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Positive Montenegro skin test among patients with sporotrichosis in Rio De Janeiro

Mônica Bastos de Lima Barros[a,*], Armando Schubach[a], Antônio Carlos Francesconi-do-Valle[a], Maria Clara Gutierrez-Galhardo[a], Tânia Maria Pacheco Schubach[a], Fátima Conceição-Silva[b], Mariza de Matos Salgueiro[a], Eliame Mouta-Confort[a], Rosani Santos Reis[a], Maria de Fátima Madeira[a], Tullia Cuzzi[a], Leonardo Pereira Quintella[a], Janaína Pinho da Silva Passos[c], Maria José Conceição[d], Mauro Célio de Almeida Marzochi[e]

[a] *Instituto de Pesquisa Clínica Evandro Chagas (IPEC), Fundação Oswaldo Cruz (Fiocruz), Av. Brasil 4365, Manguinhos, Rio de Janeiro CEP 21045-900, Brazil*
[b] *Lab. de Imunidade Celular e Humoral, Instituto Oswaldo Cruz (IOC), Fiocruz CEP 21045-900, Brazil*
[c] *Biomanguinhos, Fiocruz, Av. Brasil 4365, Manguinhos, Rio de Janeiro CEP 21045-900, Brazil*
[d] *IOC, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Av. Brigadeiro Trompovsky s/n, Rio de Janeiro CEP 21941-590, Brazil*
[e] *Secretaria Municipal de Saúde, Rua Afonso Cavalcanti 455, Cidade Nova, Rio de Janeiro CEP 20211-110, Brazil*

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Abstract

We studied 52 patients with sporotrichosis confirmed by isolation of *Sporothrix schenckii* and reactivity to the Montenegro skin test (MST) during an ongoing outbreak of this mycosis in Rio de Janeiro. The objective was to emphasize the importance of parasitological confirmation and the possibility of incorrect diagnosis based on the lesion's appearance, epidemiological information, and immunological tests. The antigen used for the MST was conserved in either thimerosal 1:10,000 (group 1) or 0.4% phenol (group 2). Nineteen patients (39%) in group 1 and seven (12%) in group 2 presented an induration $\geq$10 mm ($p < 0.001$). Sera from three patients (6.7%) reacted to indirect immunofluorescence (IIF) for leishmaniasis, while sera from 10 patients (22.2%) reacted to enzyme-linked immunosorbent assay (ELISA). Fifteen patients (28.8%) presented up to two lesions, with a predominance of ulcers. Forty-four patients (84.6%) were treated with itraconazole. In the differential diagnosis between sporotrichosis and cutaneous leishmaniasis, the possibility of co-infection, allergy to the reagent diluent, and cross-reactions should be further investigated, especially in regions with limited laboratory facilities.

Keywords: Sporotrichosis; Skin tests; Thimerosal; Leishmaniasis

* Corresponding author. Tel.: +55 21 25984263; fax: +55 21 25909988.
E-mail address: monica@ipec.fiocruz.br (M.B. de Lima Barros).

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1. Introduction

Sporotrichosis is a mycosis caused by the dimorphic fungus *Sporothrix schenckii*, which grows saprophytically as a mould in association with dead or decaying plant material (Rippon, 1988). Sporotrichosis is thus acquired mainly during outdoor leisure activities or as an occupational hazard for workers who have frequent contact with plant material or soil (Kwon-Chung and Bennet, 1992). Following traumatic implantation in the skin, the organism can cause cutaneous or subcutaneous infection, which commonly shows regional lymphatic spread. The disease occasionally persists as a fixed cutaneous ulcer at the site of inoculation (Kwon-Chung and Bennet, 1992). In Brazil, sporotrichosis has been reported especially in the States of São Paulo and Rio Grande do Sul (Freitas et al., 1965; Lopes et al., 1999). Until recently in the State of Rio de Janeiro, the disease only occurred occasionally (Gonçalves and Peryassu, 1954). However, beginning in 1998, a zoonotic epidemic has been detected involving humans, dogs, and cats (Barros et al., 2001, 2004; Schubach et al., 2004).

American tegumentary leishmaniasis (ATL), caused by *Leishmania (Viannia) braziliensis*, is endemic in Rio de Janeiro, where an epidemiological pattern of intra- and peri-domiciliary transmission predominates, linked to adaptation of the vector, *Lutzomyia intermedia*, to the modified environment (Marzochi and Marzochi, 1994). The disease normally affects humans, dogs, and horses (Aguilar et al., 1987). In cutaneous leishmaniasis (CL) or the cutaneous form of ATL, the ulcer is usually painless, single, and rounded, with well-demarcated, raised, and erythematous borders and a granulating and reddened base. Lymphangitis and regional adenitis may precede or accompany the skin lesions (Barral et al., 1995). With or without treatment, lesions usually heal within months or years (Costa et al., 1990).

In the differential diagnosis between CL and sporotrichosis, it is necessary to demonstrate the presence of the amastigotes in tissue or isolation of the parasite in culture of material obtained from the skin lesion (Kostman and DiNubile, 1993; Heller and Swartz, 1994; Tobin and Jih, 2001). However, at primary health care services in economically underprivileged regions, the differential diagnosis between sporotrichosis and CL is made on the basis of epidemiological evidence, clinical appearance of the lesion, the Montenegro skin test (MST), and occasionally serology for ATL. Within this context, specific therapy is indicated with either meglumine antimoniate, the drug of first choice for treatment of CL (Ministério da Saúde, 2000), or with iodides or itraconazole for sporotrichosis (Kauffman et al., 2000). A favorable therapeutic response is usually accepted as the criterion for diagnostic confirmation.

In the present study, we report the frequency of positive MST among patients with sporotrichosis during an ongoing outbreak of this mycosis in Rio de Janeiro. In addition, we describe the clinical and epidemiological features and the results of additional diagnostic tests for cutaneous leishmaniasis, with the objective of verifying the possibility of an erroneous diagnosis based on the lesion's appearance, epidemiological information, and immunological tests.

2. Material and methods

The study was approved by the Research Ethics Committee of the Oswaldo Cruz Foundation (Fiocruz). Records of 107 patients with culture-proven sporotrichosis treated from 1998 to 2001 at the Evandro Chagas Clinical Research Institute, Oswaldo Cruz Foundation were reviewed.

2.1. Diagnosis

Patients were subjected to the following protocol: initial clinical evaluation, MST, and collection of surface secretion obtained with a swab from the exudative lesions or draining tracts and purulent or seropurulent content aspirated from the non-ulcerated gummy lesions, as previously described (Barros et al., 2004). When the initial mycological examination of the secretion was negative, an incisional skin biopsy was obtained from the borders of active lesions. The specimen was divided into three fragments: the first was fixed in 10% buffered formalin and stained with hematoxylin–eosin, Wade, PAS, and Grocott for histopathological examination. This fragment was also used for Giemsa-stained impression slides (Luna, 1968). The second fragment was cultured in enriched blood agar medium (NNN) in order to detect the presence of *Leishmania promastigotes* (Chang and Hendricks, 1985), and the third fragment was seeded

on Sabouraud dextrose 2% agar with chloramphenicol and mycobiotic agar (DIFCO). Isolates were subcultured on potato-dextrose-agar medium (DIFCO) at 25 °C for macroscopic and microscopic morphological studies. Dimorphism of *S. schenckii* was demonstrated by conversion to the yeast-lfike form on BHI agar medium (DIFCO) at 37 °C (Barros et al., 2001). Serum samples obtained from MST-reactive patients were used to evaluate the presence of anti-*Leishmania* antibodies (IIF and ELISA).

2.1.1. Montenegro skin test (MST)

Leishmanin produced by Biomanguinhos, Fiocruz and containing 40 μg protein nitrogen/ml was used. From 1998 to 2000, 1:10,000 thimerosal was used as a preservative (group 1), but was replaced with 0.4% phenol in 2001 (group 2). After local asepsis with 70% alcohol, 0.1 ml of the antigen was injected by the intradermal route on the anterior surface of the forearm. The skin response was measured 48 h after injection. The induration was marked with a ballpoint pen, measured in millimeter, traced on moistened paper, and recorded on the patient's clinical record. An induration of 5 mm or more in the largest diameter was considered positive (Sokal, 1975; Melo et al., 1977). The tests were performed at the beginning of the clinical investigation, before the results of mycological examination.

2.1.2. Serological methods

2.1.2.1. Indirect immunofluorescence (IIF). We used the indirect immunofluorescence kit produced by Biomanguinhos for the tests, according to the manufacturer's instructions. Readings were obtained with an epifluorescence microscope. Antibody titers observed at ≥1:40 dilutions were considered positive (Furtado, 1972; Camargo, 1973).

2.1.2.2. Enzyme-linked immunosorbent assay (ELISA). We used antigen from the soluble promastigote abstract, strain MHCM/BR/76/JOF, and peroxidase-conjugated anti-human IgG (Sigma). The reaction was measured with a spectrophotometer for plate reading using a 492-nm filter. The cut-off between reactive and non-reactive samples was calculated on the basis of the mean reading of negative sera plus 2 S.D. (Voller et al., 1976; Guimarães et al., 1983; Badaro et al., 1986).

2.2. Treatment

Treatment used itraconazole, administered orally (100 mg/day). Criteria for discontinuation of medication were: regression of infiltration and erythema, absence of secretion, and lesion epithelialization. Patients were followed up for 6–12 months after healing of lesions.

2.3. Statistical analysis

Data were analyzed statistically by the non-parametric chi-square test.

3. Results

All 107 patients submitted to the MST were from the Greater Rio de Janeiro Metropolitan Area. Thimerosal was used as the preservative for 49 patients (group 1) and phenol for 58 (group 2). Fifty-two patients (48.6%) reacted to the test and comprised the series for the present study.

3.1. Epidemiological data

Of the 52 patients with positive tests, 37 (71.1%) were females, and the age range was 8–71 years (median = 41). Twenty-eight (53.8%) lived in areas with active ATL transmission (Duque de Caxias, Mangaratiba, Campo Grande, and Jacarepaguá). Eighteen (34.6%) denied any local trauma that might suggest inoculation, and some reported that the disease had first appeared as "an insect bite", suggesting leishmaniasis. Four patients without a history of trauma reported the presence of a dog with ulcerated skin lesions in the household. Thirty-four patients (65.4%) reported trauma, 28 of whom having been scratched or bitten by cats with sporotrichosis.

3.2. Clinical presentation

Twenty-nine patients (55.8%) presented with the cutaneous–lymphatic form and 15 (28.8%) presented with the fixed form with up to two lesions and a predominance of ATL-like ulcers. The eight remaining patients (15.4%) presented with more than three fixed lesions or widespread cutaneous lesions. Evolution

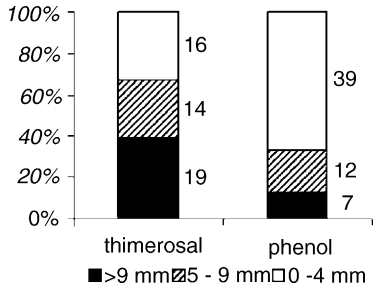

Fig. 1. Montenegro skin test in patients with sporotrichosis according to type of preservative used and intensity of reaction.

of the lesions ranged in time from 1 to 108 weeks (median = 6 weeks).

3.3. Reaction to MST

Thirty-three patients ($n = 49$, 67.4%) in group 1 and 19 ($n = 58$, 32.8%) in group 2 presented a positive test, with a statistically significant difference between the two groups ($p < 0.001$) (Fig. 1).

3.4. Intensity of reaction to MST

Nineteen patients (38.7%) in group 1 and 7 (12.1%) in group 2 presented an induration of 10 mm or more ($p < 0.001$) (Fig. 1). Thirteen patients ($n = 29$, 44.8%) with the cutaneous–lymphatic form and nine ($n = 15$, 60%) with the fixed form with up to two lesions presented a reaction of 10 mm or more.

3.5. Serology

Serology was performed in 45 of the 52 patients with a positive MST: sera from 10 patients (22.2%) reacted to Elisa, while sera from three (6.7%) reacted to IIF with titers higher than 1:40. A patient with chronic Chagas' disease reacted to MST, Elisa, and IIF (1:40).

3.6. Histopathological study

Histopathological examination of a lesion fragment was performed in 24 patients (46.1%): a granulomatous chronic inflammatory process was observed in 20, as compared to a nonspecific chronic inflammatory process in four. Amastigote forms of *Leishmania* were not visualized in any cases, but yeast-like forms were visualized in seven. Culture for *Leishmania* was performed on the 24 fragments and showed 100% negativity.

3.7. Clinical course

Six patients presented spontaneous regression of lesions, two abandoned follow-up after the diagnosis, and the remaining 44 (84.6%) were cured by treatment with itraconazole, used for 5–36 weeks (median = 12). All continued to be cured during follow-up.

4. Discussion

Cutaneous sporotrichosis and ATL share various clinical and epidemiological characteristics. Approximately, 250 cases of ATL are reported every year in the State of Rio de Janeiro, originating from both peripheral urban areas and rural zones (Ministério da Saúde, 2000). During the current sporotrichosis epidemic, most of the patients were from areas with unfavorable socio-economic conditions and inadequate health services, a factor which can hinder a precise diagnosis.

Some patients reported that the disease had first appeared as an "insect bite", in keeping with reports by other authors (Lober et al., 1980). Fifteen patients (28.8%) presented up to two skin lesions, which could suggest a diagnosis of CL. Although the cutaneous–lymphatic form is the most common presentation of sporotrichosis, fixed lesions may also occur (Sampaio et al., 1954). In another study conducted in Rio de Janeiro, 25.3% of the patients with sporotrichosis presented fixed lesions (Barros et al., 2004). On the other hand, *Leishmania* can disseminate through blood and lymph vessels, causing the sporotrichoid form, thus denoted because of its similarity to the lesions caused by *S. schenckii* (Kubba et al., 1987; Agudelo et al., 1999; Tobin and Jih, 2001).

During the present study, 65 patients with ATL were treated who tested positive with the following frequencies: MST 98.3%, ELISA 55.5%, and IIF 42.5%. Among the 52 patients with sporotrichosis and a positive MST, 22.2% also presented positive serology. *S. schenckii* and *Leishmania* co-infection may partially explain the positivity detected by MST, IIF, and ELISA, since 53.8% of the patients reported that they lived in or frequented areas with active ATL transmission. Cross-

reactivity between *Trypanosoma cruzi* and *Leishmania* antigens is a well-known phenomenon (Carvalho et al., 1987; Vexenat et al., 1996). A similar cross-reaction could be taking place between *S. schenckii* and *Leishmania*. In agreement with this hypothesis, experimentally obtained polyclonal anti-*Leishmania* rabbit serum (Sartori et al., 1991) was able to identify yeast-like structures of *S. schenckii* when used for immunohistochemistry (Schubach et al., 2001), although this finding was not confirmed by others (Salinas et al., 1990).

Sporothrix schenckii was isolated from all cases, but *Leishmania* was not detected in any case, although histopathological analyses and culture for *Leishmania* spp. of skin biopsy fragments were performed in 46% of the patients. Thus, some cases of co-infection with *S. schenckii* and *Leishmania* spp. may have escaped diagnosis. Association of the two pathogens in the same host has already been reported (Agudelo et al., 1999). In Colombia, rural dwellers habitually use thorns or wooden splinters to remove crusts from lesions or to drain them, which could facilitate entry of the fungus and promote co-infection in endemic areas (Agudelo et al., 1999).

Although sporotrichosis may be suggested by the presence of fungal structures in tissues or exudates by direct examination, the definitive diagnosis of *S. schenckii* infection requires isolation of the organism from culture at 25 °C and its conversion to the yeast-like form at 37 °C (Kwon-Chung and Bennet, 1992). Diagnosis of ATL can be established by the demonstration of amastigote forms by direct examination or by histology, or of promastigote forms obtained in cultures of lesion material (Weigle et al., 1987; Schubach et al., 2001). Giemsa-stained skin lesion smears should be interpreted with caution, since the rounded or spindle-shaped yeast-like structures of *S. schenckii* may be confused with amastigote forms of *Leishmania* (Castrejon et al., 1995). Histopathological features of sporotrichosis and ATL are similar, usually consisting of diffuse granulomatous dermatitis. Cell remnants observed on sections stained with hematoxylin–eosin mimic both of these parasites. However, careful analysis of both histological sections and direct examination under high magnification shows that amastigotes are rounded or piriform, with a weakly stained cytoplasm, a frequently eccentric nucleus, and a kinetoplast seen as a basophilic dot or bar. On the other hand, yeast-like elements of *S. schenckii* are positively stained by PAS and silver, which does not occur with amastigotes. Since both species can infect hamsters (Gonzalez de Polania et al., 1990), this precaution should be maintained when using animal inoculation as a diagnostic method.

The lesions healed in all patients followed up in this study, six of whom required no treatment. When a definitive diagnosis cannot be established, interpretation of the therapeutic response presents limitations: itraconazole has been used as a therapeutic option for the treatment of ATL (Santos et al., 1995; Amato et al., 2000). On the other hand, patients with sporotrichosis erroneously treated with meglumine antimoniate may be cured (Baptista et al., 1952; Belliboni and Patricio, 1956). When evaluating a particular case, spontaneous regression cannot be ruled out (Marsden et al., 1984; Costa et al., 1990; Barros et al., 2003) during the course of erroneous treatment.

Part of the positive MST results may be explained by allergy to the reagent diluent. Allergy to thimerosal among Brazilian patients led the manufacturer to change the formulation to phenol during the study period (Hansson and Moller, 1974; Marzochi et al., 1998; Paranhos-Silva et al., 2001). A reduction of positive tests from 67.4 to 32.8% after thimerosal was replaced with phenol as a preservative indicates that this change was appropriate.

A high frequency of patients with sporotrichosis presenting positive MST and serology for leishmaniasis was detected. In the differential diagnosis between sporotrichosis and CL, caution should be exercised in the interpretation of results, especially in regions with limited laboratory facilities where the diagnosis is exclusively based on clinical–epidemiological evidence and MST. The possibility of co-infection, allergy to the reagent diluent, and cross-reactions should be further investigated. It is important to point out that an erroneous diagnosis of ATL in patients with sporotrichosis leads to the patient's unnecessary exposure to the toxicity of antimonial treatment, as well as to the adoption of incorrect measures for epidemiological surveillance and control.

Even in regions with only basic health services, efforts should be made to prioritize the etiological diagnosis of infectious and parasitic diseases. Especially in endemic areas, the establishment of reference centers to receive clinical specimens for diagnosis, to provide professional training, and to support laboratory tests are other key measures for promoting adequate diagnosis.

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References

Agudelo, S.P., Restrepo, S., Velez, I.D., 1999. Cutaneous new world leishmaniasis–sporotrichosis co-infection: report of three cases. J. Am. Acad. Dermatol. 40, 1002–1004.

Aguilar, C.M., Rangel, E.F., Grimaldi Filho, G., Momem, H., 1987. Human, canine and equine leishmaniasis caused by *Leishmania (Braziliensis) braziliensis* in an endemic area in the State of Rio de Janeiro. Mem. Inst. Oswaldo Cruz. 82, 143.

Amato, V.S., Padilha, A.R., Nicodemo, A.C., Duarte, M.I., Valentini, M., Uip, D.E., Boulos, M., Neto, V.A., 2000. Use of itraconazole in the treatment of mucocutaneous leishmaniasis: a pilot study. Int. J. Infect. Dis. 4, 153–157.

Badaro, R., Reed, S.G., Barral, A., Orge, G., Jones, T.C., 1986. Evaluation of the micro enzyme-linked immunosorbent assay (ELISA) for antibodies in American visceral leishmaniasis: antigen selection for detection of infection-specific responses. Am. J. Trop. Med. Hyg. 35, 72–78.

Baptista, L., Belliboni, N., Castro, R., 1952. Caso de esporotricose tratado pelo antimoniato de N-Metilglucamina. Rev. Paul. Med. 41, 24–27.

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

Barros, M.B.L., Schubach, A.O., Francesconi Do Valle, A.C., Gutierrez Galhardo, M.C., Conceição-Silva, F., Schubach, T.M., Reis, R.S., Wanke, B., Marzochi, K.B., Conceição, M.J., 2004. Cat-transmitted sporotrichosis epidemic in Rio de Janeiro, Brazil: description of a series of cases. Clin. Infect. Dis. 38, 529–535.

Barros, M.B.L., Schubach, A.O., Gutierrez-Galhardo, M.C., Schubach, T.M.P., Reis, R.S., Conceição, M.J., Francesconi-do-Valle, A.C., 2003. Sporotrichosis with widespread cutaneous lesions—a report of 24 cases related to transmission by domestic cats in Rio de Janeiro, Brazil. Int. J. Dermatol. 42, 677–681.

Barros, M.B.L., Schubach, T.M., Gutierrez Galhardo, M.C., Schubach, A., Monteiro, P.C., Reis, R.S., Zancope-Oliveira, R.M., Lazera, M., Cuzzi-Maya, T., Blanco, T.C., Marzochi, K.B., Wanke, B., Valle, A.C., 2001. Sporotrichosis: an emergent zoonosis in Rio de Janeiro. Mem. Inst. Oswaldo Cruz. 96, 777–779.

Belliboni, N., Patricio, L., 1956. Tratamento da esporotricose pelo glucantime. Considerações a respeito de dois casos. Rev. Hosp. Clin. Fac. Med. São Paulo. 11, 118–120.

Camargo, M., 1973. Introdução as técnicas de imunofluorescência. Manual. Instituto de Medicina Tropical de São Paulo, São Paulo.

Carvalho, E.M., Reed, S.G., Johnson Jr., W.D., 1987. Cross-reactivity between *Trypanosoma cruzi* and Leishmania antigens in the lymphocyte blastogenesis assay. Trans. R. Soc. Trop. Med. Hyg. 81, 82–84.

Castrejon, O.V., Robles, M., Zubieta Arroyo, O.E., 1995. Fatal fungaemia due to *Sporothrix schenckii*. Mycoses 38, 373–376.

Chang, K.P., Hendricks, L.D., 1985. Laboratory cultivation and maintenance of Leishmania. In: Chang, K.P., Bray, R.S. (Eds.), Leishmaniasis. Elsevier, Amsterdam, NY, Oxford, pp. 214–244.

Costa, J.M., Vale, K.C., Franca, F., Saldanha, A.C., da Silva, J.O., Lago, E.L., Marsden, P.D., Magalhaes, A.V., e Silva, C.M., Serra Neto, A., et al., 1990. Spontaneous healing of leishmaniasis caused by *Leishmania (Viannia) braziliensis* in cutaneous lesions. Rev. Soc. Bras. Med. Trop. 23, 205–208.

Freitas, D., Moreno, G., Saliba, A., Bottino, J., Mós, E., 1965. Esporotricose em cães e gatos. Rev. Fac. Med. Vet. São Paulo. 7, 381–387.

Furtado, T., 1972. Diagnóstico Laboratorial da Leishmaniose Tegumentar Americana. An. Bras. Dermatol. 47, 211–228.

Gonçalves, A.P., Peryassu, D., 1954. A esporotricose no Rio de Janeiro (1936–1953). Hospital 46, 9–24.

Gonzalez de Polania, L.A., Alzate, A., Saravia, N., 1990. Experimental behavior of *Sporothrix schenckii* and *Leishmania mexicana* in hamsters. Rev. Inst. Med. Trop. São Paulo 32, 319–324.

Guimarães, M.C., Celeste, B.J., Camargo, M.E., Diniz, J.M., 1983. Seroepidemiology of cutaneous leishmaniasis from Ribeira do Iguape Valley. IgM and IgG antibodies detected by means of an immunoenzymatic assay (ELISA). Rev. Inst. Med. Trop. São Paulo 25, 108–112.

Hansson, H., Moller, H., 1974. Thimerosal reaction and cell-mediated immunity. N. Engl. J. Med. 290, 1202.

Heller, H.M., Swartz, M.N., 1994. Nodular lymphangitis: clinical features, differential diagnosis and management. Curr. Clin. Top. Infect. Dis. 14, 142–158.

Kauffman, C.A., Hajjeh, R., Chapman, S.W., 2000. Practice guidelines for the management of patients with sporotrichosis. Clin. Infect. Dis. 30, 684–687.

Kostman, J.R., DiNubile, M.J., 1993. Nodular lymphangitis: a distinctive but often unrecognized syndrome. Ann. Intern. Med. 118, 883–888.

Kubba, R., el-Hassan, A.M., Al-Gindan, Y., Omer, A.H., Kutty, M.K., Saeed, M.B., 1987. Dissemination in cutaneous leishmaniasis. I. Subcutaneous nodules. Int. J. Dermatol. 26, 300–304.

Kwon-Chung, K., Bennet, J., 1992. Sporotrichosis. In: Kwon-Chung, K., Bennet, J. (Eds.), Medical Mycology. Lea and Febiger, Philadelphia, pp. 707–729.

Lober, C., Kaplan, R., Herron, C., 1980. *Sporothrix schenckii* inoculation on the abdomen. South Med. J. 73, 1637–1638.

Lopes, J., Alves, S., Mari, C., Brum, L., Westphalen, J., Altermann, M., Prates, F., 1999. Epidemiology of sporotrichosis in the central region of Rio Grande do Sul. Rev. Soc. Bras. Med. Trop. 32, 541–545.

Luna, L.G. (Ed.), 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill, New York.

Marsden, P.D., Tada, M.S., Barreto, A.C., Cuba, C.C., 1984. Spontaneous healing of *Leishmania (Braziliensis) braziliensis* skin ulcers. Trans. R. Soc. Trop. Med. Hyg. 78, 561–562.

Marzochi, K.B., Marzochi, M.A., Silva, A.F., Grativol, N., Duarte, R., Confort, E.M., Modabber, F., 1998. Phase 1 study of an inactivated vaccine against American tegumentary leishmaniasis in normal volunteers in Brazil. Mem. Inst. Oswaldo Cruz. 93, 205–212.

Marzochi, M.A.C., Marzochi, K.B.F., 1994. Tegumentary and visceral leishmaniasis in Brazil. Emerging anthropozoonosis and possibilities for their control. Cad. S. Pub. 10, 359–375.

Melo, M.N., Mayrink, W., Costa, C.A., Magalhães, P.A., Dias, M., Williams, P., Araújo, F.G., Coelho, M.V., Batista, S.M., 1977. Standardization of the Montenegro antigen. Rev. Inst. Med. Trop. Sao Paulo 19, 161–164.

Ministério da Saúde, 2000. Manual de Controle da Leishmaniose Tegumentar Americana, Brasília.

Paranhos-Silva, M., Pontes-de-Carvalho, L.C., de Sa Oliveira, G.G., Nascimento, E.G., dos-Santos, W.L., 2001. Skin reactions to thimerosal and Leishmania in dogs from a leishmaniasis endemic area: it is better to keep them apart. Mem. Inst. Oswaldo Cruz. 96, 679–681.

Rippon, J., 1988. Sporotrichosis. In: Rippon, J. (Ed.), Medical mycology—The pathogenic fungi and the pathogenic actinomycetes. Saunders, W.B. Company, Philadelphia, pp. 325–352.

Salinas, G., Valderrama, L., Palma, G., Montes, G., Saravia, N.G., 1990. Detection of amastigotes in cutaneous and mucocutaneous leishmaniasis using the immunoperoxidase method, using polyclonal antibody: sensibility and specificity compared with conventional methods of diagnosis. Mem. Inst. Oswaldo Cruz. 84, 53–60.

Sampaio, S., Lacaz, C., Almeida, F., 1954. Aspectos clínicos da esporotricose em São Paulo. Rev. Hosp. Clin. Fac. Med. São Paulo 9, 391–402.

Santos, I., Santos, I.B., Montenegro, D., Lemos, E.R., Perereira, T., 1995. Use of itraconazole in 26 patients with American tegumentary leishmaniasis. An. Bras. Dermatol. 70, 103–107.

Sartori, A., Roque Barreira, M.C., Coe, J., Campos Neto, A., 1991. Immune complex glomerulonephritis in experimental kala-azar. II: detection and characterization of parasite antigens and antibodies eluted from kidneys of *Leishmania donovani*-infected hamsters. Clin. Exp. Immunol. 87, 386–392.

Schubach, A., Cuzzi-Maya, T., Oliveira, A.V., Sartori, A., de Oliveira-Neto, M.P., Mattos, M.S., Araujo, M.L., Souza, W.J., Haddad, F., Perez Mde, A., Pacheco, R.S., Momen, H., Coutinho, S.G., de Almeida Marzochi, M.C., Marzochi, K.B., da Costa, S.C., 2001. Leishmanial antigens in the diagnosis of active lesions and ancient scars of American tegumentary leishmaniasis patients. Mem. Inst. Oswaldo Cruz. 96, 987–996.

Schubach, T.M., Schubach, A.O., Okamoto, T., Barros, M.B.L., Figueiredo, F.B., Cuzzi, T., Fialho Monteiro, P.C., Reis, R.S., Perez, M.A., Wanke, B., 2004. Evaluation of an epidemic of sporotrichosis in cats: 347 cases (1998–2001). JAVMA 224, 1623–1629.

Sokal, J.E., 1975. Measurement of delayed skin test responses. N. Engl. J. Med. 293, 501–502.

Tobin, E.H., Jih, W.W., 2001. Sporotrichoid lymphocutaneous infections: etiology, diagnosis and therapy. Am. Fam. Phys. 63, 326–332.

Vexenat, A.C., Santana, J.M., Teixeira, A.R., 1996. Cross-reactivity of antibodies in human infections by the kinetoplastid protozoa *Trypanosoma cruzi*, *Leishmania chagasi* and *Leishmania (Viannia) braziliensis*. Rev. Inst. Med. Trop. São Paulo 38, 177–185.

Voller, A., Bortlett, A., Batwell, D.E., 1976. Enzyme immunoassays for parasite diseases. Trans. R. Soc. Trop. Med. Hyg. 70, 98–106.

Weigle, K.A., de Davalos, M., Heredia, P., Molineros, R., Saravia, N.G., D'Alessandro, A., 1987. Diagnosis of cutaneous and mucocutaneous leishmaniasis in Colombia: a comparison of seven methods. Am. J. Trop. Med. Hyg. 36, 489–496.