

# Frequency of autoantibodies and serum complement levels in patients with visceral or cutaneous leishmaniasis

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## ABSTRACT

**Introduction:** Leishmaniasis is a chronic infectious disease whose spectrum can vary from isolate cutaneous involvement with oligosymptomatic manifestations to systemic involvement with clinically important manifestations. The development of the infection of each type of leishmaniasis (visceral or cutaneous) depends on a complex and intriguing interaction between virulence factors of the pathogen and the immune response of the host. Analysis of sera of with *Leishmania* infection demonstrates the presence of autoantibodies against cellular and humoral components, besides circulating immune complexes and anti-IgG antibodies (rheumatoid factor). Patients with visceral leishmaniasis can present symptoms that mimic Systemic Lupus Erythematosus (SLE), hindering early diagnosis and treatment. **Objectives:** To identify the profile of autoantibodies and complement levels of patients with visceral or cutaneous leishmaniasis and to correlate their clinical presentation to those of patients with SLE. **Methods:** The presence of autoantibodies and complement levels of 90 patients, 45 with visceral leishmaniasis and 45 with cutaneous leishmaniasis, was determined. Results: The presence of statistically significant autoantibodies in patients with visceral leishmaniasis included: antinuclear antibody (ANA), positive (4.4%) or in low titers (8.9%), and IgG anticardiolipin antibody, positive (17.8%) or undetermined (8.9%). A reduction in C3 levels was also seen in 17.8% of the patients and anti-*Leishmania* antibodies > 1/80 in all patients with visceral leishmaniasis. **Conclusions:** Visceral leishmaniasis can have a positive correlation with the presence of autoantibodies, possibly by triggering a predominantly humoral, systemic, type Th2 response, representing an obligatory differential diagnosis with SLE, especially in endemic areas.

**Keywords:** autoantibodies, complement levels, visceral leishmaniasis, cutaneous leishmaniasis, systemic lupus erythematosus.

## INTRODUCTION

Leishmaniasis is one of the most important infectious diseases in the world, especially in tropical and subtropical regions. Approximately 12 million people in 88 countries are infected (most in developing nations), with two million new infections per year, and 60 thousand deaths per year.<sup>1,2,3</sup>

Leishmaniasis is a chronic infectious disease that can present a spectrum that ranges from isolate cutaneous involvement with oligosymptomatic manifestations to systemic involvement with clinical important manifestations. The development of the infection in each clinical type of

leishmaniasis (visceral or cutaneous) depends on a complex and intriguing interaction among virulence factors of the pathogen and the immune response of the host.<sup>1</sup>

The causative agents of human leishmaniasis are protozoa of the *Kinetoplastida* order, *Trypanosomatidae* family, genus *Leishmania*, and four different clinical types are identified according to the infecting species and the immune response of the host: visceral leishmaniasis, cutaneous leishmaniasis, diffuse cutaneous leishmaniasis, and cutaneous-mucous leishmaniasis.<sup>4</sup>

The etiological agents of visceral leishmaniasis are tripanosomidae of the genus *Leishmania*. In Brazil, *Leishmania*

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(*Leishmania*) *chagasi* is the species commonly isolated in patients with visceral leishmaniasis.<sup>3</sup>

In the Americas, 11 dermatropic species of *Leishmania* are recognized as causative agents of human cutaneous leishmaniasis and eight species have been described in animals<sup>4</sup>. However, seven species have been identified in Brazil, six of the subgenus *Viannia* and one of the subgenus *Leishmania*. The three main species include: *L. (V.) braziliensis*, *L. (V.) guyanensis*, and *L. (L.) amazonensis* and, more recently, the species *L. (V.) lainsoni*, *L. (V.) naiffi*, *L. (V.) lindenberg*, and *L. (V.) shawi* were identified in the states in the North and Northeast regions of Brazil.<sup>2,4,5</sup>

In visceral leishmaniasis, the intracellular parasite causes intense parasitism in the reticuloendothelial system, affecting the liver, spleen, bone marrow, and lymph nodes. It causes expressive changes in cellular and humoral response, with deficiency of gamma-interferon (IFN- $\gamma$ ), increased production of tumor necrosis factor (TNF- $\alpha$ ) and other interleukins, besides polyclonal hypergammaglobulinemia.<sup>6</sup>

The clinical presentation of visceral leishmaniasis is characterized by hepatosplenomegaly, fever, paleness, asthenia, weight loss, tachycardia, coughing, epistaxis, bleeding of the gums, myalgia, arthralgia, and adenopathy, among others; those symptoms can also be seen in systemic lupus erythematosus (SLE).<sup>7</sup>

The main signs and symptoms observed in cutaneous leishmaniasis include: Cutaneous leishmaniasis: ulcerated, painless lesion with elevated borders, central granulation tissue, with or without exudate. The lesion can be single, multiple, disseminated, or diffuse. Mucous leishmaniasis: nasal obstruction, elimination of crusts, epistaxis, dysphagia, odinophagia, hoarseness, dyspnea, and coughing; destructive lesions might be present, especially in the nasal and oral cavities, pharynx, and larynx.<sup>4</sup>

Systemic lupus erythematosus is a multifactorial, autoimmune, chronic inflammatory disease with a polymorphic clinical presentation. It is associated with immunologic dysfunction with polyclonal activation of B lymphocytes and autoantibodies against nuclear antigens, some of which participate in immune-mediated tissue lesions. Due to its polymorphic presentation, it can be mistaken with several infectious diseases, including visceral leishmaniasis.<sup>8</sup>

Some authors described cases of patients with leishmaniasis who fulfilled the clinical criteria for SLE established by the American College of Rheumatology (ACR), with clinical and laboratorial manifestations that included cytopenia, polyclonal hypergammaglobulinemia, changes in UA (from hematuria to massive proteinuria), positive antinuclear antibody (ANA)

arthralgia, and hepatomegaly. However, they also presented splenomegaly, elevated titers of C reactive protein (CRP), normal complement levels, and negative anti-DNA and other autoantibodies, which are not commonly in patients with SLE. Based on the detection of parasites in bone marrow macrophages and positive serology for leishmaniasis, visceral leishmaniasis was diagnosed. All clinical manifestations resolved after institution of the specific treatment for leishmaniasis.<sup>9,10</sup>

Latent leishmaniasis can progress to active disease secondary to changes in immunological responses, and immunosuppression induced by SLE or its treatment can change kalazar into a rapidly progressive disease.<sup>11</sup>

In 1983, the first case of visceral leishmaniasis complicating SLE was described in a Chinese woman.<sup>12</sup> Since then, several cases of visceral leishmaniasis as a cause of fever of unknown origin and cytopenia in patients with a prior diagnosis of SLE, which contributes for the confusion and difficulty in the differential diagnosis of both disorders, have been reported.<sup>13,14,15</sup>

In patients with SLE, infections represent the most common causes of death and it is frequently difficult to differentiate a concomitant infectious disease from SLE activity, since both clinical presentations can be similar.<sup>12,14,15</sup>

Both disorders can present cutaneous manifestations, which is another complicating factor in their differential diagnosis. Several studies have described a type of cutaneous leishmaniasis, called lupoid leishmaniasis, characterized by the dissemination of the initial node to form a plaque, simulating discoid lupus.<sup>16,17,18</sup>

Patients with visceral leishmaniasis can also develop renal manifestations, identical to those caused by SLE, secondary to the immunologic dysfunction, including the impairment of the renal function, changes in UA, and proteinuria.<sup>19</sup> The development of focal or diffuse mesangial and membranoproliferative glomerulonephritis, increase in mesangial matrix, and, on electron microscopy, the presence of deposits of IgG and IgM immunocomplexes and C3.<sup>20</sup>

Several possibilities can explain the development of autoantibodies in visceral leishmaniasis. Hypergammaglobulinemia is common in patients with SLE and it is present in all patients with kalazar due to the large production of IgG, IgM, and IgA antibodies, after polyclonal activation of B lymphocytes, generating specific and non-specific antibodies, besides autoantibodies expressed in low levels under normal conditions.<sup>21</sup>

Molecular mimetism between *Leishmania* antigens and ribonucleoproteins is another hypothesis raised by Granel *et al.*,<sup>22</sup> who found the production of autoantibodies, such as anti-

Sm, anti-RNP, anti-SSA, anti-SSB, and antiphospholipid, in patients with *Leishmania* infection. A third mechanism for the induction of autoantibodies in leishmaniasis can be attributed to the release and exposure of antigens, previously hidden, during tissue damage and breakage of cells of the host.<sup>22</sup>

The objective of this study was to identify the profile of autoantibodies and complement consumption in patients with cutaneous or visceral leishmaniasis, and to correlate the types of disease presentation (visceral or cutaneous) with the presence of autoantibodies and complement consumption.

## PATIENTS AND METHODS

This is a transversal study. From March 2006 to March 2008, 203 patients, 138 males and 65 females, with the diagnosis of leishmaniasis (ICD 10: B55) seen on different subspecialty clinics of the University Hospital of the Medical School of Universidade Federal de Mato Grosso do Sul were referred to the Infectious Disease Department of the same hospital. Ninety of those patients were selected by judgment sampling, 45 with visceral leishmaniasis and 45 with cutaneous leishmaniasis, according to the criteria listed below.

Preliminary patient selection was based on a review of the medical records of patients seen at the Infectious Diseases Department of the University Hospital of Universidade Federal de Mato Grosso do Sul from March 2006 to March 2008.

Patients should fulfill the following criteria: Diagnosis of visceral leishmaniasis with the presence of fever, cytopenia, and visceromegaly, besides serologic confirmation by indirect immunofluorescence (IIF) with titers  $\geq 1:80$ , and the presence of *Leishmania sp.* on microscopic examination of the bone marrow or in cultures.

Diagnosis of cutaneous leishmaniasis with suggestive lesions in the skin or mucous membranes and involvement of lymph nodes, besides laboratorial diagnosis based on parasitologic (direct testing, histopathological, or culture) and immunological (Montenegro skin test, indirect immunofluorescence, or ELISA) tests.

Exclusion criteria were as follows: patients with other infectious diseases, malignant tumors, and autoimmune diseases.

Patient identification and clinical information on the disease were collected from the medical records of each patient and, if necessary, complemented by interviewing the patients.

The Ethics on Research Committee of the Universidade Federal de Mato Grosso do Sul (CEP/UFMS) approved the use of information from medical records, and patients signed an informed consent.

## Serum Samples

Sera of patients previously selected, frozen and stored at the Central Laboratory of Campo Grande, MS, Brazil (LACEN, from the Portuguese) or at the Clinical Laboratory of the University Hospital of the Universidade Federal de Mato Grosso do Sul, were used in this study.

Before testing, the sera were identified, thawed, and divided in aliquots for posterior determination of antinuclear antibodies, rheumatoid factor (RF), antiphospholipid antibodies, and complement levels.

## Determination of Autoantibodies

Indirect immunofluorescence, using HEP-2 cells (FAAR technique) as the substrate, was used to evaluate the presence of ANA, and the interpretation followed the criteria of the II Brazilian Consensus on Antinuclear Antibody.<sup>23</sup>

Dilutions ranging from 1/40 to 1/160, depending on the lamp of the microscope (20W, 50W, 100W), were used for triage, and the final reading was done on the last dilution in which the pattern was clearly observed.<sup>23</sup>

Sera with titers below 1/80 were considered negative; those with titers between 1/80 to 1/160 were considered undetermined; and those with titers greater than 1/160 were considered positive and they were diluted until the fluorescence became negative.

Anti-native DNA – IIF using *Crithidia luciliae* as the substrate. Enzyme immunoassay was used as counterproof; levels above 55 IU/mL were considered positive, between 35 and 55 IU/mL were considered undetermined, and below 35 IU/mL, negative.

ELISA, using specific kits according to the instructions of the manufacturer (Hemagen Diagnostics, Inc), was used to determine the presence of anti-Sm, anti-RNP, anti-Ro (SSA), and anti-La (SSB). Sera with levels twice above the cut-off level were considered positive.

Anticardiolipin IgM and IgG – Enzyme immunoassay was used; titers greater than 30.0 IU/mL, for IgG, and 15.0 IU/mL, for IgM, were considered positive. Titers between 10 and 30 IU/mL, for IgG, and 10 to 15 IU/mL, for IgM, were considered undetermined; and levels below 10 IU/mL for both were considered negative. The solution of purified cardiolipin of the Sigma Laboratory was used as the substrate.

Rheumatoid Factor – The presence of RF was determined by nephelometry; titers above 40 IU/mL were considered positive, and those below this level were considered negative.

The levels of C3 were determined by immunoturbidimetry using reference levels between 90.0 and 180.0 mg/dL;

levels below 90.0 mg/dL were considered a reduction in this complement fraction.

Immunoturbidimetry was used to determine the levels of C4 with reference levels between 10.0 and 40.0 mg/dL; levels below 10.0 mg/dL were considered a reduction in C4.

### Statistical Analysis

Means were calculated for socio-demographic parameters.

The frequency of autoantibodies, total and for each one, was determined in the sera of patients and compared with the data in the literature.

The non-parametric Mann-Whitney test was used to compare age, and C3 and C4 levels in patients with visceral and cutaneous leishmaniasis.

Fisher's Exact test was used to evaluate the relationship between the type of leishmaniasis and gender, origin of the patient, and tests results (anti-*Leishmania*, anti-Sm, anti-RNP, anti-SSA, anti-SSB, and anticardiolipin antibodies, RF, and C3 and C4). The same test was used to evaluate the type of leishmaniasis and the coincidence between ANA and anticardiolipin IgM, anticardiolipin IgG, RF, C3, and C4.

The Chi-square test was used to evaluate the relationship between the type of leishmaniasis and antinuclear antibody, anti-DNA antibody, and anticardiolipin antibody.

The Z-test was used to compare the percentage of positive cases, among patients with leishmaniasis evaluated in this study, and anti-*Leishmania* antibody and C3 levels. The same test was used to compare the percentage of coinciding results between antinuclear antibody and anticardiolipin IgM and IgG, C3, and C4.

The results of the remaining parameters evaluated in this study are presented as descriptive statistics or in tables and charts.

The software SigmaStat, version 2.0, was used for the statistical analysis, and differences and relationships were considered significant when P was lower than 0.05.<sup>24</sup>

## RESULTS

### Socio-Demographic Data

In this study, 90 patients were evaluated; 50% (n = 45) had visceral leishmaniasis and 50% (n = 45) cutaneous leishmaniasis. The age of the patients ranged from 12 and 76 years, with a mean of 35.43 ± 16.79 years (mean ± standard deviation).

The age of patients with visceral leishmaniasis ranged from 12 to 76 years, with a mean of 35.67 ± 19.55 years. As for patients with cutaneous leishmaniasis, their age ranged from 14 to 67 years, with a mean of 35.20 ± 13.72 years. Differences between

the ages of patients with visceral and cutaneous leishmaniasis were not observed (Mann-Whitney test, P = 0.58).

Among patients with visceral leishmaniasis (n = 45), 48.9% (n = 22) were females and 51.1% (n = 23) were males. Among patients with cutaneous leishmaniasis, 51.1% (n = 23) were females and 48.9% (n = 22) were males. Differences between the gender of patients with visceral and cutaneous leishmaniasis were not observed (Fisher's exact test, P = 1.00).

### Origin

Among patients with visceral leishmaniasis (n = 45), 62.2% (n = 28) were from the Campo Grande area and the remaining 37.8% (n = 17) were from other areas of the state of Mato Grosso do Sul. Among patients with cutaneous leishmaniasis (n = 45), 68.9% (n = 31) were from the Campo Grande area and the remaining 31.1% (n = 14) from other areas of Mato Grosso do Sul. Significant differences between the type of leishmaniasis and the origin of patients were not observed (Fisher's exact test, p = 0.52).

### Anti-*Leishmania* Antibodies

All patients with visceral leishmaniasis, 100% (n = 45), tested positive for anti-*Leishmania* antibodies (> 1/80). On the other hand, 37.8% (n = 17) of the patients with cutaneous leishmaniasis tested negative for anti-*Leishmania* antibodies (< 1/80) and 62.2% (n = 28) were positive (> 1/80). A significant difference between the type of leishmaniasis and the titers of

**Table 1**

Origin of the patients with visceral or cutaneous leishmaniasis according to the micro-region of the state of Mato Grosso do Sul

Micro-region	Leishmaniasis	
	Visceral	Cutaneous
Alto Taquari	00 (0.0%)*	00 (0.0%)
Aquidauana	05 (11.1%)	05 (11.1%)
Baixo Pantanal	00 (0.0%)	00 (0.0%)
Bodoquena	01 (2.2%)	00 (0.0%)
Campo Grande	28 (62.2%)	31 (68.9%)
Cassilândia	00 (0.0%)	00 (0.0%)
Dourados	04 (8.9%)	03 (6.7%)
Iguatemi	03 (6.7%)	02 (4.4%)
Nova Andradina	00 (0.0%)	00 (0.0%)
Paranaíba	00 (0.0%)	00 (0.0%)
Três Lagoas	04 (8.9%)	04 (8.9%)
<b>Total</b>	<b>100.0% (n = 45)</b>	<b>100.0% (n = 45)</b>

\*Absolute frequency (relative frequency).

anti-*Leishmania* antibodies was observed (Fisher's exact test,  $P < 0.001$ ), and the frequency of positivity was higher in patients with visceral leishmaniasis (Z-test,  $P < 0.001$ ).

#### ANA

Among patients with visceral leishmaniasis ( $n = 45$ ), 86.7% ( $n = 39$ ) tested negative for the presence of antinuclear antibody (ANA), in 8.9% ( $n = 04$ ) it was undetermined, and in 4.4% ( $n = 02$ ) it was positive. In the cutaneous leishmaniasis group, the ANA was negative in 100% ( $n = 45$ ) of the patients. A statistically significant relationship was observed according to the Chi-square test ( $P = 0.04$ ).

#### Anti-native DNA

Among patients with visceral leishmaniasis ( $n = 45$ ), 93.3% ( $n = 42$ ) tested negative for anti-native DNA, in 2.2% ( $n = 1$ ) it was undetermined, and in 4.5% ( $n = 2$ ) it was positive. Among patients with cutaneous leishmaniasis, anti-native DNA was negative in all patients (100%,  $n = 45$ ). A significant relationship between the type of leishmaniasis and anti-native DNA was not observed (Chi-square test,  $P = 0.21$ ).

#### Anti-Sm antibody

All patients in this study ( $n = 90$ ), regardless of the type of leishmaniasis (visceral or cutaneous), tested negative for anti-Sm antibody (Fisher's exact test,  $P = 1.00$ ).

#### Anti-RNP antibody

As for anti-RNP antibody, all patients evaluated in this study, except for one (1.1%) who had visceral leishmaniasis, presented an undetermined result (between 5 and 9 IU/mL on enzyme immunoassay), and all the others (98.9%,  $n = 89$ ) had a negative test (Fisher's exact test,  $P = 1.00$ ).

#### Anti-Ro (SSA) antibody

All patients evaluated in this study, regardless of the type of leishmaniasis (visceral or cutaneous), tested negative for anti-SSA antibody (Fisher's exact test,  $P = 1.00$ ).

#### Anti-La (SSB) antibody

Only one patient (1.1%) with visceral leishmaniasis tested positive for anti-SSB antibody, and the remaining (98.9%,  $n = 89$ ) tested negative (Fisher's exact test,  $P = 1.00$ ).

#### Anticardiolipin IgM and IgG antibodies

Among patients with visceral leishmaniasis ( $n = 45$ ), 6.7% ( $n = 3$ ) were positive for anticardiolipin IgM antibody. Among patients with cutaneous leishmaniasis, 100% ( $n = 45$ ) tested negative for anticardiolipin IgM antibody (Fisher's exact test,  $P = 0.12$ ).

Out of 45 patients with visceral leishmaniasis, the anticardiolipin IgG antibody was undetermined in 8.9% ( $n = 4$ ) and in 17.8% ( $n = 08$ ) it was positive. Among patients with cutaneous leishmaniasis, only one (2.2%) tested positive for anticardiolipin IgG antibody. A significant relationship between the type of leishmaniasis and the result of the anticardiolipin IgG antibody was observed (Chi-square test,  $P = 0.004$ ).

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#### RHEUMATOID FACTOR

Among patients with visceral leishmaniasis ( $n = 45$ ), 24.4% ( $n = 11$ ) tested positive for RF. The rheumatoid factor was positive in 8.9% ( $n = 4$ ) of the patients with cutaneous leishmaniasis (Fisher's exact test,  $P = 0.09$ ).

#### Levels of C3 and C4 complement

Among patients with visceral leishmaniasis ( $n = 45$ ), 13.3% ( $n = 6$ ) had C3 levels below 90 mg/dL. Among patients with cutaneous leishmaniasis, all (100%,  $n = 45$ ) had C3 levels equal or above 90 mg/dL. A significant relationship between the type of leishmaniasis and C3 levels was observed (Fisher's exact test,  $P = 0.01$ , and Z-test,  $P = 0.03$ ).

None of the patients evaluated in this study, regardless of the type of leishmaniasis, had C4 levels below 10 mg/dL. Table 2 presents the data regarding the relative and absolute frequency of patients with visceral and cutaneous leishmaniasis, according to the parameters evaluated in this study.

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#### DISCUSSION

In the present study, we tried to minimize biases by selecting a medium-size study population (90 patients), statistically similar, in which half had a diagnosis of visceral leishmaniasis and the other half, cutaneous leishmaniasis. Thus, it was possible to observe the production of autoantibodies and complement in each type of the disease without other interfering factors.

Significant differences regarding age or gender were not observed between patients with visceral and cutaneous leishmaniasis; patients had a mean age of 35.43 years and approximately 50% were females. In 1990, Fernandes<sup>25</sup>

**Table 2**

Demographic and laboratorial parameters evaluated in patients with visceral (n = 45) or cutaneous (n = 45) leishmaniasis

Parameter	Leishmaniasis		P
	Visceral	Cutaneous	
<b>Gender</b>			
Female	22 (48.9%)*	23 (51.1%)	1.00 <sup>1</sup> NS
Male	23 (51.1%)	22 (48.9%)	
<b>Micro-region</b>			
Campo Grande	28 (62.2%)	31 (68.9%)	0.52 <sup>1</sup> NS
Other	17 (37.8%)	14 (31.1%)	
<b>Anti-<i>Leishmania</i> antibody</b>			
Negative	00 (0.0%)	17 (37.8%)	< 0.001 <sup>1</sup> Sig.
Positive	45 (100.0%)	28 (62.2%)	
<b>Antinuclear antibody (ANA)</b>			
Negative	39 (86.7%)	45 (100.0%)	0.04 <sup>2</sup> Sig.
Undetermined	04 (8.9%)	00 (0.0%)	
Positive	02 (4.4%)	00 (0.0%)	
<b>Rheumatoid factor</b>			
Negative	34 (75.6%)	41 (91.1%)	0.09 <sup>1</sup> NS
Positive	11 (24.4%)	04 (8.9%)	
<b>C3 level</b>			
□ 90mg/dL	39 (86.7%)	45 (100.0%)	0.01 <sup>1</sup> Sig.
< 90mg/dL	06 (13.3%)	00 (0.0%)	
<b>C4 level</b>			
□ 10mg/dL	45 (100.0%)	45 (100.0%)	1.00 <sup>1</sup> NS
< 10mg/dL	00 (0.0%)	00 (0.0%)	

\*Absolute frequency (relative frequency); NS = non-significant; Sig.= significant; <sup>1</sup>p according to Fisher's exact test; <sup>2</sup>p according to Chi-square test.

**Table 3**

Frequency of anti-dsDNA, anti-Sm, anti-RNP, anti-SSA, anti-SSB, and anticardiolipin (IgM e IgG) antibodies in patients with visceral (n= 45) or cutaneous (n = 45) leishmaniasis

Parameter	Leishmaniasis		P
	Visceral	Cutaneous	
<b>Anti-DNA</b>			
Negative	42 (93.3%)	45 (100.0%)	0.21 <sup>2</sup> NS
Undetermined	01 (2.2%)	00 (0.0%)	
Positive	02 (4.4%)	00 (0.0%)	
<b>Anti-Sm antibody</b>			
Negative	45 (100.0%)	45 (100.0%)	1.00 <sup>1</sup> NS
Positive	00 (0.0%)	00 (0.0%)	
<b>Anti-RNP antibody</b>			
Negative	44 (97.8%)	45 (100.0%)	1.00 <sup>1</sup> NS
Positive	01 (2.2%)	00 (0.0%)	
<b>Anti-SSA antibody</b>			
Negative	45 (100.0%)	45 (100.0%)	1.00 <sup>1</sup> NS
Positive	00 (0.0%)	00 (0.0%)	
<b>Anti-SSB antibody</b>			
Negative	44 (97.8%)	45 (100.0%)	1.00 <sup>1</sup> NS
Positive	01 (2.2%)	00 (0.0%)	
<b>Anticardiolipin IgM antibody</b>			
Negative	42 (93.3%)	45 (100.0%)	0.12 <sup>1</sup> NS
Positive	03 (6.7%)	00 (0.0%)	
<b>Anticardiolipin IgG antibody</b>			
Negative	33 (73.3%)	44 (97.8%)	0.004 <sup>2</sup> Sig.
Undetermined	04 (8.9%)	00 (0.0%)	
Positive	08 (17.8%)	01 (2.2%)	

\*Absolute frequency (relative frequency); NS = non-significant; Sig.= significant; <sup>1</sup>p-value according to Fisher's exact test; <sup>2</sup>p-value according to Chi-square test.

observed an incidence of 53 cases of cutaneous-mucous leishmaniasis in the region of Nioaque (MS) from August 1987 to July 1988 with a predominance of patients younger than 20 years (37.7%), but he did not observe a predominance of one gender among affected individuals.

In an epidemiological study on cutaneous leishmaniasis in the area of Corguinho (MS), Nunes *et al.*<sup>26</sup> observed that individuals with ages between 22 and 78, mainly males (75% of the cases), were most affected by leishmaniasis.

As for gender, the literature indicates that males are more susceptible to visceral and cutaneous leishmaniasis, probably due to recreational and occupational activities in this group, whose individuals have a greater tendency to enter the woods and be exposed to the natural habitat of phlebotominae.<sup>27,28</sup>

On the other hand, it has been observed, recently, a change in the pattern of the incidence of leishmaniasis, in the state of

Mato Grosso do Sul and in the country, with the expansion and important urbanization of the disease, which might contribute for the similar exposure of both genders to the disease.<sup>29</sup>

The micro-regions of the state where patients in this study came from are in agreement with the data of the Health Department of the state of Mato Grosso do Sul that indicate a greater incidence of visceral leishmaniasis in the counties of Campo Grande, Três Lagoas, and Aquidauana from 2003 to 2007.<sup>30</sup>

In the present study, all patients with visceral leishmaniasis tested positive for anti-*Leishmania* antibodies (> 1/80). As for cutaneous leishmaniasis, 37.8% tested negative for anti-*Leishmania* antibodies and 62.2% tested positive. Schubach *et al.*<sup>31</sup> found anti-*Leishmania* antibodies in 52.5% of patients with active cutaneous disease. Silveira *et al.*<sup>27</sup> found this antibody in 64.4% of 955 patients with cutaneous leishmaniasis. Studying patients with visceral leishmaniasis, Atta *et al.*<sup>32</sup>

also found anti-*Leishmania* antibodies in 100% of the cases; however, only nine patients were included in his study. Pappas *et al.*<sup>33</sup> found positive serology in 98% of 42 patients with visceral leishmaniasis. In the literature, the predominance of anti-*Leishmania* antibodies in visceral leishmaniasis can be explained by the evidence of Th2 response in this disease, with polyclonal activation of B cells and production of specific anti-*Leishmania* and other antibodies with different specificities.<sup>32,34</sup>

Among patients with visceral leishmaniasis, the ANA was undetermined in 8.9% of the cases and positive in 4.4% of the cases (titers above 1/160). Other studies comparing the presence of ANA in patients with visceral or cutaneous leishmaniasis were not found in the literature. It should be considered that this test is positive, in low titers, in 5% of the normal population and in up to 13% of the individuals older than 50 years.<sup>23</sup> Several possibilities can explain the presence of autoantibodies in visceral leishmaniasis. The most accepted hypothesis is the presence of hypergammaglobulinemia, seen in almost all patients with visceral leishmaniasis, secondary to the increased production of IgG, IgM, and IgA antibodies, is due to the polyclonal activation of B lymphocytes leading to the production of specific antibodies and autoantibodies that, under normal conditions, are expressed in low titers.<sup>21</sup> There is evidence that soluble *Leishmania major*- and *L. donovani*-derived antigens are mitogenic and trigger the production of autoantibodies.<sup>21</sup>

Undetermined anti-native DNA was seen in 2.2% of the cases and it was positive in 4.4% of the patients with visceral leishmaniasis. A significant relationship between the type of leishmaniasis and the presence of anti-native DNA was not observed. However, even though the result was not statistically significant, it is diagnostically relevant, since it is the widespread belief that anti-native DNA is almost exclusive of patients with SLE and very rare in normal individuals.<sup>35</sup> Pathogenetically, the development of anti-DNA is correlated with specific clinical presentations and also suggests the diagnosis of SLE. The result can be explained by cross-reactivity of anti-DNA antibodies that can be seen in IIF with *Crithidia luciliae*. A positive test is seen in patients with leishmaniasis with the formation of atypical images and fluorescence in other structures of the parasite.<sup>36</sup>

Granel *et al.*<sup>22</sup> reported the case of a patient with visceral leishmaniasis who was positive for anti-DNA antibodies, which disappeared after treatment with corticosteroids, antimonials, and posterior use of liposomal amphotericin B. In a study with 23 patients with visceral leishmaniasis and 14 with cutaneous leishmaniasis, Argov *et al.*<sup>21</sup> did not observe the presence of anti-DNA despite the production of other autoantibodies in those patients.

All patients evaluated in the present study, regardless of the type of leishmaniasis (visceral or cutaneous), tested negative for anti-Sm antibodies. Surprisingly, Argov *et al.*<sup>21</sup> observed the presence of anti-Sm antibodies in 7% of patients with cutaneous leishmaniasis and in 83% of the patients with visceral leishmaniasis. The sera were from South American (especially Brazil), African, and Asian (especially India) patients. Molecular mimicry between *Leishmania sp.* antigens and ribonucleoproteins is one of the hypotheses raised by Granel *et al.*<sup>22</sup> to explain the presence of those autoantibodies (anti-Sm, anti-RNP, anti-SSA, and anti-SSB) in infected patients. The possibility that Sm, RNP, SSB, and *Leishmania* share similar antigenic determinants is raised due to the inhibition of autoantibodies against those nuclear antigens by intact *Leishmania* promastigotes.<sup>21</sup>

As for anti-RNP antibodies in the patients evaluated in the present study, only one (1.1%) patient with visceral leishmaniasis presented an undetermined level and the others were negative. In the study of Argov *et al.*<sup>21</sup>, 14% of patients with cutaneous leishmaniasis and in 86% of those with visceral leishmaniasis were positive for anti-RNP antibodies.

All patients evaluated in the present study, regardless of the type of leishmaniasis (visceral or cutaneous), tested negative for anti-SSA antibodies. As for anti-SSB antibodies, only one patient (1.1%) with visceral leishmaniasis tested positive and all others were negative. In a study by Argov *et al.*<sup>21</sup>, 25% of patients with cutaneous leishmaniasis tested positive for anti-SSA and anti-SSB antibodies, and 36% and 73% of patients with visceral leishmaniasis, respectively.

Among patients with visceral leishmaniasis, 6.7% were positive for anticardiolipin IgM antibodies. In the same group, 17.8% were positive for anticardiolipin IgG antibodies. We did not find in the literature any studies on anticardiolipin antibodies for comparison. The literature reports the presence of anticardiolipin antibodies in other infectious diseases.<sup>37</sup> Repka *et al.*<sup>38</sup> observed those antibodies in 8.34% of patients with paucibacillary leprosy and 80.77% of non-treated patients with multibacillary leprosy. The origin of anticardiolipin antibodies in patients with infections has been widely debated and several hypotheses have been suggested. Among them we should mention: non-specific polyclonal activation of B cells; ability of the infectious agent to bind to endogenous phospholipids making them immunogenic; damage of the endothelium by the infectious agent, exposing phospholipid epitopes that trigger the immunologic response; and cross reaction of anti-DNA antibodies giving rise to anticardiolipin antibodies.<sup>38</sup>

The presence of anticardiolipin antibodies in patients with leishmaniasis is important since those antibodies are related

with the etiopathogenic mechanisms of venous and arterial thrombosis.<sup>37</sup> In 2006, Terrazzano *et al.*<sup>39</sup> studied 33 dogs naturally infected by *Leishmania infantum* and observed that 63.3% of them had antiplatelet antibodies and half of those animals had thrombocytopenia and clinical signs of moderate to severe disease.

Among patients with visceral leishmaniasis, 24.4% tested positive for RF, while 8.9% of the patients with cutaneous leishmaniasis were positive for RF. Those autoantibodies are frequently associated with rheumatoid arthritis (RA), but they can also be observed in other autoimmune diseases, as well as in some infections, such as leishmaniasis. The rheumatoid factor of patients with RA differs from the anti-IgG IgM antibodies seen in infectious diseases.<sup>40</sup>

Chabanne *et al.*<sup>41</sup> detected, on ELISA, high titers of IgM and IgA RF, 45% and 30%, respectively, in dogs with visceral leishmaniasis.

Atta *et al.*<sup>40</sup> found high titers of IgM RF (100 IU/mL) in 90% of patients with visceral leishmaniasis. Surprisingly, anti-CCP (citrullinated) IgG was also present in 30% of the same patients. It is believed that those antibodies are highly specific for the diagnosis of RA and, currently, they are considered good predictive markers of early RA. This production of anti-CCP antibodies could be caused by citrullination of proteins of the host during *Leishmania* infection and it could represent a new aspect of the immunopathogenesis of visceral leishmaniasis.<sup>40</sup>

The presence of high titers of circulating immunocomplexes has been reported in patients with visceral leishmaniasis and SLE.<sup>42,43</sup> Signs of activation of the complement cascade were observed in a study of patients with visceral leishmaniasis and, in 43.4% of the cases, it was associated with a simultaneous reduction in the levels of C3 and C4, along with changes in CH100, suggesting activation of the classical pathway.<sup>19</sup>

In the present study, we observed only a reduction in C3 levels in patients with visceral leishmaniasis, suggesting activation of the alternative pathway.<sup>44</sup> Corroborating those data, Stebut<sup>45</sup> observed activation of the complement system in experimental models of leishmaniasis, especially by opsonization of the surface of the parasite with C3b.

It is known that some intracellular pathogens use complement regulatory and receptors molecules as a means to gain access to the cells, such as *Leishmania*, which develops mechanisms to stimulate its own phagocytosis, promoting the consumption of complement fractions.<sup>45</sup> The release and exposure of antigens and complement activation during tissue damage and rupture of the cells of the host is another mechanism involved in complement consumption in leishmaniasis.<sup>22</sup>

## CONCLUSION

Statistically significant autoantibodies in patients with visceral leishmaniasis include:

- positive ANA (4.4%) or low titers (8.9%);
- anticardiolipin IgG positive (17.8%) or undetermined (8.9%).

A reduction in serum C3 levels was also observed in 17.8% of the patients, and anti-*Leishmania* antibodies (> 1/80) present in all patients with visceral leishmaniasis.

In cutaneous leishmaniasis, significant changes in the frequency of autoantibodies or complement levels were not observed.

We recommend investigating the possibility of visceral leishmaniasis in patients with a suspected diagnosis of SLE, especially in endemic areas for this disease, due to the clinical and laboratorial similarity between both diseases, including the development of anti-dsDNA and anticardiolipin antibodies.

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