Caso Clínico

Leishmaníase do Novo Mundo: O Papel da Microscopia Confocal no Diagnóstico e Seguimento - Dermatologia Tropical

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RESUMO – A leishmaníase cutânea pode confundir-se com outras infeções em áreas endémicas sobreponíveis e o tratamento atempado previne a sua disseminação. Os exames histopatológico e parasitológico, nem sempre acessíveis, confirmam o diagnóstico em diferido. Em contraste, a microscopia confocal de reflectância (MCR) permite a observação em tempo real com resolução celular até à derme papilar. Observámos um homem de 59 anos, brasileiro, por placas e tumores ulcerados disseminados nas extremidades. Clinicamente, o diagnóstico diferencial incluía leishmaníase e outras infeções com disseminação linfocutânea. Em MCR, observou-se a característica imagem de «ovos em ninho de aves» característica de leishmaníase cutânea. O diagnóstico de leishmaníase foi confirmado em biópsia cutânea, identificando-se a espécie *Leishmania guyanensis* no exame parasitológico. Após tratamento com anfotericina B lipossómica, a reavaliação com MCR permitiu corroborar a cura clínica, numa imagem de «ninho vazio». Em conclusão, a MCR, de forma não invasiva, apoia o diagnóstico e seguimento da leishmaníase cutânea.
PALAVRAS-CHAVE – Dermoscopia; Leishmania; Leishmaníase Cutânea/diagnóstico; Microscopia Confocal.

New World Leishmaniasis: The Role of Confocal Microscopy in Diagnosis and Follow-up - Tropical Dermatology

ABSTRACT – *Cutaneous leishmaniasis may mimic other infections in overlapping endemic areas and timely treatment prevents dissemination of the parasite. The required histopathological and microbiological examinations are not always available, and can only give a deferred confirmation of the diagnosis. In contrast, reflectance confocal microscopy (RCM) allows real-time visualization till the level of papillary dermis. A 59-year-old Brazilian male presented with ulcerated plaques and tumors on the extremities. The clinical differential diagnosis included leishmaniasis and other infections with lymphocutaneous pattern of dissemination. RCM showed the characteristic picture of «eggs in a bird's nest» which has been described in cutaneous leishmaniasis. The diagnosis of leishmaniasis was later confirmed by skin biopsy, in which Leishmania guyanensis was identified by parasitological examination. After treatment with liposomal amphotericin B, reassessment with RCM corroborated the clinical cure, showing an «empty nest» picture. In conclusion, RCM noninvasively provides useful information for diagnosis and follow-up of cutaneous leishmaniasis.*
KEYWORDS – *Dermoscopy; Leishmania; Leishmaniasis, Cutaneous/diagnosis; Microscopy, Confocal.*

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DOI: https://dx.doi.org/10.29021/spdv.76.4.972

Recebido/*Received*
15 Julho/*July* 2018
Aceite/*Accepted*
17 Setembro/*September* 2018

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INTRODUCTION

Cutaneous leishmaniasis is distributed worldwide and can mimic other cutaneous infections in the same endemic areas.[1] Unlike visceral leishmaniasis, which is concentrated in particular regions of Asia, Sudan and Brazil, cutaneous leishmaniasis is evenly distributed in Western Asia, the Mediterranean region, and Latin America.[2] A recent rise in non-endemic areas is attributable to international travel, whether by immigrants, refugees, tourists, or soldiers.[3] Early recognition of leishmaniasis allows a prompt treatment, which may prevent dissemination of the infection. However, the required histopathological examination and microbiological studies are not always available; furthermore, the aforementioned classical diagnostic tools give only a deferred confirmation of the diagnosis. In contrast, reflectance confocal microscopy (RCM) allows for a real-time visualization of skin structures with cellular resolution, down to the level of papillary dermis.

In cutaneous leishmaniasis, intracytoplasmic forms of the parasites (amastigotes) are mostly found in the papillary dermis. The findings of RCM in two cases of cutaneous leishmaniasis have been described[4,5] but the role of RCM for monitoring after treatment has hitherto not been assessed.

CASE REPORT

A 59 year-old male Brazilian patient, Fitzpatrick phototype V, presented to the Emergency Department of our Hospital with ulcerated plaques on the extremities. He had lived for 2 months near Tefé region, by the river Solimões, in the Amazon forest and was seeking political asylum in Portugal.

Six weeks earlier, a painless ulcer appeared on the right leg and subsequently new lesions progressively developed on both legs and arms. On physical examination, ulcerated plaques and tumors were seen on the extremities in a lymphocutaneous dissemination pattern (Fig. 1); inguinal and axillary non-tender 1 to 2 cm lymph nodes were palpable; involvement of mucosa was not apparent and the patient was afebrile.

The clinical differential diagnosis included leishmaniasis and other infections with lymphocutaneous ("sporotrichoid") dissemination pattern, namely sporotrichosis, mycobacteriosis, nocardiosis and yaws.

The immediately available diagnostic tools were performed: dermoscopy and RCM. On dermoscopy, besides ulceration, yellow tear-shaped globules and polymorphous vessels were identified (Fig. 2a). RCM showed the picture of «eggs in a bird's nest» (Fig. 2b,c), characteristically ascribed to cutaneous leishmaniasis. A skin biopsy was performed to histopathological and microbiological examination. Histologically, in H&E and Giemsa, macrophages were present within a dermal granulomatous infiltrate containing cytoplasmic amastigotes, which were positively stained by immunohistochemistry with anti-Leishmania antibody (Fig. 3). Direct parasitological examination and culture identified a Leishmania parasite. DNA-based methods (PCR-ITS1-RFLP and sequencing) identified the sub-genus Viannia, and *Leishmania guyanensis* was speciated by MLST analysis.

A chest radiography and abdominal ultrasound revealed no abnormalities. Mycological studies were negative for fungi (direct examination and culture of a skin sample); serological tests were negative for Leishmania, HIV and Treponema.

Diagnosed with disseminated New World cutaneous leishmaniasis, the patient was treated with intravenous liposomal amphotericin B (3 mg/kg/d) for 10 days, with no relevant toxicity. Within 2 weeks, a scale-crust replaced the ulcerated lesions; healing was complete 6 weeks after treatment, leaving postinflammatory hyperpigmented patches. Reassessment with RCM corroborated the clinical cure, showing the disappearance of the characteristic structures, in an «empty nest» picture (Fig. 4). One year after treatment, there was no recurrence.

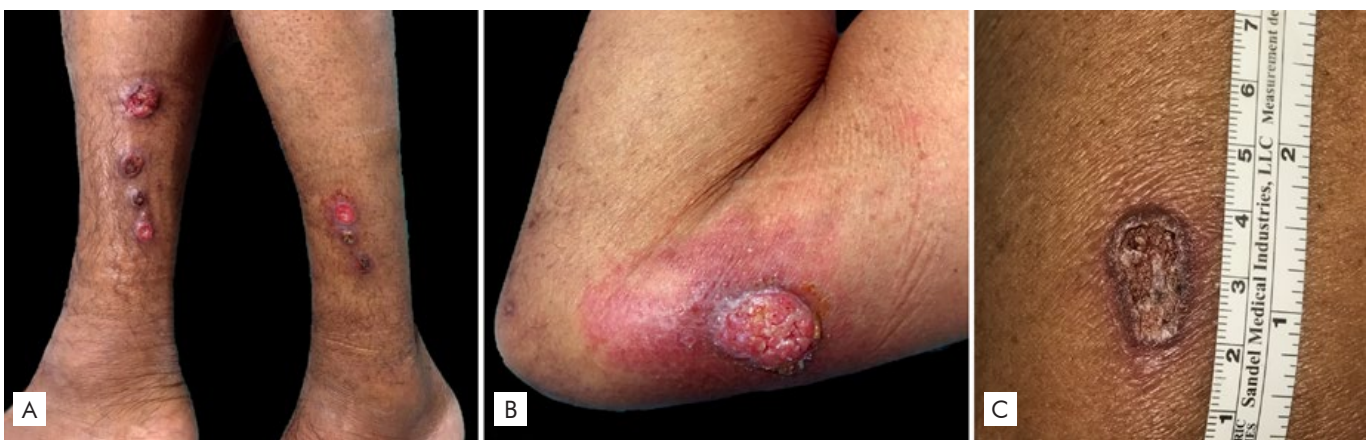

Figure 1 - New World cutaneous leishmaniasis: clinical presentation. Ulcerated plaques and tumors, with erythematous and violaceous "volcano-like" borders and cartilaginous consistency. A lymphocutaneous dissemination pattern is present on the legs (**A**), and lesions are also seen on the right (**B**) and left (**C**) arms.

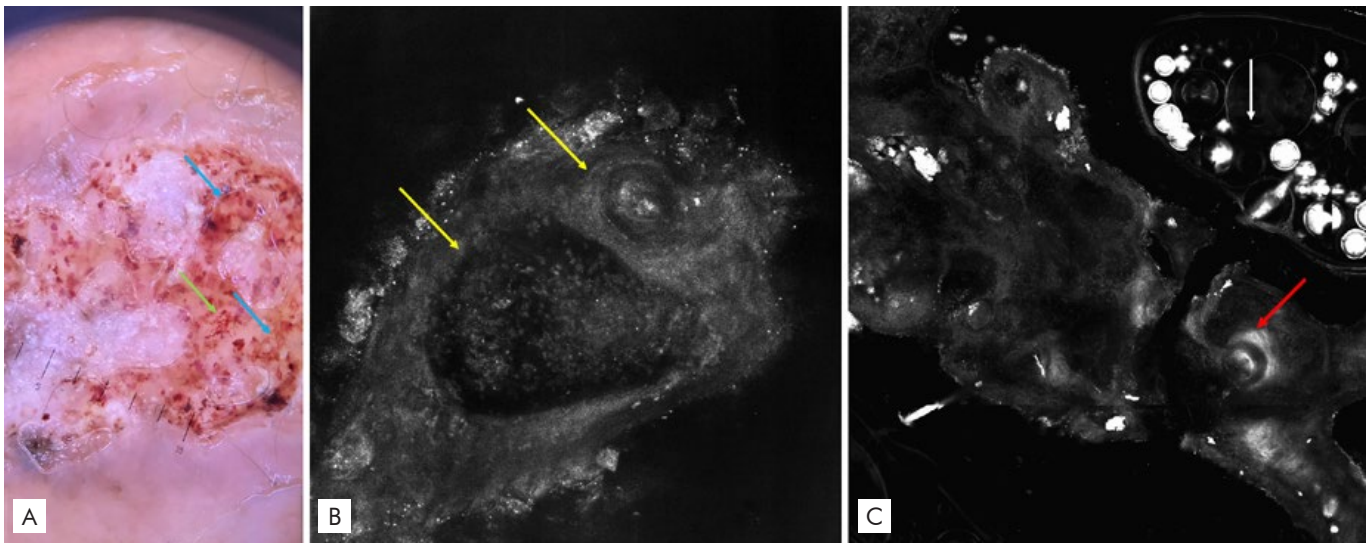

Figure 2 - New World cutaneous leishmaniasis: dermoscopy and confocal microscopy (Vivascope 1500™) performed on the left arm lesion (Fig. 1c). Dermoscopy (**A**) revealed ulceration, yellow globules: "yellow drops" (blue arrows) and polymorphous vessels (green arrow). Confocal microscopy images of the papillary dermis ((**B**) basic image 0.5 x0.5 mm, and (**C**), Mosaic image 3x3 mm) show hyperreflexive perifollicular fibers (red arrow) surrounding granulomas and multinucleated giant cells, together forming the picture of "eggs in a bird's nest" (yellow arrow); erosions (white arrow).

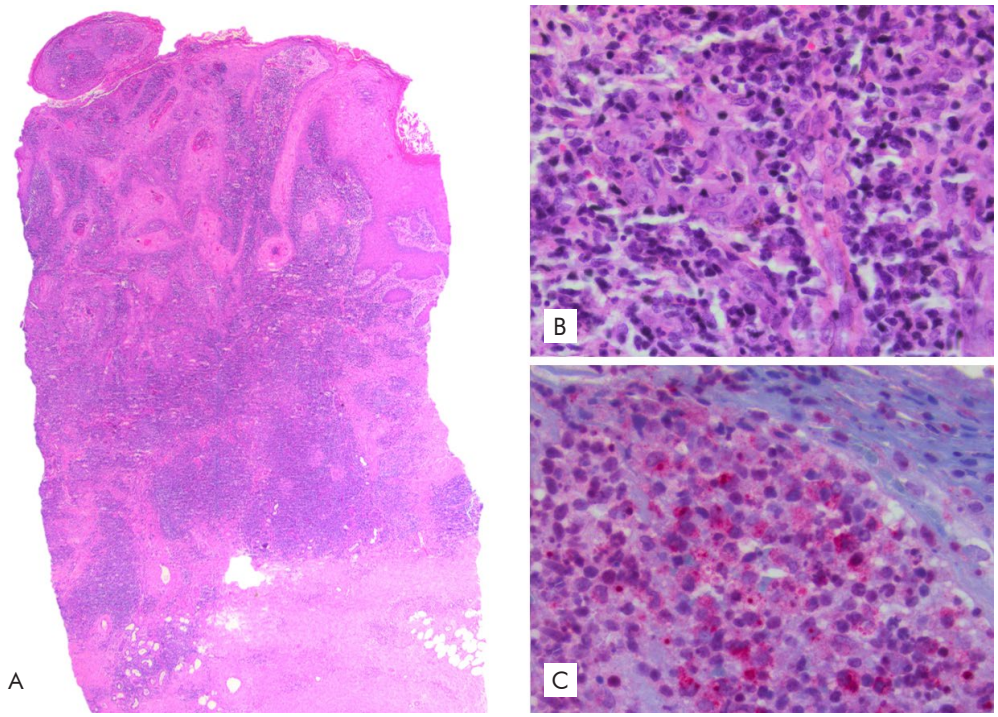

Figure 3 - New World cutaneous leishmaniasis: skin biopsy. Diffuse dermal granulomatous inflammatory infiltrate (**A**), rich in plasma cells, and covered by pseudoepitheliomatous epidermal hyperplasia; (**B**) basophilic cytoplasmic inclusion bodies in dermal histiocytes, corresponding to amastigotes (hematoxylin-eosin, original magnification x16 and x400, respectively); (**C**) positive immunohistochemistry staining with anti-Leishmania antibody (original magnification x400).

DISCUSSION

Leishmaniasis is endemic to more than 90 countries worldwide and world travel has brought the parasite to nonendemic regions.[1] The intracellular protozoa of the genus Leishmania is transmitted to humans by sandflies and is estimated to cause the ninth largest disease burden among individual infectious diseases.[2] The severity of the parasitic infection varies from a single self-healing painless skin

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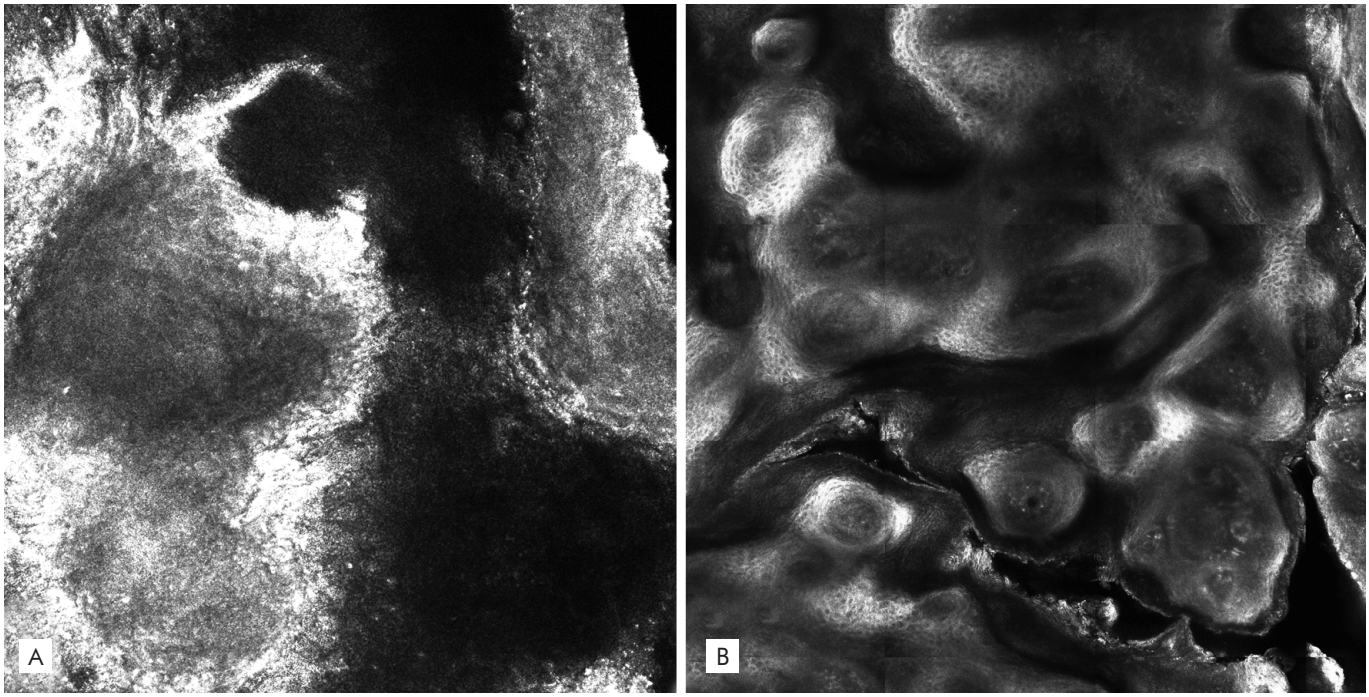

Figure 4 - New World cutaneous leishmaniasis: follow-up with confocal microscopy (Vivascope 1500™ (**A**) Basic image 0.5 x0.5 mm, (**B**) Mosaic image 2x2 mm). One month after treatment, confocal microscopy examination confirmed the disappearance of the previously identified structures: the picture of an "empty nest" in the papillary dermis.

ulcer to a life-threatening systemic infection, called visceral leishmaniasis. Cutaneous leishmaniasis is widely distributed, with about one third of cases occurring in each of these three regions: the Americas, and the Mediterranean basin, Middle East and Central Asia[2], according to which it is classified as New World and Old World leishmaniasis, respectively.

The diagnosis of leishmaniasis can be challenging because it may mimic both infectious and malignant conditions. The treatment for leishmaniasis is often toxic; therefore, diagnostic confirmation is highly suggested. Differentiation between conditions that mimic cutaneous leishmaniasis may require microbiologic and histologic evaluation, as well as molecular studies.6 Unfortunately, diagnostic sensitivity of histopathology is only about 50% to 70%[7,8] and the parasite can only be identified in 70% of cases of cutaneous leishmaniasis and 50% of cases of mucocutaneous leishmaniasis, even in experienced centers.[9,10] Diagnosis may also be achieved via polymerase chain reaction (PCR) studies on skin samples, serologic assays, isoenzyme analysis, and monoclonal antibody analysis.[1] The CDC recommends using several techniques and obtaining multiple specimens from different lesions - or different portions of the same lesion - in order to increase diagnostic sensitivity.[11]

Dermatoscopic evaluation may provide helpful clues to support a clinical suspicion of cutaneous leishmaniasis.[12] Dermoscopy in cutaneous leishmaniasis typically shows generalized erythema (in virtually all lesions), reddish-yellow structureless areas with a pinkish halo, intermingled with drop or tear-shaped yellow globules named "yellow tears" (40% to 53%), corresponding to keratin plugs; and a combination of different vascular patterns (88%), often with branching focused telangiectasias at the periphery. Other dermoscopic features include hyperkeratosis (50%), central erosion/ulceration (46%), and a white, starburst-like pattern corresponding to parakeratotic hyperkeratosis (19% to 39%).[4,5,12,13] The characteristic "yellow tears", central erosion and polymorphous vessels were observed in this case.

RCM is a noninvasive real-time imaging technique for examination of the skin at a cellular resolution,[14] that allows cytomorphological features to be assessed to a depth of 350 μm, which corresponds to the papillary dermis. The clinical spectrum of application of RCM keeps expanding progressively, and increasing evidence supports its diagnostic value in different cutaneous infections, like parasitoses and infestations.[15-18]

In the present case, RCM allowed for immediate noninvasive visualization of the characteristic aspects of cutaneous leishmaniasis in a patient with a relatively broad clinical differential diagnosis. Moreover, RCM was able to accurately assess and corroborate the efficacy of treatment – a finding which has not yet been described.

This case's findings on RCM were in accordance with previous reports of cutaneous leishmaniasis[4]: a) granulomas, looking similar to hair follicles but smaller and disconnected from the skin surface, are observed; b) the most striking features in the dermis are hyperreflecting interwoven fibers

forming roundish structures; c) within these, follicles and granulomas present as bright oval structures, giving the appearance of "eggs in a bird's nests". Other RCM features of cutaneous leishmaniasis include intradermal mixed inflammatory infiltrate, linear and comma-shaped vessels and multinucleated giant cells, amorphous material and "brick-like" structures.[5]

In conclusion, leishmaniasis, one of the "neglected diseases," remains a worldwide health problem with many different and complex clinical presentations. RCM provides useful information for the initial approach to a suspected case of cutaneous leishmaniasis. As a bedside real-time imaging technique, RCM may guide the selection of ancillary studies in order to efficiently confirm the diagnosis. As a noninvasive method, RCM may be also useful for the follow-up and monitoring of treatment efficacy in cutaneous leishmaniasis.

Acknowledgements

The authors thank Professor Lenea Campino, MD PhD, President of the Scientific Council and Head of Leishmaniasis Department in Instituto de Higiene e Medicina Tropical for the laborious and successful work of speciation. The authors also thank Dr Tiago Marques, MD, specialist in Infectious Diseases in Hospital de Santa Maria, for his collaboration in the treatment and follow-up of the patient.

Conflitos de interesse: Os autores declaram não possuir conflitos de interesse.
Suporte financeiro: O presente trabalho não foi suportado por nenhum subsídio ou bolsa.
Confidencialidade dos dados: Os autores declaram ter seguido os protocolos do seu centro de trabalho acerca da publicação dos dados de doentes.
Protecção de pessoas e animais: Os autores declaram que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pelos responsáveis da Comissão de Investigação Clínica e Ética e de acordo com a Declaração de Helsínquia da Associação Médica Mundial.

Conflicts of interest: *The authors have no conflicts of interest to declare.*
Financing Support: *This work has not received any contribution, grant or scholarship.*
Confidentiality of data: *The authors declare that they have followed the protocols of their work center on the publication of data from patients.*
Protection of human and animal subjects: *The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).*

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