American cutaneous leishmaniasis: use of a skin test as a predictor of relapse after treatment

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While relapses following clinical cure of American cutaneous leishmaniasis are frequent, no test has been described until now to predict such relapses. A cohort of 318 American cutaneous leishmaniasis patients was followed up for two years after treatment with meglumine antimoniate, during which time 32 relapses occurred, 30 in the first year and two in the second (accumulated risk: 10.5%). No association was found between these relapses and the parasite-specific antibody response before and after treatment, or between the relapses and stratification by sociodemographic and clinical characteristics. However when *Leishmania* was used as antigen, patients with a negative skin test at the time of diagnosis presented a 3.4-fold higher risk (hazard risk = 3.4; 95% confidence interval, 1.7–7.0) of American cutaneous leishmaniasis relapse, compared with patients with a positive response. This result shows that the skin test can be a predictor of American cutaneous leishmaniasis relapse after treatment.

Keywords: leishmaniasis, cutaneous; skin tests; recurrence; cohort studies; Brazil.

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Introduction

Approximately 10% of patients treated for American cutaneous leishmaniasis caused by *Leishmania (Viannia) braziliensis* experience relapses (*1*) and, in a few cases, this results in the mucocutaneous form of the disease. Reactivation of a resistant parasite population after treatment is the probable explanation of relapses present either in the same place as the earlier lesion or — as a result of haematogenic dissemination — in other parts of the body such as the oropharynx or nose (*2*). Recurring infections, unlike relapses, are rare and produce lesions in new sites (*3*).

Cure after treatment for American cutaneous leishmaniasis has been defined mainly by the absence of clinical symptoms because there is still no reliable way to ascertain if treatment has been fully effective (*4*). Parasitological monitoring of treatment is very difficult because of the scarcity of amastigotes in the lesions and the difficulties in cultivating the parasites. The polymerase chain reaction (PCR) technique has improved this monitoring and is able to detect about 80% of *Leishmania* DNA in scars of patients up to 8 years after treatment (*5*). However, it appears that the occurrence of relapses depends not only on the persistence of parasites, but also the intrinsic pathogenic characteristics of the parasites and/or the immune response of the patient. According to WHO recommendations (*4*), treatment should be monitored by quantitative serological tests, because a decrease in serum antibody titres may reflect clinical improvement and cure, even though standard criteria have yet to be proposed. Resolution of infection depends predominantly on cell-mediated immune mechanisms (*2*). The skin test with *Leishmania* antigen provides a delayed-type hypersensitivity reaction and is a good marker of the cellular immune response in leishmaniasis. A weak cell-mediated immune response in American cutaneous leishmaniasis patients could be a risk factor for relapse (*3*).

This paper presents the results of the first cohort study designed to investigate clinical and laboratory predictors of American cutaneous leishmaniasis relapse after successful treatment. The study was carried out in Belo Horizonte, Brazil, where *Leishmania (Viannia) braziliensis* is responsible for 96% of American cutaneous leishmaniasis cases and *Leishmania (L.) amazonensis* for only 4% (*6*).

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Materials and methods

Study design

A consecutive cohort of American cutaneous leishmaniasis patients was assembled, treated and followed up in the same institution. After giving written informed consent, the patients were interviewed and examined by the same physician (*V.M.A.P.*), following a predefined protocol containing information on sociodemographic factors (sex, age, skin colour, birthplace, schooling, income, occupation, and place of residence) and clinical factors (clinical presentation and time of American cutaneous leishmaniasis infection, number and site of lesions, family history of American cutaneous leishmaniasis, presence of other medical conditions, and previous treatment). Clinical evaluations were performed before and immediately after treatment and every six months for a period of two years after treatment. A relapse after treatment was defined as: (a) the occurrence of a new lesion inside the borders of a previous scar, (b) the occurrence of a new mucosal lesion in patients with a cutaneous scar, or (c) the reactivation of a scar in the site of a previous mucosal lesion. Serological tests were performed before and 10 days after treatment. Individuals were followed up until either the end of the study or the date of relapse or, for those who finished the study before its completion, at the last examination before the end of the study. At the end of the study, a report was given to each patient, together with instructions to return if the lesion was reactivated.

Subjects

All patients were drawn from the outpatient clinic of the René Rachou Research Centre, Oswaldo Cruz Foundation, a reference medical service for cutaneous and visceral leishmaniasis in Belo Horizonte, Brazil. Criteria for inclusion in this study were (a) the presence of a typical skin and/or mucosal lesion, (b) no history of antimonial use, and (c) a positive result in at least one of the following laboratory examinations: skin test, indirect immunofluorescence tests, and skin biopsy for presence of parasites. All procedures were performed on the first day of examination.

The delayed-type hypersensitivity reaction was assessed in all patients by a skin test using antigen prepared from suspensions of a soluble extract of *Leishmania (L.) amazonensis* (International Code Number IFLA/BR/1967/PH8) promastigotes with a nitrogen concentration of 40 μg/ml under conditions of good manufacturing practice. A cutaneous response of $\geqslant 5$ mm in diameter, 48 h after the intradermal injection of 0.1 ml of antigen, was considered a positive test. Indirect immunofluorescence tests for detecting IgG against *Leishmania* were carried out for samples from 334 patients and repeated for 282 patients, 10 days after complete remission of the lesions; titres of $\geqslant 1{:}80$ were considered positive. The presence of *Leishmania* amastigotes was investigated in Giemsa-stained smears from skin biopsies of the lesions, taken aseptically with local anaesthesia, from 139 patients using a 4-mm punch.

Treatment

All patients were treated with intramuscular meglumine antimoniate (Glucantime)—dosage of 15 mg $Sb^{5+} \cdot kg^{-1} \cdot day^{-1}$ for cutaneous leishmaniasis patients and 20 mg $Sb^{5+} \cdot kg^{-1} \cdot day^{-1}$ for mucocutaneous leishmaniasis patients for at least 20 and 30 days, respectively (*7*).

Statistical analysis

Variation in the distribution of indirect immunofluorescence test titres was compared using Kruskal–Wallis one-way analysis of variance. Kaplan–Meier curves showing the probability of no relapse during follow-up were calculated for the total cohort and for each specific factor investigated. They were tested for heterogeneity using the log-rank test. Cox's proportional hazard regression model was used to determine the independent effect of the different factors on relapse (*8*). The assumption on proportionality of hazards was checked graphically by plotting the logarithm of the hazards. The analysis was conducted using Stata statistical software (*9*).

Results

All patients were treated regardless of whether or not they were included in the study sample. The treatment showed high efficacy: the lesions healed in 94.8% of the patients by the end of the treatment and in the remaining 5.2% within 30 days after the end of treatment. The short duration of the disease — 75% of the patients showed lesions that had evolved over a period of under 6 months — may have contributed to the efficacy of treatment. A total of 225 patients (58.7%) complained of drug side-effects such as arthralgia (10.2%), nausea (9.2%), myalgia (5.5%) and skin rash (3.4%). The side-effects were similar to those described in the literature (*10*), and did not lead to suspension of treatment. No cardiac arrhythmia or sudden death was observed (*11*).

Fifty-two out of 442 patients did not meet the criteria for inclusion in this study and seven refused to participate. Of the remaining 383 patients, 318 (83%) were successfully followed up after treatment. There was no significant difference between these participants and the 65 who were not followed up with regard to any of the factors investigated.

Table 1 shows the risk of American cutaneous leishmaniasis relapse after treatment according to sociodemographic, clinical and laboratory factors. The cohort consisted of 207 men and 111 women, with ages ranging from 6 months to 80 years (mean, 35.4 $\pm$ 18.9 years). Twenty-six patients presented other infectious diseases at the time of examination: 21 with influenza, 4 with pneumonia, and 1 with chicken-pox. There were 32 relapses, 26 occurring within 6 months, four between 7 and 12 months, and

Table 1. **Risk of American cutaneous leishmaniasis relapse after treatment according to sociodemographic, clinical and laboratory criteria**

Factors	No. of patients[a] (*n* = 32)	No. with relapse (*n* = 286)	No. with no relapse	Hazard risk[b]
Skin Test[c]				
Positive	240 (79)	17	223	1.00
Negative	63 (21)	14	49	3.44 (1.70–6.99)
Sex				
Male	207 (65)	24	183	1.00
Female	111 (35)	8	103	0.59 (0.27–1.32)
Age (years)				
0–9	29 (9)	3	26	1.00
10–50	212 (67)	24	188	1.13 (0.34–3.75)
>50	77 (24)	5	72	0.64 (0.15–2.69)
Skin colour				
Non-white	180 (57)	21	159	1.00
White	138 (43)	11	127	0.66 (0.32–1.38)
Schooling (years)				
0	59 (18)	8	51	1.00
1–4	158 (50)	13	145	0.58 (0.24–1.41)
>4	101 (32)	11	90	0.80 (0.32–2.00)
Income[d]				
< US$ 100	45 (15)	4	41	1.00
US$ 100–200	158 (51)	17	141	1.22 (0.41–3.64)
>US$ 200	103 (34)	7	96	0.77 (0.23–2.65)
Occupation				
Rural	71 (22)	5	66	1.00
Non-rural	247 (78)	27	220	1.63 (0.65–4.12)
ACL form[e]				
Cutaneous	300 (94)	31	269	1.00
Mucocutaneous	18 (6)	1	17	0.52 (0.01–3.86)
Number of lesions				
1	218 (69)	20	198	1.00
>1	100 (31)	12	88	1.41 (0.68–2.88)
Duration of disease (months)				
< 3	131 (41)	19	112	1.00
3–5	118 (37)	6	112	0.34 (0.13–0.84)
6–11	40 (13)	5	35	0.84 (0.31–2.25)
⩾ 12	29 (9)	2	27	0.45 (0.10–2.92)
Other diseases				
No	292 (92)	29	263	1.00
Yes	26 (8)	3	23	1.18 (0.36–3.87)
IFAT[f,g]				
Positive	220 (79)	24	196	1.00
Negative	60 (21)	3	57	(0.66–7.29)
Decrease of IFAT titres[f]				
No	131 (50)	14	117	1.00
Yes	132 (50)	13	119	(0.43–1.95)

[a] Values in parentheses are percentages.

[b] Values in parentheses are 95% confidence intervals.

[c] No information on 1 relapse and 14 non-relapses.

[d] No information on 4 relapses and 8 non-relapses.

[e] ACL = American cutaneous leishmaniasis.

[f] No information on 5 relapses and 33 non-relapses.

[g] IFAT = indirect immunofluorescence test.

two in the second year after treatment. Twenty-nine out of the 32 relapses occurred in the same site as the previous cutaneous lesion in patients with cutaneous leishmaniasis (Fig. 1). Two other patients with cutaneous leishmaniasis presented both a cutaneous relapse and new mucosal lesions. Only one patient with mucocutaneous leishmaniasis showed reactivation of a healed mucosal lesion.

The accumulated risk for relapse was 10.5% (95% CI: 7.5–14.6). Kaplan–Meier estimates showed

an increased risk of relapse in persons with a negative delayed-type hypersensitivity response at the time of diagnosis (Fig. 2). No significant association was found between relapse and any of the sociodemographic and clinical aspects considered. The geometric mean of the inverse of indirect immunofluorescence test titres was 89.1 at diagnosis and 38.9 ten days after treatment, with a significant decrease in indirect immunofluorescence test titres following treatment (P <0.001). However, this reduction was not associated with American cutaneous leishmaniasis relapse (Table 1). The Cox multivariate regression analysis showed that the negative delayed-type hypersensitivity response at the time of diagnosis was the only factor associated with relapse, even after adjustment for possible confounding variables (hazard risk = 3.4; 95% CI: 1.7–7.0) (Table 1).

Discussion

Only a small proportion of patients in this study were involved in rural activities, which supports the evidence in favour of re-emergence of American cutaneous leishmaniasis as an endemic infection in the crowded urban areas of Brazil (*12, 13*). Compliance with treatment was remarkably high among all the patients treated. The low income and educational level in the study cohort is a common feature among the participants in prospective studies of infectious diseases in developing countries (*14*). However, the internal validity of our results was not affected by the losses for follow-up because of the high homogeneity of the study population. The observed accumulated risk of relapse (10.5%) is similar to the rates found in other endemic areas in Brazil (*1*). Most of the relapses (30/32) occurred within one year, which reinforces the WHO recommendation that patients should be followed up for at least one year after treatment (*4*). Thirty out of the 32 relapses were cutaneous leishmaniasis patients, treated with 15 mg Sb^{5+} • kg^{-1}• day^{-1} of meglumine antimoniate, as recommended by the Brazilian National Health Authorities (*7*). It may be desirable to increase the dose used for cutaneous leishmaniasis cases caused by *L. braziliensis* to 20 mg Sb^{5+} • kg^{-1}• day^{-1}, as already suggested by WHO (*4*). No correlation between the humoral immune response and American cutaneous leishmaniasis relapse was observed. The significant decrease in indirect immunofluorescence test titres probably reflects the reduction in parasite antigen stimulation after treatment.

The sensitivity and specificity of the skin test for diagnosis of American cutaneous leishmaniasis are good. The increased risk of relapse observed in patients with a negative skin test reflects the role of cell-immune mechanisms in the control of American cutaneous leishmaniasis infection. Experimental evidence for these mechanisms exists for mice where the occurrence of relapse due to immunosuppression of clinically cured animals, previously infected with *L. mexicana*, has been observed (*15*). A negative delayed-type hypersensitivity response in cutaneous leishmaniasis and mucocutaneous leishmaniasis cases before treatment may indicate that the patient has not triggered the immune response and that the drug therapy could promote cure, as defined by the absence of clinical symptoms, without preventing relapse. The incidence of relapse in 7% of patients with a positive delayed-type hypersensitivity response suggests that other factors, not evaluated in this study, are also involved in the risk of relapse. The use of the skin test as a predictor of American cutaneous leishmaniasis relapse is expected to have an important public health impact because the test is safe, inexpensive and widely used for diagnosis of American cutaneous leishmaniasis. The test may also indicate the need to treat patients who have a negative delayed-type hypersensitivity response at the time of diagnosis with a higher dose or for a longer period of drug administration, in order to prevent relapse. Further investigations in endemic areas where other species of *Leishmania* coexist are now required to assess the consistency of our findings. ■

Fig. 1. **Relapse of a previously healed lesion in the leg of a patient with cutaneous leishmaniasis.** Arrows indicate ulcerated lesions

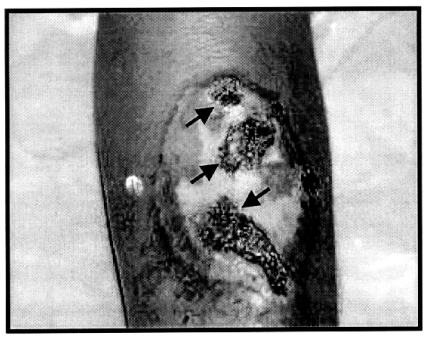

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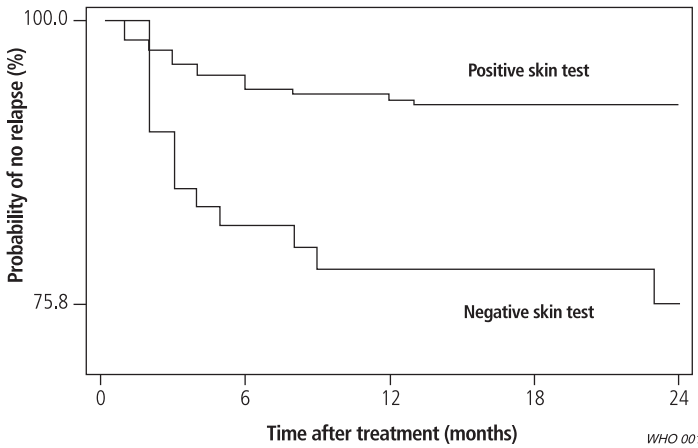
Fig. 2. **Kaplan–Meier estimates of the probability of no relapse after treatment for American cutaneous leishmaniasis.** Estimates are based on data for 17 relapses in 240 patients with a positive delayed-type hypersensitivity (DTH) and 14 relapses in 63 patients with a negative DTH. The difference between strata was statistically significant ($P = 2.0 \times 10^{-4}$ in the log-rank test)



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Résumé

Leishmaniose cutanée américaine : un test cutané comme indicateur prédictif des rechutes après traitement

Au Brésil, la leishmaniose cutanée américaine, due à *Leishmania (Viannia) braziliensis*, donne des rechutes chez environ 10 % des malades traités. En l'absence de critère fiable, la guérison après traitement de la leishmaniose cutanée américaine est principalement définie d'après les données cliniques. Cet article présente les résultats d'une étude de suivi de deux ans portant sur les rechutes chez 318 malades traités par l'antimoniate de méglumine. Le consentement éclairé a été recueilli par écrit et les malades ont été interrogés et examinés par le même médecin, suivant un protocole préétabli comportant la collecte d'informations sur les facteurs socio-démographiques (sexe, âge, couleur de la peau, lieu de naissance, niveau d'études et de revenus, profession, lieu de résidence) et cliniques (tableau clinique et durée de la leishmaniose cutanée américaine, nombre et localisation des lésions, antécédents familiaux de leishmaniose cutanée américaine, présence d'autres affections et traitements antérieurs). Une réaction d'hypersensibilité retardée a été recherchée à l'aide d'un test cutané utilisant un antigène préparé à partir de suspensions de promastigotes morts. Des tests sérologiques ont été effectués avant le traitement et 10 jours après celui-ci.

Tous les malades ont été traités par l'antimoniate de méglumine par voie intramusculaire (Glucantime à la dose de 15 mg $Sb^{5+} \cdot kg^{-1} \cdot jour^{-1}$ pour la leishmaniose cutanée et 20 mg $Sb^{5+} \cdot kg^{-1} \cdot jour^{-1}$ pour la leishmaniose cutanéo-muqueuse pendant au moins 20 et 30 jours respectivement. La rémission des lésions a été complète chez tous les patients. Un examen clinique a été réalisé avant le traitement, immédiatement après puis tous les 6 mois pendant 2 ans. Une rechute après traitement se définissait comme suit : *a*) survenue d'une nouvelle lésion à l'intérieur d'une ancienne cicatrice, *b*) survenue d'une nouvelle lésion muqueuse chez des patients porteurs d'une cicatrice cutanée, ou *c*) réactivation d'une cicatrice à l'emplacement d'une ancienne lésion muqueuse. Des courbes de Kaplan-Meier montrant la probabilité de l'absence de rechute pendant la période de suivi ont été établies pour l'ensemble de la cohorte et pour chacun des facteurs étudiés. Le modèle de régression de Cox à risques proportionnels a été utilisé pour déterminer l'effet indépendant des différents facteurs sur la survenue d'une rechute.

La cohorte se composait de 207 malades de sexe masculin et 111 de sexe féminin, âgés de 6 mois à 80 ans (moyenne 35,4 $\pm$ 18,9 ans), dont 300 (94,3 %) étaient atteints de leishmaniose cutanée et 18 (5,7 %) de leishmaniose cutanéo-muqueuse. Le niveau d'études et de revenus de la plupart des malades était faible. Seuls 71 d'entre eux (22,3 %) travaillaient dans l'agriculture ou la sylviculture. On a observé 32 rechutes, dont 26 pendant les 6 mois suivant le traitement, 4 entre 7 et 12 mois après le traitement et 2 au cours de la deuxième année de suivi. Vingt-neuf rechutes ont eu lieu à l'emplacement d'une lésion cutanée antérieure chez les malades atteints de la forme cutanée. Deux autres malades atteints de cette forme ont présenté à la fois une rechute cutanée et de nouvelles lésions muqueuses. Seul un malade atteint de leishmaniose cutanéo-muqueuse a présenté une réactivation d'une ancienne lésion muqueuse cicatrisée. La plupart des rechutes (30/32) sont survenues dans l'année qui a suivi le traitement, ce qui confirme la recommandation de l'OMS (1990) selon laquelle les patients doivent être suivis pendant au moins un an après le traitement. Le risque cumulatif de rechute était de 10,5 %. Il n'y avait pas d'association entre les rechutes et la réponse en anticorps spécifiques du parasite avant et après traitement, ni entre les rechutes et la stratification selon les caractéristiques socio-démographiques et cliniques des malades.

Le test cutané est basé sur une réaction d'hypersensibilité retardée et constitue un bon marqueur de la réponse immunitaire cellulaire dans la leishmaniose. Chez les malades dont le test cutané était négatif au moment du diagnostic, le risque de rechute de leishmaniose cutanée américaine était 3,4 fois plus élevé que chez ceux dont le test était positif. L'utilisation du test cutané comme indicateur prédictif des rechutes de leishmaniose cutanée américaine devrait avoir un impact important en santé publique car ce test est sans danger, peu coûteux et largement utilisé pour le diagnostic de la maladie. Il peut également signaler la nécessité, pour empêcher les rechutes, de traiter par des doses plus élevées ou pendant plus longtemps les malades n'ayant pas présenté de réaction d'hypersensibilité retardée au moment du diagnostic. De nouvelles investigations dans des zones d'endémie où coexistent d'autres espèces de *Leishmania* sont maintenant nécessaires pour confirmer nos observations.

Resumen

Leishmaniasis cutánea americana: uso de una prueba cutánea como factor predictivo de las recaídas tras el tratamiento

La leishmaniasis cutánea americana, causada por *Leishmania (Viannia) brazifensis*, da lugar a recaídas en aproximadamente un 10% de los pacientes tratados en el Brasil. A falta de un criterio fiable, las curaciones postratamiento de la leishmaniasis cutánea americana se han definido principalmente atendiendo a los datos clínicos. En este artículo se presentan los resultados de un estudio de seguimiento de dos años de las recaídas sufridas por una cohorte de 318 pacientes aquejados de leishmaniasis cutánea americana después de ser tratados con antimoniato de meglumina. Se obtuvo el consentimiento informado por escrito y los pacientes fueron entrevistados y examinados por el mismo médico, con arreglo a un protocolo predefinido con información sobre factores sociodemográficos (sexo, edad, color de la piel, lugar de nacimiento, educación y nivel de ingresos, ocupación y lugar de residencia) y factores clínicos (cuadro clínico y momento de aparición de la leishmaniasis cutánea americana, número de lesiones y lugar de las mismas, historia familiar de leishmaniasis cutánea americana, concurrencia de otras afecciones y tratamiento anterior). Se evaluó la respuesta de hipersensibilidad retardada mediante una prueba cutánea en la que se empleó antígeno preparado a partir de suspensiones de promastigotes muertos. Se realizaron pruebas serológicas antes del tratamiento y 10 días después del mismo.

Todos los pacientes fueron tratados con antimoniato de meglumina intramuscular (Glucantime®): dosis de 15 mg de $Sb^{5+} * kg^{-1} * día^{-1}$ en los pacientes con leishmaniasis cutánea, y de 20 mg de $Sb^{5+} * kg^{-1} * día^{-1}$ en los pacientes con leishmaniasis mucocutánea, durante al menos 20 y 30 días, respectivamente (7). Hubo remisión completa de las lesiones en todos los pacientes. Se realizaron evaluaciones clínicas antes e inmediatamente después del tratamiento, y cada seis meses durante dos años después del tratamiento. Se definió como recaída postratamiento toda: (a) aparición de una lesión nueva dentro de los límites de una cicatriz anterior, (b) aparición de una nueva lesión mucosa en pacientes con una cicatriz cutánea, o (c) reactivación de una cicatriz en el lugar de una lesión mucosa previa. Se trazaron las curvas de Kaplan-Meier, que muestran la probabilidad de que no se produzca ninguna recaída durante el seguimiento, para el conjunto de la cohorte y para cada factor específico investigado, y se utilizó el modelo de riesgo instantáneo proporcional de Cox para determinar el efecto independiente de los diferentes factores de recidiva.

La cohorte estaba constituida por 207 hombres y 111 mujeres, con edades de entre 6 meses y 80 años (media = 35,4 $\pm$18,9 años); 300 (94,3%) pacientes sufrían leishmaniasis cutánea, y 18 (5,7%) leishmaniasis mucocutánea. El nivel de educación y de ingresos de la mayoría de los pacientes era bajo. Sólo 71 (22,3%) realizaban tareas agrícolas o silvícolas. Se registraron 32 recaídas, 26 dentro de los 6 primeros meses de tratamiento, 4 al cabo de entre 7 y 12 meses, y 2 durante el segundo año tras el tratamiento. Veintinueve de las 32 recaídas se manifestaron en el mismo lugar afectado por la lesión cutánea previa en los pacientes con leishmaniasis cutánea. Otros dos pacientes con leishmaniasis cutánea presentaron tanto una recaída cutánea como nuevas lesiones mucosas. Sólo en un paciente con leishmaniasis mucocutánea se observó una reactivación de una lesión mucosa cicatrizada. La mayoría de las recaídas (30/32) ocurrieron en el término de un año, lo que corrobora la recomendación de la OMS (1990) de que se debe seguir a los pacientes durante al menos un año después del tratamiento. El riesgo acumulado de recaída fue del 10,5%. No se observó ninguna relación entre la recaída y la respuesta de producción de anticuerpos específicos contra el parásito antes y después del tratamiento, o entre la recaída y la estratificación por características sociodemográficas y clínicas.

La prueba cutánea provoca una reacción de hipersensibilidad retardada y es un buen marcador de la respuesta inmunitaria celular en la leishmaniasis. Los pacientes con una prueba cutánea negativa en el momento del diagnóstico presentaron un riesgo 3,4 veces mayor de recaída que los que habían dado positivo. Se prevé que el uso de la prueba cutánea como factor predictivo de recidiva de leishmaniasis cutánea americana tendrá gran repercusión en la salud pública, ya que la prueba es segura y económica, amén de ampliamente usada para el diagnóstico de la leishmaniasis cutánea americana. La prueba parece indicar también que a los pacientes en los que no se observa ninguna reacción de hipersensibilidad retardada en la prueba cutánea en el momento del diagnóstico se les debe tratar con una dosis mayor o durante más tiempo para prevenir las recaídas. Es necesario realizar ahora nuevas investigaciones en las zonas endémicas en que coexisten otras especies de *Leishmania* a fin de evaluar la coherencia de estos resultados.

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