Front. Med. 2012, 6(4): 395–405
DOI 10.1007/s11684-012-0231-5

RESEARCH ARTICLE

Utility of transbronchial biopsy in the diagnosis of lymphangioleiomyomatosis

Riffat Meraj[1], Kathryn A. Wikenheiser-Brokamp[2], Lisa R. Young[3], Sue Byrnes[4], Francis X. McCormack (✉)[1]

[1] *Division of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, University of Cincinnati School of Medicine, Cincinnati, OH 45529, USA;* [2] *Pathology and Laboratory Medicine, Cincinnati Children's Hospital Medical Center and University of Cincinnati School of Medicine, Cincinnati, OH 45529, USA;* [3] *Division of Pediatric Pulmonary Medicine, Department of Pediatrics, Vanderbilt University, Nashville, TN 37232, USA;* [4] *The LAM Foundation, Executive Drive, Cincinnati, OH 45241, USA*

Abstract **Pulmonary lymphangioleiomyomatosis (LAM) is a rare cystic lung disease that targets women during their reproductive years. A confident diagnosis can often be based on clinical grounds, but diagnostic certainty requires pathological analysis. Although surgical lung biopsy is considered the gold standard for obtaining tissue in patients with diffuse lung disease, it is also associated with higher morbidity and mortality than alternative, less invasive techniques. The objective of our study was to examine the utility of transbronchial biopsy in the diagnosis of LAM. We conducted two online surveys of over 1 000 LAM patients registered with the LAM Foundation who were accessible by email. Transbronchial biopsy specimens were subsequently collected and reviewed by an expert pathologist to validate the diagnosis. We found that transbronchial biopsy has a yield of approximately 60% in patients with LAM. We conclude that transbronchial biopsy may be a safe and effective method for establishing the diagnosis of LAM, obviating the need for surgical lung biopsy in more than half of LAM patients.**

Keywords lymphangioleiomyomatosis; lymphangiomyomatosis; multicystic lung disease; diffuse cystic lung disease; transbronchial biopsy; perivascular epithelioid cell tumor (PEComa); HMB-45

Introduction

Lymphangioleiomyomatosis (LAM) is a cystic lung disease caused by smooth muscle cell proliferation, infiltration and tissue remodeling [1]. LAM occurs in up to 40% of women with tuberous sclerosis complex (TSC-LAM) and in a non-heritable sporadic form (S-LAM) that affects about 5 women per million. Although LAM is widely considered to be an interstitial lung disease (ILD) by clinicians, the pathology community has classified LAM as a low-grade malignant neoplasm since 2004 [2,3]. Genetic techniques have demonstrated that cells which comprise recurrent LAM lesions in the allograft of transplanted LAM patients arise from the recipient, consistent with a metastatic mechanism for the disease [4,5].

Because the disease is rare and the signs and symptoms can be non-specific, the diagnosis of LAM is often elusive and delayed, and many patients are treated for several years for asthma or chronic bronchitis before LAM is considered [6]. Even when the disease presents in a prototypical fashion with spontaneous pneumothorax, the diagnosis is typically delayed until after the first recurrence [7,8].

A clinically confident diagnosis of LAM can be made in patients who present with a characteristic high-resolution CT scan (HRCT) pattern of lung cysts and either TSC, chylous pleural effusion, chylous ascites, angiomyolipoma, lymphangioleiomyoma or lymph nodes involved by LAM [9] or a serum VEGF-D level of greater than 800 pg/ml [10]. The diagnosis can also be made on cytological grounds, based on transcutaneous needle biopsy of axial lymph nodes or lymphangioleiomyomas or the finding of LAM cells or LAM cell clusters in chylous fluid collections [11]. Approximately one third of patients present without the criteria for the clinical, serological, cytological and radiological based diagnoses mentioned above, however [10]. In those cases, if the patient and physician wish to achieve diagnostic certainty, lung biopsy is required.

Through the first half of the last century, all lung biopsies were obtained surgically by performing thoracotomy. Endoscopic procedures were subsequently developed that have

Received September 4, 2012; accepted October 8, 2012
Correspondence: frank.mccormack@uc.edu

become highly effective diagnostic tools. In 1965, Andersen and colleagues at the Mayo Clinic described the technique of transbronchial biopsy in 13 patients using the rigid bronchoscope [12]. In the late 1960s, the flexible fiberoptic bronchoscope (FB) was introduced and over time transbronchial biopsy through a channel in the FB became a widely-employed diagnostic technique for lung cancer and selected diffuse lung disorders including granulomatous lung diseases [13]. In many of the interstitial lung diseases, however, accurate diagnosis requires tissue specimens that are large enough to characterize pathological patterns or capture lesions in more patchy processes. Video-assisted thoracoscopic biopsy was first described in the 1980–1990s [14], and is now the procedure of choice for surgical lung biopsy when diagnostic certainty is required and less invasive techniques are not feasible or not likely to be successful.

Surgical lung biopsy has higher diagnostic yield compared to transbronchial lung biopsy but is also associated with higher morbidity and mortality. Complications of surgical lung biopsy include postoperative respiratory failure, prolonged air-leak, infection, chronic pain and death [15–17]. Surgical lung biopsy for the diagnosis of interstitial lung disease has a postoperative mortality rate of 2% to 4.5% [12]. Transbronchial biopsy, in contrast, has a much more favorable risk profile in most cases. Pneumothorax, hemorrhage and infection all occur in less than 5% of patients [13,18,19–25], and death occurs in less than 0.12% – 0.24% of patients [20,21]. Although transbronchial lung biopsy in experienced hands is considered a safe and well-tolerated procedure, caution is advised in patients with bleeding diatheses, respiratory failure, need for positive pressure ventilation and pulmonary hypertension. One must also consider the projected diagnostic yield before exposing patients to transbronchial biopsy, since unsuccessful procedures invariably lead to subsequent procedures with the associated cumulative risk. Little is known about the risk of complications from transbronchial biopsy in patients with LAM.

The yield of transbronchial biopsy in diagnosing LAM is generally considered to be low [26]. We report here that LAM can be diagnosed in up to 60% of patients using tissue specimens obtained transbronchially, and thus transbroncial biology is a plausible diagnostic approach in selected patients being evaluated for LAM.

Materials and methods

All patients 18 years of age or older who were registered with the LAM Foundation, a patient advocacy organization in the United States, were invited to perform two online surveys (Fig. 1). The first survey was sent to all patients with email access and was followed by phone interviews. A second online survey, refined based on the responses to the first questionnaire, was conducted using an online survey instrument (SurveyMonkey. Com, LLC, Palo Alto, CA, USA) to identify more patients who had undergone transbronchial biopsy and for the purpose of obtaining additional slides from the identified procedures for our review. The LAM patients who reported they had undergone transbronchial biopsy in the first survey were excluded from the second survey.

Institutional review board (IRB) approval was obtained from University of Cincinnati (IRB #:10-10-26-01). Written consents were obtained and a copy of the consent was mailed to the patients for their records. Attempts were made to collect both stained and unstained slides of the lung tissue obtained transbronchially for blinded histological review by the study pathologist.

Results

The schema that describes the study is depicted in Fig. 1. The first survey was electronically delivered to 847 LAM Foundation registered patients. Of the 217 patients who responded to the survey, 52 reported a history of transbronchial biopsy and 14% reported complications, including pneumothorax (6%), bleeding (4%), chest pain (2%), and pneumonia (2%). Phone interviews were then conducted for the 52 positive respondents. After excluding 8 patients who could not be contacted, 3 patients who withdrew consent and 15 patients who through phone discourse were found not to have had transbronchial biopsy for the express purpose of making a diagnosis of LAM, it was determined that only 26 of the initial 52 affirmative responders had undergone transbronchial biopsy for bona fide diagnostic purposes. Of those 26 patients, 15 reported that the procedure was diagnostic for LAM, indicating a yield of 58%.

Through time, additional patients registered with the LAM Foundation. A second online survey with questions modified to remove identified ambiguities was sent to 1 082 LAM Foundation registered patients, excluding the 52 patients who reported having had transbronchial biopsies in the first survey, but otherwise overlapping extensively with the original cohort. Of the 380 patients who responded, 37 patients reported they had undergone transbronchial biopsy for diagnostic purposes and 20 (54%) were self-reported to be positive for LAM. Because the primary purpose of the second survey was to obtain additional biopsy specimens for blinded pathological review, and because the questions had been refined to reduce ambiguity, phone interviews were not conducted.

Collectively, 35 of 63 patients identified through these two surveys had self-reported, diagnostic transbronchial biopsies indicating a diagnostic yield of 56%.

A vigorous attempt was made to collect transbronchial biopsies from both of these survey participant groups. Thirty two hospital pathology departments were contacted to inquire about the availability of transbronchial biopsy specimens, and tissue release forms were faxed to 29 hospitals in the United

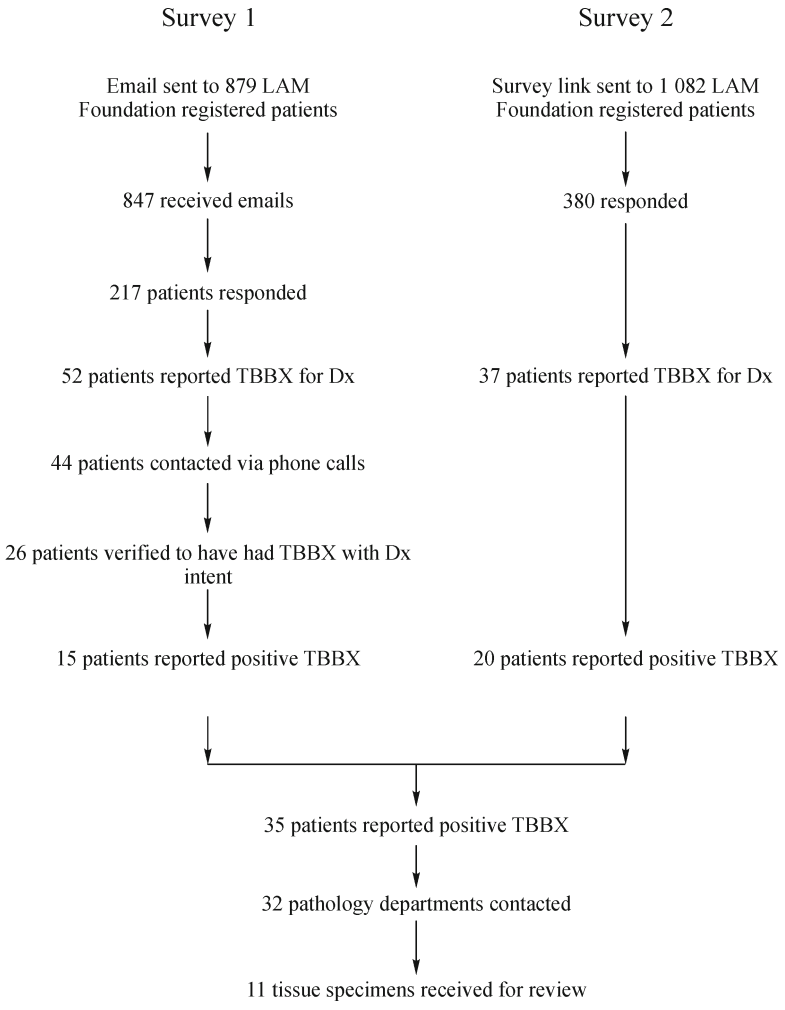

Fig. 1 Study schema.

States and Canada. A total of 11 transbronchial biopsy cases were retrieved and pathologically reviewed for this study. One case was excluded from the analysis because the procedure was conducted in a transplanted patient to rule out LAM recurrence and recurrence was never unambiguously documented or excluded. Neither unstained slides or blocks could be obtained on the remaining 18 patients, in most cases because the hospital was not responsive or the samples were no longer available. Hematoxylin and eosin stained histological sections were reviewed for all cases. The biopsies were considered diagnostic if the two key histologic features of LAM were present, namely cystic changes and LAM cell infiltrations (Table 1, Figs. 2 and 3). Note was also made as to whether hemosiderin laden macrophages were present in the air spaces given the common occurrence of this finding in LAM. Indeed, hemosiderin laden macrophages were identified in all cases examined. The presence of hemosiderin laden macrophages, however, was not used as a criterion to determine whether the biopsy was diagnostic since this is a relatively non-specific finding that can be seen in association with many pulmonary pathologic entities. Cystically dilated airspaces detected histologically correlate with the diffuse cysts in LAM lungs seen radiographically and by gross pathologic examination (Fig. 4). Cystic spaces were present in 5 of the 6 diagnostic biopsies and none of the non-diagnostic biopsies. LAM cells were characterized as round, oval or spindled cells growing in haphazardly arranged bundles rather than the orderly arrangement of smooth muscle cells normally present around airways and vessels (Figs. 2 and 3). There was one diagnostic case that lacked definitive cysts and LAM cells by routine histologic evaluation of H&E stained slides. Diagnosis in this case was aided by special stains. Immunohistochemically stained slides were received for 4 cases, and HMB-45 and smooth muscle actin immunohistochemical staining was performed on 1 additional case at the time of review. Immunohistochemical staining highlighted rare HMB-45 positive cells with cytologic features of LAM cells in the biopsy that lacked histologic evidence of definitive cystic changes and LAM cells in H&E stained sections, rendering a diagnosis of LAM in this

Table 1 Transbronchial biopsy results in patients with a confirmed diagnosis of LAM

Patient ID	Age (year)	Clinical history	Diagnostic	Diagnostic features
2	29	Not provided	Yes	LAM cells (+) cysts (+) Hemosiderin (+) IMH: HMB-45 (scattered cells +) SMA (focally +)
3	37	Possible LAM	No	LAM cells (−), Cysts (?) Hemosiderin (+) IMH: HMB-45 (−) SMA (focally +), PR (focally +)
4	40	Honeycomb lung; rule out LAM, eosinophilic granuloma Clinical diagnosis: LAM	No	LAM cells (−), Cysts (−) Hemosiderin (+) IMH: None
7	35	Not provided	Yes	LAM cells (+), Cysts (+) Hemosiderin (+) IMH: None
8	39	History of LAM s/p lung transplant with increased dypsnea and CT showing increased septal lines; ?Rejection, ?PTLD, ?LAM	Yes	LAM cells (?), Cysts (−) Hemosiderin (+) IMH: HMB-45 (2 slides; 1 [−], 1 [rare +]) Desmin (focally +), ER (focally +), PR (rare +)
9	50	8 years of progressive dyspnea, cysts on chest CT, PFTs showing obstruction. Patient is a non-smoker with normal alpha-1 antitrypsin levels; rule out LAM	No	LAM cells (−), Cysts (−) Hemosiderin (+) IMH: None
10	37	Not provided	Yes	LAM cells (+), Cysts (+) Hemosiderin (+) IMH: None
11	##	Cystic lung disease; rule out LAM	Yes	LAM cells (+), Cysts (+) Hemosiderin (+) IMH: HMB-45 (scattered +) SMA (+), Desmin (+)
12	40	Cystic lung disease; assess for LAM	Yes	LAM cells (+), Cysts (+) Hemosiderin (+) IMH: HMB-45 (scattered +) SMA (+), ER (focally +)
13	41	Cystic lung disease concerning for LAM	No	LAM cells (−), Cysts (−) Hemosiderin (not examined) IMH: None

##, the age of the patient was not disclosed.

previously non-diagnostic biopsy. Of the 5 total biopsies with HMB-45 immunohistochemical staining available, 4 contained subpopulations of HMB-45 positive LAM cells. The one HMB-45 negative biopsy was judged to be non-diagnostic. LAM cell proliferations were also highlighted by positive immunohistochemical staining for the smooth muscle markers, smooth muscle actin and desmin, in selected cases (Fig. 3).

The outside facilities, including consultation results, reported that the biopsy was diagnostic for LAM in 6 of 10 cases (60%). Our review confirmed the outside interpretation in 6 cases and conflicted with the outside interpretation in 3 cases. An additional case of a transbronchial biopsy of a transplanted lung from a patient with known LAM was interpreted as "consider possibility of recurrent LAM" by the outside facility, and considered diagnostic of LAM upon review. Of note, the original slides received for review in this case were not diagnostic of LAM, including an HMB-45 stained slide which was negative; however, a second HMB-45 stained slide was subsequently received and reviewed yielding rare HMB-45 positive cells with cytologic features of LAM cells that were not represented on the initial slides. The 3 cases wherein there was a discrepancy between the original and review diagnosis included: (1) one case with no clinical information provided that was interpreted as "mild chronic interstitial inflammation; mild, focal interstitial fibrosis; intraalveolar siderophages; negative for granuloma; negative for malignancy" that was considered diagnostic of LAM upon review (Fig. 2 E–F), and (2) 2 cases from patients with a clinical diagnosis of LAM or possible LAM in which the outside pathology report indicated a diagnosis of "features suspicious for LAM" and "LAM," respectively. Importantly, the diagnosis of LAM in this second instance was made after examination of additional deeper histologic sections that were not received for review. Overall, pathologic evaluation of the 11 cases received for review led to the determination that 6 of

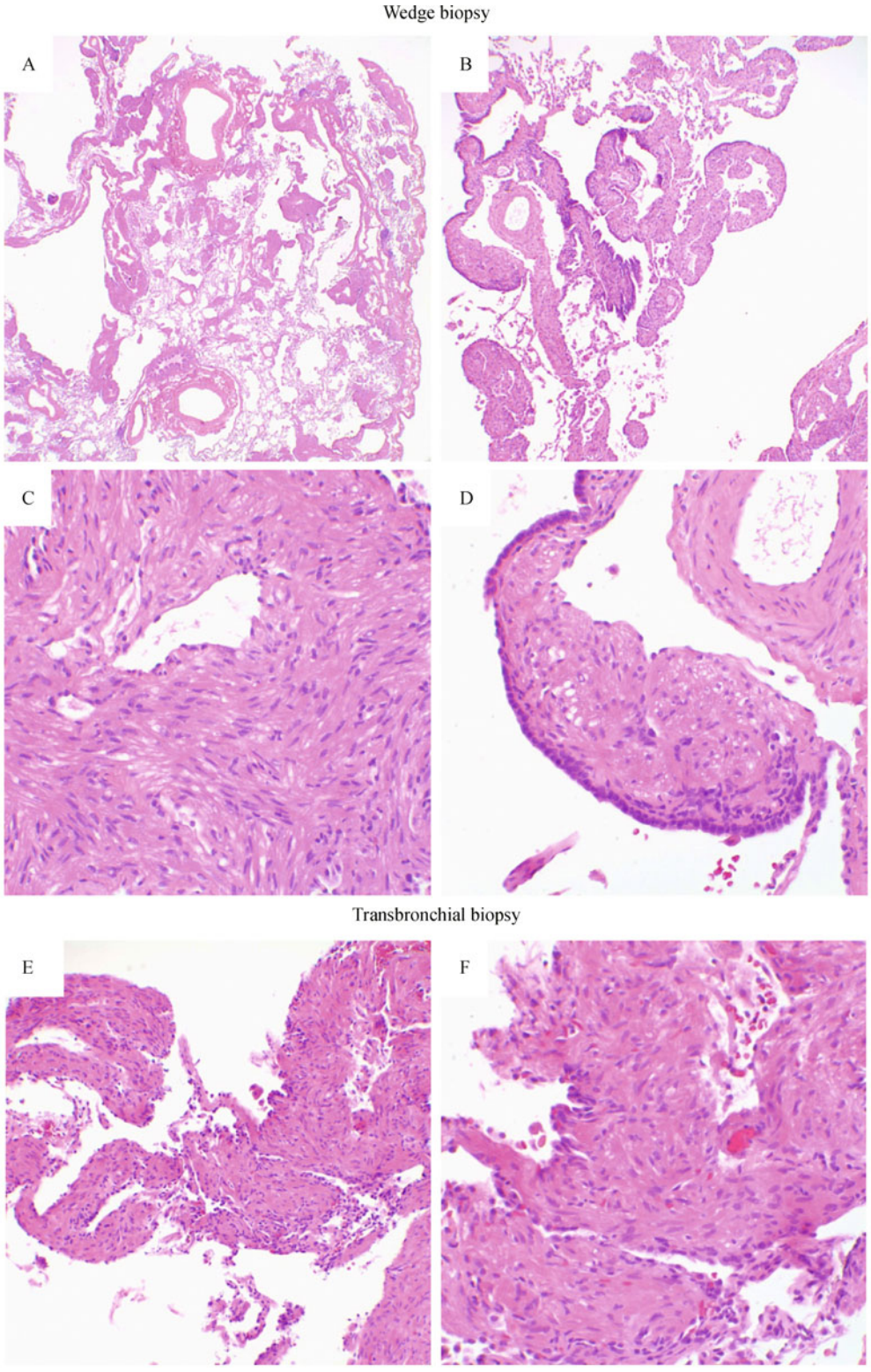

Fig. 2 Surgical lung wedge biopsy (A–D) and transbronchial biopsy (E–F) from a patient who underwent both procedures. Both biopsies have diagnostic histologic features of LAM including cysts (A–B, E) with LAM cell bundles at the periphery. The LAM cells are morphologically heterogeneous with a spectrum ranging from spindle-shaped cells (C) to larger round to oval epithelioid cells (D). Hematoxylin and eosin stain. Original magnifications: (A) 20 ×; (B) 100 ×; (C, D, F) 400 ×; (E) 200 ×.

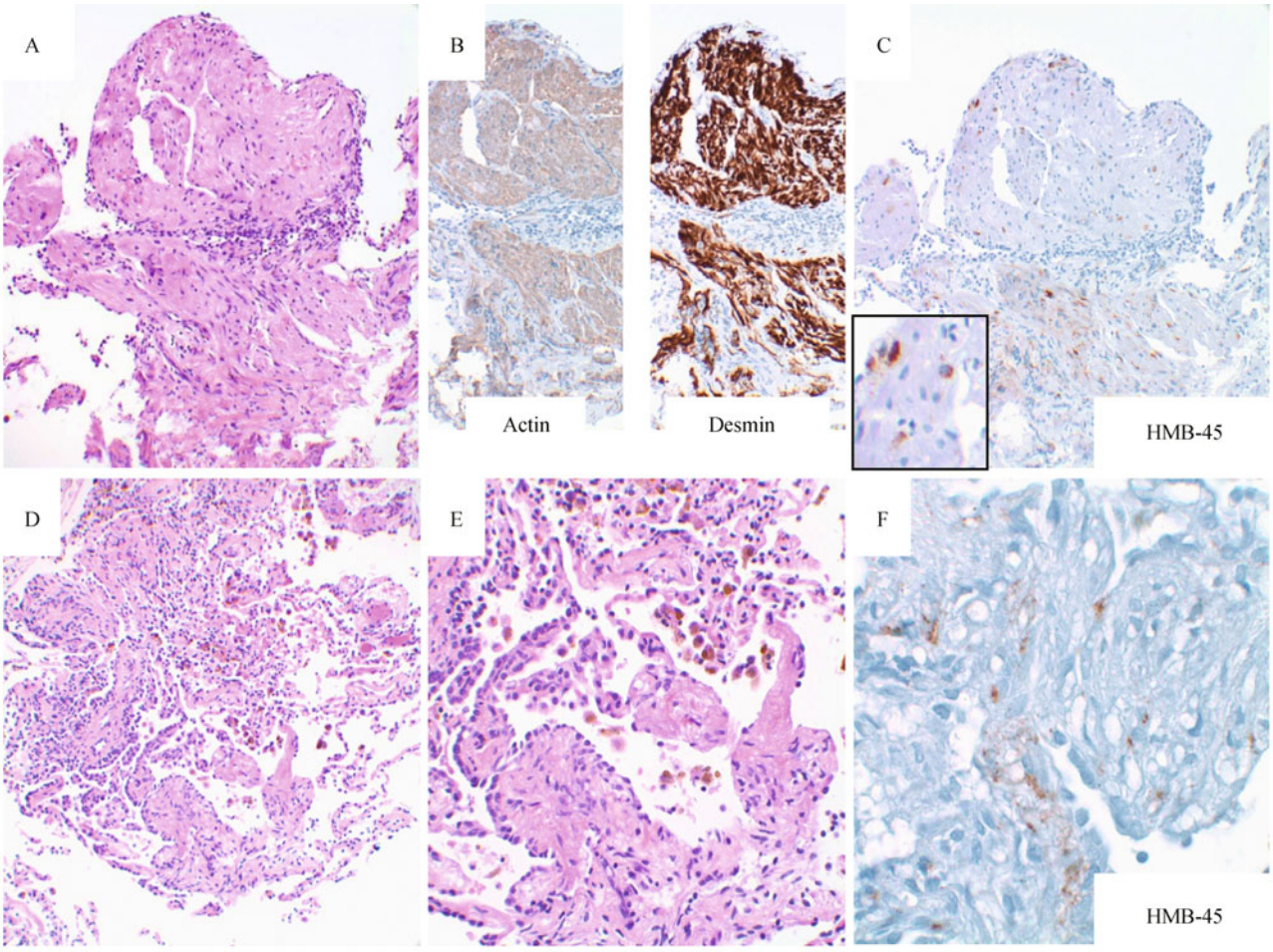

Fig. 3 Diagnostic transbronchial biopsies from two LAM patients (A–C and D–F) showing haphazardly arranged LAM cell proliferations surrounding enlarged airspaces (A, D, E). The LAM cells are diffusely positive for the smooth muscle markers, actin and desmin (B), and a subpopulation of LAM cells are positive for HMB-45 (C, F) by immunohistochemical stains. Hemosiderin laden macrophages are frequently present within airspaces (D, E). Hematoxylin and eosin stain (A, D, E) or immunohistochemical stains (B, C, F). Original magnifications: (A–D) 200 ×; (E) 400 ×; (F) 1 000 ×.

10 transbronchial biopsies were diagnostic for LAM, indicating a diagnostic yield of 60% (Fig. 5). Of the four cases that were nondiagnostic, the diagnosis of LAM was based on typical cystic change on HRCT and surgical biopsy results in two, markedly elevated serum VEGF-D of 2 296 pg/ml in one, and the presence of angiomyolipoma in one.

Discussion

LAM is typically included on lists of diffuse lung diseases that can occasionally be diagnosed using transbronchial biopsy through a fiberoptic bronchoscope. Although case reports and small case series over the past several decades have reported that transbronchial biopsy can be diagnostic in 20%–85% of patients with LAM [27–34], the pulmonary community has been slow to adopt this approach, and in practice the technique is only infrequently employed for this purpose. We believe that our results and those of others support the diagnostic utility of the test.

In an early series of 5 patients with LAM who underwent transbronchial biopsy, the atypical smooth muscle cells that we identified were initially misinterpreted as fibrocytes [35]. When 4 of these early cases were subsequently reviewed by an expert LAM pathologist, 3 out of 4 were found to be diagnostic for LAM and the 4th was deemed to be suggestive of the diagnosis. In 1996, Naalsund *et al.* presented a case series of patients with LAM [36]. Transbronchial lung biopsy was conclusive in 4 of 5 patients. In 2 patients the changes were initially described as interstitial fibrosis, and were verified to be consistent with LAM after the suspicion of LAM was communicated to the pathologist and additional staining was performed. The 5th patient required surgical

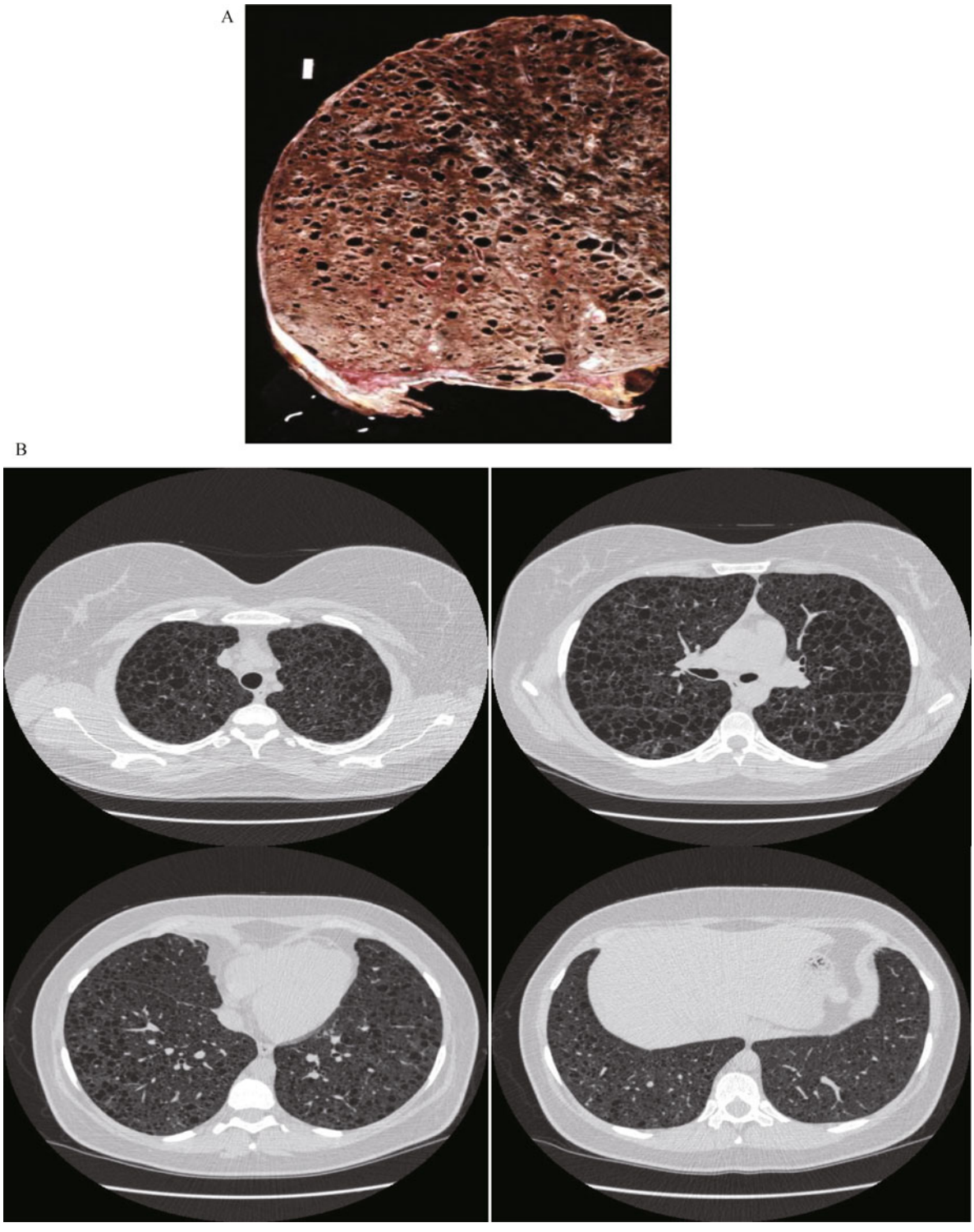

Fig. 4 (A) Gross pathologic image of LAM lung. Grossly, LAM lungs are enlarged and diffusely cystic, with dilated airspaces as large as 2.0 cm in diameter. (B) HRCT showing diffuse thin walled cysts in a patient with LAM. Diagnosis was confirmed with transbronchial biopsy.

lung biopsy to confirm the diagnosis. The CT of the chest was typical for LAM in 3 of the 4 patients diagnosed with LAM. All patients had experienced at least one episode of pneumothorax in their lifetime, and pulmonary function impairment was moderate to severe, with FEV1 ranging from 43% to 83% of predicted and DLCO from 24% to 58% of predicted [36]. Torre and Harari found that a transbronchial biopsy approach to the diagnosis of LAM was successful in

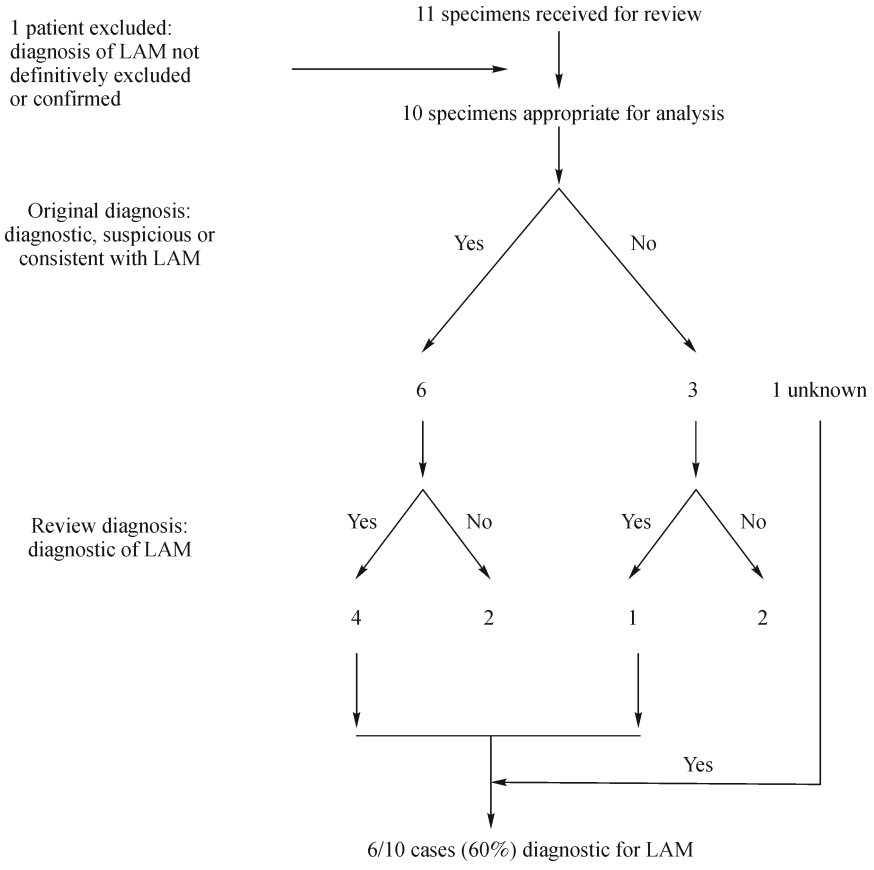

Fig. 5 Summary of pathologic review of received transbronchial biopsies from LAM patients.

six of seven patients with high clinical-radiological suspicion for LAM [37]. In the remaining case the diagnosis of LAM was confirmed after surgical lung biopsy. They reported one pneumothorax and no other complications in this cohort. Ling Ye *et al.* reported in a review article that 97 of 108 patients in their series from China were diagnosed with pulmonary biopsies [38]. In 49 patients, the diagnostic pulmonary biopsies were obtained transbronchially. The overall yield of transbronchial biopsy was not reported in this study, but this series clearly demonstrates the effectiveness of this approach. Transbronchial biopsy has also been reported to be diagnostic in lung transplant patients with recurrence of LAM in the allograft [39].

In our study, all diagnostic biopsies exhibited abnormal proliferations of smooth muscle cells, and HMB-45 staining was demonstrated in a subpopulation of these cells in four cases. HMB-45 is an antibody raised to a melanoma related antigen called gp-100 that is expressed in LAM cells [27], and is positive in melanoma and adrenal pheochromocytomas from patients with multiple endocrine neoplasia type 2A [40]. The fraction of LAM cells that stain with HMB-45 is highly variable and often on the order of only 10%–20%. For larger biopsy specimens, many pathologists believe that the histological findings of LAM are sufficiently distinctive to allow for definitive diagnosis in the appropriate clinical setting. Thus, it is possible to make the diagnosis of LAM on large specimens even in the rare cases that are HMB-45 negative. For small samples obtained by transbronchial biopsy, HMB-45 staining improves diagnostic sensitivity and specificity [27,41]. It is therefore important for the bronchoscopist to communicate clinical suspicion of LAM to the pathologist so that appropriate immunohistochemical analysis can be planned. It is also important to note that sampling error can arise and result in a negative biopsy in cases with a paucity of LAM cell proliferations. In general, in order to optimize the diagnostic yield of transbronchial biopsy in patients with diffuse lung disease, at least five to six specimens from unique sites should be obtained during bronchoscopy and larger biopsy specimens enhance diagnostic sensitivity and specificity [42]. LAM specific studies of optimal biopsy numbers and sizes have not been performed. Importantly, examination of additional histologic levels of the biopsy, especially in the setting of a high clinical suspicion for LAM, may be fruitful in providing a definitive diagnosis as was the case in one of the samples provided to us, and thus spare the patient additional diagnostic procedures. Emerging data suggest that LAM cell expression of cathepsin-K or MART-1 may also be useful for diagnosis by identifying

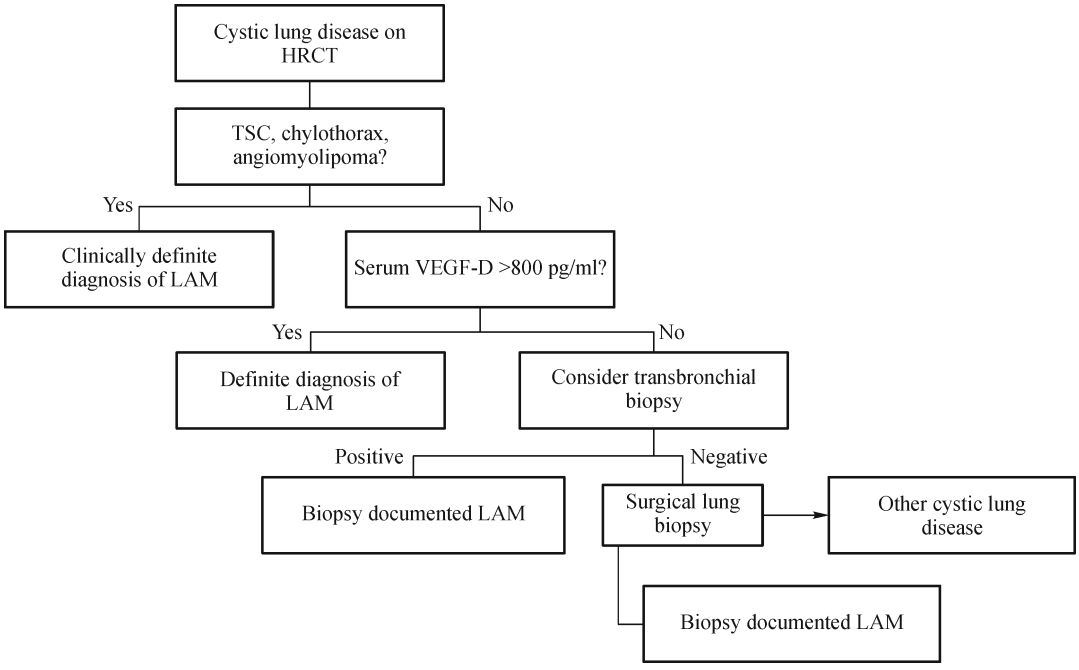

Fig. 6 Proposed diagnostic algorithm for females with cystic lung disease.

LAM cells in smaller transbronchial biopsy specimens [43,44]. Estrogen and progesterone receptor staining in LAM cells provide additional adjunct markers for diagnosis that may one day also be useful for guiding therapy [45].

Routine use of transbronchial biopsy for the diagnosis of LAM cannot be widely recommended until the safety of the procedure in patients with diffuse cystic lung disease is better understood. Transbronchial biopsy does not generally affect pulmonary function, unless pneumothorax, bleeding or infection occurs. There is a general sense among pulmonary physicians that the risk of pneumothorax may be higher in patients with cystic lung disease, however, this has never been formally studied or substantiated in a definitive way. The major risk, pneumothorax and need for chest tube drainage, is an expected outcome of surgical biopsy. The major hazard of transbronchial biopsy, therefore, is a failure to be vigilant for pneumothorax after the procedure. To optimize safety, a post procedure chest radiograph should be routinely obtained in patients with LAM. The series by Torre and Harari is small but suggests that the complication rate may be comparable to other diffuse lung disease populations [37]. Another area that needs to be further explored is the relationship between the cyst number and distribution to the diagnostic yield of biopsy. LAM is likely more difficult to detect using transbronchial biopsy in early stages when the degree of LAM cell infiltration and cystic change is low, although this remains to be tested. Determining the risks and benefits of transbronchial biopsy in LAM will require prospective collection of data in an organized manner, perhaps through LAM networks such as those established in the United States, the UK and Japan.

There were several limitations to this study including the small sample size and the limited clinical data that were available from the study population. Biases introduced by non-response, self-selection, and inaccurate patient recall could certainly have affected the results. In addition, despite our best efforts, tissue blocks or unstained slides were available for review and/or HMB-45 staining in only a subset of patients.

We conclude that transbronchial biopsy has an acceptable diagnostic yield in patients with LAM and propose a diagnostic algorithm for females with cystic lung disease that includes the use of the diagnostic biomarker, serum VEGF-D [10] (Fig. 6). A less invasive, bronchoscopic attempt at making the diagnosis of LAM prior to referring patients with cystic lung disease for surgical lung biopsy may be justified. This may be particularly true for patients who are considering use of mammalian target of rapamycin (mTOR) inhibitors [46] and need to confirm the diagnosis before exposure to the drug toxicities of everolimus or sirolimus, or other patients who do not want to be subjected to the risk of VATS. Additional studies to determine the diagnostic yield of transbronchial biopsy at various disease stages, and to better define the procedure risks are needed.

Acknowledgements

We would like to thank the LAM Foundation for their assistance

with contacting patients and hospitals, and Thomas V. Colby, MD Professor of Laboratory Medicine and Pathology at Mayo Clinic Foundation.

Abbreviations

LAM, lymphangioleiomyomatosis; VATS, video-assisted thoracic surgery; IMH, immunohistochemistry; SMA, smooth muscle actin; ER, estrogen receptor; PR: progesterone receptor; PTLD, post-transplant lymphoproliferative disorder; HRCT: high resolution CT; DLCO, carbon monoxide diffusion capacity; PFT, pulmonary function test; TBBX, transbronchial biopsy; Dx, diagnosis.

References

1. McCormack FX. Lymphangioleiomyomatosis: a clinical update. Chest 2008; 133(2): 507–516
2. Travis WD, Colby TV, Corrin B, Shumosato Y, Brambilla E. Histological Typing of Lung and Pleural Tumors. Berlin: Springer. 1999
3. Travis WD, Brambilla E, Muller-Hermlink HK, Harris CC. Pathology and Genetics: Tumours of the Lung, Pleura, Thymus and Heart. Lyon: IARC. 2004
4. Karbowniczek M, Astrinidis A, Balsara BR, Testa JR, Lium JH, Colby TV, McCormack FX, Henske EP. Recurrent lymphangiomyomatosis after transplantation: genetic analyses reveal a metastatic mechanism. Am J Respir Crit Care Med 2003; 167(7): 976–982
5. Bittmann I, Dose TB, Müller C, Dienemann H, Vogelmeier C, Lohrs U. Lymphangioleiomyomatosis: recurrence after single lung transplantations. Hum Pathol 1997; 28(12): 1420–1423
6. Taveira-DaSilva AM, Steagall WK, Moss J. Lymphangioleiomyomatosis. Cancer Contr 2006; 13: 276–285
7. Almoosa KF, McCormack FX, Sahn SA. Pleural disease in lymphangioleiomyomatosis. Clin Chest Med 2006; 27(2): 355–368
8. Johnson SR, Tattersfield AE. Clinical experience of lymphangioleiomyomatosis in the UK. Thorax 2000; 55(12): 1052–1057
9. Johnson SR, Cordier JF, Lazor R, Cottin V, Costabel U, Harari S, Reynaud-Gaubert M, Boehler A, Brauner M, Popper H, Bonetti F, Kingswood C, Review Panel of the ERS LAM Task Force. European Respiratory Society guidelines for the diagnosis and management of lymphangioleiomyomatosis. Eur Respir J 2010; 35 (1): 14–26
10. Young LR, Vandyke R, Gulleman PM, Inoue Y, Brown KK, Schmidt LS, Linehan WM, Hajjar F, Kinder BW, Trapnell BC, Bissler JJ, Franz DN, McCormack FX. Serum vascular endothelial growth factor-D prospectively distinguishes lymphangioleiomyomatosis from other diseases. Chest 2010; 138(3): 674–681
11. Hirama M, Atsuta R, Mitani K, Kumasaka T, Gunji Y, Sasaki S, Iwase A, Takahashi K, Seyama K. Lymphangioleiomyomatosis diagnosed by immunocytochemical and genetic analysis of lymphangioleiomyomatosis cell clusters found in chylous pleural effusion. Intern Med 2007; 46(18): 1593–1596
12. Andersen HA, Miller WE, Bernatz PE. Lung biopsy: transbronchoscopic, percutaneous, open. Surg Clin North Am 1973; 53(4): 785–793
13. Levin DC, Wicks AB, Ellis JH Jr. Transbronchial lung biopsy via the fiberoptic bronchoscope. Am Rev Respir Dis 1974; 110(1): 4–12
14. Mack MJ, Aronoff RJ, Acuff TE, Douthit MB, Bowman RT, Ryan WH. Present role of thoracoscopy in the diagnosis and treatment of diseases of the chest. Ann Thorac Surg 1992; 54(3): 403–408, discussion 407–409
15. Kreider ME, Hansen-Flaschen J, Ahmad NN, Rossman MD, Kaiser LR, Kucharczuk JC, Shrager JB. Complications of video-assisted thoracoscopic lung biopsy in patients with interstitial lung disease. Ann Thorac Surg 2007; 83(3): 1140–1144
16. Messika J, Parquin F, Puyo P, Sage E, Bonnette P, Rivaud E, Catherinot E, Couderc LJ, Chapelier A. Lung surgery in haematological patients: useful? hazardous? Rev Pneumol Clin 2011; 67(4): 220–225 (in French)
17. Quadrelli S, Lyons G, Ciallella L, Iotti A, Chertcoff J. Lung biopsy for the diagnosis of interstitial lung disease. Medicina (B Aires) 2007; 67(6 Pt 2): 691–697 (in Spanish)
18. Hanson RR, Zavala DC, Rhodes ML, Keim LW, Smith JD. Transbronchial biopsy via flexible fiberoptic bronchoscope; results in 164 patients. Am Rev Respir Dis 1976; 114(1): 67–72
19. Prakash UBS. Bronchoscopy. New York: Raven Press. 1994
20. Herf SM, Suratt PM, Arora NS. Deaths and complications associated with transbronchial lung biopsy. Am Rev Respir Dis 1977; 115(4): 708–711
21. Simpson FG, Arnold AG, Purvis A, Belfield PW, Muers MF, Cooke NJ. Postal survey of bronchoscopic practice by physicians in the United Kingdom. Thorax 1986; 41(4): 311–317
22. Frazier WD, Pope TL Jr, Findley LJ. Pneumothorax following transbronchial biopsy. Low diagnostic yield with routine chest roentgenograms. Chest 1990; 97(3): 539–540
23. de Fenoyl O, Capron F, Lebeau B, Rochemaure J. Transbronchial biopsy without fluoroscopy: a five year experience in outpatients. Thorax 1989; 44(11): 956–959
24. Anders GT, Johnson JE, Bush BA, Matthews JI. Transbronchial biopsy without fluoroscopy. A seven-year perspective. Chest 1988; 94(3): 557–560
25. Hernández Blasco L, Sánchez Hernández IM, Villena Garrido V, de Miguel Poch E, Nuñez Delgado M, Alfaro Abreu J. Safety of the transbronchial biopsy in outpatients. Chest 1991; 99(3): 562–565
26. Schwarz MI, King TE. Interstitial Lung Disease. Toronto, Ont.: B.C. Decker. 1988
27. Bonetti F, Chiodera PL, Pea M, Martignoni G, Bosi F, Zamboni G, Mariuzzi GM. Transbronchial biopsy in lymphangiomyomatosis of the lung. HMB45 for diagnosis. Am J Surg Pathol 1993; 17(11): 1092–1102
28. Hayashida M, Seyama K, Inoue Y, Fujimoto K, Kubo K; Respiratory Failure Research Group of the Japanese Ministry of Health, Labor, and Welfare. The epidemiology of lymphangioleiomyomatosis in Japan: a nationwide cross-sectional study of presenting features and prognostic factors. Respirology 2007; 12 (4): 523–530
29. Chu SC, Horiba K, Usuki J, Avila NA, Chen CC, Travis WD, Ferrans VJ, Moss J. Comprehensive evaluation of 35 patients with lymphangioleiomyomatosis. Chest 1999; 115(4): 1041–1052
30. Kitaichi M, Nishimura K, Itoh H, Izumi T. Pulmonary lymphangioleiomyomatosis: a report of 46 patients including a clinicopathologic study of prognostic factors. Am J Respir Crit Care Med 1995;

151(2 Pt 1): 527–533

31. Høie J, Jørstad SO, Odegaard A. Pulmonary lymphangioleiomyomatosis. Tidsskr Nor Laegeforen 1995; 115(10): 1230–1232
32. Benden C, Rea F, Behr J, Corris PA, Reynaud-Gaubert M, Stern M, Speich R, Boehler A. Lung transplantation for lymphangioleiomyomatosis: the European experience. J Heart Lung Transplant 2009; 28(1): 1–7
33. Seyama K, Kira S, Takahashi H, Ohnishi M, Kodama Y, Dambara T, Kobayashi J, Kitamura S, Fukuchi Y. Longitudinal follow-up study of 11 patients with pulmonary lymphangioleiomyomatosis: diverse clinical courses of LAM allow some patients to be treated without anti-hormone therapy. Respirology 2001; 6(4): 331–340
34. Lim KE, Tsai YH, Hsu Yl Y, Hsu Wc W. Pulmonary lymphangioleiomyomatosis. High-resolution CT findings in 11 patients and compared with the literature. Clin Imaging 2004; 28(1): 1–5
35. Taylor JR, Ryu J, Colby TV, Raffin TA. Lymphangioleiomyomatosis. Clinical course in 32 patients. N Engl J Med 1990; 323(18): 1254–1260
36. Naalsund A, Johansen B, Foerster A, Kolbenstvedt A. When to suspect and how to diagnose pulmonary lymphangioleiomyomatosis. Respirology 1996; 1(3): 207–212
37. Torre O, Harari S. The diagnosis of cystic lung diseases: a role for bronchoalveolar lavage and transbronchial biopsy? Respir Med 2010; 104(Suppl 1): S81–S85
38. Ye L, Jin M, Bai C. Clinical analysis of patients with pulmonary lymphangioleiomyomatosis (PLAM) in mainland China. Respir Med 2010; 104(10): 1521–1526
39. Chen F, Bando T, Fukuse T, Omasa M, Aoyama A, Hamakawa H, Fujinaga T, Shoji T, Sakai H, Hanaoka N, Wada H. Recurrent lymphangioleiomyomatosis after living-donor lobar lung transplantation. Transplant Proc 2006; 38(9): 3151–3153
40. Unger PD, Hoffman K, Thung SN, Pertsemlides D, Wolfe D, Kaneko M. HMB-45 reactivity in adrenal pheochromocytomas. Arch Pathol Lab Med 1992; 116(2): 151–153
41. Serrano Pardo R, Sotelo Rodríguez MT, Alvarez Martínez C, Ballestin Carcavilla C, Larrú Cabrero E. Pulmonary lymphangioleiomyomatosis (LAM). Diagnostic usefulness of immunohistochemical markers. Arch Bronconeumol 1998; 34(10): 509–511(in Spanish)
42. Curley FJ, Johal JS, Burke ME, Fraire AE. Transbronchial lung biopsy: can specimen quality be predicted at the time of biopsy? Chest 1998; 113(4): 1037–1041
43. Chilosi M, Pea M, Martignoni G, Brunelli M, Gobbo S, Poletti V, Bonetti F. Cathepsin-k expression in pulmonary lymphangioleiomyomatosis. Mod Pathol 2009; 22(2): 161–166
44. Zhang X, Travis WD. Pulmonary lymphangioleiomyomatosis. Arch Pathol Lab Med 2010; 134(12): 1823–1828
45. Ferrans VJ, Yu ZX, Nelson WK, Valencia JC, Tatsuguchi A, Avila NA, Riemenschn W, Matsui K, Travis WD, Moss J. Lymphangioleiomyomatosis (LAM): a review of clinical and morphological features. J Nippon Med Sch 2000; 67(5): 311–329
46. McCormack FX, Inoue Y, Moss J, Singer LG, Strange C, Nakata K, Barker AF, Chapman JT, Brantly ML, Stocks JM, Brown KK, Lynch JP 3rd, Goldberg HJ, Young LR, Kinder BW, Downey GP, Sullivan EJ, Colby TV, McKay RT, Cohen MM, Korbee L, Taveira-DaSilva AM, Lee HS, Krischer JP, Trapnell BC; National Institutes of Health Rare Lung Diseases Consortium; MILES Trial Group. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. N Engl J Med 2011; 364(17): 1595–1606