Lung (2019) 197:139–146
https://doi.org/10.1007/s00408-018-00191-3

INTERSTITIAL LUNG DISEASE

Concentration of Serum Vascular Endothelial Growth Factor (VEGF-D) and Its Correlation with Functional and Clinical Parameters in Patients with Lymphangioleiomyomatosis from a Brazilian Reference Center

Alexandre Franco Amaral[1] · Martina Rodrigues de Oliveira[1] · Olívia Meira Dias[1] · Fábio Eiji Arimura[1] · Carolina Salim Gonçalves Freitas[1] · Milena Marques Pagliarelli Acencio[1] · Vanessa Adélia de Alvarenga[1] · Ronaldo Adib Kairalla[1] · Carlos Roberto Ribeiro Carvalho[1] · Bruno Guedes Baldi[1] · Tuberous Sclerosis, Lymphangioleiomyomatosis and Angiomyolipoma Study Group, Universidade de Sao Paulo, Brazil

Received: 7 November 2018 / Accepted: 24 December 2018 / Published online: 8 January 2019

Abstract

Introduction Serum vascular endothelial growth factor-D (VEGF-D) is a lymphangiogenic growth factor that is considered a valuable tool in the diagnosis of lymphangioleiomyomatosis (LAM). Previous studies have reported a wide variability in VEGF-D serum levels in LAM patients and it seems to be associated with pulmonary impairment and lymphatic involvement.

Methods We conducted a cross-sectional study from 2009 to 2017 that evaluated VEGF-D serum levels in a cohort of LAM patients who were never treated with mTOR inhibitors and compared them to healthy age-matched volunteers. Clinical and functional parameters were assessed and correlated with their respective serum VEGF-D levels.

Results One hundred and four patients were included in the analysis. Serum VEGF-D levels were higher in LAM patients compared to healthy controls: 796 (404–1588) versus 162 (117–232) pg/mL, respectively ($p < 0.001$). Patients with tuberous sclerosis complex–LAM, TSC–LAM (20%), had higher levels of VEGF-D when compared to patients with sporadic LAM (80%) [1005 (641–2732) vs. 772 (370–1383), $p = 0.05$]. Serum VEGF-D levels were weakly correlated with DL_{CO} ($r = -0.26$, $p = 0.001$) and lymphatic involvement was more frequent in those with serum VEGF-D levels equal or above 800 pg/mL (35% vs. 13%, $p = 0.02$).

Conclusions In LAM, serum VEGF-D is weakly associated with lung function impairment and strongly associated with lymphatic involvement. VEGF-D is validated for use in Brazilian patients with LAM whose characteristics must be accounted for when evaluating their serum VEGF-D levels.

Keywords Interstitial lung disease · Lymphangioleiomyomatosis · Pulmonary function tests · Vascular endothelial growth factor

Introduction

Lymphangioleiomyomatosis (LAM) is a rare neoplastic disease, mainly affecting women of reproductive age, and is characterized by the proliferation of atypical muscle cells (known as LAM cells) around the airways, blood vessels, and lymphatics. LAM may be sporadic (S-LAM) or associated with tuberous sclerosis complex (TSC–LAM) and disease progression can result in vascular and airway obstruction, cyst formation, and lung destruction [1–3]. The main clinical features of LAM include progressive dyspnea, pneumothorax, chylothorax, abdominal tumors, such as angiomyolipomas and lymphangioleiomyomas, and, occasionally, hemoptysis [1–5].

Alexandre Franco Amaral, Martina Rodrigues de Oliveira, Fábio Eiji Arimura, Carolina Salim Gonçalves Freitas, Carlos Roberto Ribeiro Carvalho, and Bruno Guedes Baldi—on behalf of Tuberous Sclerosis, Lymphangioleiomyomatosis and Angiomyolipima Study Group, Universidade de Sao Paulo, Brazil.

The members of Tuberous Sclerosis, Lymphangioleiomyomatosis and Angiomyolipoma Study Group are listed in Acknowledgements section.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00408-018-00191-3) contains supplementary material, which is available to authorized users.

✉ Bruno Guedes Baldi
bruno.guedes2@terra.com.br

Extended author information available on the last page of the article

LAM cells have smooth muscle characteristics on histology and can present *TSC1* or *TSC2* gene mutations [6]. These culminate in constitutive activation of the mechanistic target of rapamycin (mTOR) signaling pathway which regulates essential cell functions such as growth, motility, and survival [7]. LAM cells also express vascular endothelial growth factor-D (VEGF-D), a lymphangiogenic growth factor, which has an important role in LAM cell spread into lymphatics, thereby contributing to metastasis [8, 9].

Serum levels of VEGF-D are increased in most LAM patients. In an appropriate clinical and radiological context, serum levels higher than 800 pg/mL seem to have a high specificity for LAM diagnosis, helping to distinguish it from other cystic lung diseases [1, 10–13]. Serum VEGF-D thresholds to differentiate LAM patients from healthy women vary between studies, and range from 440 to 1239 pg/mL [13–15]. Additionally, in patients who present only lung cysts without extrapulmonary features, serum VEGF-D levels below 800 pg/mL do not exclude LAM. Furthermore, a consensus of experts proposed that for patients with cystic lung abnormalities characteristic of LAM and without other confirmatory clinical or extrapulmonary radiologic features, serum VEGF-D levels over 800 pg/mL may be used to establish a diagnosis of LAM, before lung biopsy, due to its high specificity [1].

Serum VEGF-D levels seem to be associated with disease severity [11, 16–18] and with lymphatic involvement [13–15]. A previous study found higher concentrations of VEGF-D in patients with TSC–LAM and in those with S-LAM with lymphangiomas compared to patients without such findings [17]. This shows its potential association with clinical phenotypes of LAM. Additionally, high serum VEGF-D levels > 800 pg/mL at the time of diagnosis were associated with lower diffusing capacity of carbon dioxide (DL_{CO}) [15, 17] and lower blood oxygenation in S-LAM [15], suggesting its role as a biomarker of disease severity. Other studies also showed a correlation of serum VEGF-D levels with the extent of pulmonary cysts on computed tomography (CT) scans [13, 15, 19].

Since serum VEGF-D levels can be elevated before functional impairment sets in, this serum marker may be useful in making therapeutic decisions especially in patients who are unable to undergo pulmonary function tests (PFTs) due to cognitive impairment, e.g., some patients with TSC–LAM. Nevertheless, treatment indication based solely on serum VEGF-D levels lacks definitive evidence and is not recommended by most of the recent guidelines [1]. Finally, VEGF-D is a useful biomarker for assessing the course of the disease and the response to treatment over the long term. In the Multicentric International LAM Efficacy of Sirolimus (MILES) Trial, LAM patients treated with sirolimus, an mTOR inhibitor, had decreased VEGF-D serum concentrations compared to placebos, confirming the role of VEG-D as a marker for treatment response [16]. Further studies are still required to assess the role of serum VEGF-D as a prognostic biomarker. However, a recent study assessing the long-term effects of treatment with sirolimus failed to demonstrate the correlation between the magnitude of reduction in serum VEGF-D levels with improvement in PFT parameters, such as forced expiratory volume in the first second (FEV_1) or DL_{CO} [20].

The correlation of serum VEGF-D levels in Brazilian patients with LAM with clinical and functional parameters has not been established yet. Therefore, we aimed to (1) compare serum VEGF-D levels between LAM patients and age-matched healthy female subjects; (2) compare serum levels of VEGF-D in patients with S-LAM and TSC–LAM; (3) correlate serum VEGF-D levels with clinical and functional parameters, such as time since diagnosis, renal angiomyolipoma, lymphatic involvement (chylothorax, chylous ascites, and/or lymphangioleiomyoma), walking distance, and minimum peripheral oxygen saturation (SpO_2) on a standardized six-minute walk test (6MWT), FEV_1, and DL_{CO}.

Methods

Study Subjects

This cross-sectional study included patients with definitive LAM diagnoses based on previously described criteria [21] that were followed at the Interstitial Lung Disease outpatient clinic of the Pulmonary Division of a tertiary reference center at University of São Paulo, Brazil, from 2009 to 2017. Patients were clinically stable for at least 6 weeks and were never treated with mTOR inhibitors. Clinical (age, time from diagnosis, number and percentage of patients with TSC, pneumothorax, renal angiomyolipoma, chylothorax, chylous ascites, and lymphangioleiomyomas) and functional data were obtained within 6 months of blood collection for the determination of serum VEGF-D levels. Forty age-matched female healthy subjects were also enrolled as a control group. The study protocol was approved by the local research ethics committee (protocol number 4147/14/127) and signed informed consent was obtained from each patient.

Pulmonary Function Tests

PFTs were performed following ATS/ERS guidelines [22–24]. Spirometry was performed using a calibrated pneumotachograph (Medical Graphics Corporation, St. Paul, MN, USA) while lung volumes and DL_{CO} were measured

using a body plethysmograph (Elite Dx, Elite Series; Medical Graphics Corporation). Forced vital capacity (FVC), FEV_1, FEV_1/FVC, total lung capacity (TLC), residual volume (RV), RV/TLC, and DL_{CO} were obtained. Predicted values were derived from the Brazilian population [25, 26].

Serum VEGF-D Assessment

Serum levels of VEGF-D were measured using commercially available enzyme-linked immunosorbent assay kits (Quantikine Human VEGF-D Immunoassay) and the tests were carried out according to the manufacturer's instructions (ELISA; R&D Systems, Minneapolis, MN, USA). The lower detection limit of the assay was 15.6 pg/mL.

Six-Minute Walk Test

A 6MWT was performed according to the standards of the American Thoracic Society [27]. Walking distance in a 30-m corridor and minimum SpO_2 by pulse oximetry were recorded, the latter using a standard portable device (Nonin Onyx Model 9500, Nonin Medical Inc., Plymouth, MN, EUA).

Statistical Analysis

Data are reported as the mean ± SD for variables with normal distribution, as the median (25th–75th percentiles) for variables with non-normal distribution, or as numbers (percentiles). The unpaired *t* test or the Mann–Whitney *U* test was used to compare continuous variables, whereas categorical variables were compared using the Fisher's exact or Chi-square tests. Spearman's rank correlation coefficients were calculated to measure the degrees of association between serum VEGF-D levels and clinical and functional parameters. A p value of <0.05 was considered statistically significant. Data were analyzed using SigmaStat version 3.5 (Systat Software, Inc., San Jose, CA, USA).

Results

Clinical Features and Pulmonary Function Tests

One hundred and four female patients, with a mean age of 43 ± 11 years, were included in the study. The duration from the time of diagnosis to sample collection ranged from 0 to 23 years with a median of 1 year. TSC was present in 20% of patients and more than half of them had renal angiomyolipoma, while 47% of them had a history of pneumothorax and about a quarter had some evidence of lymphatic involvement (Table 1).

Table 1 Clinical and functional features of patients with LAM and comparison with control group

	LAM ($n=104$)	Control group ($n=40$)	p
Clinical features			
Age (years)	43 ± 11	43 ± 11	0.94
Time from diagnosis (years)	1 (0–5)		
Presence of TSC	21 (20.2%)		
Pneumothorax	49 (47.1%)		
Chylothorax	15 (14.4%)		
Renal angiomyolipoma	59 (56.7%)		
Lymphangioleiomyoma	16 (15.4%)		
Chylous ascites	4 (3.8%)		
Lymphatic involvement[a]	25 (24%)		
Pulmonary function tests			
FEV_1 (L)	2.14 ± 0.78		
%predicted	75 ± 26		
FVC (L)	2.98 ± 0.68		
%predicted	88 ± 18		
FEV_1/FVC	0.70 ± 0.19		
RV (L)	1.98 ± 0.84		
%predicted	131 ± 50		
TLC (L)	5.05 ± 0.86		
%predicted	104 ± 17		
RV/TLC	0.39 ± 0.13		
DL_{CO} (mL/min/mmHg)	17.9 ± 6.8		
%predicted	74 ± 29		
Six-minute walk test			
Distance (m)	496 ± 111		
Minimum SpO_2 (%)	90 ± 12		
VEGF-D serum levels (pg/mL)	796 (404–1588)	162 (117–232)	< 0.001

Values are the mean ± SD, median (25th–75th percentiles) or percentage (%)

DL_{CO} lung diffusing capacity for carbon monoxide, *FEV_1* forced expiratory volume in the first second, *FVC* forced vital capacity, *LAM* lymphangioleiomyomatosis, *RV* residual volume; *SpO_2* oxyhemoglobin saturation by pulse oximetry, *TLC* total lung capacity, *TSC* tuberous sclerosis complex, *VEGF* vascular endothelial growth factor-D

[a]Lymphatic involvement: chylothorax, chylous ascites, and/or lymphangioleiomyoma

The results of PFTs of the whole sample are described in Table 1. FEV_1 was 75 ± 26% of predicted normal values, with a FEV_1/FVC ratio of 0.70 ± 0.19. Air trapping, represented by a RV of 131 ± 50% of predicted value, was a common finding. None of the patients exhibited a restrictive functional pattern, and DL_{CO} was mildly reduced (74 ± 29% of predicted values). The distance walked was 496 ± 111 meters and minimum SpO_2 was 90 ± 12%.

Most patients had S-LAM (80%), who were slightly older than patients with TSC–LAM; however, this difference was not statistically significant ($p = 0.09$). Furthermore, there were no differences in the time from diagnosis to sample collection, in the frequency of previous pneumothorax, or in lymphatic involvement between the S-LAM and TSC–LAM groups. All patients with TSC–LAM had renal angiomyolipoma (Table 2).

S-LAM patients presented with a slightly more severe disease compared to TSC–LAM patients, signified by a greater obstructive functional impairment with a lower FEV_1/FVC ratio and higher RV and RV/TLC values. There were no differences in walking distance or minimum SpO_2 between the two groups (Table 2).

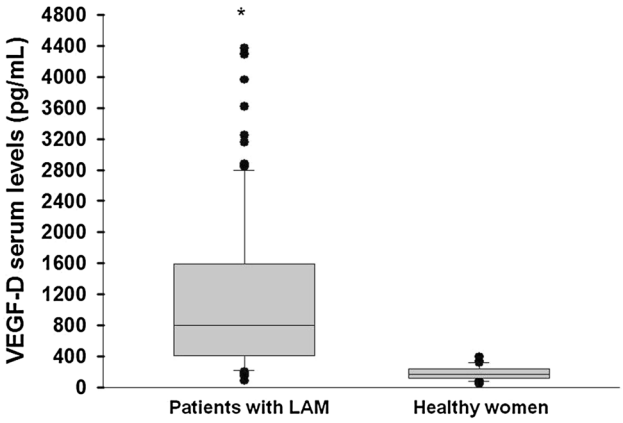

Fig. 1 Box plots of the VEGF-D serum levels compared between patients with LAM and healthy controls. Boxes represent the 25th to 75th percentiles. The median is the horizon dark line within the box. *$p < 0.001$

Table 2 Comparison of patients with LAM associated with TSC ($n = 21$) with those with sporadic LAM ($n = 83$)

	LAM-TSC ($n = 21$)	Sporadic LAM ($n = 83$)	*p*
Clinical features			
Age (years)	39 ± 12	44 ± 11	0.09
Time from diagnosis (years)	1 (1–4)	2 (0–5)	0.85
Pneumothorax	10 (48%)	39 (47%)	0.85
Chylothorax	2 (10%)	13 (16%)	0.71
Renal angiomyolipoma	21 (100%)	38 (46%)	< 0.001
Lymphangioleiomyoma	2 (10%)	14 (17%)	0.62
Chylous ascites	0	4 (5%)	0.70
Lymphatic involvement[a]	5 (24%)	20 (24%)	0.80
Pulmonary function tests			
FEV_1 (L)	2.24 ± 0.70	2.11 ± 0.80	0.5
%predicted	77 ± 22	75 ± 27	0.67
FVC (L)	2.76 ± 0.54	3.03 ± 0.71	0.10
%predicted	81 ± 14	90 ± 18	0.04
FEV_1/FVC	0.79 ± 0.16	0.68 ± 0.19	0.01
RV (L)	1.39 (1.24–1.84)	1.90 (1.51–2.42)	0.01
%predicted	109 (91–147)	123 (101–154)	0.16
TLC (L)	4.54 ± 0.57	5.18 ± 0.88	0.04
%predicted	95 ± 13	106 ± 18	0.02
RV/TLC	0.31 (0.29–0.39)	0.38 (0.33–0.47)	0.04
DL_{CO} (mL/min/mmHg)	19.7 ± 6.5	17.5 ± 6.9	0.23
%predicted	80 ± 34	73 ± 28	0.32
Six-minute walk test			
Distance (m)	540 (432–614)	495 (449–561)	0.29
Minimum SpO_2 (%)	92 ± 8	88 ± 12	0.18
VEGF-D serum levels (pg/mL)	1005 (641–2732)	772 (370–1383)	0.05

Values are the mean ± SD, median (25th–75th percentiles) or percentage (%)

DL_{CO} lung diffusing capacity for carbon monoxide, *FEV_1* forced expiratory volume in the first second, *FVC* forced vital capacity, *LAM* lymphangioleiomyomatosis, *LAM–TSC* lymphangioleiomyomatosis associated with tuberous sclerosis complex, *RV* residual volume, *SpO_2* oxyhemoglobin saturation by pulse oximetry, *TLC* total lung capacity, *TSC* tuberous sclerosis complex, *VEGF* vascular endothelial growth factor-D

[a]Lymphatic involvement: chylothorax, chylous ascites, and/or lymphangioleiomyoma

VEGF-D Serum Levels

Serum VEGF-D levels were significantly higher in patients with LAM compared to age-matched controls: 796 (404–1588) and 162 (117–232) pg/mL, respectively (Table 1; Fig. 1). No woman in the healthy control group had a serum VEGF-D measurement above the 800 pg/mL threshold. Patients with TSC–LAM also had higher levels of serum VEGF-D compared to patients with S-LAM, and this difference was marginally significant ($p=0.05$, Table 2). There were no correlations between serum VEGF-D levels and time from diagnosis to sample collection or the majority of functional parameters, except for a weak correlation with DL_{CO} (% of predicted), $r=-0.26$, $p=0.001$ (Supplementary Table 1).

Adopting the previously established cut-off serum VEGF-D value of 800 pg/mL, which has a high specificity for the diagnosis of LAM [1], we divided the patients into two groups based on distinct difference in serum VEGF-D levels (Table 3). Patients with lower serum VEGF-D levels tended to be older than those with higher levels. Lymphatic involvement was commoner in the group with serum VEGF-D levels ≥ 800 pg/mL (35% vs. 13%, $p=0.02$). However, no differences were detected in functional or 6MWT parameters between the groups (Table 3).

Table 3 Comparison of patients with LAM with VEGF-D ≥ 800 pg/mL ($n=52$) with those with VEGF-D < 800 pg/mL ($n=52$)

	LAM with VEGF-D ≥ 800 pg/mL ($n=52$)	LAM with VEGF-D < 800 pg/mL ($n=52$)	*p*
Clinical features			
Age (years)	41 ± 11	45 ± 11	0.05
Time from diagnosis (years)	1 (0–5)	1.5 (0–4.5)	1.00
Presence of TSC	11 (21%)	10 (19%)	1.00
Pneumothorax	24 (46%)	25 (48%)	1.00
Chylothorax	10 (19%)	5 (10%)	0.26
Renal angiomyolipoma	26 (50%)	33 (63%)	0.23
Lymphangioleiomyoma	12 (23%)	4 (8%)	0.06
Chylous ascites	2 (4%)	2 (4%)	0.61
Lymphatic involvement[a]	18 (35%)	7 (13%)	0.02
Pulmonary function tests			
FEV_1 (L)	2.11 ± 0.80	2.16 ± 0.77	0.77
%predicted	74 ± 27	77 ± 26	0.53
FVC (L)	3.01 ± 0.70	2.94 ± 0.68	0.63
%predicted	89 ± 18	88 ± 18	0.79
FEV_1/FVC	0.69 ± 0.20	0.71 ± 0.18	0.56
RV (L)	1.76 (1.27–2.10)	1.85 (1.44–2.71)	0.26
%predicted	123 (99–140)	117 (101–159)	0.53
TLC (L)	5.02 ± 0.94	5.08 ± 0.79	0.74
%predicted	104 ± 20	104 ± 15	0.85
RV/TLC	0.37 (0.29–0.42)	0.37 (0.32–0.47)	0.47
DL_{CO} (mL/min/mmHg)	16.9 ± 7.4	18.9 ± 6.2	0.14
%predicted	69 ± 31	79 ± 28	0.08
Six-minute walk test			
Distance (m)	500 ± 102	490 ± 121	0.67
Minimum SpO_2 (%)	88 ± 10	90 ± 14	0.53
VEGF-D serum levels (pg/mL)	1588 (1079–2587)	404 (239–573)	< 0.001

Values are the mean ± SD, median (25th–75th percentiles) or percentage (%)

DL_{CO} lung diffusing capacity for carbon monoxide, *FEV_1* forced expiratory volume in the first second, *FVC* forced vital capacity, *LAM* lymphangioleiomyomatosis, *RV* residual volume, *SpO_2* oxyhemoglobin saturation by pulse oximetry, *TLC* total lung capacity, *TSC* tuberous sclerosis complex, *VEGF* vascular endothelial growth factor-D

[a]Lymphatic involvement: chylothorax, chylous ascites, and/or lymphangioleiomyoma

Discussion

This study provided data on serum levels of VEGF-D and its related parameters in a large sample of patients with LAM who were followed at a Brazilian reference center. The main findings of this study include the following: (1) serum VEGF-D levels were higher in patients with LAM compared to healthy controls; (2) patients with TSC–LAM had slightly greater levels of serum VEGF-D in comparison to those with S-LAM; (3) there was a weak association between serum levels of VEGF-D and DL_{CO}; (4) lymphatic involvement was more frequent in those with higher levels of serum VEGF-D.

The role of VEGF-D has gained importance in the management of patients with LAM. This is because it is currently considered a diagnostic tool that may preclude the need for further invasive procedures, such as is frequently required for a definite diagnosis of the disease to be made [28]. However, data supporting its role have not been assessed in the Brazilian population. Although this biomarker has a high specificity for the diagnosis of LAM, especially when it reaches values above 800 pg/mL, and can also predict response to treatment, its role as a prognostic biomarker has not been completely established [1, 16].

Discrepancies in the levels of VEGF-D encountered in our LAM patients might reflect true differences between populations. Specifically, levels seemed to be somewhat lower when compared to those found in previous studies and with patients from other countries (Table 4) [11, 13, 15, 17, 18]. Interpreting these findings based solely on ethnic or genetic background is incredibly challenging, since our center receives referrals from the entire country, which has a complex history of miscegenation. The cut-off value of 800 pg/mL, albeit conservative, appears to be an appropriate diagnostic value for the Brazilian population, as no healthy control subject had values above this threshold. However, it lacks sensitivity and a serum level below this value is not enough to definitively exclude the diagnosis of LAM in an appropriate clinical setting.

Other demographic and functional characteristics may help to explain the lower levels of serum VEGF-D that were found in our study. Lymphatic involvement, reported in up to 69%

Table 4 Comparison of VEGF-D serum levels and clinical and functional features between different studies

	Current study (2018)	Young (2010)	Xu (2013)	Glasgow (2009)	Radzikowska (2015)	Seyama (2006)
n	104	84	78	111 (only S-LAM)	48	44 (only S-LAM)
VEGF (pg/mL)	796 (404–1588)		3842 (2491–> 4000)	1869 ± 145		1069 (809–1412)
VEGF (TSC–LAM)	1005 (641–2732)	3465 (1970–7195)			2682 ± 1347	
VEGF (S-LAM)	772 (370–1383)	1175 (780–2013)		1869 ± 145	1281 ± 791	1069 (809–1412)
VEGF (control group)	162 (117–232)	309 (211–433)	405 (245–528)	657 ± 43		295 (262–333)
Age (years)	43 ± 11	43 ± 12	41 ± 8	51		37 ± 11
Time from diagnosis (years)	1 (0–5)				> 6	
TSC–LAM (%)	20.2	33.3			25	
Pneumothorax (%)	47.1				50	
Chylothorax (%)	14.4				20.8	
Renal angiomyolipoma (%)	56.7			36	43.8	
Lymphangioleiomyomas (%)	15.4					
Chylous ascites (%)	3.8					
Any lymphatic involvement (%)	24			69		
FEV1 (% of predicted)	75 ± 26	64 ± 23	64 ± 27			
DL_{CO} (% of predicted)	74 ± 29		48 ± 21			

Values are the mean ± SD, median (25th–75th percentiles) or percentage (%)

VEGF-D vascular endothelial growth factor, *TSC–LAM* lymphangioleiomyomatosis associated with tuberous sclerosis, *S-LAM* sporadic lymphangioleiomyomatosis, DL_{CO} lung diffusing capacity for carbon monoxide, FEV_1 forced expiratory volume in the first second

of patients in the study by Glasgow and colleagues, was only present in 24% of our patients [15]. There were also differences in disease severity, since FEV_1 and DL_{CO} were only mildly reduced in our cohort compared to reports of moderate impairment described by other authors [11, 13]. Time from diagnosis to sample collection may also explain this difference, since most of our patients had been recently diagnosed when subjected to a blood draw compared, for example, to patients in the study by Glasgow and coauthors [15]. Thus, our study adds to existing evidence, with data from a different population, supporting the diagnostic role of the biomarker. However, the lower levels of VEGF-D we encountered also corroborate the need to adapt more conservative cut-off values, since reference values, based on best specificity and sensitivity from one study, may not be reproducible in other centers.

The importance of VEGF-D as a disease severity marker is also highlighted in our study. We were able to find a weak association between DL_{CO} and levels of serum VEGF-D, which corroborates findings in previous studies [15, 17, 18]. It also adds to the notion that higher levels reflect greater pulmonary impairment, which is reinforced by the correlation with the extent of cysts on high-resolution CT scans as has been described previously [13, 17, 19]. However, unlike in other studies [11, 13, 15, 18], there was no association of VEGF-D with other functional parameters, such as FEV_1, walked distance, and SpO_2. Additionally, its prognostic role in assessing the risk of disease progression and death remains uncertain.

Lymphatic involvement was also commoner in patients with higher serum levels of VEGF-D, which not only reflects the very nature of the biomarker, but is also in line with other reports demonstrating an association with chylous effusions and/or lymphangioleiomyoma [11, 13, 15, 17]. TSC–LAM patients had slightly higher values of VEGF-D, which has already been found by other authors; however, there was no association with the prevalence of renal angiomyolipoma, which is in line with reports from other centers [11, 13, 15].

Our study has several limitations that need to be addressed. Although our study was conducted in a single center, we included 104 patients, which constituted the second greatest casuistic in the assessment of VEGF-D in patients with LAM. Being a reference center, data from our hospital can be extrapolated to the whole Brazilian population. These data are consistent with findings from other authors worldwide, hence reinforcing its validity. Another limitation is that we did not assess serum VEGF-D levels in patients with other cystic diseases. However, this biomarker has been reported to be in lower levels in such diseases in comparison to LAM [11, 12], and the control group allowed a fair comparison to avoid the risk of finding false positive results in our LAM population. As also previously highlighted, we are not able to exclude the possibility that sample collection, storage, assay conditions, and analysis could be responsible for the results obtained [11, 13]. However, the comparison with a control group minimized such potential limiting factors.

In conclusion, our study adds to the body of evidence that puts VEGF-D as a relevant diagnostic and disease severity biomarker in patients with LAM and reinforces its use in a broader population, including Brazil. In LAM, serum VEGF-D is only weakly associated with lung function impairment and is more associated with lymphatic involvement. The characteristics of patients with LAM may explain the varying levels of serum VEGF-D found in studies from several countries.

Acknowledgements Other members of the Tuberous Sclerosis, Lymphangioleiomyomatosis and Angiomyolipoma Study Group include the following: Bruno Eduardo Pedroso Balbo, Elieser Hitoshi Watanabe, Luciana Paula Samorano, Luiz Fernando Onuchic, Maria Cecília da Matta Rivitti-Machado, Maria Luiza Giraldes de Manreza, Maurício Dener Cordeiro, Patrícia Takahashi, and Zilda Najjar Prado de Oliveira.

Funding Financial support for this study was provided by Novartis Biociências S.A. Novartis had no participation whatsoever in the design, management, and analysis of data or in the decision to publish this study.

Compliance with Ethical Standards

Conflict of interest The authors received no refunds or financial benefits and have no conflicts of interest to declare.

References

1. McCormack FX, Gupta N, Finlay GR, Young LR, Taveira-DaSilva AM, Glasgow CG et al (2016) Official American Thoracic Society/Japanese Respiratory Society Clinical Practice Guidelines: Lymphangioleiomyomatosis Diagnosis and Management. Am J Respir Crit Care Med 194(6):748–761
2. Glassberg MK (2004) Lymphangioleiomyomatosis. Clin Chest Med 25(3):573–582, vii
3. Ryu JH, Moss J, Beck GJ, Lee JC, Brown KK, Chapman JT et al (2006) The NHLBI lymphangioleiomyomatosis registry: characteristics of 230 patients at enrollment. Am J Respir Crit Care Med 173(1):105–111
4. Freitas CSG, Baldi BG, Jardim C, Araujo MS, Sobral JB, Heiden GI et al (2017) Pulmonary hypertension in lymphangioleiomyomatosis: prevalence, severity and the role of carbon monoxide diffusion capacity as a screening method. Orphanet J Rare Dis 12(1):74
5. Baldi BG, Salim C, Freitas G, Araujo MS, Dias OM, Pereira DAS et al (2014) Clinical course and characterisation of lymphangioleiomyomatosis in a Brazilian reference centre. Sarcoidosis Vasc Diffus Lung Dis 31(2):129–135
6. Carsillo T, Astrinidis A, Henske EP (2000) Mutations in the tuberous sclerosis complex gene TSC2 are a cause of sporadic pulmonary lymphangioleiomyomatosis. Proc Natl Acad Sci USA 97(11):6085–6090
7. Sengupta S, Peterson TR, Sabatini DM (2010) Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. Mol Cell 40(2):310–322

8. Kumasaka T, Seyama K, Mitani K, Sato T, Souma S, Kondo T et al (2004) Lymphangiogenesis in lymphangioleiomyomatosis: its implication in the progression of lymphangioleiomyomatosis. Am J Surg Pathol 28(8):1007–1016
9. Nascimento ECT, Baldi BG, Mariani AW, Annoni R, Kairalla RA, Pimenta S et al (2018) Immunohistological features related to functional impairment in lymphangioleiomyomatosis. Respir Res 19:83
10. Radzikowska E, Jagus P, Skoczylas A, Sobiecka M, Chorostowska-Wynimko J, Wiatr E et al (2013) Role of serum vascular endothelial growth factor D in discrimination of patients with polycystic lung diseases. Pol Arch Med Wewn 123(10):533–538
11. Young LR, Vandyke R, Gulleman PM, Inoue Y, Brown KK, Schmidt LS et al (2010) Serum vascular endothelial growth factor-D prospectively distinguishes lymphangioleiomyomatosis from other diseases. Chest 138(3):674–681
12. Young LR, Inoue Y, McCormack FX (2008) Diagnostic potential of serum VEGF-D for lymphangioleiomyomatosis. N Engl J Med 358(2):199–200
13. Xu KF, Zhang P, Tian X, Ma A, Li X, Zhou J et al (2013) The role of vascular endothelial growth factor-D in diagnosis of lymphangioleiomyomatosis (LAM). Respir Med 107(2):263–268
14. Chang WYC, Cane JL, Blakey JD, Kumaran M, Pointon KS, Johnson SR (2012) Clinical utility of diagnostic guidelines and putative biomarkers in lymphangioleiomyomatosis. Respir Res 13:13–34
15. Glasgow CG, Avila NA, Lin JP, Stylianou MP, Moss J (2009) Serum vascular endothelial growth factor-D levels in patients with lymphangioleiomyomatosis reflect lymphatic involvement. Chest 135(5):1293–1300
16. Young L, Lee HS, Inoue Y, Moss J, Singer LG, Strange C et al (2013) 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(6):445–452
17. Radzikowska E, Jagus P, Sobiecka M, Chorostowska-Wynimko J, Wiatr E, Kus J et al (2015) Correlation of serum vascular endothelial growth factor-D concentration with clinical presentation and course of lymphangioleiomyomatosis. Respir Med 109(11):1469–1475
18. Seyama K, Kumasaka T, Souma S, Sato T, Kurihara M, Mitani K et al (2006) Vascular endothelial growth factor-D is increased in serum of patients with lymphangioleiomyomatosis. Lymphat Res Biol 4(3):143–152
19. Baldi BG, Araujo MS, Freitas CSG, da Silva Teles GB, Kairalla RA, Dias OM et al (2014) Evaluation of the extent of pulmonary cysts and their association with functional variables and serum markers in lymphangioleiomyomatosis (LAM). Lung 192(6):967–974
20. Taveira-DaSilva AM, Jones AM, Julien-Williams P, Stylianou M, Moss J (2018) Long-term effect of sirolimus on serum vascular endothelial growth factor D levels in patients with lymphangioleiomyomatosis. Chest 153(1):124–132
21. Johnson SR, Cordier JF, Lazor R, Cottin V, Costabel U, Harari S et al (2010) European Respiratory Society guidelines for the diagnosis and management of lymphangioleiomyomatosis. Eur Respir J 35(1):14–26
22. Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V et al (2005) Standardisation of the single-breath determination of carbon monoxide uptake in the lung. Eur Respir J 26(4):720–735
23. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A et al (2005) Standardisation of spirometry. Eur Respir J 26(2):319–338
24. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F et al (2005) Standardisation of the measurement of lung volumes. Eur Respir J 26(3):511–522
25. Neder J, Andreoni S, Peres C, Nery LE (1999) Reference values for lung function tests. III. Carbon monoxide diffusing capacity (transfer factor). Braz J Med Biol Res 32(6):729–737
26. Pereira CA, Sato T, Rodrigues SC (2007) New reference values for forced spirometry in white adults in Brazil. J Bras Pneumol publicação Of da Soc Bras Pneumol e Tisilogia 33(4):397–406
27. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories (2002) ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 166(1):111–117
28. Gupta N, Finlay GA, Kotloff RM, Strange C, Wilson KC, Young LR et al (2017) Lymphangioleiomyomatosis Diagnosis and Management: High-Resolution Chest Computed Tomography, Transbronchial Lung Biopsy, and Pleural Disease Management. An Official American Thoracic Society/Japanese Respiratory Society Clinical Practice Guideline. Am J Respir Crit Care Med 196(10):1337–1348

Affiliations

Alexandre Franco Amaral[1] · Martina Rodrigues de Oliveira[1] · Olívia Meira Dias[1] · Fábio Eiji Arimura[1] · Carolina Salim Gonçalves Freitas[1] · Milena Marques Pagliarelli Acencio[1] · Vanessa Adélia de Alvarenga[1] · Ronaldo Adib Kairalla[1] · Carlos Roberto Ribeiro Carvalho[1] · Bruno Guedes Baldi[1] · Tuberous Sclerosis, Lymphangioleiomyomatosis and Angiomyolipoma Study Group, Universidade de Sao Paulo, Brazil

[1] Divisao de Pneumologia, Instituto do Coracao, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Avenida Doutor Enéas de Carvalho Aguiar, 44, São Paulo, São Paulo 05403-900, Brazil