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Disease Severity Prediction by Spirometry in Adults with Visceral Leishmaniasis from Minas Gerais, Brazil

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Abstract. Visceral leishmaniasis (VL) is associated with interstitial pneumonitis according to histology and radiology reports. However, studies to address the functional impact on respiratory function in patients are lacking. We assessed pulmonary function using noninvasive spirometry in a cross-sectional study of hospitalized adult VL patients from Minas Gerais, Brazil, without unrelated lung conditions or acute infections. Lung conditions were graded as normal, restrictive, obstructive, or mixed patterns, according to Brazilian consensus standards for spirometry. To control for regional patterns of lung function, we compared spirometry of patients with regional paired controls. Spirometry detected abnormal lung function in most VL patients (70%, 14/20), usually showing a restrictive pattern, in contrast to regional controls and the standards for normal tests. Alterations in spirometry measurements correlated with hypoalbuminemia, the only laboratory value indicative of severity of parasitic disease. Abnormalities did not correlate with unrelated factors such as smoking or occupation. Clinical data including pulmonary symptoms and duration of therapy were also unrelated to abnormal spirometry findings. We conclude that the severity of VL is correlated with a restrictive pattern of lung function according to spirometry, suggesting that there may be interstitial lung involvement in VL. Further studies should address whether spirometry could serve as an index of disease severity in the management of VL.

INTRODUCTION

Visceral leishmaniasis (VL) in Brazil is caused by *Leishmania* (*Leishmania*) *chagasi*, syn. of *Leishmania* (*Leishmania*) *infantum*. VL has become an epidemic in some areas of Brazil including Minas Gerais. The disease can present with diverse clinical syndromes ranging from asymptomatic infection to severe disease, with fatalities due to hemorrhages and infection.[1–3] VL affects organs rich in macrophages, which serve as the main host cell for the parasites, and also results in altered immune responses indirectly affecting the kidneys and lungs.[4] Pulmonary involvement is characterized by interstitial pneumonitis according to histopathological findings in autopsies,[5] and is also associated with common acute bacterial infections due to the immune dysregulation caused by this disease.[6] In experimental hamster models, interstitial pneumonitis of varied intensity is often observed histologically in the alveolar septae, without associated parasites.[6,7] A similar observation has been made in the lungs of infected dogs, the common reservoir for VL in Brazil.[8] Canine VL is associated with fibrosis of the lung, but pulmonary parasites are rarely observed.[9] There are associated functional changes in the respiratory system. Hemodynamic alterations caused by severe anemia and low serum protein content could also affect lung physiology.

Spirometry is a noninvasive technique that detects functional changes in lung dynamics.[10] Spirometry measurements can lead to characterization of lung function as obstructive disease, often associated with the loss of alveoli, or restrictive disease due to mechanical alterations of alveolar septae and decreased elasticity of lung tissue. Patient cooperation is needed for these tests, but VL patients are often ambulatory and not bedridden.[11] Spirometry has been used to predict respiratory infection in cystic fibrosis patients. This noninvasive technology can be used in the clinical management of patients with pulmonary involvement to other diseases.[12]

We hypothesized that evaluation of pulmonary function using spirometry could indicate the type and severity of lung disease due to VL, in the absence of acute pulmonary infections. Herein, we studied spirometry data from adult patients with VL in an epidemic area of Brazil. We evaluated the correlation between pulmonary function and VL disease severity, by comparing spirometry measures in VL subjects, Brazilian normal standards, and regional paired controls.

PATIENTS AND METHODS

This cross-sectional study documented pulmonary function of 20 hospitalized patients diagnosed with VL, confirmed by parasitological diagnosis by the identification of amastigotes in bone marrow aspirates. Screening tests for VL included indirect immunofluorescence assays or immunochromatographic tests (Kalazar Detect; InBios International, Inc., Seattle, WA) prior to validation with bone marrow aspirates. Patients were older than 16 years and able to sign an informed consent statement. We excluded VL patients diagnosed with human immunodeficiency virus (HIV) coinfection, or with acute lung infections. This study was approved by the Research Ethics Committee of the Faculty of Medicine, University of São Paulo, according to Protocol no. 081/12.

Spirometry data from VL subjects were also compared with data from local control subjects, to avoid bias related to regional variations in spirometry measurements. Subjects

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TABLE 1
Laboratory hematological data from visceral leishmaniasis patients from Minas Gerais, Brazil

Laboratory test	Samples available	Mean ± SD	Minimum	Maximum
Erythrocytes (millions/mm^3)	20	$3.3 \times 10^6 \pm 0.73$	2.0×10^6	4.2×10^6
Hemoglobin (g/dL)	20	8.40 ± 1.62	5.70	10.90
Hematocrit (%)	20	26.05 ± 4.85	16.60	32.00
Leukocytes (cells/mm^3)	20	2,550 ± 1,198	610	4,720
Albumin (g/dL)	17	2.99 ± 0.51	1.80	3.70
Globulin (g/dL)	17	4.86 ± 1.95	2.80	11.40
Total protein (g/dL)	17	7.85 ± 2.11	5.10	14.60
A/G ratio	17	0.87 ± 0.18	0.28	0.93

A/G = albumin/globulin ratio; SD = standard deviation.

in the control group were selected by randomly pairing spirometry files of the same hospital patients according to age and sex during the same period of treatment. Among the eligible controls, two files were randomly selected form each control group by an external independent collaborator. Those files constituted a non-*Leishmania* patient group that could be considered as a random sample of subjects with regional nonpulmonary diseases other than VL, requiring. This is useful for defining a regional disease that affects spirometry results, and avoiding bias due to regional changes in lung function.

Spirometry method. Spirometry was performed on cooperative, awake, and ambulatory patients. No bronchodilators were used. A microQuark PC-based spirometer (Cosmed, Rome, Italy) was used in this research. The parameters included forced volume capacity (FVC), forced expiration volume in the first second (FEV1), Tiffeneau index (FEV1/FVC), and a forced expiratory flow of 25–75% (FEF 25–75%). Predicted values used in this study to evaluate results were based on the II Consensus of Spirometry.[13] Spirometry patterns were defined as restrictive or obstructive according to curves, and individual patient values were compared with expected test results in the healthy Brazilian population. In brief, pulmonary function was considered restrictive with a higher than normal FEV1/FVC ratio and lower than normal FVC and FEV1, but with a normal FEF of 25–75% compared with expected Brazilian values.[13] The lung physiology pattern was considered obstructive when FEV1/FVC was lower than expected Brazilian reference standards.[10,12]

Statistical analysis. We performed a quantitative comparison between groups using Student's *t* test, if variance was found to be normally distributed. For qualitative variables, the absolute (*n*) and relative (%) frequencies were calculated, and the difference between proportions was estimated by Fisher's exact test. Correlation between events was evaluated with Spearman's rank correlation. The difference was considered significant when the probability of equality was less than 5% ($P < 0.05$) in two-tailed assays. GraphPad Prism version 5 (Graph Pad Software, La Jolla, CA) was the statistical software used for these analyses.

RESULTS

VL descriptive study. We analyzed spirometry data from 20 VL patients (17 men and three women) with a mean age of 35 ± 14 years (range: 15–71 years). All patients had splenomegaly, and 18 of 20 (90%) had hepatomegaly. The main symptoms at admission included fever (100%), weight loss (95%), weakness (90%), cough (45%), pallor (30%), jaundice (25%), edema (25%), and hemorrhagic events (5%).

The duration of drug treatment of VL ranged from 6 to 30 days. Ten of 20 (50%) patients required blood transfusion during therapy. The mean transfusion volume was 600 ± 346.41 mL. Transfusion was necessary for only more severely affected patients. All patients had negative serology for HIV-1 and 2. Pentavalent antimonial drugs were used for VL treatment in 70% of the patients. Amphotericin B was used in 20% ($N = 4$); two received the liposomal formulation, and two received the deoxycholate emulsion form. The remaining patients were treated with both types of drugs in separate treatments plans. The mean blood counts, total serum proteins, and the albumin/globulin ratio values for all patients are presented in Table 1.

Respiratory symptoms, most commonly cough, were present in nine patients (40%): five (25%) had productive coughs and three (15%) had a dry cough. There was only one reported case of dyspnea. Eleven of 20 (55%) patients had a smoking history; however, only three (15%) were active smokers. The mean smoking duration was 12.05 years

TABLE 2
Mean quantitative results of spirometry from hospitalized adult VL patients compared with RC patients in Minas Gerais, Brazil

	VL		RC		
Variable	Mean ± SD	% Predicted	Mean ± SD	% Predicted	VL vs. RC groups
FVC (L) examination	3.56 ± 1.15	77.5%	3.87 ± 0.84	86.4%	NS
FVC (L) predicted	4.59 ± 0.84	$P < 0.01$	4.48 ± 0.77	$P < 0.01$	
FEV1 (L) examination	3.17 ± 1.01	82.5%	3.32 ± 0.71	88.6%	NS
FEV1 (L) predicted	3.84 ± 0.75	$P < 0.05$	3.76 ± 0.67	$P < 0.01$	
FEF 25–75% (L/s) examination	4.57 ± 1.71	96.0%	4.00 ± 1.19	103.1%	NS
FEF 25–75% (L/s) predicted	4.76 ± 1.59	NS	3.88 ± 0.99	NS	
FEV1/FVC (%) examination	92.6 ± 6.4	112.3%	86.6 ± 6.1	104.7%	$P < 0.001$
FEV1/FVC (%) predicted	82.8 ± 2.7	$P < 0.001$	82.7 ± 2.9	$P < 0.01$	

FEF 25–75% = forced expiratory flow of 25–75%; FEV1 = forced expiration volume in the first second; FVC = forced volume capacity; RC = regional control; SD = standard deviation; VL = visceral leishmaniasis. Significance assessed by two-tailed unpaired *t* test.

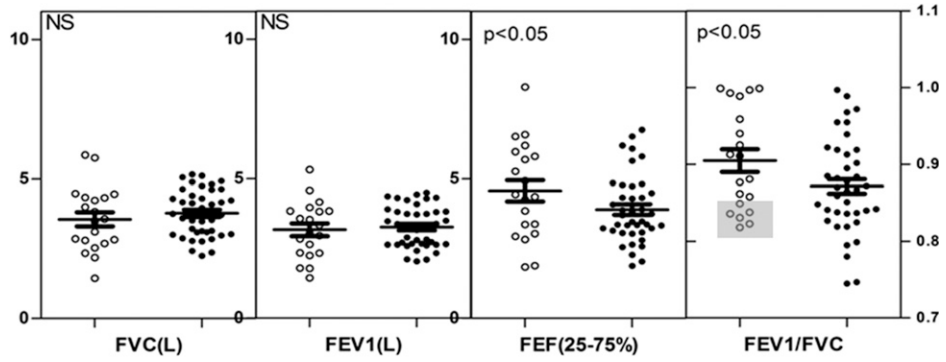

FIGURE 1. Spirometry quantitative data from adult visceral leishmaniasis (VL) patients from Minas Gerais, Brazil. Open dots: VL patients; closed dots: regional control patients; standard bars: standard error of the mean; shaded area: normal spirometry VL patients. Significance expressed in each graph was obtained with two-tail Student's *t* test after variance assessment.

(range: 05–54 years). The mean number of cigarettes smoked per day was 12.85 (range: 2–25).

Fourteen of 20 (70%) patients had abnormal spirometry results. Only the restrictive-disorder pattern was observed in VL patients. The magnitude of restrictive lung disease was low in nine (45%) and moderate in five (25%) of the 20 patients (Table 2).

Regional comparison. We compared these spirometry data from VL patients with a regional control group of hospitalized patients, as described in the Patients and Methods section. This group was analyzed to control for regional pulmonary function changes that could alter the spirometry data. These data are presented in Figure 1. As expected, the paired groups had the same age and sex ratio. VL patients had a broad distribution in lung volumes and flux (FVC and FEV1). Functional measures, including FEF 25–75% and FEV1/FVC, were greater in VL patients compared with the regional patient control group.

Grouping VL patients according to alterations in spirometry. We divided VL patients into subgroups according to their patterns of pulmonary function detected by spirometry (normal or restrictive). No statistically significant differences were observed between the duration of symptoms before and after hospitalization in patients with normal versus abnormal spirometry (Table 3). Most laboratory data were similar between the groups (N versus R *P* values in the table), with the exception of serum protein levels (Table 4). Total globulin levels were similar between the groups, but total serum protein and albumin levels were significantly reduced in the serum of VL patients with abnormal compared with normal spirometry. Examination of specific spirometry measurements revealed significant correlations between serum albumin and spirometry values, with lower serum albumin values correlating with lower FVC and FEV1, corrected for the Brazilian population normal values (last two columns of Table 4). Measurement of the liver enzyme alanine aminotransferase (ALT) also correlated with FVC and with FEV1 measurements, although there was not a significant association between ALT and other measurements of liver function among VL patients with normal versus restrictive spirometry measurements. Measures of anemia including total erythrocyte counts, hemoglobin, and hematocrit approached but did not reach statistical significance. Thus, some measurements of disease severity correlated with, or were associated with abnormal pulmonary function as evaluated by spirometry.

DISCUSSION

Patients included in this study had clinical and laboratory features diagnostic of VL. The laboratory features were similar to those typically described in the literature, including

TABLE 3

Comparison of examination and predicted spirometry values from visceral leishmaniasis adult patients from Minas Gerais, Brazil, sorted by normal and restrictive spirometry patterns related to Brazilian standards

		Visceral leishmaniasis patients			
		Normal spirometry		Restrictive spirometry	
Spirometric variable		Mean ± SD	O vs. P *P* value*	Mean ± SD	O vs. P *P* value*
FVC (L)	Observed	4.79 ± 0.82	NS	3.03 ± 0.82	< 0.001
	Predicted	4.78 ± 1.01		4.51 ± 0.79	
FEV1 (L)	Observed	4.24 ± 0.63	NS	2.72 ± 0.76	< 0.001
	Predicted	4.01 ± 0.86		3.77 ± 0.73	
FEF 25–75% (L/s)	Observed	5.65 ± 0.73	NS	4.11 ± 1.82	NS
	Predicted	5.10 ± 1.95		4.61 ± 1.46	
FEV1/FVC %	Observed	93.25 ± 4.49	< 0.05	92.82 ± 7.27	< 0.001
	Predicted	84.12 ± 1.81		82.23 ± 2.81	

FEF 25–75% =forced expiratory flow of 25–75%; FEV1 = forced expiration volume in the first second; FVC = forced volume capacity; NS = nonsignificant; SD = standard deviation.
*Two-tailed Students *t* test was performed for observed vs. predicted data.

TABLE 4

Comparison of the available laboratory data between normal and restrictive groups to assess differences between sorted groups and their correlation with spirometry values, expressed as percentage of observed/predicted data individual data

Laboratory test	Visceral leishmaniasis patients sorted by spirometry results					Correlation of entire data with % observed/predicted spirometry measures	
	Normal		N vs. R	Restrictive		FVC (L) Pearson *r*	FEV1 (L) Pearson *r*
Variable	*N*	Mean ± SD	*P* value	*N*	Mean ± SD	*P* value	*P* value
Erythrocytes (million/mm^3)	6	3.62 ± 0.41	NS	14	3.23 ± 0.83	0.34; NS	0.39; $0.1 < P < 0.05$
Hemoglobin (g/dL)	6	9.28 ± 1.20	NS	14	8.02 ± 1.66	0.37; NS	0.40; $0.1 < P < 0.05$
Hematocrit (%)	6	28.5 ± 3.3	NS	14	25.0 ± 5.1	0.36; NS	0.39; $0.1 < P < 0.05$
Leukocytes (/mm^3)	6	2,478 ± 1,123	NS	14	2,581 ± 1,269	–0.09; NS	–0.10; NS
Platelets (×10E^5/mm^3)	6	1.30 ± 0.46	NS	14	1.31 ± 0.90	–0.08; NS	–0.05; NS
Total protein (g/dL)	5	9.50 ± 2.91	< 0.05	12	7.25 ± 1.32	0.41; NS	0.31; NS
Albumin (g/dL)	5	3.55 ± 0.10	< 0.05	12	2.83 ± 0.52	0.75; $P < 0.001$	0.75; $P < 0.001$
Globulin (g/dL)	5	5.92 ± 3.13	NS	12	4.43 ± 1.10	0.21; NS	0.10; NS
A/G ratio	5	0.67 ± 0.24	NS	12	0.66 ± 0.16	0.24; NS	0.29; NS
Conjugated bilirubin (mg%)	5	0.25 ± 0.14	NS	9	0.56 ± 0.59	–0.36; NS	0.38; NS
Unconjugated bilirubin (mg%)	5	1.10 ± 1.38	NS	9	0.54 ± 0.47	0.19; NS	0.07; NS
ALT (U)	6	48.7 ± 37.3	NS	14	97.6 ± 84.9	–0.53; $P < 0.05$	–0.52; $P < 0.05$

A/G ratio = albumin/globulin ratio; ALT = alanine aminotransferase; FVC = forced volume capacity; SD = standard deviation. Comparison between sorted group values by two-tailed *t* test. Pearson correlation significance estimated using two-tailed assays.

anemia, leucopenia, thrombocytopenia, pancytopenia, hypoalbuminemia, and hyperglobulinemia. Abnormally high hepatic enzyme levels were also noted as described in recent reviews of VL patients.[14]

Dry cough was a common pulmonary manifestation in VL patients evaluated in this report. A smoking history was also present in a large fraction of the patients. Long-term smoking is associated with air trapping and dynamic hyperinflation due to airway obstruction, leading to a loss of elastic retraction in the lung and muscular and skeletal adaptations of the thoracic wall. Longitudinal studies of smokers demonstrated an increase in pulmonary distensibility with a corresponding increase in all absolute pulmonary volumes.[15] However, there was no statistically significant difference noted between the history of tobacco use and changes in spirometry measurements in these patients. Most of our patients with abnormal spirometry exhibited a restrictive pattern of pulmonary function, distinguishing them from pathologic changes in smokers who usually exhibit an obstructive pattern. Our evaluations suggested there were no regional changes in lung function that could account for spirometry patterns, according to a control group of non-VL hospitalized patients matched age and sex with VL subjects. This was an important control, since our subjects were located close to a large population in an area with mining. Even though environmental conditions in this region could lead to pulmonary abnormalities, our regional patient control group suggested that an environmental effect might not be the cause of abnormal spirometry results in the VL subjects we evaluated.

All VL patients with abnormal spirometry findings exhibited the same pattern of altered function, with decreased pulmonary volumes (decreased FVC; decreased FEV1 in some). No patients had an FEF 25–75% value below the lower limit. The higher FEV1/FVC values were similar to those found in other pulmonary disorders classified as restrictive lung disease. A reduced pulmonary volume indicates changes in pulmonary compliance, since compliance is volume dependent. Restrictive lung disease is physiologically characterized by a reduction in total lung capacity, due to some lung disorders or a restricted chest wall.[10] The most common causes of restrictive lung disease include fibrotic changes in lung tissue or infiltrative diseases that can be inflammatory (reviewed in Gan and others[16]). Lung compliance is also reduced in pulmonary edema (transudative and exudative) or pulmonary fibrosis, or in conditions leading to alveolar deposits similar to the findings observed in autopsies of VL patients.[5] In studies of patients with VL that underwent lung biopsies, the lungs were congested and enlarged macroscopically. The intra-alveolar septae were thickened as a result of an inflammatory infiltrate comprising mainly lymphocytes, macrophages, and plasma cells. Interstitial cells were also increased in size due to the presence of lipid vacuoles, leading to mild edema and congestion in septal capillaries.[8]

The duration of symptoms, smoking history, length of treatment, need for blood transfusion, and respiratory symptoms did not correlate significantly with the spirometry results in patients with VL. Transfusion was associated with alterations in FVC and FEV1, but these changed in the same direction without altering the FVC/FEV1 ratio[17], which was the main abnormality observed in our patients. Serum albumin level was the only laboratory test that was significantly associated with abnormal spirometry findings. Patients with hypoalbuminemia also exhibited reduced pulmonary volume measurements. We cannot claim that this alteration was due to alterations in osmotic pressure since the mean total serum protein was normal. As in prior reports, globulin levels were increased in most VL patients, but the large variability made this difficult to compare with normal controls. We hypothesize that low albumin levels could be indicative of severe immunological dysfunction in VL, and that hypoalbuminemia could be an indicator of disease severity in VL patients. This phenomenon could be indirectly related to parasite burden in other organs such as spleen and liver, given that only intact parasites were not observed in alveolar septae or pulmonary macrophages.

In conclusion, most VL patients in this small study exhibited changes in pulmonary function that suggested restrictive ventilatory disorders. These changes could be related to VL-induced pulmonary inflammation associated with the intensity of disease progression and an

inflammatory host response. Although this is a small study, the data suggest that, similar to cystic fibrosis patients,[12] spirometry might be a marker of disease severity in individual patients. This might be used for disease monitoring during therapy. VL can be a recrudescent disease in some immunodeficient patients, as observed in some chronic patients in India.[18] Thus, we suggest that the use of noninvasive spirometry deserves evaluation as an inexpensive measure of disease progression or response to therapy that could be useful in some patients with severe disease. Our study suggests that the utility of spirometry as an index of disease severity in VL patients deserves further evaluation using large cohorts of patients in future studies.

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