Tuberous sclerosis complex for the pulmonologist

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Shareable abstract (@ERSpublications)
Tuberous sclerosis complex is associated with diverse pulmonary manifestations including LAM, multiple micronodular pneumocyte hyperplasia and chylous effusions. LAM occurs in 30–40% of adult females with tuberous sclerosis complex. https://bit.ly/3iLqZ08

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Abstract
Tuberous sclerosis complex (TSC) is a rare multisystem genetic disorder affecting almost all organs with no sex predominance. TSC has an autosomal-dominant inheritance and is caused by a heterozygous mutation in either the TSC1 or TSC2 gene leading to hyperactivation of the mammalian target of rapamycin (mTOR). TSC is associated with several pulmonary manifestations including lymphangioleiomyomatosis (LAM), multifocal micronodular pneumocyte hyperplasia (MMPH) and chylous effusions. LAM is a multisystem disorder characterised by cystic destruction of lung parenchyma, and may occur in either the setting of TSC (TSC-LAM) or sporadically (S-LAM). LAM occurs in 30–40% of adult females with TSC at childbearing age and is considered a nonmalignant metastatic neoplasm of unknown origin. TSC-LAM is generally milder and, unlike S-LAM, may occur in males. It manifests as multiple, bilateral, diffuse and thin-walled cysts with normal intervening lung parenchyma on chest computed tomography. LAM is complicated by spontaneous pneumothoraces in up to 70% of patients, with a high recurrence rate. mTOR inhibitors are the treatment of choice for LAM with moderately impaired lung function or chylous effusion. MMPH, manifesting as multiple solid and ground-glass nodules on high-resolution computed tomography, is usually harmless with no need for treatment.

Introduction
Tuberous sclerosis complex (TSC) is a rare multisystem genetic disorder that can potentially affect all organs. TSC is the second most frequent phakomatosis after neurofibromatosis type I. During fetal life, it results in dysfunction of cell differentiation, proliferation and migration leading to various clinical manifestations that may be present at birth or manifest themselves during childhood or adulthood [1].

The first case of TSC may have been described in 1835 by a French dermatologist named Pierre François Olive Rayer who reported facial angiofibromas, calling them “facial vegetations” at that time. In 1862, the German pathologist, Friedrich Daniel von Recklinghausen reported cardiac rhabdomyomas and cortical tubers in a child. The French eponymous term “sclérose tubéreuse de Bourneville” was then coined after the French neurologist Désiré-Magloire Bourneville, who first described cortical tubers. Over the years,
physicians from different specialities contributed to the accumulated knowledge of what we know today by the term TSC, first used in 1942 by Sylvan Moolten [2].

TSC is characterised by hamartomas in different organs including the brain, lungs, skin, kidneys, heart and eyes. However, manifestations are not universal and usually follow an age-related expression pattern; some manifestations may be obvious at birth (for example, some of the skin lesions), while others do not appear until adulthood, such as renal angiomyolipomas (rAMLs) and lymphangioleiomyomatosis (LAM). As single organ manifestations may vary from one patient to another, they often remain undiagnosed until TSC is identified. Interplay and coordination of physicians from different specialties are necessary in all cases, hence the need for multidisciplinary discussion [3].

**TSC and LAM: the success of registry-based clinical research**

A rare disease is defined, from an epidemiological and regulatory perspective, as any condition determined to be of low prevalence or number of cases. In the European Union, this is defined as not more than five affected persons per 10 000 [4], while in the United States it is defined as <200 000 patients affected, in Japan as <50 000 and in Australia <2000 [5]. Low prevalence, scarcity of knowledge and phenotype heterogeneity have restricted research into rare diseases, including both TSC and LAM. Registries of rare diseases were implemented to describe the natural history and outcomes of such diseases, to support research and to establish a database for evaluating drugs and other therapies; in addition, these seek to connect patients, families and clinicians [6].

The Groupe d’Étude et de Recherche sur les Maladies “Orpheline” Pulmonaires (GERM“O”P) was founded in 1993 by Jean-François Cordier. It served as a French registry for rare pulmonary diseases and is considered to be the first registry for LAM [7]. Its name was changed recently to “Orpha.Lung”, standing for orphan lung diseases. Over the years, registries have made possible retrospective studies of clinical and radiological manifestations [8], epidemiological studies [7], descriptions of the natural course of disease [8, 9] and the assessment of specific questions such as pregnancy in patients with LAM [10]. Furthermore, some progress has been made through retrospective surveys of members of LAM patient associations, such as the impact of air travel on the risk of pneumothorax [11]. In addition, other national registries and foundations have been set up, leading to the Multicenter International Lymphangioleiomyomatosis Efficacy of Sirolimus (MILES) trial in 2011, in which the LAM Foundation was instrumental [12].

For TSC, the “TuberOus SClerosis registry to increase disease Awareness” (TOSCA) was established in 2014 as the first international disease registry with the specific aim to gather clinical data and address knowledge gaps in the natural history and management of TSC. It comprises a core section, including diagnostic characteristics, and a “petal projects” section, exploring and focusing on specific disease manifestations (e.g. epilepsy, renal and pulmonary features, genetics) [13, 14]. For example, using the TOSCA registry data, Jansen et al. [15] evaluated the characteristics of subependymal giant cell astrocytoma (SEGA) in patients with TSC, and observed that they also occur in adults. Nabbout et al. [16] assessed the burden of epilepsies associated with TSC. Kingswood et al. [17] studied the clinical characteristics of rAMLs in patients with TSC, and described a higher frequency in females and in patients with TSC2 mutations.

**Epidemiology**

The prevalence of TSC is estimated to be one case per 6–10 000 live births (or 1:25 000 population) [18–20], with no difference across ethnic groups or sex predominance. Prevalence is age-dependent, prevailing in the young: 1:15 000 in children aged < 5 years, 1:20 000 in individuals aged <30 years and 1:25–29 000 in those aged <65 years [21].

While approximately 40–50% of female patients with TSC have cystic lung changes suggestive of LAM on systematic computed tomography (CT) of the chest [22–24], only a minority (≈10%) have symptomatic LAM; not all cystic lung lesions in TSC patients are due to LAM [25].

While LAM is the main pulmonary manifestation of TSC, it may also occur sporadically (S-LAM). The prevalence of S-LAM, estimated to be between 1:100 000 and 1:400 000 of the general population [26, 27], is theoretically lower than TSC-LAM; however, S-LAM is more common than TSC-LAM in the American registry [28]. This discrepancy may reflect a milder presentation of TSC-LAM, frequently diagnosed at a pre-clinical stage through systematic screening; indeed, most LAM patients requiring medical intervention have the sporadic form of the disease [29].
The probability of developing LAM in females with TSC increases with age until the menopause. In a retrospective study of 101 female patients with TSC aged >15 years, the frequency of LAM increased by 8% every year; LAM was present in 27% of patients aged <21 years, and in 81% of those aged >40 years [23]. TSC-LAM is diagnosed at a younger age (<35 years), probably due to active screening. The mean age at presentation of S-LAM is 35 years [28, 30, 31] but nearly 10% of the cases are diagnosed after menopause [30]. Of note, LAM is the inaugural event in 28% of TSC patients [32].

Although LAM is classically considered a disease of females of childbearing age, it may, rarely, affect males with TSC, unlike S-LAM which occurs exclusively in females. Systematic CT screening studies in adult males with TSC using chest and lung bases of abdominal CT found cystic lung disease in 10–38% of the subjects, although virtually none of them had clinical pulmonary manifestations [33–35]. In contrast, multifocal micronodular pneumocyte hyperplasia (MMPH), another pulmonary manifestation of TSC, occurs with no sex preference.

**Genetics and pathophysiology**

**Genes and rare variants**

TSC is an autosomal dominant disorder caused by heterozygous pathogenic variants in either the TSC1 (chromosome 9q34) or the TSC2 (chromosome 16p13) gene [36], coding respectively for hamartin and tuberin. These pathogenic variants cause constitutive activation of the mammalian target of rapamycin (mTOR) pathway, with consequent dysregulation of cell growth [37]. Of note, two-thirds of cases are caused by de novo pathogenic mutations [2, 38].

In 2012, the identification of a pathogenic mutation was incorporated into the international criteria for the diagnosis of TSC [1]. Since then, molecular testing for TSC1 and TSC2 mutations has become more widely available worldwide, thereby facilitating diagnostic, screening and counselling strategies. Only a small number of pathogenic variants are frequently found in affected individuals, thus making genotype-phenotype correlation difficult. The Leiden Open Variation Database (LOVD) is a free and regularly updated web-based open-source database founded by the Leiden University Medical Centre in the Netherlands, which hosts many of the known TSC1 and TSC2 variants together with their assigned significance (benign, pathogenic or unknown significance) (https://databases.lovd.nl/shared/genes?search_name=tuberous%20sclerosis). It enables physicians and geneticists to interpret the results of the genetic studies in patients with TSC. LVOD catalogued 11 866 variants with 1121 unique variants for TSC1 and 3251 unique variants for TSC2. Penetrance is almost complete. Mutations within TSC1 are predominantly small truncating nonsense and insertion or deletion mutations, whereas TSC2 mutations are more frequently missense mutations and large rearrangements [39]. TSC manifestations tend to be less severe, especially in the brain, kidney and lung [40]. However, variability exists in disease expression, even among family members carrying the same mutation.

Pathogenic variants can be found in TSC2 in ~70% of patients with a clinical diagnosis of TSC, and in TSC1 in ~10%; the remaining 20% of patients have no mutation identified or carry a variant of unknown significance [41, 42]. However, recent technologies such as next-generation sequencing and RNA-based approaches have identified somatic mosaicism or intronic splicing variants affecting TSC1 or TSC2 [39]. It is therefore unlikely that a third gene is involved.

**Impact of mutations on cell signalling**

TSC1 and TSC2 are tumour-suppressor genes. A pathogenic mutation that results in loss of heterozygosity (LOH) disables the inhibitory function of the gene and leads to hamartoma formation [43, 44] through constitutive activation of the mTOR pathway. Hamartoma development in TSC follows Knudson’s two-hit tumour-suppressor gene model. After inheriting a pathogenic germline variant in one of the TSC genes, a somatic mutation inactivates the remaining wild-type allele [45] and results in LOH, causing angiofibromas, LAM, AMLs and renal cell carcinoma. In contrast, S-LAM and sporadic AMLs are caused by somatic first-hit and second-hit mutations inactivating each wild-type allele of TSC2.

Hamartin and tuberin (encoded by TSC1 and TSC2, respectively), together with a third protein encoded by the TBC1D7 gene, form a heterotrimeric complex called the TSC protein complex [37]. This is the main negative regulator of Ras homologue enriched in brain (RHEB), itself an activator of mTOR complex-1 (mTORC1) [46], a protein complex mainly composed of mTOR [47] and regulatory-associated protein of mTOR (RAPTOR). Activation of the mTORC1 complex is implicated in extensive metabolic reprogramming, including stimulation of nucleotide synthesis, protein translation, lipid synthesis, angiogenesis, lymphangiogenesis and cell invasion, as well as downregulation of autophagy and apoptosis. This “canonical” RHEB-mTORC1 signalling pathway is hyperactivated in patients with TSC carrying
inactivating mutations of TSC1 or TSC2 and can be targeted by rapamycin and other mTOR inhibitors. In addition, putative "noncanonical" signalling pathways may be involved in TSC, which may be independent of TSC2, mTORC1 or RHEB [39].

**From TSC to LAM**

LAM is considered to be caused by the proliferation of abnormal LAM cells, found profusely in the lungs, but also in the lymph nodes, blood, uterus and chylous fluid. Morphologically, LAM cells are nearly identical to AML cells and share some characteristics with smooth-muscle cells.

LAM cells are characterised by biallelic inactivating mutations of either TSC1 or TSC2; in TSC-LAM, one mutation is considered germinal and the other somatic, whereas in S-LAM both mutations are somatic. Hyperactivation of the RHEB-mTORC1 signalling pathway is responsible for mTOR-dependent cell proliferation and potentially for cell migration [48]. LAM cells produce lymphangiogenic growth factors (vascular endothelial growth factor (VEGF)-C and -D) and growth factor receptors (VEGFR-2 and VEGFR-3). LAM cells express particularly VEGFR-3 [49], and lymphatic endothelial cells express VEGFR-3 and VEGFR-2 [50]. These agents are thought to promote lymphatic endothelial cell recruitment and budding of LAM cell clusters into the lymphatic lumen up to the pulmonary microvasculature, and then contribute to the destruction of the lung parenchyma and chaotic remodelling called "frustrated lymphangiogenesis" (figure 1) [25, 51, 52]. It has been demonstrated that greater lymphatic activity is associated with more severe disease as measured by the LAM histologic score [53]. LAM, either sporadic or associated with TSC, is therefore considered a low-grade, metastasising nonmalignant neoplasm of unknown origin [54], with similar mutations found in LAM and AML cells [55]. This theory would explain how LAM can recur after lung transplantation. Of note, TSC mutations are found only in a proportion of patients with S-LAM [56], although this may be due to mosaicism or technical limitations.

**Origin of LAM cells**

The origin of LAM cells remains elusive. Although different theories have been proposed (uterus, AML, ovary, neural crest), there is accumulating evidence suggesting that they may originate from the uterus. The greater incidence of LAM in females of reproductive age suggests a putative role for sex hormones in disease pathogenesis [57]. Menses influence patient symptoms and disease activity slows after menopause [9, 58, 59]. Moreover, uterine leiomyomas share some characteristics with LAM cells with regards to oestrogen and progesterone receptor expression [60], and when metastasising to the lungs they cause cystic lesions, although distinct from those seen in LAM [61]. Furthermore, LAM cells can be found in the uterus. Experimental inactivation of TSC2 in uterine cells in mice causes myometrial tumours in the lungs [62]. Recently, Guo et al. [55], using single-cell transcriptomic analysis, demonstrated the existence of unique mesenchymal cells, termed LAM-core cells, which carried similar gene mutations and gene expression within lung and uterine LAM lesions in patients with S-LAM, with close similarity to myometrial and stromal cells from normal human and mouse uteri. This observation strongly supports the theory of the uterus as a source of LAM cells in the lung. Although these findings are compelling, another source must coexist to explain TSC-LAM in males.

**Histology**

According to the World Health Organization (WHO) classification of lung tumours [63], LAM is considered a PEComatous tumour arising from perivascular epithelioid cells. It consists of a proliferation of plump spindle-shaped cells with typically pale eosinophilic cytoplasm called LAM cells, which carry TSC1 or TSC2 gene mutation. These cells are usually present in the pulmonary interstitium along the blood and lymphatic vessels of the lung and along the axial lymphatics of the thorax and abdomen. There are two types of LAM cells within the lungs [64]. Small spindle-shaped cells that are likely to react with antiproliferation cell nuclear antigen antibodies, and epithelioid-like cells with abundant cytoplasm. The latter react with HMB-45, a monoclonal antibody that detects a pre-melanosomal protein called gp100. Although the gp100 HMB-45 staining is classically used for the diagnosis of LAM, tyrosinase-related protein (TRP-1) is more widely expressed and could be more sensitive [65]. Both small spindle-shaped cells and epithelioid-like cells react with antibodies against smooth-muscle cell-specific antigens (e.g. $\alpha$-actin, desmin and vimentin) [66]. The destruction of elastic fibres and collagen in the basal membrane of LAM cells mediated by the secretion of matrix metalloproteinases (-2 and -9) results in cyst formation [67, 68]. Sex hormone receptors are invariably present in LAM cells [69, 70]. Interestingly, the smooth muscle cells from rAMLs have identical structure and immunohistochemical profile as LAM cells [71].

**Clinical manifestations**

Patients with TSC present with a broad spectrum of clinical manifestations that may vary from absent or mild symptoms, to severe and debilitating disease with multiple organ involvement (figure 2). Clinical
FIGURE 1 From tuberous sclerosis complex (TSC) to lymphangioleiomyomatosis (LAM), proposed schema of pathophysiology. RHEB: Ras homologue enriched in brain; mTOR: mammalian target of rapamycin; mTORC1: mTOR complex-1; RAPTOR: regulatory-associated protein of mTOR; VEGF: vascular endothelial growth factor; VEGFR: vascular endothelial growth factor receptor; AML: angiomylipoma; MMP: matrix metalloproteinase.
manifestations have an age-related expression, thus warranting periodic and thorough follow-up. Some features appear in childhood and do not progress into adulthood (for example, cardiac rhabdomyoma), some may develop only in adults (such as LAM), while others are present throughout the individual’s lifespan (namely facial angiofibroma). TSC2 mutation tends to cause a more severe clinical disease than other mutations, especially with regards to neurological, renal and pulmonary features. Furthermore, no TSC manifestation is pathognomonic; a combination of certain features makes the diagnosis more reliable. Diagnostic criteria for TSC were first proposed in 1998 at the first International Tuberous Sclerosis Consensus Conference, and were updated in 2012 [1].

**Orocutaneous manifestations**

Dermatological and oral lesions account for four of the 11 major criteria and three of the six minor criteria. Knowledge and recognition of skin features is therefore necessary for the diagnostic process. These include hypomelanotic macules (or hypopigmented macules, ash-leaf spots), present in 90% of TSC patients, which represent the earliest visible sign of TSC at birth. Angiofibromas, present in ~75% of patients, are hamartomatous nodules involving vascular and connective tissues, especially in the central areas of the face. Ungual fibromas (or Koenen tumours) are hamartomatous fibromas that affect up to 80% of patients, more commonly observed on the feet. Shagreen patches are more specific for TSC, present in ~50% of patients, and are described as connective tissue hamartomas most common in the lower back [72]. As for oral lesions, dental enamel pits may be encountered in virtually all TSC patients, and oral fibromas in approximately half of them [39].

**Renal manifestations**

The more frequent renal manifestation of TSC is rAMLs, although they can also be found in the liver, in the uterus and, exceptionally, in the lungs [73–75]. The prevalence of rAMLs increases with age, being present in 9% of patients aged <2 years and in 79% of those aged >40 years. The TOSCA registry revealed that rAMLs were found in 52% of patients, with a higher frequency in females (58% versus 42% in males) and in carriers of TSC2 mutations (59% versus 33% in TSC1). Symptoms include pain, bleeding, hypertension and impaired renal function, although most patients are asymptomatic [17]. Medical or invasive management prior to any complication intends to prevent life-threatening haemorrhages and avoid

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**FIGURE 2** Extrathoracic manifestations of tuberous sclerosis complex. a) Shagreen patch; b) angiofibromas; c) ungual fibroma (arrow); d) oral fibroma (arrow) and dental enamel pits (dotted arrow); e) bilateral renal angiomyolipoma (asterisks); f) retroperitoneal lymphangioma (arrow).
nephrectomy and renal insufficiency. Renal AMLs are also found in patients with S-LAM (∼50%), but are usually unilateral and smaller. It is therefore recommended to suspect and evaluate for TSC if a LAM patient presents with bilateral rAMLs [26]. Furthermore, 30–35% of patients with TSC develop multiple renal cysts, and as many as 5% of them meet the criteria for polycystic kidney disease [17, 39, 73]. In addition, 2% of TSC patients present with an overlap between autosomal dominant polycystic kidney disease and TSC, due to a contiguous gene deletion of TSC2-PKDI, the two genes lying adjacent to one another on chromosome 16p13.3 [76]. Renal cell carcinoma (RCC) is seen in 2–4% of TSC patients, in contrast to 1% of the general population. It is seen more often with TSC2 mutation, and occurs in younger patients (age 30–40 years versus 55–60 years), with a female predominance. TSC-associated RCC is often multiple and bilateral, with histological heterogeneity within the same patient. Various histological types have been described: papillary, clear cell, chromophobe and oncocytoma more frequently seen. Compared to rAMLs, RCC grows faster and has no lipid content. Immunohistochemically, AML is HMB-45 positive and RCC is rather cytokeratin positive [77, 78].

**Neurological and neuropsychiatric manifestations**

The neurological and neuropsychiatric features of TSC generate the greatest burden of the disease. The majority of affected individuals have structural brain lesions that include cortical tubers, subependymal nodules, SEGA, radial migration lines and retinal astrocytic hamartomas, among others. Cortical tubers (from which derives the name of the disease) are present in ∼90% of the patients with a predilection for the frontal lobes. SEGA develops in up to 25% of patients [15], usually in the first two decades of life [39]. It is considered a slow-growing lesion; grade I according to the WHO classification. Epilepsy is present in 70–90% of affected individuals [16, 79], and usually appears within the first year of life. TSC-associated neuropsychiatric disorders include intellectual disability, developmental delay, autism spectrum disorders and mood disorders [80]. Although globally underrecognised, up to two-thirds of patients suffer from mental health issues, 50% have intellectual impairment and 40% suffer from autism [39]. Of note, cognitive impairment may impact the diagnostic process and overall management.

**Thoracic manifestations**

LAM is the most common pulmonary manifestation in adult patients with TSC, especially females. Other thoracic features include MMPH, pneumothoraces and a variety of lymphatic manifestations (figure 3). TSC-LAM is more often asymptomatic than S-LAM (it is virtually asymptomatic in males [39]) and more frequently diagnosed through active screening. When symptomatic, the most common feature is dyspnoea.
Spontaneous pneumothorax occurs in 45–60% of cases [14, 81]. Chest pain, fatigue, cough and chyloptysis (expectoration of chylous material) are less frequently seen (table 1) [28, 81].

MMPH is a rare pulmonary manifestation of TSC and refers to nodular proliferation of type II pneumocytes along the alveolar septa. It is a histologically distinct entity from LAM, staining negative for HMB45 and positive for phospho-S6. However, MMPH lesions also show LOH for TSC genes and activation of mTOR pathway, implying a pathophysiology similar to LAM [82]. MMPH was first described in 1991 in a 38-year-old female with TSC [83]. Its exact prevalence is unknown, although it has been estimated to range between 40% and 60% [84]. Similar to TSC, MMPH has no sex predominance [85]. On high-resolution computed tomography (HRCT), MMPH appears as multiple solid, semi-solid or ground-glass nodules, which range in size from 1 to 10 mm and are usually upper-lobe predominant and peripheral [86]. MMPH is thought to be indolent and nonprogressive [87], although data are scarce. It needs to be differentiated from pre-malignant pulmonary lesions that may be found in association with LAM, such as atypical adenomatoid hyperplasia, especially when found in S-LAM [88]. Interestingly, a young male with TSC had MMPH lesions that disappeared upon treatment with an mTOR inhibitor, which was introduced for rAMLs [89].

Spontaneous pneumothorax affects between 55% and 73% of patients with LAM during their lifetime with an estimated relapse rate in the absence of surgery of 70% [8, 28, 31, 84, 90]. Pneumothorax is the presenting symptom in 40% of cases [26]; inaugural pneumothorax is associated with a younger age at diagnosis and a better outcome, possibly due to early diagnosis [91, 92]. Patients with LAM have 1000 times greater risk for pneumothorax than the general population; the annual incidence is 8% after the first symptoms and 5% after LAM diagnosis [11]. LAM-associated pneumothorax should be treated surgically, given that conservative management is associated with a higher rate of recurrence [90]. The latest American Thoracic Society (ATS)/Japanese Respiratory Society (JRS) guidelines suggest that patients with LAM be offered ipsilateral pleurodesis after their first pneumothorax rather than waiting for a first recurrence [93]. Pleurodesis reduces the risk of relapse by ~50%, but this risk remains much higher than after pleurodesis for primary spontaneous pneumothorax [11]. There is no difference between chemical or surgical pleurodesis; however, the pleural adhesions induced by the surgical techniques make the pleura difficult to dissect and more prone to intraoperative bleeding in a later procedure, such as lung transplantation. Total pleural coverage has been suggested as an alternative [94]. Of note, none of these

![Image](https://doi.org/10.1183/16000617.0348-2020)

| TABLE 1 Relevant features in tuberous sclerosis complex (TSC)-associated lymphangioleiomyomatosis (LAM) compared to sporadic lymphangioleiomyomatosis (S-LAM) |
|---------------------------------------------------------------|---------------------------------------------------------------|
| **TSC-LAM**                                                  | **S-LAM**                                                    |
| Genetics                                                     | Both TSC1 and TSC2 germinal mutations                         |
|                                                              | TSC2 mutations more prevalent                                 |
| Epidemiology                                                 | Younger age at diagnosis                                     |
|                                                              | Reported in males (virtually asymptomatic)                   |
| Clinical presentation                                        | Less symptomatic                                             |
|                                                              | Spontaneous pneumothorax more frequent                       |
|                                                              | Systematic screening in TSC patients                          |
| Imaging                                                      | Milder cystic lung disease                                   |
|                                                              | Coexistence of MMPH                                           |
|                                                              | Hepatic and renal AMLs (especially bilateral) more common    |
|                                                              | Extrathoracic TSC features                                   |
| Lung physiology                                              | Normal lung function more frequent                            |
|                                                              | Airflow obstruction and diffusion abnormalities possible      |
| Serum VEGF-D                                                 | Elevated (>800 pg·mL$^{-1}$) in >95% of patients             |
| Indications for mTOR inhibitors                               | For patients with LAM with abnormal/declining lung function  |
|                                                              | For selected patients with LAM with problematic chylous       |
|                                                              | effusions                                                    |
| Prognosis                                                    | Generally milder disease                                     |
|                                                              | Lung transplantation more frequent                            |

VEGF: vascular endothelial growth factor; mTOR: mammalian target of rapamycin; AML: angiomyolipoma; MMPH: multifocal micronodular pneumocyte hyperplasia.
procedures should be considered a contraindication for lung transplantation [93, 95]. Furthermore, sirolimus has been shown to be promising in the prevention of recurrent pneumothorax in patients with LAM [96].

Lymphatic manifestations include chylothorax, chylous lung congestion, mediastinal lymphadenopathy and mediastinal and/or retroperitoneal lymphangioleiomyomomas [84]. Chyle fluid collections are found in 30% and in the peritoneal space in 10% of LAM patients. Direct invasion or obstruction of the thoracic duct by LAM cell clusters induces a reflux of chylous fluid into the lymphatic vessels of the mediastinum and lungs, causing chylothorax and parenchymal chylous congestion [25]. The latter presents as interstitial infiltrates, interlobular septal thickening and ground glass opacities, with or without consolidations [97]. Other aetiologies must be taken into account in the differential diagnosis, such as drug-induced pneumonitis and infections, especially *Pneumocystis* pneumonia, in patients treated with mTOR inhibitors. TSC patients are at higher risk of recurrent aspiration, especially those with important cognitive impairment or treated with antiepileptic drugs [84].

**Diagnosis**

**Diagnosis of TSC**

A diagnosis of TSC is established with a high level of confidence (definite TSC) if a pathogenic variant in one of the TSC genes, two major clinical features, or one major and two or more minor clinical features are present. A possible diagnosis of TSC is made if either only one major feature or only two minor features are encountered (tables 2 and 3) [1]. Routine genetic testing is generally not needed to confirm or rule out TSC, but may be useful in individuals who do not fulfil the criteria and for counselling purposes.

| Genetic diagnostic criteria | Either TSC1 or TSC2 pathogenic mutation in DNA from normal tissue |
|-----------------------------|---------------------------------------------------------------|
| Clinical diagnostic criteria | Major features                                               |
|                             | Hypomelanotic macules (≥3, ≥5 mm diameter)                   |
|                             | Angiofibromas (≥3) or fibrous cephalic plaque                 |
|                             | Ungual fibromas (≥2)                                        |
|                             | Shagreen patch                                              |
|                             | Multiple retinal hamartomas                                 |
|                             | Cortical dysplasias (includes tubers and cerebral white matter radial migration lines) |
|                             | Subependymal nodules                                        |
|                             | Subependymal giant cell astrocytoma                         |
|                             | Cardiac rhabdomyoma                                         |
|                             | LAM¶,+                                                      |
|                             | Angiomyolipomas (≥2)                                        |
|                             | Minor features                                              |
|                             | “Confetti” skin lesions                                      |
|                             | Dental enamel pits (>3)                                     |
|                             | Intraoral fibromas (≥2)                                     |
|                             | Retinal achromic patch                                       |
|                             | Multiple renal cysts                                         |
|                             | Nonrenal hamartomas                                         |
| Diagnosis                   | Genetic criteria                                            |
|                             | Definite                                                   |
|                             | OR                                                         |
|                             | Two major features                                          |
|                             | OR                                                         |
|                             | One major feature with ≥2 minor features                    |
|                             | Possible                                                   |
|                             | Either one major feature                                    |
|                             | OR                                                         |
|                             | ≥2 minor features                                           |

LAM: lymphangioleiomyomatosis. ¶: defined as a mutation that clearly inactivates the function of the TSC1 or TSC2 proteins (e.g. out-of-frame insertion/deletion or nonsense mutation), prevents protein synthesis (e.g. large genomic deletion) or is a missense mutation whose effect on protein function has been established by functional assessment. Other TSC1 or TSC2 variants whose effect on function is less certain do not meet these criteria, and are not sufficient to make a definite diagnosis of TSC; ¶: see table 3; +: a combination of LAM and angiomyolipomas without other features does not meet criteria for a definite diagnosis. Information from [1].
Diagnosis of LAM

The presence of TSC paves the way to a clinical diagnosis of LAM in presence of a characteristic HRCT, which is defined as the “presence of multiple, bilateral, round, well-defined, relatively uniform, thin-walled cysts in a diffuse distribution” [26, 93]. The intervening lung parenchyma is usually normal; however, in TSC patients, ill-defined ground-glass opacities corresponding to MMPH or septal thickening with or without ground-glass opacities consistent with chylous congestion may be present (table 3). The latest ATS/JRS guidelines recommend that clinical diagnosis of LAM should be established using the least invasive approach [93, 98]. Diagnosis of S-LAM may be more challenging than TSC-LAM and requires the presence of one of the associated features besides characteristic HRCT, such as rAMLs, serum VEGF-D of >800 pg·mL\(^{-1}\), chylous effusion, lymphangiomatoses, demonstration of LAM cells in pleural fluid or lymph node aspiration/biopsy or histopathological confirmation after a lung biopsy or retroperitoneal/pelvic mass biopsy (figure 4). A thorough clinical history and physical examination has a pivotal role to rule out other alternative diagnosis for cystic lung disease, such as metastatic carcinoma, emphysema, light-chain deposition disease, Sjögren syndrome, pulmonary Langerhans cell histiocytosis and Birt–Hogg–Dubé syndrome [99–101].

Pulmonary function tests

Pulmonary function tests (PFTs) are an important tool for diagnosis, follow-up and management of patients with LAM. In a retrospective series from a LAM registry, airway obstruction was the most common physiological pattern (57.3%), while normal and restrictive patterns were present in 33.9% and 11.4%, respectively. Low diffusing capacity of the lung for carbon monoxide (\(D_L^{CO}\)) was found in 56.9% of patients, and 17% of patients showed a positive bronchodilator response [28]. In a series of 143 patients, airway obstruction was similarly predominant (68%) and 16% had a mixed physiology. Reversible airway obstruction was present in a quarter of patients. Interestingly, positive bronchodilator response was associated with a more severe airway obstruction, an accelerated rate of forced expiratory volume in 1 s (FEV\(_1\)) decline and a solid rather than cystic pattern of LAM lesions at histology. However, \(D_L^{CO}\) correlated better than FEV\(_1\) with LAM histologic score, a predictor of poor outcome. A positive bronchodilator response and a low \(D_L^{CO}\) may predict a rapid decline of lung function and a worse outcome [102, 103]. A positive bronchodilator response was not associated with a reduction of dynamic hyperinflation or improvement of exercise capacity following bronchodilator treatment [104]. Thereby, the role of bronchodilator therapy in such patients remains unclear.

FEV\(_1\) declines at a rate of \(\sim\)70–140 mL·year\(^{-1}\), with a tendency to a slower rate after menopause [12, 59]. Although their disease may be milder at diagnosis, patients with TSC-LAM have a similar rate of annual lung function decline [32]. A recent study compared incidental cases of S- versus TSC-LAM and found a similar pulmonary disease extent on HRCT and severity on PFTs. After adjusting for selection bias, Di MARCO et al. [29] concluded that patients with TSC- and S-LAM have indistinguishable natural history.

Implications of diagnosis, screening and surveillance

Patients with TSC have a significantly shorter survival than the general population [38]; their estimated life expectancy is reduced to 70 years [105]. Leading causes of death include renal disease, sudden unexpected death in epilepsy and LAM [38, 106, 107]. TSC-LAM patients have a shorter life expectancy (63 years) when compared to TSC patients without LAM [23, 105]. A Canadian study reported a higher mortality

| TABLE 3 Diagnostic criteria for lymphangioleiomyomatosis (LAM) |
|---------------------------------------------------------------|
| Compatible clinical history AND Characteristic HRCT of the chest AND One or more of: |
| Presence of TSC |
| Renal angiomyolipoma(s) |
| Elevated serum VEGF-D >800 pg·mL\(^{-1}\) |
| Chylous effusion (pleural or ascites) confirmed by tap and biochemical analysis of the fluid |
| Lymphangioleiomyomas (lymphangiomatoses) |
| Demonstration of LAM cells or LAM cell clusters on cytological examination of effusions or lymph nodes |
| Histopathological confirmation of LAM by lung biopsy or biopsy of retroperitoneal or pelvic masses |
| HRCT: high-resolution computed tomography; TSC: tuberous sclerosis complex; VEGF: vascular endothelial growth factor. Reproduced and modified from [93] with permission. |
Clinical suspicion of LAM

- Yes
- No

HRCT chest with features characteristic of LAM

- Yes
- No

Consider alternative diagnosis

Detailed clinical evaluation confirms the presence of TSC

- Yes
- No

Confirmed diagnosis of TSC-LAM

Obtain:
1) Serum VEGF-D
2) Non-contrast CT or MRI abdomen/pelvis
3) Chylous fluid/node/mass aspiration (if applicable)

Are any of the following present?
1) Serum VEGF-D ≥800 pg·mL–1
2) Renal AMLs or lymphangioleiomyomas
3) Positive cytology

- Yes
- No

Confirmed diagnosis of LAM

Is histopathological confirmation desired/required?

- Yes
- No

Continue close monitoring with serial PFTs every 3–4 months

Transbronchial lung biopsy with characteristic features of LAM

- Yes
- No

Confirmed diagnosis of LAM

Surgical lung biopsy

Confirmed diagnosis of LAM

FIGURE 4 Proposed algorithm for the diagnosis of lymphangioleiomyomatosis (LAM) in a patient with compatible clinical history. HRCT: high-resolution computed tomography; TSC: tuberous sclerosis complex; VEGF: vascular endothelial growth factor; CT: computed tomography; MRI: magnetic resonance imaging; AML: angiomyolipoma; PFT: pulmonary function test. #: suspect LAM clinically in young to middle-aged female patients presenting with worsening dyspnoea and/or pneumothorax/chylothorax. Most patients with LAM will have an obstructive defect on PFTs. Some patients, especially early in their disease course, may be asymptomatic and have normal PFTs. ¶: characteristic HRCT features of LAM include the presence of multiple, bilateral, round, well-defined, relatively uniform, thin-walled cysts in a diffuse distribution. The intervening lung parenchyma often appears normal on HRCT. Other associated features that can be seen on HRCT in some patients with LAM include the presence of chylous pleural effusion, pneumothorax, ground-glass opacity suggestive of chylous congestion or multiple tiny nodules characteristic of multifocal micronodular pneumocyte hyperplasia (in patients with TSC-LAM). *: referral to a TSC centre should be considered if there is uncertainty regarding the diagnosis of TSC. Features suggestive of TSC include the presence of any of the following: subungual fibromas, facial angiofibromas, hypomelanotic macules, confetti lesions, Shagreen patches, positive family history of TSC, history of seizures or cognitive impairment, or presence of cortical dysplasias, subependymal nodules and/or subependymal giant cell astrocytomas on brain imaging. Routine brain imaging is not indicated if clinical suspicion for TSC is low. Detailed diagnostic criteria for TSC to
Pulmonary hypertension

Pulmonary hypertension is a known complication of LAM, albeit with limited published data regarding its prevalence and characteristics. The prevalence is estimated to be ∼7% [110–112]. Pre-capillary pulmonary hypertension is usually mild with a mean pulmonary artery pressure of 29–32 mmHg, and is identified after a mean 9 years after the diagnosis of LAM. Haemodynamic features correlate with PFTs, especially $FEV_1$ and $DL_{CO}$ [111, 112]. In light of these data, the 6th World Symposium on Pulmonary Hypertension proposed that pulmonary hypertension in LAM be classified under pulmonary hypertension due to lung diseases/hypoxia group (group 3) instead of group 5 (unclear and/or multifactorial mechanisms) [113]. A screening echocardiogram is recommended in patients with severe disease, in those with long-term oxygen therapy and in those considered for lung transplantation [26].

Particular considerations

Pregnancy in patients with LAM

LAM cells frequently express receptors for oestrogen and progesterone. Consequently, it was hypothesised that LAM is a hormone-dependent disease and females were recommended to avoid pregnancy for fear of disease progression or complications due to increased oestrogen load during pregnancy. Case reports
suggest that LAM may be exacerbated by exogenous oestrogens [114]. COHEN et al. [58] found that females who had never been pregnant or diagnosed with LAM after pregnancy had better FEV$_1$ and $D_L$CO than those who were diagnosed before and during their pregnancy. In a series of 50 patients with LAM, JOHNSON and TATTERSFIELD [30] reported pregnancy in 28 patients, of whom three were diagnosed before pregnancy and four during their pregnancy. Of these seven pregnancies, five were complicated by chylous effusion ($n=2$) and pneumothoraces ($n=3$). The overall incidence of complications was 11 times higher during pregnancy. TAVEIRA-DASILVA et al. [10] retrospectively assessed lung function and CT scans before and after pregnancy in 16 females with LAM. Five patients suffered pneumothoraces during pregnancy, of which four were bilateral. There was an increased rate of loss of lung function, with mean±SD FEV$_1$ deteriorating from 77±19% to 64±25% predicted and $D_L$CO from 66±26% to 57±26% predicted. 10 patients had greater rates of decline after pregnancy compared to their pre-conception state. Cyst scores worsened in all cases where a CT scan was available. It was not possible to predict the impact of pregnancy according to pre-pregnancy lung function [10]. Despite the need to address this subject, data remain scarce and limited to case reports and retrospective observations. Preconception counselling and management should be based on a case-by-case discussion.

Air travel

Air travel is a known risk factor for pneumothorax in LAM patients. A lower atmospheric pressure increases the volume of pulmonary cysts, conceivably facilitating their rupture with consecutive pneumothorax. The risk of pneumothorax in LAM patients is ~1000 times higher than in the general population, and about three times higher after air travel as compared to baseline [11]. The risk is significantly higher during the first 30 days following the flight [11]. Interestingly, air travel-related pneumothorax may not always be symptomatic [115]. Despite this, there is no specific recommendation with regards to air travel in patients with LAM and it is not systematically contraindicated. Patients should be educated about signs and symptoms of pneumothorax prior to flying and advised to seek medical advice if suggestive symptoms occur [26, 116]. The safety of air travel following a pneumothorax in patients with LAM has not been studied. The British Thoracic Society recommends confirming the resolution on a chest radiograph and to wait a further 7 days before the next flight [116].

Treatments

mTOR inhibitors

Up until 2008, management of LAM was primarily supportive, referring to smoking cessation programmes, vaccination and avoidance of exogenous oestrogens, as well as education regarding air travel, pneumothorax and pregnancy [26]. The identification of TSC1 and TSC2 mutations in TSC, and somatic mutations in S-LAM led to a better understanding of the pathophysiology. The identification of the dysregulation in the hamartin–tuberin complex of mTORC1, which lies downstream of the signalling pathway related to proliferation and cell growth foreshadowed the therapeutic potential of mTOR inhibitors for diverse TSC manifestations. To date, sirolimus is the only mTOR inhibitor approved for patients with LAM by the United States Food and Drug Administration, European Medicines Agency and Pharmaceuticals and Medical Devices Agency of Japan. The latest ATS/JRS guidelines recommend sirolimus use in patients with LAM with abnormal and/or declining lung function and in selected cases of chylous effusions [98].

Sirolimus was first studied in a small population of patients displaying rAMLs, either with TSC or S-LAM [117]. In this nonrandomised, open-label trial, sirolimus reduced the size of rAMLs and increased FEV$_1$ during the 12-month active treatment period. Other subsequent studies reported similar results in S-LAM patients [118–120]. The MILES trial was the first randomised, placebo-controlled, double-blind trial that demonstrated the efficacy of sirolimus in patients with LAM and moderate pulmonary impairment (FEV$_1$ <70%) [12]. During the 12-month treatment period, sirolimus stabilised lung function, with a between-group difference in the mean change of FEV$_1$ of 153 mL (11%). In addition, it improved quality of life and symptoms, and decreased serum VEGF-D levels. These benefits were lost when sirolimus was discontinued during the following 12-month observation period, during which lung function deteriorated at the same rate as in the placebo arm. These benefits were maintained at 4 years in a retrospective study in patients with S-LAM, with mild adverse events and persisting decreased levels of VEGF-D [121]. A population study of the MILES trial patients suggested that VEGF-C may be a promising biomarker to assess treatment response to sirolimus, although less promising for the diagnosis of LAM [122]. In addition, sirolimus has demonstrated additional benefits in other pulmonary manifestations, such as chylous effusion [123], chylous congestion [97] (resulting often in a complete resolution) and in abdominal lymphangiomas [124]. Whether sirolimus decreases the risk of pneumothorax is unknown, although an observational study of five females with LAM and recurrent pneumothorax treated with sirolimus for 5–36 months showed some promising results [96].
Aphthous ulcers or stomatitis were observed in the majority of patients and resolved with dose reduction or topical therapy. Diarrhoea and infectious complications, mainly pulmonary, were also common. Drug-induced pneumonitis, although not reported in the MILES trial, is a frequent side-effect in renal transplant patients and has also been described in LAM patients treated with sirolimus. It should be suspected, among other diagnoses, in patients presenting with worsening dyspnoea and ground-glass opacities on HRCT [125]. Approximately half of patients have an elevation of serum lipid levels due to sirolimus [117]. If there was an indication to treat the hyperlipidaemia, the use of simvastatin rather than atorvastatin has been shown to be well tolerated and might have an added benefit of controlling the disease [126].

Everolimus, another mTOR inhibitor, was associated with an improvement of FEV\textsubscript{1}, an increase in 6-min walk distance, a stabilisation of forced vital capacity (FVC) and a reduction of VEGF-D serum level in patients with FEV\textsubscript{1} 30–90% predicted [127]. In addition, it showed benefits in patients with other manifestations of TSC, such as SEGAs (by decreasing tumour size by >50% [128]), rAMLs, cutaneous features and resistant epilepsy [129, 130]. A recent meta-analysis supports the latter findings; mTOR inhibitors were associated with improved lung function and reduced size of rAMLs [131]. Compared to sirolimus, everolimus has a shorter half-life and better bioavailability, making it an interesting option for patients awaiting transplantation [132].

Longer studies are warranted to evaluate long-term effects of mTOR inhibitors. In the MILES trial, only patients with an FEV\textsubscript{1} <70% of predicted were eligible [12]; whether patients with better lung function would also benefit from sirolimus is still unknown. Interestingly, in a prospective cohort of 47 patients with progressive lung impairment secondary to LAM, patients with a higher FEV\textsubscript{1} at baseline and shorter disease duration had a significantly higher FEV\textsubscript{1} improvement [133]. Finally, care must be taken when prescribing mTOR inhibitors in patients already receiving antiepileptic drugs, given the risk of drug–drug interactions.

Investigational treatments

Doxycycline, a tetracycline antibiotic, has been studied in the treatment of LAM because of its inhibitory effects on production and activity of MMPs that promote lung destruction in LAM. However, a 2-year randomised double-blind trial of 23 female patients failed to demonstrate a difference in the decline in FEV\textsubscript{1} (the primary end-point), FVC, \textit{DLCO}, shuttle walk distance or quality of life [132], despite a decrease in urine MMP levels [134]. The ATS/JRS guidelines advise against its use in LAM patients [98].

Hormone therapy has been investigated because of the putative role of sex hormones in disease pathogenesis. No randomised controlled trial has studied progesterone and observational studies are overall negative, although data are conflicting. A prospective study of 11 patients showed that triptorelin, a gonadotrophin-releasing hormone inducing gonadal suppression in pre-menopausal females, did not preclude the loss of lung function, while causing loss of bone mineral density [135]. The routine use of hormonal therapy is not recommended [26, 98].

Lung transplantation

Lung transplantation is recommended for patients with LAM who reach a New York Heart Association functional class III or IV with limited exercise capacity and severely reduced lung function [26]. LAM is a rare cause for lung transplantation (1%). Recent observations showed an improved survival rate; up to 73.7% at 10 years [136] and a median survival of 12 years [137] following transplantation. TSC itself should not be considered a contraindication, although serious comorbidities are much more frequent than in patients with S-LAM [138], namely cognitive impairment.

Cases of LAM recurrence following lung transplantation have been reported [139, 140], consistent with LAM being a benign low-grade metastatic neoplasm. In clinical practice, with the advent of mTOR inhibitors, few patients may still require lung transplantation. Of note, sirolimus use before transplantation has been associated with impaired wound healing and bronchial dehiscence, eventually leading to death [141, 142]. Low-dose sirolimus may be continued in patients listed for lung transplantation, discontinued at the time of transplantation and resumed if necessary post-transplantation [143]. A recent cohort study of 13 patients suggested that, in selected patients, the introduction of sirolimus within the first month was safe [144]. However, more studies are needed to determine the optimal timing for resumption of sirolimus. The rationale to resume sirolimus after transplantation is to treat chylous effusion, to reduce LAM recurrence rate and to manage extrapulmonary manifestations [145].
**Future perspectives**

Although mTOR inhibitors are effective at stabilising lung function, the rate of lung function decline resumes once the drug is discontinued [12], since mTOR inhibitors are cytostatic rather than cytotoxic on LAM cells and do not cure the disease. Other therapeutic strategies are under study. LAM cells express several antigens found in melanoma, including gp100, MART-1, TRP-2 and TRP-1. The first three are effective targets for T-cells in melanoma, while the latter is a humoral target antigen [65]. These antigens evade immune detection through unknown mechanisms. It has been hypothesised that they may be targeted with melanoma immunotherapy via either immune checkpoint blockade or cytotoxic lymphocytes.

A marked reactivity for programmed death ligand-1 (PD-L1) in LAM nodules found in human lung as compared to control has been shown [146]. In a mouse model of LAM, a significantly increased number of T-cells in the lungs compared with controls, associated with an upregulation of PD-1–PD-L1 in LAM nodules has been evidenced. Furthermore, treatment with anti-PD-1 antibodies resulted in a longer survival in LAM mice, suggesting an interesting therapeutic avenue [147].

LAM cells are susceptible to cytotoxic, gp100 reactive and major histocompatibility complex (MHC) class I CD8 T-cells in *vivo* [65]. Based on this, Han *et al.* [148] tested a proof-of-concept with adoptive transfer of specific T-cells in *vivo* as a possible treatment for LAM. They developed a murine model of pulmonary LAM and injected gp100-TCR-specific T-cells isolated from MHC complex-matched mice into their circulation. This procedure reduced, but did not eliminate, the tumour burden. The addition of anti-PD-1 antibody further decreased the number of LAM cells [148].

Other ways of improving immune responses to melanosomal antigens, and thus bypass T-cell exhaustion, have been studied in the context of melanoma, such as cancer vaccine immunotherapy, showing promise that may be translated to LAM [146].

**Conclusion**

TSC is a multifaceted genetic disorder with substantial heterogeneity. Pulmonary manifestations are common, especially LAM, which is one of the leading causes of morbidity and mortality in TSC. Pneumothorax is quite frequent and characterised with frequent recurrence even after surgery. mTOR inhibitors are the only approved treatment for patients with progressive or moderate-to-severe LAM, thus changing the therapeutic landscape in LAM and other TSC manifestations, notably rAMLs. Immunotherapy warrants evaluation in LAM, in the hope of extending the benefits of treatment beyond the clinical stabilisation already obtained with mTOR inhibitors.
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