Sensitivity of Lymph Node Aspiration in Localized Cutaneous Leishmaniasis Due to *Leishmania (Viannia) braziliensis*

Gustavo Adolfo Sierra Romero+, Raimunda Nonata Ribeiro Sampaio, Vanize de Oliveira Macêdo, Philip Davis Marsden

Núcleo de Medicina Tropical, Universidade de Brasília, Campus Universitário, Asa Norte, 70919-970, Brasília, DF, Brasil

Twenty nine patients with localized cutaneous leishmaniasis had lymph node and skin ulcer aspirations for culture of Leishmania *with the modified Marzochi´s vacuum aspiratory technique. Sensitivity of lymph node aspiration was 58.6% and 34.5% for skin ulcer aspiration (P=0.06). Combined sensitivity of the two methods was 79.3%. There was no agreement between methods (Kappa Index = -0.084;* $CI_{95\%}$ *-0,45; 0,28) showing the potential complementary roles in diagnostic approach.*

Key words: *Leishmania (Viannia) braziliensis* - lymphadenopathy - skin ulcer culture - lymph node culture

Lymph node aspiration for *Leishmania* culturing was initially evaluated in visceral leishmaniasis and it showed good sensitivity when compared to bone marrow and splenic aspirations (Ho et al. 1948, Siddig et al. 1988).

Recently, attention has been focused in lymphadenopathy associated with cutaneous leishmaniasis; lymph node involvement was shown to be extremely common in *Leishmania (Viannia) braziliensis* (L.V.b.) infections (Barral et al. 1992, Sousa et al. 1995). Onset of lymphatic symptoms can appear before, concommitant or after skin ulceration and in rare occasions it could be the only sign of infection (Barral et al. 1995). Despite the importance of this type of clinical involvement there are no systematic studies on the sensitivity of lymph node aspiration compared to skin ulcer aspiration for *Leishmania* culture in patients with L.V.b. infection to assure the relative merit of each procedure. Lymph node aspiration could be advantageous considering a lower degree of contamination when compared to skin ulceration which is usually infected or colonized by fungi or bacteria. We performed aspiration cultures from skin and enlarged lymph nodes in patients with L.V.b. infections using a modified technique following the original principles suggested by Marzochi et al. (1993).

MATERIALS AND METHODS

The study population was composed of 131 patients who were attended at the Corte de Pedra basic health unit, Presidente Tancredo Neves municipality, State of Bahia, Brazil, from August to November 1996. Eligible patients had less than six suspected skin ulcers and a positive Montenegro skin test. Pregnant women, children under eight , patients with more than five lesions, with a history of previous cutaneous lesions with typical scars suggestive of old leishmaniasis; mucosal involvement or history of anti-leishmanial treatment were excluded. Lesion number for inclusion was less than six to differentiate clearly the localized clinical syndrome from disseminated cutaneous leishmaniasis (Carvalho et al. 1994). The Montenegro skin test was performed with 0.1 ml *L.* (*Leishmania*) a*mazonensis* (MHOM/BR/86/BA 125) antigen (250 μg/ml). Reaction of 5 mm in diameter or greater, measured 48-72 hr after intradermic injection, were recorded as positive.

Eighty seven (76.9%) out of 113 eligible patients had history of lymphadenopathy and 70 patients had adenomegaly (lymph node enlargement > 1 cm in diameter) satellite to skin lesions during the first visit. Twenty nine (41.4%) accepted the aspiration procedures signed our informed consent form and constituted the sample evaluated.

Culture media were prepared using blood agar base no. 2 (DIFCO cod. 0696-17) with 15% defibrinated rabbit blood which was added after fusion of agar at 50°C. Gentamicin (100 μg/ml) and

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+ Corresponding author. Fax: +55-61-273.2811. E-mail: gromero@unb.br
deceased
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5-fluorocytosine (100 μg/ml) were added and the mixture was distributed in 10 ml glass tubes (Vacutainer^R). The tubes were covered with rubber caps and vacuum resumed by aspirating 15 ml with a 20 ml syringe using a 22 gauge needle. A liquid phase of 0.3 ml isotonic saline with gentamicin (100 μg/ml) was added immediately before the aspiration puncture by injection through the rubber cap with a 1 ml syringe and a 22 gauge needle. All injection procedures were performed through the rubber cap after cleaning it with 0.2% iodinated alcohol.

Aspiration puncture was made with the commercial Vacutainer^R needle holder and 21 gauge needles as for the blood extraction technique. The puncture site was cleaned with 0.2% iodinated alcohol and local anesthesia was induced with 0.3 ml lidocaine (2%) injected with a 1 ml syringe and a 13 gauge needle. The procedure was performed through intact skin in the ulcer border at a 20^o angle or directly into the enlarged lymph node with a rotatory movement and finally the needle was pulled out allowing air to come into then needle with the material. The material which adhered to the glass wall was washed toward the solid phase using the liquid phase. The procedure was repeated at the same place three times in three separate tubes which were labeled as first, second and third aspirates for skin lesions and in two tubes for lymphatic lesions, labeled first and second aspirates. When the patient had more than one ulcer only one was aspirated usually the one with the most recent onset. The largest lymph node was preferred for aspiration.

Culture tubes were kept at 22-28^{o}C and were observed every day using an inverted microscope for 28 days. When positive, new tubes were inoculated to obtain parasites for cryopreservation. Culture aspiration and observation of leishmanial growth were performed by one researcher avoiding inter-observer variations. All isolates were characterized using monoclonal antibodies at Instituto Evandro Chagas, Belém, State of Pará, Brazil.

All patients were treated in the basic health unit of Corte de Pedra with conventional drugs recommended by the Ministry of Health. The study was part of a research project approved by the Ethics Comitee of the University of Brasília.

Group comparisons were processed using Epi Info 6.04 software.

RESULTS

Table I shows the characteristics of 87 patients with a positive history of lymph node enlargement and 29 patients in whom we did culture procedures. The sample was representative of the population studied. Three patients showed lymphangitic involvement associated with adenomegaly. Lymph node enlargement was usually painless except in those with lymphangitis.

Forty out of 70 patients with adenomegaly during the first evaluation had only one enlarged node. The mean number of lymph nodes in this group was 1.9 (SD=1.5) and the mean duration of adenomegaly was 6.2 weeks (SD=4.6).

Lymph node cultures showed greater sensitivity when compared to skin ulcer cultures but the difference did not achieve statitiscal significance. Any combination of two aspirates of skin ulcer showed the same sensitivity (34.5%). Three aspirates from skin ulcers gave better results (44.8% sensitivity). There was no difference in time to obtain a positive culture Table II. Combined sensitivity of the two aspiration methods was 79.3% ($CI_{95\%}$ 59.7; 91.3). Seven patients had positive cultures from skin and lymph nodes, six and ten patients had exclusively positive cultures from skin

TABLE I

Characteristics of patients with history of lymph node enlargement associated with localized cutaneous leishmaniasis (N=87) and patients who did aspiration procedures (N=29)

Characteristic	N=87 [a]	N=29 [a]	Statistical test	P value
Mean age (years)	21.0 (14.6)	20.7 (10.8)	0.01[b]	0.91
Sex (male)	60.0 (69)	22 (75.9)	0.50[c]	0.48
Mean skin lesion number	1.5 (0.4)	1.4 (0.6)	0.84[b]	0.37
Mean duration of cutaneous disease (weeks)	6.1 (7.5)	4.2 (2.0)	2.15[b]	0.14
Mean duration of lymphatic disease (weeks)	5.6 (4.5)	5.1 (2.2)	0.79[b]	0.38
Onset related to appearance of skin ulceration				
before	35 (40.2)	12 (41.4)	0.01[c]	0.91
concommitant	43 (49.4)	14 (48.3)	0.01[c]	0.91
after	9 (10.3)	2 (6.9)	0.03[c]	0.85
Mean Montenegro skin test diameter (mm)	17.8 (6.9)	20.3 (7.4)	2.75[b]	0.10

a: standard deviation of the means and % of categorical variables; *b:* T test; *c:* χ^2 test.

TABLE II

Lymph node and skin culture comparisons in 29 patients with localized cutaneous leishmaniasis and lymphadenopathy

Characteristic	Lymph node culture[a] ($CI_{95\%}$)	Skin ulcer culture[b] ($CI_{95\%}$)	Statistical test	P value
Sensitivity	58.6% (39.1-75.9)	34.5% (18.6-54.3)	3.40[c]	0.06
Mean growth´s time	8.8 days (6.9-10.7)	8.6 days (5.2-12)	0.02[d]	0.89

a: data from combination of two lymph node aspirates; *b:* data from combination of the first and second skin ulcer aspirates; *c:* χ^2 test; *d:* T test.

and lymph node aspirations respectively and six patients had negative cultures. Kappa Index was -0.08 ($CI_{95\%}$ -0,45; 0,28) showing no agreement between methods. The contamination rate for lymph node aspiration was zero and only one tube from a skin ulcer aspiration showed fungal growth.

All isolates were characterized as L.V.b. and all patients had a confirmed parasitological diagnosis by at least one of the following methods: histopathological sections, hamster inoculation or imprint of skin biopsy material (data not shown).

DISCUSSION

Our observations about lymph node involvement in L.V.b. infection confirm its magnitude and focus attention on the important role of this manifestation in diagnostic procedures. Our data are important because they represent the first attempt to assure the relative merit of lymph node aspiration for diagnosis of localized cutaneous leishmaniasis in a well characterized prospectively evaluated group of patients with L.V.b. infection.

Vacuum aspiratory puncture of lymph nodes constitutes a practical approach for diagnosis of localized cutaneous leishmaniasis. Our data suggest at least comparable sensitivity to skin ulcer aspiration yield. Low contamination rates could be attributed to media composition, vacuum aspiratory technique reducing exposure of culture medium during inoculation and thorough cleaning procedures before puncture. Lack of agreement between methods brings up the possibility of potential complementary roles in diagnostic approach.

We recommend the combined approach of lymph node and skin ulcer aspiration for diagnosis of localized cutaneous lesihmaniasis using the modiefied Marzochi´s vacuum aspiratory technique.

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REFERENCES

Barral A, Barral-Netto M, Almeida R, de Jesus AR, Grimaldi Jr. G, Netto EM, Santos I, Bacellar O, Carvalho EM 1992. Lymphadenopathy associated with *Leishmania braziliensis* cutaneous infection. *Am J Trop Med Hyg 47: 587-592.*

Barral A, Guerreiro J, Bomfim G, Correia D, Barral Netto M, Carvalho EM 1995. Lymphadenopathy as the first sign of human cutaneous leishmaniasis infection by *Leishmania braziliensis. Am J Trop Med Hyg 53: 256-259.*

Carvalho EM, Barral A, Costa JML, Bittencourt A, Marsden P 1994. Clinical and immunopathological aspects of disseminated cutaneous leishmaniasis. *Acta Trop 56: 315-325.*

Ho EA, Soong TH, Li Y 1948. Comparative merits of sternum spleen and liver punctures in the study of human visceral leishmaniasis. *Trans R Soc Trop Med Hyg 41: 629-636.*

Marzochi MCA, Teixeira PC, Marzochi KBF, Conceição NF, Coutinho W, Brito DB 1993. Vacuum aspiratory puncture system for *Leishmania* culturing, isolation and transport, preliminary report. *Rev Inst Med Trop São Paulo 35: 301-303.*

Siddig M, Ghalib H, Shillington DC, Petersen EA 1988. Visceral leishmaniasis in the Sudan: comparative parasitological methods of diagnosis. *Trans R Soc Trop Med Hyg 82: 66-68.*

Sousa AQ, Parise ME, Pompeu MML, Filho JMC, Vasconcelos IAB, Lima JWO, Oliveira EG, Vasconcelos AW, David JR, Maguire JH 1995. Bubonic leishmaniasis: a common manifestation of *Leishmania (Viannia) braziliensis* infection in Ceará, Brazil. *Am J Trop Med Hyg 53: 380-385.*