Respiratory Medicine xxx (2015) 1–7

Contents lists available at ScienceDirect

Respiratory Medicine

journal homepage: www.elsevier.com/locate/rmed

Correlation of serum vascular endothelial growth factor-D concentration with clinical presentation and course of lymphangioleiomyomatosis

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ARTICLE INFO

Article history:
Received 21 May 2015
Received in revised form
7 September 2015
Accepted 8 September 2015
Available online xxx

Keywords:
Lymphangioleiomyomatosis
Lymphangioma
Pulmonary function tests
Renal angiomyolipomas
Tuberous sclerosis
VEGF-D

ABSTRACT

Background: Increased serum vascular endothelial growth factor D (VEGF-D) concentration has been accepted as a diagnostic marker in lymphangioleiomyomatosis (LAM). The study was performed to evaluate the correlation of VEGF-D with clinical presentation and course of LAM.
Material: The study group comprised of 48 women with LAM (27 with sLAM, 9 with sLAM and lymphangioma (sLAM-LYM) and 12 patients with TSC/LAM). Patients were assessed at the time of VEGF-D examination, and pulmonary function parameters were compared with those, obtained one year before. VEGF-D serum concentration was measured by ELISA method.
Results: Patients with TSC/LAM and sLAM-LYM displayed higher concentrations of VEGF-D than patients with sLAM (2682 ± 1347 pg/mL and 2223 ± 1184 pg/mL vs.1281 ± 791 pg/mL; $p = 0.0002$, $p = 0.009$) respectively. Patients with sLAM and VEGF-D concentration <800 pg/mL (sLAM-L) had better lung function as assessed by FEV1 (2.38 ± 0.88 L vs. 1.75 ± 0.8 L; $p < 0.015$) and *DL*,CO (5.8 ± 2.25 vs. 3.93 ± 1.74 mL/min/mmHg; $p < 0.028$), had higher blood oxygenation, then those with VEGF-D >800 pg/mL (sLAM-H). Significant yearly increase of TLC (390 ± 700 mL; $p < 0.021$) and RV (340 ± 790 mL; $p < 0.03$), and decrease of distance in 6MWT (−30 ± 50 m; $p = 0.04$) were observed in sLAM-H group. Lung function parameters remained constant in sLAM-L patients. Patients with sLAM-H displayed higher yearly decline of FVC (120 vs. 50 mL; $p = 0.035$) and increase of TLC (390 vs. −80 mL; $p = 0.038$) and RV (340 vs. 90 mL; $p = 0.045$) than sLAM-L patients. Negative correlations between VEGF-D concentration and *DL*,CO, PaO2, PaCO2, and positive with HRCT grading, and desaturation in 6MWT were noticed in sLAM patients without lymphangioma.
Conclusions: Serum VEGF-D is the useful biomarker of LAM extension, and might also prove predictive towards therapeutic decision.

1. Introduction

Lymphangioleiomyomatosis (LAM) is a rare polycystic lung disease, which affects mainly women in childbearing age. Disease occurs in two forms: as a sporadic lymphangioleiomyomatosis (sLAM) or associated with tuberous sclerosis complex (TSC/LAM) [1–3].

LAM is caused by proliferation of abnormal smooth muscle-like cells (LAM cells), that results in polycystic destruction of lungs, formation of renal angiomyolipomas, lymphadenopathy, cystic lymphangiomas, abdominal lymphangioleiomyomas, chylothorax, and chyloperitoneum [2,4]. It is a low–grade metastasizing neoplasm spreading through lymphatic routes and belongs to the group of the PEComas (perivascular epithelioid cell tumours) [4]. It is currently believed, that the key molecular mechanism driving LAM development and progression is the loss of TSC1 or TSC2 gene function, resulting in the mTOR signalling pathway activation and subsequently dysregulation of cell growth, survival and motility

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http://dx.doi.org/10.1016/j.rmed.2015.09.005
0954-6111/

up-regulation [5]. Higher expression of lymphangiogenic growth factors, particularly VEGF-D can play an important role in development of LAM. VEGF-D, a ligand for the lymphatic growth factor receptor VEGFR-3/Flt-4, is one of proteins, which can direct the metastatic process in LAM [6,7]. Considerable number of publications has provided evidence suggesting, that serum VEGF-D concentration might prove useful as a diagnostic biomarker of the LAM. It has been repeatedly demonstrated, that serum VEGF-D level over 800 pg/mL clearly distinguishes LAM from other polycystic lung diseases (specificity 90–100%; sensitivity 73%–87%) [8–12]. Moreover, a higher VEGF-D serum concentration has been indicative for patients with more advanced disease, those with TSC/LAM and patients with LAM and lymphangioma [8,11,12].

Recently, usefulness of this biomarker in assessment of mTOR inhibitor treatment was presented. Bissler et al., in the EXIST-2 study, demonstrated the straight correlation of VEGF-D decreasing and loss of angiomyolipoma volume during the therapy with everolimus in patients with angiomyolipomas in the course of TSC or sLAM [13]. Similarly, in MILES study significant decreasing of VEGF-D serum concentration was observed in patients with LAM treated with sirolimus in comparison to the placebo group. Additionally, a correlation between VEGF-D serum concentration and severity of the disease was shown [14].

LAM is characterized by the wide spectrum of the natural history and clinical presentation. There are young patients with severe respiratory impairment while others present with stable, or only slightly progressing disease [2,15]. The mTOR inhibitors are currently considered the most effective therapy for patients with advancing form of the disease. They are potent immunosuppressants with well described adverse effects; some potentially serious, although generally acceptable and manageable [13,16,17]. According to European Respiratory Society LAM guidelines, therapy with mTOR inhibitors should be considered in patients with significant loss of lung function and progressive disease, with rapid decline in lung function parameters [2]. However, considering relatively low short-term sensitivity of lung function testing in lung function decline assessment, these criteria might result in the significant delay of therapy. Therefore, we believe that considering serum VEGF-D level as an additional factor, apart from clinical status assessment and pulmonary function tests might prove worthwhile in therapeutic decision making process.

The study was performed to evaluate the correlation of VEGF-D with clinical presentation and course of LAM.

2. Material and methods

2.1. Study population

The study group comprised of 36 (77%) women with sporadic lymphangioleiomyomatosis (sLAM) (mean age 47.8 ± 10.5 years) and 12 (23%) patients with TSC/LAM (mean age 37 ± 8.5 years). Within sLAM group, there were 9 (19%) patients, who additionally had retroperitoneal lymphangioma (sLAM-LYM). Patients with sLAM were stratified according to the VEGF-D serum concentration, into the subgroups with low (<800 pg/mL) (sLAM-L) and high (>800 pg/mL) (sLAM-H) VEGF-D levels. There were no current smokers in the study group, 32 (67%) patients were nonsmokers and 16 (33%) were ex-smokers.

The detailed demographic and clinical characteristics of study group are presented in Table 1.

All patients enrolled into the study fulfilled the criteria of definite LAM according to European Respiratory Society guidelines. Final diagnosis of LAM was based on clinical and radiological examination, and was confirmed by histological assessment of tissue material from the open lung biopsy in 35 cases. Biopsy of abdominal lesions or renal angiomyolipoma (AML) was performed in 7 patients. Twelve patients met the criteria of definite TSC, and in 9 cases diagnosis was additionally confirmed by histological examination. Only in two patients the diagnosis of sLAM was based on typical clinical and radiological findings, presence of bilateral renal angiomyolipomas, and high (above 800 pg/mL) serum VEGF-D concentration.

For the analysis of delay in LAM diagnosis and disease duration, the date of first symptoms, date of diagnosis, and date of VEGF-D measurement were recorded.

Pulmonary function tests were performed according to the joint guidelines of the American Thoracic Society and European Respiratory Society [18]. The lung volumes were measured by body plethysmography (Jeager MasterScreen software version 4.65; Wuerzburg; Germany) and diffusing capacity for carbon monoxide (*D*L,CO) using the single breath technique. Absolute values and percentages of predicted values measured at the time of VEGF-D measurement and on the visit one year before were analysed.

The six minute walk test (6MWT) was performed according to recommendations [19]. All pulmonary function tests were done at the time of VEGF-D assessments and compared with values obtained during previous visit one year earlier.

The lung involvement in HRCT was graded according to proposition of Avila et al. In the first group cystic lesions involved less than 30%, in second 30–60%, and in third over 60% of the equally divided three zones of the lungs [20].

2.2. VEGF-D assessment

Serum VEGF-D concentrations were measured by quantitative sandwich enzyme immunoassay technique (R&D Systems, Minneapolis, MN, USA), accordance to manufacturer recommendations as presented earlier. Briefly, 5 mL of blood collected in serum separator tubes, and allowed to clot for 30 min at 4 °C, were centrifuged at 2500 rpm for 10 min. Serum samples were aliquoted and stored at −70 °C for future analysis. The spectrophotometric reader Tecan Infinite M200 (Austria) was used to assess the optical density at 450 nm. All measurements were performed in duplicates. Test sensitivity ≥31.3 pg/mL, range 125–4000 pg/mL allowed reliable VEGF-D estimation in human serum [21].

The study was supported by the National Tuberculosis and Lung Diseases Research Institute grant No 7.20. The local Bioethics Committee approved the study and patients signed informed consent.

3. Statistical methods

Statistical analyses were performed using Statistica 10 (StatSoft Inc., Tulsa, OK, USA).

The Mann–Whitney U-test, Student's t-test for dependent and independent means, Wilcoxon test, Cochran and Cox test for the comparative analysis were applied.

Anova and Anova Kruskal–Wallis tests were used for comparison of more than two groups and the results were verified with post–hoc Tukey's and Dunn's tests respectively.

Linear regression model was used to assess the relation between VEGF-D serum concentration and age in the time of examination, time with symptomatic disease, results of pulmonary function tests, 6MWT and blood gas parameters.

Pearson r or Spearman-R coefficient was used to measure the strength of association between two variables.

The chi-square test (or its appropriate modification) was used for proportion assessment.

A value of probability (p) of <0.05 was considered to indicate statistical significance. All p values are two-sided and unadjusted for multiple testing.

Please cite this article in press as: E. Radzikowska, et al., Correlation of serum vascular endothelial growth factor-D concentration with clinical presentation and course of lymphangioleiomyomatosis, Respiratory Medicine (2015), http://dx.doi.org/10.1016/j.rmed.2015.09.005