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Early View

Research letter

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Methacholine reactivity in LAM is inversely related to FEV_1 and VEGF-D

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To the Editor;

Lymphangioleiomyomatosis (LAM) is a multisystem disease characterized by cystic lung destruction, leading to respiratory failure, and associated with kidney (e.g., angiomyolipomas (AML)) and lymphatic involvement (e.g., lymphangioleiomyomas, chylous effusions) [1, 2]. LAM occurs sporadically or in association with Tuberous Sclerosis Complex (TSC), an autosomal-dominant disorder characterized by mutations of the *TSC1* or *TSC2* genes. Lung destruction results from the proliferation of LAM cells, which possess neoplastic properties and are found in LAM lung nodules, in association with fibroblasts, mast cells, lymphocytes and lymphatic endothelial cells [3, 4]. LAM patients may show increases in serum levels of the lymphangiogenic factor, vascular endothelial growth factor-D (VEGF-D), a LAM biomarker used in differential diagnosis of cystic lung diseases and to identify LAM patients likely to respond to sirolimus treatment [5-7].

Cystic lung destruction in LAM is associated with decline in FEV_1 and/or DLco, reduction in the 6-minute walk test and an abnormal cardiopulmonary exercise test [3, 8]. About a third of LAM patients exhibit an intermittent bronchodilator response to β-adrenergic agonists, which is associated with the presence of LAM lung nodules and a more rapid decline in FEV_1 [3, 9] . Although LAM airways showed evidence of airway inflammation [3], histopathology did not correlate with bronchodilation in response to β-adrenergic agonists [8].

Bronchodilator responsiveness is characteristic of patients with asthma, a clinically heterogeneous disease marked by differences in histopathology and responses to therapeutic agents [10] . In some patients with asthma-like symptoms, airway hyper-reactivity may be established by responsiveness to methacholine [11], a non-selective muscarinic receptor

agonist that acts on the parasympathetic nervous system and a bronchoprovocative agent that may cause shortness of breath and wheezing in susceptible individuals.

Given the LAM histopathology, we questioned whether the bronchodilator responsiveness seen in some LAM patients occurred in association with methacholine responsiveness, as seen in asthma. If so, we asked whether methacholine responsiveness was preferentially associated with other biomarkers of LAM severity and drug responsiveness, e.g., VEGF-D, FEV_1, bronchodilators.

Forty-three LAM patients participated prospectively in the methacholine study. Diagnosis of LAM was obtained by VATS (11 patients), transbronchial biopsy (1), CT and AML (20), CT and TSC (7), and CT and lymphatic involvement (4). Patients were excluded if they had a history of asthma, baseline $FEV_1 < 1$ L, history of cigarette smoking, or treatment of LAM with sirolimus or other mTOR inhibitors. The study was approved by the Ethical Committee of Ospedale San Giuseppe, Milan. Written, informed consent was obtained from all patients.

Lung volumes, flow rates and single-breath DLco were measured (Vmax 229, SensorMedics, Yorba Linda, CA), according to ATS recommendations [12]. Patients abstained from using inhaled bronchodilators and corticosteroids for 12 h prior to testing. A positive response to bronchodilators consisted of an increase in FEV_1 of 12% over baseline and of at least 200 mL. On day 1 of the study, flow rates were measured before and after inhalation of 400 μg of salbutamol sulfate in solution. On the following day (day 2) and at the same time of day, flow rates were measured before and after nebulization of 500 μg of ipratopium bromide. On day 3, the bronchoprovocation challenge test with methacholine was performed. Patients were excluded if the FEV_1 was < 1 L. The test was conducted according to ATS recommendations

[12]. Methacholine (Lofarma Metacolina 1% solution), 30 mg was administered with progressively more concentrated solutions via nebulizer in 2 min increments; each dose was followed by reassessment of spirometry. If a patient became symptomatic, the test was interrupted. It was continued if FEV_1 did not fall > 20% from baseline. The test was stopped and considered positive when an FEV_1 decline >20% from baseline was observed. The test was considered negative when the patient did not respond to the maximum concentrations of methacholine. VEGF-D concentration was determined by a quantitative sandwich enzyme immunoassay technique [5, 6].

Fourteen of the 43 patients exhibited bronchoconstriction in response to bronchoprovocation with methacholine. We initially performed univariate analyses of possible predictors of response to methacholine challenge. P-values were derived using a t-test for continuous variables and Fisher's exact test for binary variables. Then, a multiple logistic regression was used to identify factors associated with the response to the methacholine challenge. Variables with p-value < 0.10 in the univariate analyses were considered for inclusion. All tests were two sided and no p-value adjustment for multiple comparison were made.

From the univariate analyses, FEV_1 (p=0.076), VEGF-D (p=0.036) and response to Salbutamol (p=0.040) were associated with a methacholine response. The response to Salbutamol, however, was not associated with FEV_1 or VEGF-D. LAM airways also exhibited bronchodilatation in response to ipratropium but an ipratropium response was not concordant with methacholine responsiveness (P=0.488) (see Table). Also, no factors were found to be significantly associated with the response to ipratropium. Significant predictors of a negative

methacholine challenge in the multivariate model were lack of a response to Salbutamol (p=0.019), higher levels of FEV_1 (p=0.049), and higher levels of serum VEGF-D (p=0.015). Thus, subjects who did not respond to Salbutamol, or had higher levels of FEV_1 or of VEGF-D levels tended to have a negative response to methacholine challenge. Thus, reactivity to methacholine correlated positively with β-adrenergic agonist responsiveness, and inversely with greater FEV_1 and higher serum VEGF-D levels.

As these data suggest, in LAM, like in asthma, airway hypersensitivity can be assessed by methacholine challenge. A subset of LAM patients was sensitive to methacholine. The methacholine-sensitive patients were examined for bronchodilator responsiveness with Salbutamol (albuterol), a short-acting β-adrenergic agonist and ipratropium, an anti-cholinergic agonist. The methacholine-responsive patients exhibited a statistically significant response to Salbutamol (P=0.04). The same methacholine-responsive LAM patients, however, did not show a concordant bronchodilator response to ipratropium (P=0.488). Thus, there was an apparent dissociation between the methacholine responsiveness and that of the bronchodilators.

The association of serum VEGF-D levels with methacholine sensitivity was unexpected. Serum VEGF-D levels have been shown to be a biomarker for LAM [5, 6], with levels greater than 800 pg/mL consistent with a LAM diagnosis, although LAM patients may have VEGF-D levels less than 800 pg/mL [13]. In this study, we found that higher serum VEGF-D levels were associated with reduced methacholine sensitivity in LAM patients not treated with sirolimus. Since VEGF-D is a lymphangiogenic growth factor, enhanced lymphangiogenesis may reduce methacholine sensitivity. In the MILES trial [5, 7], higher levels of VEGF-D were associated with an enhanced response to sirolimus treatment and correlated with increased rate of disease

progression; in other studies , sirolimus appears to inhibit the lymphatic component [14]. Methacholine bronchoprovocation in patients with LAM was used to assess airway reactivity. A negative methacholine challenge was associated with a lack of response to β-adrenergic agonist, higher levels of FEV_1, and unexpectedly, higher levels of the lymphangiogenic factor, VEGF-D. Our current study suggests that the lymphatic component may be involved in methacholine responsiveness.

Table: Univariate analyses of possible predictors of response to methacholine challenge

	Methacholine Challenge (N=43)				**P-value***
	Negative (N=29)		**Positive (N=14)**		
	Mean	**SEM**	**Mean**	**SEM**	
Age (yr)	37.7	2.2	43.0	3.2	0.178
FEV_1 (mL/yr)	2.4	0.1	2.1	0.1	0.076
$\%FEV_1$	84.9	3.5	77.6	3.9	0.214
DLco (ml/min/mm Hg)	14.8	0.9	13.5	1.0	0.382
%DLco	58.0	3.3	54.2	4.0	0.492
VEGF-D (pg/mL)	1396.8	233.6	771.7	167.4	0.036
	N	**%**	**N**	**%**	
Salbutamol	26	90	6	43	0.040
Ipratropium	21	72	6	43	0.488
TSC	23	79	11	79	1.000
AML	16	55	7	50	1.000
Lymphatic	25	86	11	79	0.665
Menopause	27	93	12	86	0.585
RX Hx	28	97	13	93	1.000

*P-values derived using a t-test for continuous variables and Fisher's exact test for binary variables (shown are for values either "no response" or "absence of the condition").
FEV1, forced expiratory volume in one second; DLco, diffusing capacity of lung for carbon monoxide ; VEGF-D, vascular endothelial growth factor D; TSC, Tuberous Sclerosis Complex; AML, angiomyolipoma, RX, treatment ; Hx, history .

References

1. McCormack, F.X., et al., Official American Thoracic Society/Japanese Respiratory Society Clinical Practice Guidelines: Lymphangioleiomyomatosis Diagnosis and Management. Am J Respir Crit Care Med, 2016. 194:748-61.
2. Johnson, S.R., Lymphangioleiomyomatosis. Eur Respir J, 2006. 27:1056-65.
3. Taveira-DaSilva, A.M., G. Pacheco-Rodriguez, and J. Moss, The natural history of lymphangioleiomyomatosis: markers of severity, rate of progression and prognosis. Lymphat Res Biol, 2010. 8:9-19.
4. Gupta, N., et al., 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, 2017. 196:1337-1348.
5. Young, L., 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, 2013. 1:445-52.
6. Young, L.R., et al., Serum vascular endothelial growth factor-D prospectively distinguishes lymphangioleiomyomatosis from other diseases. Chest, 2010. 138:674-81.
7. McCormack, F.X., et al., Efficacy and safety of sirolimus in lymphangioleiomyomatosis. N Engl J Med, 2011. 364:1595-606.
8. Taveira-DaSilva, A.M., 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, 2001. 164:1072-6.
9. Taveira-DaSilva, A.M., et al., Reversible airflow obstruction in lymphangioleiomyomatosis. Chest, 2009. 136:1596-1603.
10. Miller, M.R., et al., Standardisation of spirometry. Eur Respir J, 2005. 26:319-38.
11. Crapo, R.O., et al., Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med, 2000. 161:309-29.
12. American Thoracic Society. Single-breath carbon monoxide diffusing capacity (transfer factor). Recommendations for a standard technique--1995 update. Am J Respir Crit Care Med, 1995. 152:2185-98.
13. Glasgow, C.G., et al., Serum vascular endothelial growth factor-D levels in patients with lymphangioleiomyomatosis reflect lymphatic involvement. Chest, 2009. 135:1293-1300.
14. Taveira-DaSilva, A.M., et al., Changes in lung function and chylous effusions in patients with lymphangioleiomyomatosis treated with sirolimus. Ann Intern Med, 2011. 154:797-805, W-292-3.