory function. Clinical trials are warranted to assess the efficacy and safety of these inhibitors in LAM patients. Furthermore, combining MMP inhibitors with other therapeutic approaches, such as mTOR inhibitors like sirolimus, could provide synergistic benefits by addressing multiple pathological mechanisms in LAM. Additionally, LAM cells interact with other cell types, such as fibroblasts and immune cells, within the lung microenvironment.[40] These interactions are mediated through various cytokines and growth factors, creating a proinflammatory and profibrotic milieu that supports disease progression. For example, transforming growth factor-beta (TGF-β), a key fibrogenic cytokine involved in cellular proliferation, differentiation, and extracellular matrix (ECM) production, is markedly upregulated in Lymphangioleiomyomatosis (LAM).[41] This upregulation of TGF-β contributes to the

pathogenesis of LAM by promoting fibrotic processes, including the excessive deposition of ECM components such as collagen, leading to progressive fibrosis and architectural distortion of pulmonary tissues.[41,42] The resultant fibrosis and tissue remodeling impair alveolar integrity, compromise lung elasticity, and progressively reduce pulmonary function. TGF-β-driven fibrosis is thus a clear factor in the pathophysiology of LAM, contributing to the chronic decline in respiratory capacity and exacerbating clinical manifestations such as dyspnea, hypoxemia, and eventual respiratory failure.

3. Statistical Insights

Epidemiological data underscore the rarity and severity of LAM. It predominantly affects women, with an estimated prevalence of 3-7 per million people in the general population. The average age at diagnosis is approximately 35 years, with many patients experiencing a progressive decline in lung function. The median survival time from diagnosis is approximately 10 years, though this can vary widely depending on the extent of disease and response to treatment.

Pulmonary function tests in LAM patients typically show a decline in forced expiratory volume in one second (FEV1) at an average rate of 75-120 mL per year. This progressive decline highlights the need for early diagnosis and intervention to preserve lung function. Therapeutic interventions targeting the mTOR pathway, such as sirolimus (rapamycin), have shown efficacy in stabilizing lung function and reducing the size of angiomyolipomas, providing a rationale for their use in managing LAM.

4. Clinical Features

4.1 Renal Angiomyolipomas (AMLs)

Renal angiomyolipomas (AMLs) are a prominent extrapulmonary feature in LAM, affecting approximately 30-50% of patients.[43,44] These benign tumors are composed of a mixture of blood vessels, smooth muscle cells, and fat, often growing within the kidneys. While many AMLs remain asymptomatic and are discovered incidentally during imaging studies, larger tumors pose a risk for spontaneous hemorrhage, which can lead to acute abdominal or flank pain, hematuria, and even hypovolemic shock.[45] The risk of hemorrhage increases with tumor size, particularly for those larger than 4 cm in diameter.[46] Regular monitoring through ultrasound or MRI is crucial for early detection and assessment of AML growth and potential bleeding risks. When intervention is necessary, options include selective arterial embolization to reduce blood supply to the tumor, nephron-sparing surgery to remove the tumor while preserving kidney function, and mTOR inhibitors like sirolimus or everolimus[47,48]. These pharmacological agents have demonstrated effectiveness in shrinking AMLs and reducing the risk of bleeding, offering a less invasive treatment alternative for patients with multiple or large tumors.

4.2 Pleural Effusion

Pleural effusion, particularly chylous effusion, is a notable complication in LAM, occurring in approximately 20-30% of patients[22,49]. Chylous effusions occur due to thoracic duct or lymphatic vessel disruption, resulting in the leakage of lipid-rich lymphatic fluid into the pleural space. Clinically, chylous effusions in LAM are linked to symptoms such as dyspnea, pleuritic chest pain, and persistent cough, which exacerbate the already compromised lung function caused by cystic destruction. Diagnostic imaging, including chest X-rays and CT scans, detects effusions in symptomatic patients, while thoracentesis confirms the diagnosis by revealing a pleural fluid triglyceride level exceeding 110 mg/dL[50], with levels surpassing 500 mg/dL in severe cases. Management strategies include dietary modifications like a low-fat diet with medium-chain triglycerides (MCTs), which bypass lymphatic transport and reduce effusion recurrence. Although therapeutic thoracentesis offers temporary relief, effusions recur in up to 75% of cases within six months. For persistent effusions, pleurodesis shows success in 25-95% of cases[51], while pleuroperitoneal shunting achieves a 60-70% reduction in effusion size. Furthermore, mTOR inhibitors, such as sirolimus, have demonstrated efficacy in reducing chylous effusions and lymphatic involvement in up to 50-70% of LAM patients, with studies showing a 30-50% reduction in pleural effusion volume and recurrence rates. These therapeutic options underscore the need for a comprehensive, multidisciplinary approach to managing chylous effusion in LAM, addressing both the immediate respiratory symptoms and long-term disease progression.

4.3 Pneumothorax

Pneumothorax, or the collapse of a lung due to the presence of air in the pleural space, is one of the most frequent and significant complications in LAM, affecting up to 60-70% of patients[52]. This condition often presents acutely with sudden onset of chest pain, shortness of breath, and sometimes hypoxia, requiring prompt medical intervention. The recurrent nature of pneumothorax in LAM patients poses a substantial challenge, as many individuals experience multiple episodes[52], necessitating repeated medical or surgical interventions. Initial management typically involves the insertion of a chest tube to evacuate the air and allow the lung to re-expand. However, due to the high recurrence rate, more definitive treatments are often necessary. Chemical or surgical pleurodesis, which involves the introduction of a sclerosing agent or mechanical abrasion to induce pleural adhesion and prevent future lung collapse, is commonly employed. Video-assisted thoracoscopic surgery (VATS) is another option, allowing for minimally invasive intervention to repair the lung and perform pleurodesis. Despite these measures, the recurrence rate remains high at 2%-14%[53], prompting consideration of lung transplantation in severe cases where recurrent pneumothorax leads to significant morbidity and deteriorating lung function. The chronic and recurrent

nature of pneumothorax in LAM highlights the need for ongoing surveillance and proactive management strategies to improve patient outcomes and quality of life.

4.4 Lymphatic Manifestations

Lymphatic manifestations are a critical aspect of Lymphangioleiomyomatosis (LAM), significantly impacting patient morbidity and occurring in approximately 30-40% of sporadic LAM cases[54]. These manifestations include lymphangioleiomyomas, chylous effusions, lymphadenopathy, and lymphatic obstruction. Lymphangioleiomyomas are benign cystic tumors commonly found in the abdomen, retroperitoneum, and pelvis, affecting up to 29% of LAM patients[54]. These tumors can cause symptoms such as nausea, bloating, abdominal distension, and urinary issues, with their size often fluctuating diurnally[55]. Chylous effusions, including pleural effusions (30% of patients) and ascites (10% of patients), result from lymphatic obstruction and are associated with more advanced lung disease13. Lymphadenopathy, visible on CT scans in about 30% of patients, typically affects the retroperitoneal, retrocrural, or pelvic regions1. LAM lesions express lymphangiogenic growth factors VEGF-C and VEGF-D, as well as their receptors VEGFR-2 and VEGFR-3, contributing to the formation of chaotic lymphatic channels3. Serum VEGF-D is elevated in 70% of LAM patients and serves as a valuable diagnostic and prognostic biomarker3. Treatment options for lymphatic complications include sirolimus, an mTOR inhibitor that has shown effectiveness in stabilizing lung function and resolving chylous effusions[54,55]. This molecular targeted therapy is particularly promising for managing the lymphatic and chylous complications of LAM[55].

5. Diagnosis

Diagnosing lymphangioleiomyomatosis (LAM) can be a complex process due to its nonspecific clinical presentation and the occasional need for histological confirmation. There are several diagnostic methods that healthcare providers use to accurately diagnose LAM and provide appropriate treatment. Currently, LAM is diagnosed on the basis of compatible chest radiographs, pulmonary function tests (PFTs), and computed tomography (CT) findings.

Serum VEGF-D Levels in Patients with Lymphangioleiomyomatosis (LAM)
Vascular endothelial growth factor-D (VEGF-D) has emerged as a crucial biomarker in the diagnosis and management of lymphangioleiomyomatosis (LAM), a rare lung disease characterized by cystic lung lesions and smooth muscle cell proliferation[56]. VEGF-D, a lymphangiogenic growth factor, is overproduced in LAM due to tuberous sclerosis gene mutations activating the mechanistic target of the rapamycin (mTOR) pathway[1]. Elevated serum VEGF-D levels have significant diagnostic value, with a specificity of 97-100% at a threshold of 800 pg/mL when distinguishing LAM from other cystic lung diseases[57]. Studies have shown that approximately 70% of LAM patients have elevated VEGF-D levels[57,58]. This noninvasive test, performed through a simple blood draw, offers a patient-friendly alternative to invasive procedures such as surgical

lung biopsies[57,59]. The American Thoracic Society and Japanese Respiratory Society recommend serum VEGF-D testing for diagnosing LAM in women with compatible CT findings before considering surgical lung biopsy. Beyond diagnosis, serum VEGF-D levels correlate with disease severity, lymphatic involvement, and treatment response, particularly to mTOR inhibitors like sirolimus. Notably, VEGF-D levels markedly decrease during sirolimus treatment, making it a valuable tool for monitoring disease progression and therapeutic efficacy.

Serum vascular endothelial growth factor-D (VEGF-D) has emerged as a valuable biomarker for lymphangioleiomyomatosis (LAM) diagnosis and management. Clinically, elevated VEGF-D levels, typically above 800 pg/mL, demonstrate high specificity (98%) for LAM diagnosis when combined with characteristic cystic changes on high-resolution computed tomography (HRCT). However, sensitivity at this threshold is limited to 76%, indicating that normal VEGF-D levels do not exclude LAM. VEGF-D levels in LAM patients (mean 1856±1742 pg/mL) are significantly higher than in patients with other cystic lung diseases (mean 410±173 pg/mL, $p<0.001$) , and compared to healthy controls (657 ± 43 pg/mL; $p < 0.001$)[60]. This distinction aids in differentiating LAM from other pulmonary conditions, although rare false positives may occur in diseases such as Birt-Hogg-Dubé syndrome.[61] Serial VEGF-D measurements contribute to monitoring disease activity and treatment response. Notably, VEGF-D levels negatively correlate with lung function parameters such as FEV1/FVC ($rs=-0.8630$; $p=0.0269$) and %DLCO ($rs=-0.9796$; $p=0.0035$), suggesting its potential as a marker of disease severity. However, VEGF-D levels may remain elevated in transplanted patients and those receiving medical therapies, necessitating cautious interpretation in these contexts.[61]

5.1 High-Resolution Computed Tomography (HRCT)

High-resolution computed tomography (HRCT) serves as the primary noninvasive diagnostic modality, demonstrating exquisite sensitivity in detecting characteristic cystic lesions with specificity ranging from 68.75% to 72.6%[62]. Imaging reveals multiple round, well-defined cysts of variable dimensions distributed uniformly within the pulmonary parenchyma[63]. The diagnostic accuracy requires correlation with clinical presentation and ancillary diagnostic studies to differentiate from similar cystic lung pathologies[63]. Diagnostic algorithms integrate HRCT findings with clinical data and biomarkers to enhance diagnostic precision[62]. Serial HRCT examinations enable precise quantification of disease progression, assessment of therapeutic response, and early detection of potential complications such as pneumothorax or pleural effusion[63]. These comprehensive imaging evaluations guide clinical interventions, therapeutic strategies, and longitudinal management protocols.

While HRCT demonstrates high diagnostic sensitivity, its findings are not pathognomonic, necessitating a multifaceted diagnostic approach[63]. Definitive diagnosis may require clinical evaluation, serological testing, pulmonary function studies, and potentially histopathological examination. The integration of HRCT with clinical and laboratory data significantly improves overall diagnostic accuracy for various pulmonary conditions[62].

Ongoing research aims to refine HRCT techniques for better image resolution and diagnostic accuracy. Advances in imaging technology, like ultrahigh-resolution CT, may improve detection and monitoring of LAM. Integrating HRCT with other modalities, such as MRI for extrapulmonary manifestations or PET-CT for metabolic activity, could offer a more comprehensive LAM assessment. In summary, HRCT is pivotal in LAM diagnosis and management, offering high sensitivity and detailed imaging capabilities. Its noninvasive nature makes it suitable for both initial diagnosis and ongoing monitoring, significantly contributing to patient care. Interpreted alongside clinical data and other diagnostic tests, HRCT enhances diagnosis accuracy, informs treatment decisions, and helps monitor disease progression, reflecting advancements in diagnostic imaging and personalized medicine, ultimately improving outcomes for LAM patients.

5.2 Pulmonary Function Tests (PFTs)

Pulmonary Function Tests (PFTs) are indispensable for evaluating the functional impact of lymphangioleiomyomatosis (LAM) on pulmonary physiology. These comprehensive assessments typically reveal obstructive or mixed obstructive-restrictive ventilatory defects, providing critical insights into disease progression and respiratory mechanics. Characteristic findings include a reduction in forced expiratory volume in one second (FEV1) and a significant decrease in the diffusing capacity of the lung for carbon monoxide (DLCO), which serve as quantitative markers of pulmonary impairment.

The standard PFT battery comprises three primary diagnostic components: spirometry, which quantifies FEV1 and forced vital capacity (FVC); plethysmography, measuring total lung capacity (TLC) and residual volume (RV); and single-breath carbon monoxide uptake, evaluating diffusing capacity. Among these parameters, spirometry, particularly FEV1, emerges as the most critical metric for longitudinal assessment in LAM patients. Recent technological advancements have facilitated the development of portable spirometers, enabling more frequent and convenient domiciliary pulmonary function monitoring.

A comprehensive clinical evaluation remains paramount in the diagnostic algorithm, particularly for identifying concomitant tuberous sclerosis complex (TSC). Clinicians must conduct meticulous physical examinations, paying close attention to characteristic cutaneous manifestations such as facial angiofibromas, subungual

fibromas, shagreen patches, and hypomelanotic macules. These dermatological findings can provide crucial diagnostic clues and support comprehensive patient management.

While PFTs are invaluable for assessing pulmonary function, clinicians must recognize their inherent limitations. These tests may lack sensitivity for detecting early functional changes and potentially increase the risk of pneumothorax in LAM patients. Therefore, a multimodal approach integrating PFTs with high-resolution computed tomography (HRCT) offers the most comprehensive evaluation of LAM, ensuring a nuanced understanding of disease progression and individual patient characteristics.

5.3 The rs4588 Polymorphism

A significant finding from the NHLBI cohort study highlighted the critical role of genetic variation at rs4588 in lymphangioleiomyomatosis (LAM) disease progression. Women carrying the CC genotype at this locus exhibited a markedly accelerated time to death or lung transplant, with a median time of 104 months compared to 150 months for those with AA or AC genotypes.[64] This 46-month reduction in median time to death or transplant for CC genotype carriers underscores the prognostic significance of this genetic variant in LAM.[64] The rs4588 polymorphism affects the vitamin D binding protein (VTDB), encoded by the GC gene. Serum VTDB levels were found to be lower in progressive LAM compared to stable disease, correlating with diffusing capacity (DLCO). This suggests that VTDB levels may serve as a biomarker for disease severity and progression.

5.4 Clinical Implications

Despite serum VTDB levels showing no correlation with age, menopausal status, or serum VEGF-D levels, they stand out as potential markers for disease progression. This suggests that VTDB levels could serve as a critical tool in personalized treatment strategies and clinical trial designs. The ability to monitor VTDB levels may provide valuable insights into disease activity, enabling more precise and effective clinical interventions.

5.5 Treatment

The cornerstone of LAM treatment is mTOR inhibition, primarily utilizing sirolimus (rapamycin) and everolimus. These agents inhibit the constitutively activated mTOR pathway in LAM cells, mitigating dysregulated cellular proliferation. Sirolimus has demonstrated significant efficacy in preserving lung function and reducing angiomyolipoma size. The Multicenter International LAM Efficacy of Sirolimus (MILES) trial revealed a mean FEV1 gain of 1 ± 2 ml/month with sirolimus compared to a 12 ± 2 ml/month decline with placebo[32]. A meta-analysis showed significant improvements in

FEV1 and FVC after mTOR inhibitor therapy, with weighted mean differences of 0.15 L (95% CI: 0.08 to 0.22, P < 0.01) and 0.22 L (95% CI: 0.11 to 0.32, P < 0.01), respectively[65].

Everolimus has also shown promising results in LAM treatment. In a phase IIa study, everolimus treatment for 26 weeks resulted in a mean FEV1 improvement of 114 mL (95% CI: 11-217) from baseline[66]. Additionally, everolimus demonstrated efficacy in reducing VEGF-D levels, a biomarker of LAM severity, from a median of 1730 pg·mL−1 to 934.5 pg·mL−1 over 26 weeks[66]. Long-term studies have shown that sirolimus can maintain its efficacy for up to 4 years, with annual changes in FEV1 and DLCO reduced from -7.4% ± 1.4% to -0.3% ± 0.5% (P < 0.001) and -6.4% ± 0.9% to -0.4% ± 0.5% (P < 0.001), respectively [65,67]. However, it's important to note that cessation of sirolimus therapy can result in disease progression, underscoring the need for continuous treatment[65].

In addition to mTOR inhibitors, other pharmacological treatments for LAM have been investigated. For instance, combination therapies of doxycycline and sirolimus were studied in the Sirolimus and Autophagy Inhibition in Lymphangioleiomyomatosis (SAIL) trial, but no beneficial outcomes were observed that did not improve significantly beyond those previously demonstrated in the MILES trial[68]. A question of inquiry should also be why, in the SAIL trial in the group receiving 400 mg of hydroxychloroquine, the heightened dosage had no significant impact on lung function as measured by FEV1, FVC or DLCO levels. The efficacy and safety of this combination therapy of sirolimus and hydroxychloroquine remain to be further investigated. In conclusion, while sirolimus and everolimus are currently the most effective pharmacological treatments for LAM, further research is needed to optimize their use and explore novel therapeutic approaches.

6. Methods

To gather information for my analysis, I utilized PubMed, Google Scholar, and the Cochrane Library as primary search engines, with key terms including “Lymphangioleiomyomatosis,” “mTOR inhibitors,” “VEGF-D,” “angiomyolipomas,” and “chylous effusions.” Searches also included specific terms related to the mechanisms, challenges, and therapeutic applications of these topics. Search results were filtered to include peer-reviewed articles published within the last 20 years, with exceptions made for older studies that were deemed credible and relevant after cross-referencing with more recent findings. Additionally, reputable medical websites, such as those from the National Institutes of Health (NIH) and Cleveland Clinic, were consulted to corroborate information and ensure accuracy, particularly in sections addressing therapeutic applications.

Data extraction focused on identifying key findings, such as the mechanisms underlying LAM, challenges in its management, and clinical applications of targeted

therapies. Each relevant article was analyzed for details including publication date, study outcomes, and methodologies. References cited within primary articles were also reviewed and incorporated when they provided valuable insights for the analysis. The gathered information was synthesized through a narrative synthesis approach, organizing findings into key sections related to LAM's pathophysiology, therapeutic challenges, and treatment applications. This methodology facilitated a comprehensive understanding of the current knowledge and advancements in the field of Lymphangioleiomyomatosis research.

1. Harknett, E. C. *et al.* Use of variability in national and regional data to estimate the prevalence of lymphangioleiomyomatosis. *QJM Int. J. Med.* 104, 971–979 (2011).
2. McCarthy, C., Gupta, N., Johnson, S. R., Yu, J. J. & McCormack, F. X. Lymphangioleiomyomatosis: pathogenesis, clinical features, diagnosis, and management. *Lancet Respir. Med.* 9, 1313–1327 (2021).
3. Harari, S., Torre, O. & Moss, J. Lymphangioleiomyomatosis: what do we know and what are we looking for? *Eur. Respir. Rev.* 20, 034–044 (2011).
4. Johnson, S. R. & Tattersfield, A. E. Decline in Lung Function in Lymphangioleiomyomatosis: Relation to Menopause and Progesterone Treatment. *Am. J. Respir. Crit. Care Med.* 160, 628–633 (1999).
5. Wakida, K. *et al.* Lymphangioleiomyomatosis in a Male. *Ann. Thorac. Surg.* 100, 1105–1107 (2015).
6. Schiavina, M. *et al.* Pulmonary Lymphangioleiomyomatosis in a Karyotypically Normal Man without Tuberous Sclerosis Complex. *Am. J. Respir. Crit. Care Med.* 176, 96–98 (2007).
7. Kirkeby, M. H., Bendstrup, E. & Rose, H. K. Case report: If it is not asthma—think of lymphangioleiomyomatosis in younger female patients. *Front. Med.* 11, (2024).
8. Dibble, C. C. *et al.* TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1. *Mol. Cell* 47, 535–546 (2012).
9. Inoki, K., Li, Y., Zhu, T., Wu, J. & Guan, K.-L. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat. Cell Biol.* 4, 648–657 (2002).
10. Chung, J., Kuo, C. J., Crabtree, G. R. & Blenis, J. Rapamycin-FKBP specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. *Cell* 69, 1227–1236 (1992).
11. Wu, C.-W. & Storey, K. B. mTOR Signaling in Metabolic Stress Adaptation. *Biomolecules* 11, 681 (2021).
12. McCarthy, C., Gupta, N., Johnson, S. R., Yu, J. J. & McCormack, F. X. Lymphangioleiomyomatosis: pathogenesis, clinical features, diagnosis, and management. *Lancet Respir. Med.* 9, 1313–1327 (2021).
13. Achen, M. G. *et al.* Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). *Proc. Natl. Acad. Sci.* 95, 548–553 (1998).

14. Chen, J., Zhang, X. D. & Proud, C. Dissecting the signaling pathways that mediate cancer in *PTEN* and *LKB1* double-knockout mice. *Sci. Signal.* 8, (2015).
15. Parsa, A. T. *et al.* Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nat. Med.* 13, 84–88 (2007).
16. Ferrara, N. VEGF: an update on biological and therapeutic aspects. *Curr. Opin. Biotechnol.* 11, 617–624 (2000).
17. Maes, C. *et al.* Impaired angiogenesis and endochondral bone formation in mice lacking the vascular endothelial growth factor isoforms VEGF164 and VEGF188. *Mech. Dev.* 111, 61–73 (2002).
18. Almoosa, K. F., McCormack, F. X. & Sahn, S. A. Pleural Disease in Lymphangioleiomyomatosis. *Clin. Chest Med.* 27, 355–368 (2006).
19. Ryu, J. H., Doerr, C. H., Fisher, S. D., Olson, E. J. & Sahn, S. A. Chylothorax in Lymphangioleiomyomatosis*. *Chest* 123, 623–627 (2003).
20. Johnson, S. R. Lymphangioleiomyomatosis. *Eur. Respir. J.* 27, 1056–1065 (2006).
21. Ryu, J. H. *et al.* The NHLBI Lymphangioleiomyomatosis Registry: Characteristics of 230 Patients at Enrollment. *Am. J. Respir. Crit. Care Med.* 173, 105–111 (2006).
22. Johnson, S. R. Survival and disease progression in UK patients with lymphangioleiomyomatosis. *Thorax* 59, 800–803 (2004).
23. Gupta, K. *et al.* VEGF Prevents Apoptosis of Human Microvascular Endothelial Cells via Opposing Effects on MAPK/ERK and SAPK/JNK Signaling. *Exp. Cell Res.* 247, 495–504 (1999).
24. Johnson, S. R. & Tattersfield, A. E. Decline in Lung Function in Lymphangioleiomyomatosis: Relation to Menopause and Progesterone Treatment. *Am. J. Respir. Crit. Care Med.* 160, 628–633 (1999).
25. Taveira-DaSilva, A. M., Stylianou, M. P., Hedin, C. J., Hathaway, O. & Moss, J. Decline in Lung Function in Patients With Lymphangioleiomyomatosis Treated With or Without Progesterone. *Chest* 126, 1867–1874 (2004).
26. Young, L. R. *et al.* Serum Vascular Endothelial Growth Factor-D Prospectively Distinguishes Lymphangioleiomyomatosis From Other Diseases. *Chest* 138, 674–681 (2010).
27. Taveira-DaSilva, A. M. *et al.* Reversible airflow obstruction, proliferation of abnormal smooth muscle cells, and impairment of gas exchange as predictors of outcome in lymphangioleiomyomatosis. *Am. J. Respir. Crit. Care Med.* 164, 1072–1076 (2001).
28. Jeltsch, M. *et al.* Hyperplasia of Lymphatic Vessels in VEGF-C Transgenic Mice. *Science* 276, 1423–1425 (1997).
29. Stacker, S. A., Baldwin, M. E. & Achen, M. G. The role of tumor lymphangiogenesis in metastatic spread. *FASEB J.* 16, 922–934 (2002).
30. Li1,2,3, M. *et al.* Diagnostic performance of VEGF-D for lymphangioleiomyomatosis: a meta-analysis. *J. Bras. Pneumol.* e20210337 (2022) doi:10.36416/1806-3756/e20210337.
31. Thompson, C. B. Rethinking the Regulation of Cellular Metabolism. *Cold Spring Harb. Symp. Quant. Biol.* 76, 23–29 (2011).
32. McCormack, F. X. *et al.* Efficacy and Safety of Sirolimus in Lymphangioleiomyomatosis. *N. Engl. J. Med.* 364, 1595–1606 (2011).

33. Granchi, C., Bertini, S., Macchia, M. & Minutolo, F. Inhibitors of Lactate Dehydrogenase Isoforms and their Therapeutic Potentials. *Curr. Med. Chem.* 17, 672–697 (2010).
34. Shestov, A. A. *et al.* Quantitative determinants of aerobic glycolysis identify flux through the enzyme GAPDH as a limiting step. *eLife* 3, e03342 (2014).
35. Espenshade, P. J. & Hughes, A. L. Regulation of Sterol Synthesis in Eukaryotes. *Annu. Rev. Genet.* 41, 401–427 (2007).
36. Dodd, K. M., Yang, J., Shen, M. H., Sampson, J. R. & Tee, A. R. mTORC1 drives HIF-1α and VEGF-A signalling via multiple mechanisms involving 4E-BP1, S6K1 and STAT3. *Oncogene* 34, 2239–2250 (2015).
37. Ancona, S. *et al.* Differential Modulation of Matrix Metalloproteinases-2 and -7 in LAM/TSC Cells. *Biomedicines* 9, 1760 (2021).
38. Cui, N., Hu, M. & Khalil, R. A. Biochemical and Biological Attributes of Matrix Metalloproteinases. *Prog. Mol. Biol. Transl. Sci.* 147, 1–73 (2017).
39. Kirkpatrick, J. D. *et al.* Urinary detection of lung cancer in mice via noninvasive pulmonary protease profiling. *Sci. Transl. Med.* 12, eaaw0262 (2020).
40. Clements, D., Dongre, A., Krymskaya, V. P. & Johnson, S. R. Wild Type Mesenchymal Cells Contribute to the Lung Pathology of Lymphangioleiomyomatosis. *PLOS ONE* 10, e0126025 (2015).
41. Evans, S. E., Colby, T. V., Ryu, J. H. & Limper, A. H. Transforming Growth Factor-β 1 and Extracellular Matrix-Associated Fibronectin Expression in Pulmonary Lymphangioleiomyomatosis. *Chest* 125, 1063–1070 (2004).
42. Broekelmann, T. J., Limper, A. H., Colby, T. V. & McDonald, J. A. Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. *Proc. Natl. Acad. Sci.* 88, 6642–6646 (1991).
43. Avila, N. A., Kelly, J. A., Chu, S. C., Dwyer, A. J. & Moss, J. Lymphangioleiomyomatosis: Abdominopelvic CT and US Findings. *Radiology* 216, 147–153 (2000).
44. Kerr, L. A., Blute, M. L., Ryu, J. H., Swensen, S. J. & Malek, R. S. Renal angiomyolipoma in association with pulmonary lymphangioleiomyomatosis: Forme fruste of tuberous sclerosis? *Urology* 41, 440–444 (1993).
45. Thapa, N., Maharjan, S., Hona, A., Pandey, J. & Karki, S. Spontaneous rupture of renal angiomyolipoma and its management: A case report. *Ann. Med. Surg. 2012* 79, 104037 (2022).
46. Bhatt, J. R. *et al.* Natural History of Renal Angiomyolipoma (AML): Most Patients with Large AMLs >4cm Can Be Offered Active Surveillance as an Initial Management Strategy. *Eur. Urol.* 70, 85–90 (2016).
47. Watanabe, E. H. *et al.* The effect of sirolimus on angiomyolipoma is determined by decrease of fat-poor compartments and includes striking reduction of vascular structures. *Sci. Rep.* 11, 8493 (2021).
48. Stamatiou, K. N. *et al.* Combination of Superselective Arterial Embolization and Radiofrequency Ablation for the Treatment of a Giant Renal Angiomyolipoma Complicated with Caval Thrombus. *Case Rep. Oncol. Med.* 2016, 8087232 (2016).
49. Ryu, J. H. *et al.* The NHLBI Lymphangioleiomyomatosis Registry: Characteristics of

230 Patients at Enrollment. *Am. J. Respir. Crit. Care Med.* 173, 105–111 (2006).
50. Porcel, J. M. *et al.* Clinical characteristics of chylothorax: results from the International Collaborative Effusion database. *ERJ Open Res.* 9, 00091–02023 (2023).
51. Schild, H. H., Strassburg, C. P., Welz, A. & Kalff, J. Treatment options in patients with chylothorax. *Dtsch. Arzteblatt Int.* 110, 819–826 (2013).
52. Almoosa, K. F. *et al.* Management of Pneumothorax in Lymphangioleiomyomatosis. *Chest* 129, 1274–1281 (2006).
53. Ng, C. S. H., Lee, T. W., Wan, S. & Yim, A. P. C. Video assisted thoracic surgery in the management of spontaneous pneumothorax: the current status. *Postgrad. Med. J.* 82, 179–185 (2006).
54. Gupta, R., Kitaichi, M., Inoue, Y., Kotloff, R. & McCormack, F. X. Lymphatic manifestations of lymphangioleiomyomatosis. *Lymphology* 47, 106–117 (2014).
55. Johnson, S. R. Lymphangioleiomyomatosis. *Eur. Respir. J.* 27, 1056–1065 (2006).
56. Buffenstein, R., Karklin, A. & Driver, H. S. Beneficial physiological and performance responses to a month of restricted energy intake in healthy overweight women. *Physiol. Behav.* 68, 439–444 (2000).
57. Hirose, M. *et al.* Serum vascular endothelial growth factor-D as a diagnostic and therapeutic biomarker for lymphangioleiomyomatosis. *PLOS ONE* 14, e0212776 (2019).
58. Park, S. & Lee, E. J. Lymphangioleiomyomatosis and mTOR inhibitors in real world-a narrative review. *J. Thorac. Dis.* 15, 6333–6344 (2023).
59. Feemster, L. C. *et al.* Summary for Clinicians: Lymphangioleiomyomatosis Diagnosis and Management Clinical Practice Guideline. *Ann. Am. Thorac. Soc.* 14, 1073–1075 (2017).
60. Glasgow, C. G., Avila, N. A., Lin, J.-P., Stylianou, M. P. & Moss, J. Serum vascular endothelial growth factor-D levels in patients with lymphangioleiomyomatosis reflect lymphatic involvement. *Chest* 135, 1293–1300 (2009).
61. Revilla-López, E. *et al.* Lymphangioleiomyomatosis: Searching for potential biomarkers. *Front. Med.* 10, 1079317 (2023).
62. Selina, F. *et al.* Measuring Sensitivity, Specificity and Accuracy of HRCT of Chest and Comparison with Biomarkers in COVID-19 Suspected Patients: A Cross Sectional Study. *Mymensingh Med. J. MMJ* 30, 503–508 (2021).
63. Zompatori, M. *et al.* Diagnostic Imaging of Diffuse Infiltrative Disease of the Lung. *Respiration* 71, 4–19 (2004).
64. Miller, S. *et al.* The vitamin D binding protein axis modifies disease severity in lymphangioleiomyomatosis. *Eur. Respir. J.* 52, 1800951 (2018).
65. Gao, N., Zhang, T., Ji, J., Xu, K.-F. & Tian, X. The efficacy and adverse events of mTOR inhibitors in lymphangioleiomyomatosis: systematic review and meta-analysis. *Orphanet J. Rare Dis.* 13, 134 (2018).
66. Goldberg, H. J. *et al.* Everolimus for the treatment of lymphangioleiomyomatosis: a phase II study. *Eur. Respir. J.* 46, 783–794 (2015).
67. Koul, P. A. & Mehfooz, N. Sirolimus in lymphangioleiomyomatosis: A case in point for research in ‘orphan’ diseases. *Lung India Off. Organ Indian Chest Soc.* 36,

353–355 (2019).
68. El-Chemaly, S. *et al.* Sirolimus and Autophagy Inhibition in Lymphangioleiomyomatosis: Results of a Phase I Clinical Trial. *Chest* 151, 1302–1310 (2017).