Lymphology 49 (2016) 140-149

DIAGNOSTIC AND TREATMENT MONITORING POTENTIAL OF SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR-D IN LYMPHANGIOLEIOMYOMATOSIS

Y. Mou,* L. Ye,* J. Wang, M.-S. Ye, Y.-L. Song, L. Zhu, M.-L. Jin

Department of Respiratory Medicine, Zhongshan Hospital Affiliated to Fudan University, Shanghai, China

*These authors contributed equally to this study.

ABSTRACT

Lymphangioleiomyomatosis (LAM) is a rare multisystem disease occurring almost exclusively in premenopausal women and characterized by cystic lung destruction, abdominal tumors (renal angiomyolipomas (AML)), and involvement of the axial lymphatics (adenopathy, lymphangioleiomyomas). Serum vascular endothelial growth factor-D (VEGF-D), a lymphangiogenic factor, has been recently considered as a novel marker for LAM. Herein we report the diagnostic and differential diagnostic value of serum VEGF-D in LAM patients and evaluate the change of serum VEGF-D levels before and after treatment with sirolimus. The study group included 66 patients with LAM (47 definite LAM and 19 probable LAM based on European Respiratory Society guidelines), 14 patients with other polycystic lung diseases, and 20 healthy female controls. Serum VEGF-D levels were quantified by enzyme-linked immunoassay (ELISA). Serum VEGF-D levels were significantly increased in definite LAM patients compared with healthy controls (3890.3±373.3 pg/ml vs. 413.3±33.2 pg/ml, p<0.05). The optimal cutoff point for LAM diagnosis was 692.5 pg/ml with sensitivity of 97.9% and specificity of 100%. In probable LAM patients, serum VEGF-D levels were all greater than 692.5 pg/ml. Serum VEGF-D levels were significantly increased in definite LAM patients who had chylothorax compared with those without chylothorax (5153.9±598.3 pg/ml vs. 2869.8±372.8 pg/ml, p<0.05). But serum VEGF-D levels in LAM patients with/without pneumothorax, AML, and lymphangioleiomyomas were not significantly changed. Serum VEGF-D levels in definite LAM patients and patients with other cystic lung diseases were 3890.3±373.3 pg/ml and 412.6±27.5 pg/ml, respectively (p<0.05). We determined an optimal cutoff value of 688.5pg/ml, resulting in sensitivity of 97.9% and specificity of 100%. Following a median of 12-month treatment with sirolimus, serum VEGF-D levels decreased from 3135.0±909.4 pg/ml to 1731.8±621.2 pg/ml and symptoms improved. Our study found that serum VEGF-D levels were significantly higher in LAM patients compared with healthy controls and patients with other polycystic lung diseases and that the levels were further increased when complicated by chylothorax. Serum VEGF-D levels may be useful for diagnosis and differential diagnosis with high specificity and sensitivity as well as for following treatment response with sirolimus.

Keywords: lymphangioleiomyomatosis (LAM), vascular endothelial growth factor-D (VEGF-D), chylothorax, diagnostic potential, treatment monitoring

Lymphangioleiomyomatosis (LAM) is a rare, frequently fatal cystic lung disease that predominantly affects premenopausal women (1-2). LAM patients usually develop progressive dyspnea, recurrent pneumothorax, chylous pleural effusion and occasional haemoptysis (3). Cystic lung destruction results in lung function decline that leads to respiratory failure. Eventually patients need supplemental oxygen, and in some cases lung transplantation. Thin-walled homogeneously distributed cysts are found throughout the lung parenchyma on high-resolution CT (HRCT) scans (4-5). Extrapulmonary involvement includes renal angiomyolipomas (AML), lymphadenopathy, and cystic masses of the axial lymphatics (termed lymphangioleiomyomas) that can result in abdominal and pelvic lymphatic obstruction (6).

Vascular endothelial growth factor-D (VEGF-D) is one of the lymphangiogenic growth factors. There was no serum diagnostic marker available for LAM until 2006 when serum VEGF-D levels were reported elevated in LAM patients compared with healthy controls (7). Both Young et al (8) and Xu et al (9) found that VEGF-D levels were higher in LAM patients than in healthy volunteers and proposed using VEGF-D levels as a tool in the differential diagnosis of cystic lung diseases. Serum VEGF-D levels were used as one of the diagnostic criteria for LAM in a randomized, placebo-controlled, double-blinded clinical trial, called prospective analysis of the Multicenter International Lymphangioleiomyomatosis Efficacy of Sirolimus (MILES) (10). Recent study showed that the abnormalities in the tuberous sclerosis complex1/2 (TSC1/2) genes resulted in the inactivation of mammalian target of sirolimus (mTOR) (11), and this observation led to studying sirolimus in patients with LAM (12). Sirolimus, one of the mTOR inhibitors, has been shown to be effective in stabilizing lung function and reducing chylous effusions, lymphangioleiomyomas, and AMLs (13).

Our study aimed to test the diagnostic value of serum VEGF-D in diagnosis of LAM and probable LAM and to evaluate treatment response to sirolimus.

MATERIALS AND METHODS

Study Population

Study protocol was approved by the Institutional Review Board of Zhongshan Hospital affiliated to Fudan University, and all participants provided written informed consent before enrollment. All LAM patients enrolled in this study from 2009 to 2014 fulfilled the requirements of definite LAM or probable LAM diagnosis criteria of ERS (14).

The diagnosis of LAM was made by tissue biopsy and/or clinical and radiographic data. All patients underwent CT scans of the chest, abdomen, and pelvis to determine the extent of lung disease and to confirm the presence of AML, adenopathy, and lymphangioleiomyomas.

Twenty age-matched healthy female controls and 14 patients with other polycystic lung diseases (OPLDs) excluding LAM were enrolled from 2009 to 2014. The exclusion criteria for participants in healthy controls were as follows: age <18 or >60 years; serum test positive for HIV or hepatitis; inability to perform reliable pulmonary function tests; and any history of respiratory disease.

Clinical Data

All required clinical data were obtained from an interview, previous files, and records. The following data were recorded: age, sex, lung function, CT scans of chest, abdomen, and pelvis, and results of pathologic examination of tissues.

The presence or absence of lymphangioleiomyoma and adenopathy in LAM patients

TABLE 1
Characteristics of Patients

	Controls	Definite LAM	Probable LAM	OPLDs
Men/Women, n	0/20	0/47	0/19	2/12
Age, y	38.2 ± 6.7	39.4 ± 8.9	43.6 ± 9.6	49.5 ± 11.9
Pneumothorax, n	0	19	3	0
Chylothorax, n	0	21	0	0
AML, n	0	15	0	0
VEGF-D, pg/mL	413.3 ± 33.2	3890.3 ± 373.3	1380 ± 415	412.6 ± 27.5
FEV_1, L	2.52 ± 0.15	1.67 ± 0.13	1.45 ± 0.18	2.47 ± 0.29
FEV_1%pred, %	99.6 ± 6.5	64.9 ± 4.6	58.3 ± 9.3	100.3 ± 7.9
FEV_1/FVC, %	80.1 ± 6.1	63.9 ± 3.0	88.42 ± 5.1	79.1 ± 5.3
DLCO/VA%, %	98.7 ± 6.5	54.8 ± 4.8	35.84 ± 5.4	99.8 ± 6.4

was confirmed by CT scans. The CT scans were obtained at first visit and evaluated by two radiologists in the Department of Radiology, Zhongshan Hospital affiliated to Fudan University and Shanghai Medical Imaging Institute.

Diagnosis of LAM was confirmed by pathologic examination. LAM was characterized by cysts and multifocal nodular proliferation of immature smooth muscle and perivascular epithelioid cells (LAM cells). Immunohistochemistry for alpha-smooth muscle actin and HMB45 was also used as an adjunct to diagnosis (14).

VEGF-D Measurements

Serum samples were obtained at first visit. They were collected in serum separator tubes, allowed to clot for 30 min, and centrifuged at 4000 rpm for 10 min. Serum was separated and stored at -20%C before measurement. Serum VEGF-D levels were measured using the Quantikine Human VEGF-D Immunoassay (R&D Systems; Minneapolis, MN) according to the manufacturer's instructions by enzyme-linked immunosorbant assay (ELISA).

Pulmonary Function Tests

Pulmonary function test was performed using a Type Masterscreen-pFT Jaeger (Hoechberg, Germany). Forced expiratory volume in one second (FEV_1), percentage of forced expiratory volume in one second to the predicted value (FEV_1%pred), and forced expiratory volume in one second/forced vital capacity (FEV_1/FVC) were measured. Diffusing capacity for carbon monoxide (DLCO) was measured by a single-breath method and percentage of diffusing capacity for carbon monoxide/alveolar volume to the predicted value (DLCO/VA%) was obtained.

Assessments of the Effects of Sirolimus

Some LAM patients in our Center take sirolimus to improve their symptoms. In order to analyze the change in VEGF-D levels pre-treatment and post-treatment with sirolimus, serum samples from some patients were obtained pre- and post-treatment with sirolimus, and VEGF-D levels were measured by ELISA as described previously.

Statistical Analysis

All statistical analyses were done using the Statistical package for the Social Sciences (SpSS) 16.0 software (SpSS for Microsoft Windows, package version 16.0; SpSS Inc., Chicago, IL, USA). Serum VEGF-D levels were expressed as mean±SE. A Student t test

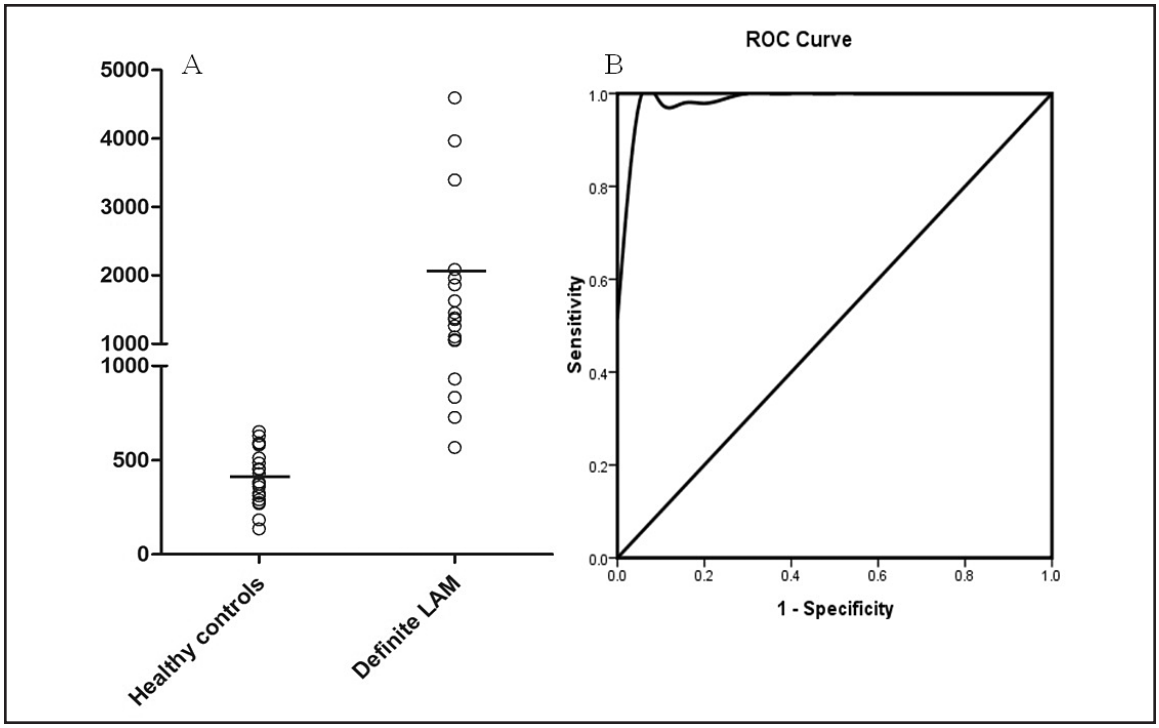

Fig. 1. Serum VEGF-D levels in patients with lymphangioleiomyomatosis (LAM) compared with healthy controls. (A) Comparison of serum VEGF-D levels in the definite LAM and health controls. (B) ROC curve for definite LAM, an optimal cutoff value is 692.5pg/ml, representing the sensitivity of 97.9% and specificity of 100.0%.

was used to evaluate the differences of serum VEGF-D levels between different groups. The ROC curve was also used to select the optimal cutoff point at which the sensitivity and specificity were maximally reached. $P<0.05$ was considered statistically significant.

RESULTS

Characteristics of Subjects

Sixty-six patients (definite LAM and probable LAM), 20 age-matched healthy female controls, and 14 patients with OPLDs were enrolled from 2009 to 2014.

According to ERS criteria, among the 66 patients, 47 patients with definite LAM were confirmed by: pathological diagnosis of lung tissue (23 cases), retroperitoneal mass and lymph node (4 cases), kidney tissues (4 cases), history of chylothorax (21 cases), chylous ascites (2 cases), AML (15 cases), or tuberous sclerosis complex (TSC, 2 cases). Of the 47 definite LAM patients, 19 presented with pneumothorax, 21 with chylothorax, 2 with chylous ascites, 15 with AML, 15 with retroperitoneal cyst and 2 with TSC-LAM accompanied by a seizure. 19 patients who were classified as probable LAM had a clinical history compatible with LAM, lung cysts on CT, but no extrapulmonary presentation or evidence of TSC (*Table 1*).

Serum VEGF-D Levels were Increased in Definite LAM Patients Compared with Healthy Controls

Serum concentrations of VEGF-D were measured in 47 definite LAM patients and 20 healthy controls. The mean value of serum VEGF-D in LAM and healthy controls was 3890.3±373.3 pg/ml and 413.3±33.2 pg/ml

respectively. Serum VEGF-D levels were significantly increased in the definite LAM patients compared with healthy controls ($p<0.000$) (*Fig. 1A*), and the ROC curve demonstrated an area under the curve of 0.995 ($p<0.000$) for definite LAM in comparison with healthy controls. The sensitivity and specificity of the optimal cutoff value 692.5 pg/ml were 97.9% and 100.0%, respectively. The ROC curve showed that VEGF-D remained an effective diagnostic test in the LAM patients (*Fig. 1B*). According to our analysis, an optimal cutoff value to diagnose LAM is 692.5 pg/ml. VEGF-D levels of all 19 probable LAM patients were over 692.5 pg/ml, thereby supporting the value of 692.5 pg/ml as an optimal cut-off based on the analysis.

Serum VEGF-D and its Related Factors

Twenty-one cases of 47 LAM patients had chylothorax, and serum VEGF-D levels were significantly increased in these patients with chylothorax compared with those without (5153.9±598.3 pg/ml vs. 2869.8±372.8 pg/ml, p=0.002) (*Fig. 2A*). Fifteen patients had AML but there was no statistically significant difference in serum VEGF-D levels in LAM patients with AML compared with those without (3209.5±494.0 pg/ml vs. 4241.7±498.9 pg/ml, p=0.193). There was no statistical difference found in the 19 LAM patients with pneumothorax compared with those without (2439.4±461.4 pg/ml vs. 3245.9±420.6 pg/ml, p=0.205) or in the 15 LAM patients with lymphangioleiomyomas compared with those without (4458.9±797.1 pg/ml vs. 3623.8±406.2 pg/ml, p=0.302) (*Figs. 2B-D*).

Serum VEGF-D and Pulmonary Function Tests

In order to investigate whether the variables of lung function (FEV_1, FEV_1%pred, FEV_1/FVC and DLCO/VA%) were correlated with serum VEGF-D levels, 30 cases of definite 47 LAM patients underwent lung function tests at the time of serum sampling. The other 17 LAM patients did not perform the lung function because they had or currently presented with a pneumothorax. There was no correlation between VEGF-D levels and FEV_1, FEV_1%pred, FEV_1/FVC and DLCO/VA% in definite LAM patients. Also, there was no correlation between VEGF-D levels and lung function of definite LAM patients with or without chylothorax.

Comparison of Serum VEGF-D Levels in Definite LAM Patients and Patients with OPLDs

Serum concentrations of VEGF-D were measured in 47 definite LAM patients, 20 healthy controls, and 14 patients with OPLDs. The mean value of serum VEGF-D in definite LAM patients and patients with OPLDs were 3890.3±373.3 pg/ml and 412.6±27.5 pg/ml, respectively ($p<0.002$) (*Fig. 3A*) and the mean value of serum VEGF-D in healthy controls and patients with OPLDs were (413.3±33.2 pg/ml vs. 412.6±27.5 pg/ml, p=0.987). The ROC curve demonstrated an area under the curve of 0.997 ($p<0.002$) for definite LAM and OPLDs. We determined an optimal cut-off value of 688.5 pg/ml providing sensitivity of 97.9% and specificity of 100% (*Fig. 3B*).

Pre- and Post-Treatment with Sirolimus in LAM Patients

Eleven of the 47 definite LAM patients were treated with sirolimus (2mg/d). Median treatment length was 12 months with a range of 5 to 48 months. During the treatment period, serum level of sirolimus was maintained between 5 and 15 ng/ml. Mouth and lip ulcers (6/11), headache (4/11), nausea (4/11), acne (1/11) and elevated cholesterol (1/11) were seen after sirolimus treatment. We found that sirolimus treatment reduced serum VEGF-D levels. VEGF-D levels in definite LAM patients post-treatment were significantly lower than pre-treatment (3135.0±909.4 pg/ml vs. 1731.8±621.2 pg/ml,

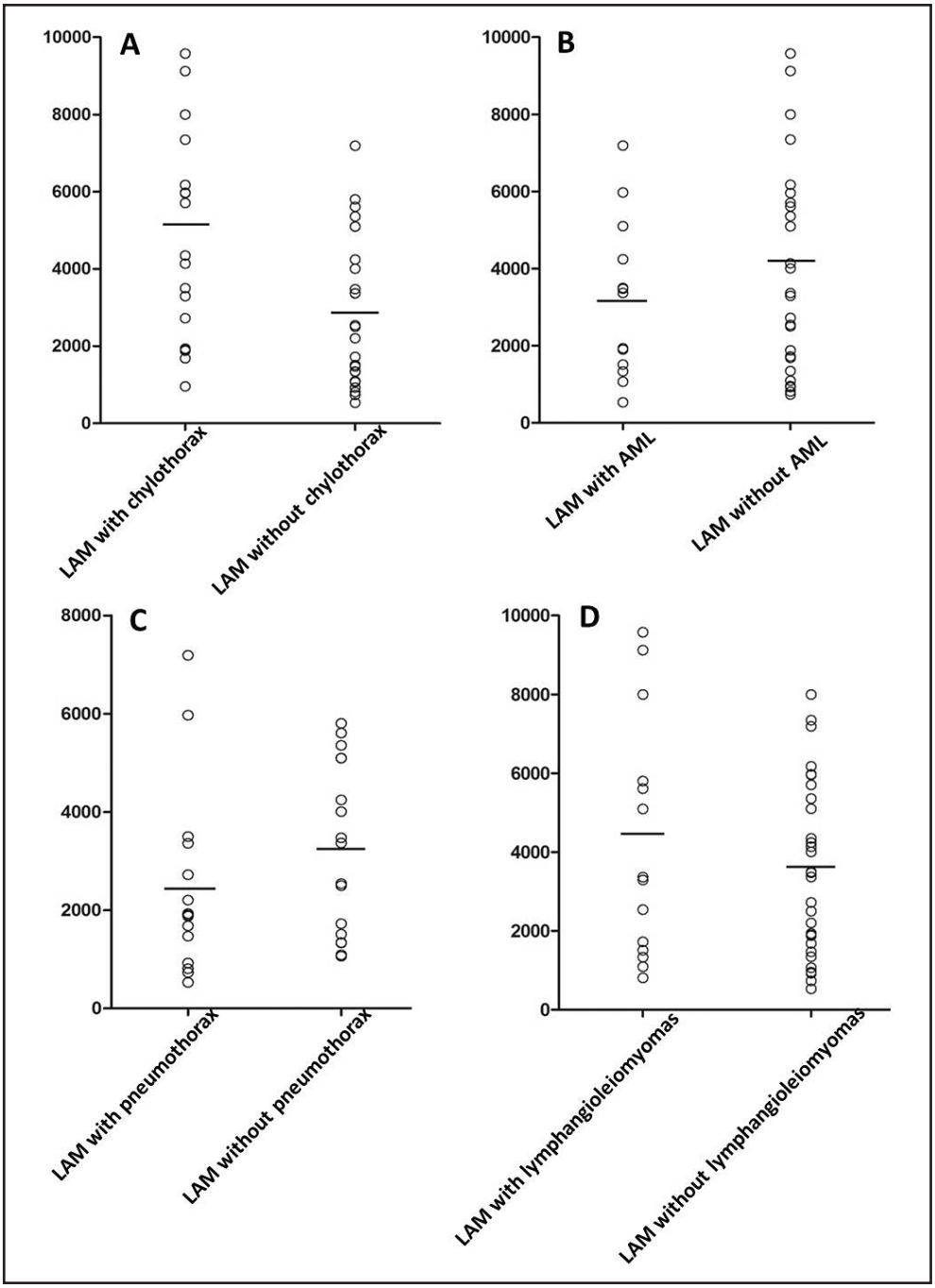

Fig. 2. Serum VEGF-D levels and its related factors. (A) Serum VEGF-D levels were significantly increased in LAM patients with chylothorax compared with those without (p=0.002). (B) There was no statistically significant difference of serum VEGF-D levels in LAM patients with AML compared with those without (p=0.193). (C) No statistically significant difference was found in LAM patients with pneumothorax compared with those without (p=0.205). (D) There was no statistically significant difference in LAM patients with lymphangioleiomyomas compared with those without (p=0.302).

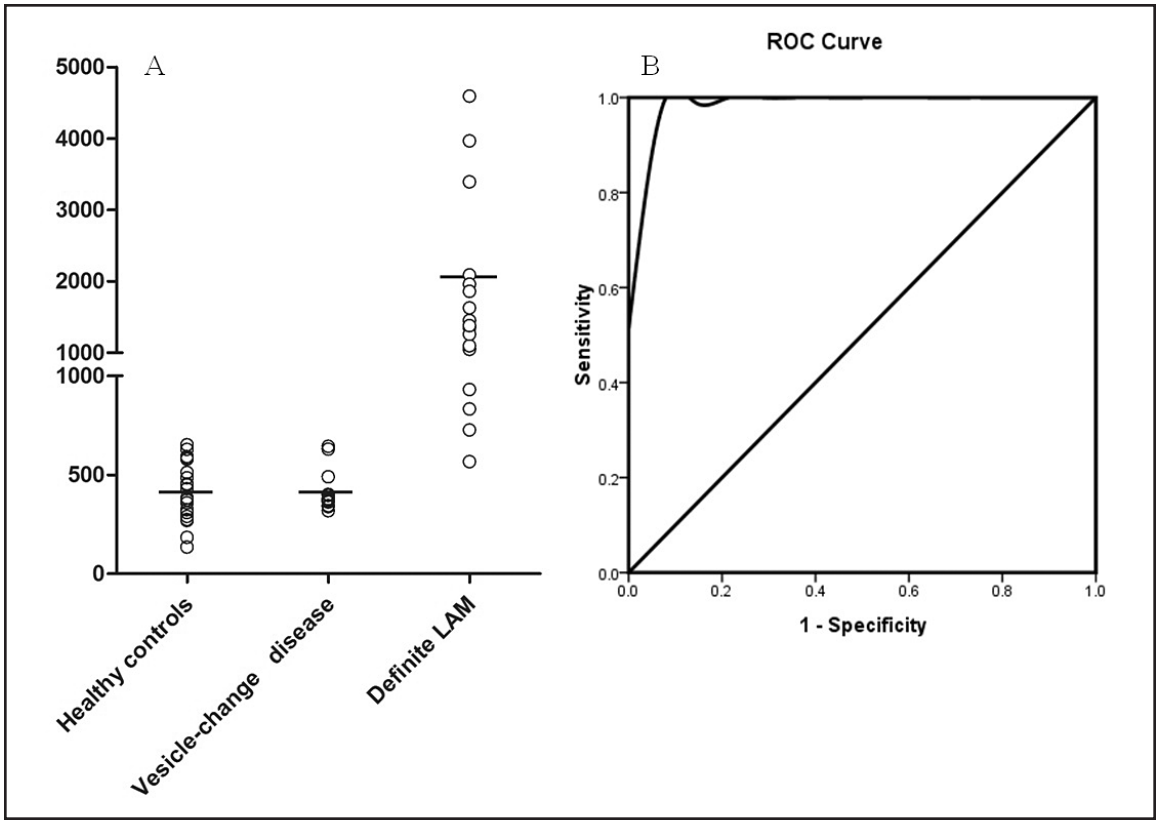

Fig. 3. Serum VEGF-D levels in patients with definite LAM as compared with other cystic lung diseases. (A) Comparison of serum VEGF-D levels in definite LAM and OPLDs. (B) ROC curve for definite LAM and OPLDs. An optimal cutoff value is 688.5pg/ml, representing the sensitivity of 97.9% and specificity of 100.0%.

p=0.046) during the 12-month treatment period. Lung function of FEV_1 post-treatment and pre-treatment were (1.34±0.15) L and (1.23±0.16) L, respectively. Oxygen saturation (SaO_2) at room air post-treatment and pre-treatment were 95.37% and 91.83%, respectively. The symptom of dyspnea was assessed by modified medical research council (mMRC) and mMRC of the 11 LAM patients improved after sirolimus treatment. No difference was found in DLCO after the treatment. Our research showed that sirolimus treatment was effective in reducing serum VEGF-D levels and improving dyspnea. Thus, symptom improvement correlated with reduced VEGF-D levels.

DISCUSSION

VEGF-D, a ligand for the lymphatic growth-factor receptor VEGFR-3/Flt-4 (also VEGFR-2/KDR), belongs to a family of growth factors that induce formation/growth of lymphatic vessels and contributes to spread of tumor cells to lymph nodes (15-18). It has been well documented that VEGF-D is the only growth factor within the VEGF family significantly correlated in to LAM patients (7-9,18). Serum levels of other VEGFs, such as VEGF-A and VEGF-C, demonstrated no significant difference in LAM patients compared with healthy controls (7). Our results show that serum VEGF-D levels are increased in patients with definite LAM compared with healthy controls and that an optimal cut-off value is 692.5 pg/ml. At this cutoff point, the overall sensitivity (97.9%)

and specificity (100%) is highest. To date, there is no consensus on a VEGF-D level cutoff point. However, 800 pg/ml has been suggested for diagnosis of LAM and this value was used for inclusion criteria of MILES (19,20). Our present study suggests a cutoff of 692.5 pg/ml as the new threshold for LAM diagnosis. Clinical diagnosis of LAM is currently based on ERS guidelines, in which lung biopsy is often used to confirm the diagnosis. However, there is a potential that serum VEGF-D levels could decrease the need for lung biopsy (8).

Of the 66 patients, 19 patients were classified as probable LAM according to ERS criteria. They had a clinical history resembling with LAM, lung cysts on CT, but no extrapulmonary features or evidence of TSC. Chang et al found that 30% of the patients remained undiagnosed according to ERS criteria, and this percentage could be reduced to 20% by adding a VEGF-D level of >800 pg/ml (21). Our determined value of 692.5 pg/ml supports this conclusion and lowers the optimal cut-off point, which should further reduce this undiagnosed rate.

The manifestations of LAM include recurrent pneumothorax, chylothorax, AML, and lymphangioleiomyomas. According to our analysis, serum VEGF-D levels were significantly increased in LAM patients with chylothorax compared with those patients without. However, there was no statistically significant difference in serum VEGF-D levels in LAM patients with pneumothorax, AML, lymphangioleiomyomas compared with those without.

One important question in LAM patients is the possible link between the serum VEGF-D levels and lung damage. Studies showed that FEV_1 and DLCO/VA% correlate with CT damage in LAM and changed over time as the disease progressed (22-24). Seyamal et al demonstrated a negative correlation between the VEGF-D levels and respiratory function tests represented by FEV_1/FVC and DLCO/VA%, but no significant correlation was observed between VEGF-D levels and FEV_1%pred (7). In our study, we did not find a correlation between VEGF-D levels and FEV_1, FEV_1%pred, FEV_1/FVC, DLCO/VA% in LAM patients. This negative result could be due to several reasons. First, FEV_1/FVC is only an indicator of pulmonary obstructive ventilation dysfunction, and it can't reflect the severity of obstructive ventilation dysfunction. Therefore, the correlation of VEGF-D levels and FEV_1/FVC has no significance. Secondly, in our study, DLCO/VA% was measured by a single-breath method, and the accuracy may not be high enough.

Radzikowska et al reported that serum VEGF-D levels were significantly higher in patients with LAM than those with OPLDs (25). Our data were consistent with their report using our optimal cutoff value of 688.5 pg/ml. This observation is of importance because VEGF-D levels may be useful as a specific biomarker to discriminate between LAM and OPLDs.

LAM is caused by proliferation of cells that have mutations or deletions in one of the tuberous sclerosis genes, TSC1 or TSC2 (26). TSC1and TSC2 genes encode hamartin and tuberin respectively, two proteins that regulate the mTOR signaling pathway involved in causing abnormal LAM cell proliferation. Activation of mTORC1 leads to cell growth and proliferation, and, therefore, sirolimus (the mTOR inhibitor) is used to block the mTORC1 signaling pathway in the treatment of LAM (27,28).

McCormack et al found that in LAM patients treated with sirolimus, reduced serum VEGF-D levels correlated with lung function stabilization, symptom relief, and improvement in the quality of life (10). Goldberg et al also found that everolimus improved some indicators of lung function and exercise capacity with reduced serum VEGF-D levels (29). Analyses from MILES study found that VEGF-D levels might be considered as not only a diagnostic biomarker but also a biomarker of lymphangioleiomyomatosis severity and treatment response (30). In our study, we found an increase of

FEV_1, SaO2, and mMRC and a decrease of serum VEGF-D levels after treatment with sirolimus, and these results support a relationship between the symptom improvement and the VEGF-D decrease. Therefore, we believe that VEGF-D might be perceived as a promising potential biomarker for monitoring and following up LAM treated with sirolimus.

CONCLUSION

In conclusion, our data confirm and expand the use of serum VEGF-D levels as a diagnostic test that can distinguish LAM and probable LAM patients from healthy controls and patients with OPLDs. Additionally, measurement of serum VEGF-D levels should potentially decrease the need for lung biopsy and also offer potential as a promising biomarker for monitoring sirolimus treatment response.

Authors' Contributions

MY collected and analyzed the data, and drafted the manuscript; YL participated in design of the study and modified the manuscript and has equal contribution with MY; WJ performed the statistical analysis; YMS carried out collection of the data; SYL participated in modification the manuscript; ZL participated in design of the study; JML conceived of the study, and participated in its design and coordination. All authors contributed to the intellectual content of the manuscript and approved the final version submitted for publication.

ACKNOWLEDGMENT

The authors wish to thank Zhang Zhifeng and WEI Su-lan at the Respirology Medicine Department of Zhongshan Hospital Affiliated with Fudan University for their contributions. Funded by Municipal Natural Science Foundation of Shanghai (14ZR1406200).

REFERENCES

1. Juvet, SC, FX McCormack, DJ Kwiatkowski, et al: Molecular pathogenesis of lymphangioleiomyomatosis: Lessons learned from orphans. Am. J. Respir. Cell Mol. Biol. 36 (2007), 398-408.
2. Johnson, SR: Lymphangioleiomyomatosis. Eur. Respir. J. 27 (2006), 1056-1065.
3. Johnson, SR: Lymphangioleiomyomatosis: Clinical features, management and basic mechanisms. Thorax. 54 (1999), 254-264.
4. Taveira-DaSilva, AM, J Moss: Clinical features, epidemiology, and therapy of lymphangioleiomyomatosis. Clin. Epidemiol. 7 (2015), 249-257.
5. McCormack, FX: Lymphangioleiomyomatosis: A clinical update. Chest 133 (2008), 507-516.
6. Avila, NA, JA Kelly, SC Chu, et al: Lymphangioleiomyomatosis: Abdominopelvic CT and US findings. Radiology 216 (2000), 147-153.
7. Seyama, K, T Kumasaka, S Souma, et al: Vascular endothelial growth factor-D is increased in serum of patients with lymphangioleiomyomatosis. Lymphat. Res. Biol. 4 (2006), 143-152.
8. Young, LR, R Vandyke, M Gulleman, et al: Serum vascular endothelial growth factor-D prospectively distinguishes lymphangioleiomyomatosis from other diseases. Chest 138 (2010), 674-681.
9. Xu, KF, P Zhang, X Tian, et al: The role of vascular endothelial growth factor-D in diagnosis of lymphangioleiomyomatosis. Respir. Med. 107 (2013), 263-268.
10. McCormack, FX, Y Inoue, J Moss, et al: Efficacy and safety of sirolimus in lymphangioleiomyomatosis. N. Engl. J. Med. 364 (2011), 1595-1606.
11. Kenerson, H, AL Folpe, TK Takayama, et al: Activation of the mTOR pathway in sporadic angiomyolipomas and other perivascular epithelioid cell neoplasms. Hum. Pathol. 38 (2007), 1361-1371.
12. Davies, DM, SR Johnson, AE Tattersfield, et al: Sirolimus therapy in tuberous sclerosis or sporadic lymphangioleiomyomatosis. N. Engl. J. Med. 358 (2008), 200-203.
13. Bissler, JJ, JC Kingswood, E Radzikowska, et al: Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): A multicentre, randomised, double-blind, placebo-controlled trial. Lancet 381 (2013), 817-824.
14. Johnson, SR, JF Cordier, R Lazor, et al: European Respiratory Society guidelines for the diagnosis and management of lymphangioleiomyomatosis. Eur. Respir. J. 35 (2010), 14-26.

15. Proczka, RM, M Malecki, J Chorostowska-Wynimko, et al: Vascular endothelial growth factor (VEGF) in patients with peripheral ischemia. J. Physiol. Pharmacol. 57 (2006), 305-311.
16. Watorek, E, M Paprocka, D Dus, et al: Endostatin and vascular endothelial growth factor: Potential regulators of endothelial progenitor cell number in chronic kidney disease. Pol. Arch. Med. Wewn. 121 (2011), 296-301.
17. Przepiera-Bedzak, H, K Fischer, M Brzosko: Serum levels of angiogenic cytokines in psoriatic arthritis and SAPHO syndrome. Pol. Arch. Med. Wewn. 123 (2013), 297-302.
18. Young, LR, Y Inoue, FX McCormack: Diagnostic potential of serum VEGF-D for lymphangioleiomyomatosis. N. Engl. J. Med. 358 (2008), 199-200.
19. McCormack, FX, Y Inoue, J Moss, et al: Efficacy and safety of sirolimus in lymphangioleiomyomatosis. N. Engl. J. Med. 364 (2011), 1595-606.
20. Young, LR, R Vandyke, PM Gulleman, et al: Serum vascular endothelial growth factor-D prospectively distinguishes lymphangioleiomyomatosis from other diseases. Chest 138 (2010), 674-81.
21. Chang, WY, JL Cane, JD Blakey, et al: Clinical utility of diagnostic guidelines and putative biomarkers in lymphangioleiomyomatosis. Respir. Res. (2012), 13:34 (online).
22. Johnson, SR, AE Tattersfield: Decline in lung function in lymphangioleiomyomatosis: Relation to menopause and progesterone treatment. Am. J. Respir. Crit. Care Med.160 (1999), 628-633.
23. Avila, NA, CC Chen, SC Chu, et al: Pulmonary lymphangioleiomyomatosis: correlation of ventilation-perfusion scintigraphy, chest radiography, and CT with pulmonary function tests. Radiology 214 (2000), 441-446.
24. Taveira-DaSilva, AM, C Hedin, MP Stylianou, et al: Reversible airflow obstruction, proliferation of abnormal smooth muscle cells, and impairment of gas exchange as predictors of outcome in lymphangioleiomyomatosis. Am. J. Respir. Crit. Care Med. 164 (2001), 1072-1076.
25. Radzikowska E, P Jagus, A Skoczylas, et al: Role of serum vascular endothelial growth factor D in discrimination of patients with polycystic lung diseases. Pol. Arch. Med. Wewn. 123 (2013), 533-538.
26. Meraj, R, KA Wikenheiser-Brokamp, LR Young, FX McCormack: Lymphangioleiomyomatosis: New concepts in pathogenesis, diagnosis, and treatment. Semin. Respir. Crit. Care Med. 33 (2012), 486-497.
27. Sengupta S, TR Peterson, DM Sabatini: Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. Mol. Cell 40 (2010), 310-322.
28. Huang, J, BD Manning: A complex interplay between Akt, TSC2 and the mTOR complexes. Bioch. Soc. Trans. 37 (2009), 217-222.
29. Goldberg, HJ, S Harari, V Cottin, et al: Everolimus for the treatment of lymphangioleiomyomatosis: A phase II study. Eur. Respir. J. 46 (2015), 783-794.
30. Young, L, HS Lee, Y Inoue, et al: Serum VEGF-D a concentration as a biomarker of lymphangioleiomyomatosis severity and treatment response: A prospective analysis of the Multicenter International Lymphangioleiomyomatosis Efficacy of Sirolimus (MILES) trial. Lancet Respir. Med. 1 (2013), 445-452.

Mei-ling Jin, MD
Department of Respiratory Medicine
Zhongshan Hospital
Affiliated to Fudan University
Shanghai, China 200032
Email: mljin118@163.com