Surgical and Experimental Pathology

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Neoplastic cells parasitized by *Mycobacterium leprae*: report of two cases of melanocytic nevus and one of basal cell carcinoma

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Abstract

Background: Leprosy is a major public health concern in many developing countries. *Mycobacterium leprae (M. leprae)*, the causative agent of leprosy, has the ability to parasitize different types of human cells and tissues. In this article, the uncommon parasitism of nevus and neoplastic and epithelial cells by *M. leprae* are described using histological sections doubly stained by Fite-Faraco (FF) and immunohistochemistry (IHC), respectively.

Case presentation: Three patients with borderline-lepromatous and lepromatous leprosy underwent excision of melanocytic nevi (cases 1–2) and basal cell carcinoma (case 3). Histological sections confirmed the clinical diagnosis of melanocytic nevus and basal cell carcinoma, but leprosy lesions were evident in adjacent tissues. FF staining and IHC (CD68, Melan A, and 34βE12) demonstrated the presence of bacilli within the nevus cells and also in the epithelial cells of the basal cell carcinoma.

Conclusions: *M. leprae* has the ability to parasitize different types of cells, including neoplastic cells. Pathologists working in settings where leprosy is endemic should be aware that some cutaneous biopsy specimens may also present with leprosy findings.

Keywords: Leprosy, Carcinoma, Basal cell, Nevus, pigmented, *Mycobacterium leprae*

Background

Leprosy is an infectious disease caused by *Mycobacterium leprae* (*M. leprae*), and it remains a major public health problem in South America, Eastern Mediterranean, Western Pacific, Africa, and South-East Asia (Rao 2017). Leprosy is an insidious and spectral disease that is clinically able to mimic inflammatory to neoplastic diseases (Talhari et al. 2015). In the Ridley and Jopling classification, there are two polar forms (i.e., tuberculoid [TT] and lepromatous [LL]) and three intermediate or borderline forms (i.e., borderline tuberculoid [BT], borderline borderline [BB], and borderline lepromatous [BL]) (Ridley and Jopling 1966; Fachin et al. 2017).

Leprosy in the tuberculoid spectrum is a disease limited to few lesions, and the tuberculoid pattern histologically appears with granulomas mainly around neural filaments and piloerector muscle (Talhari et al. 2015; Ridley and Jopling 1966). Bacilloscopy is commonly negative (0+), or positive with a low bacilloscopic index (1 or 2+) (Ridley and Jopling 1966; Fachin et al. 2017). Meanwhile, patients in the *lepromatous* spectrum present disseminated skin and systemic involvement, and granulomas are diffuse, consisting mainly of multivacuolated macrophages containing a large number of bacilli (smear microscopy of 5 to 6+) (Ridley and Jopling 1966). Previous report described the parasitism of macrophages, sweat and sebaceous glands, hair follicles, piloerector muscles, Schwann cells, and perineural and endothelial cells (Massone et al. 2015). In our leprosy referral unit, we have observed the parasitism of squamous

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epithelial cells of the epidermis and pilosebaceous follicle, myoepithelial cells of adjoining glands of the skin, and melanocytes and smooth muscle cells of the vessel wall (unpublished observations, Soares, C.T.).

Herein, we describe three cases of patients with BL or LL. Two had melanocytic nevi and one had basal cell carcinoma, all exhibiting parasitism by *M. leprae*.

Case presentation

Case 1

A 43-year-old man with unremarkable past history was referred to the Dermatology department complaining of painless nodules in his lower limbs that appeared 4 months ago. He had not undergone previous treatments for these symptoms. Clinical examination revealed multiple painless nodules in the lower limbs and diffuse infiltration of the skin associated with erythema and bilateral total madarosis.

A blackish, asymmetric, polychromic macula measuring approximately 8 mm, which was not previously seen by the patient, was found on the dorsum (Fig. 1a and b). At dermatoscopic examination, this lesion had an irregular pigment network, poorly defined borders, regression areas, and a blue-gray veil (Fig. 1c). A diagnosis of

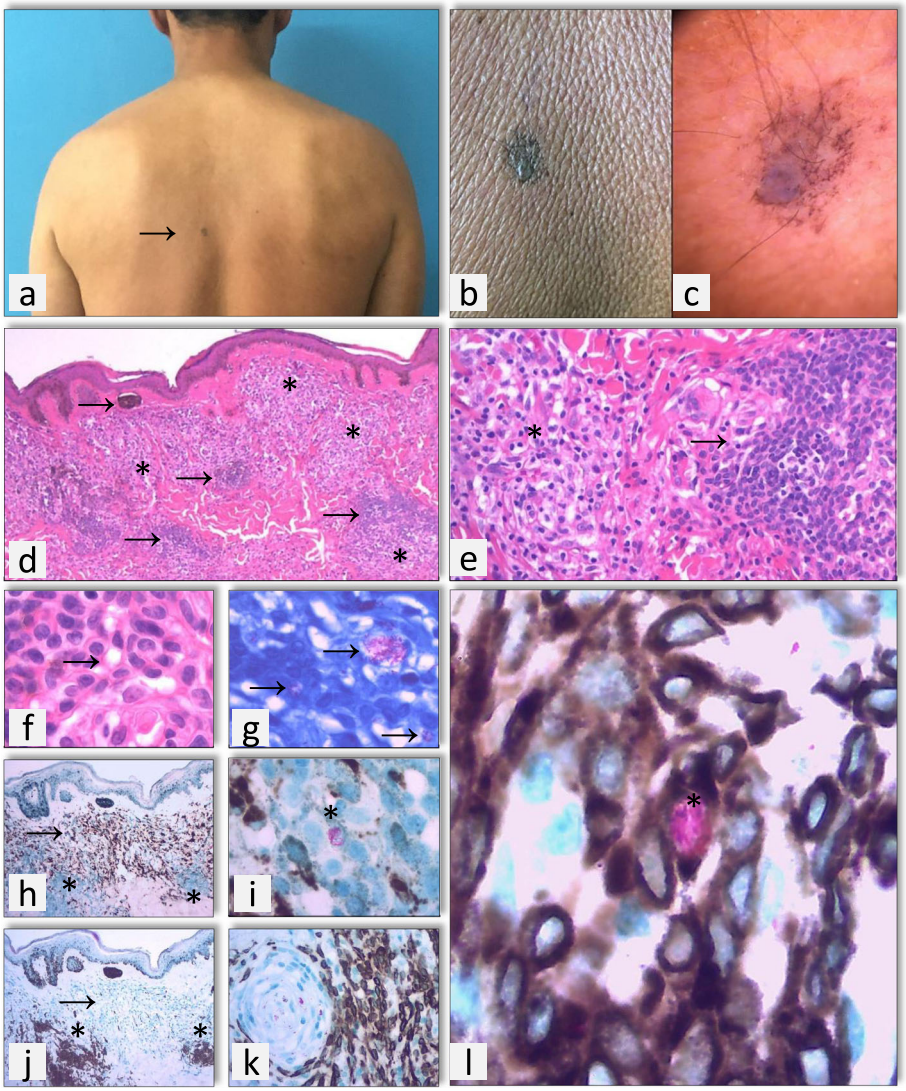

Fig. 1 Parasitism of melanocytic nevus by *M. leprae* in case 1. **a** and **b** Nevus lesion in the dorsum of a patient with leprosy BL. **c** Dermatoscopy showing the nevus lesion and adjacent skin without characteristics of a leprosy lesion. **d** and **e** Histological sections showing compound melanocytic nevus (→) and leprosy lesion (*) permeating nevus cells (HE). **f** and **g** Vacuolated nevus cells (HE) containing bacilli (FF) (→). **h** and **i** Double staining (IHC-CD68 and FF) showing leprosy lesion macrophages stained (→) and non-stained nevus cells (*) containing bacilli in the cytoplasm. **j** and **k** Double staining (IHC-Melan A and FF) showing non-immunostained leprosy lesion (→) and immunostained nevus cells (*), both containing bacilli in the cytoplasm. **l** Double staining (IHC-Melan A and FF) showing immunostained nevus cells (*) containing bacilli inside the intracytoplasmic vacuole

leprosy in the *lepromatous* pole (BL-LL) was considered, and the pigmented lesion was suspected to be a melanocytic nevus or melanoma.

The patient underwent incisional biopsies for leprosy investigation and an excisional biopsy of the melanocytic lesion. The histological analyses showed signs of active and progressing BL leprosy in both biopsy specimens.

The pigmented lesion exhibited histological characteristics of a compound melanocytic nevus; concomitantly, a BL pattern infiltrate was evident in the tissues adjacent to the nevus (Fig. 1d and e). Fite-Faraco (FF) (bacilloscopy) and double staining (immunohistochemistry [IHC] and FF) of the histological sections showed intact and fragmented bacilli in the leprosy infiltrates (5+) and also inside the melanocytic nevus cells (Fig. 1f-l).

Case 2

A 46-year-old man with a previous diagnosis of LL was referred by another Dermatology outpatient unit after completion of a 12-dose multibacillary multidrug therapy. During routine follow-up, he presented with regressive lesions, with some papules distributed throughout the body. Thus, he was indicated for a new cutaneous biopsy, with a clinical suspicion of leprosy lesion of the lepromatous pole in regression. The histological characteristics showed an LL lesion in regression associated with a melanocytic dermal nevus (Fig. 2a-c). Bacilloscopy (FF) of the histological sections and double staining (IHC and FF) revealed numerous fragmented (5+) bacilli within the macrophages of the leprosy infiltrates as well as in the cells within the melanocytic lesion (Fig. 2d-j).

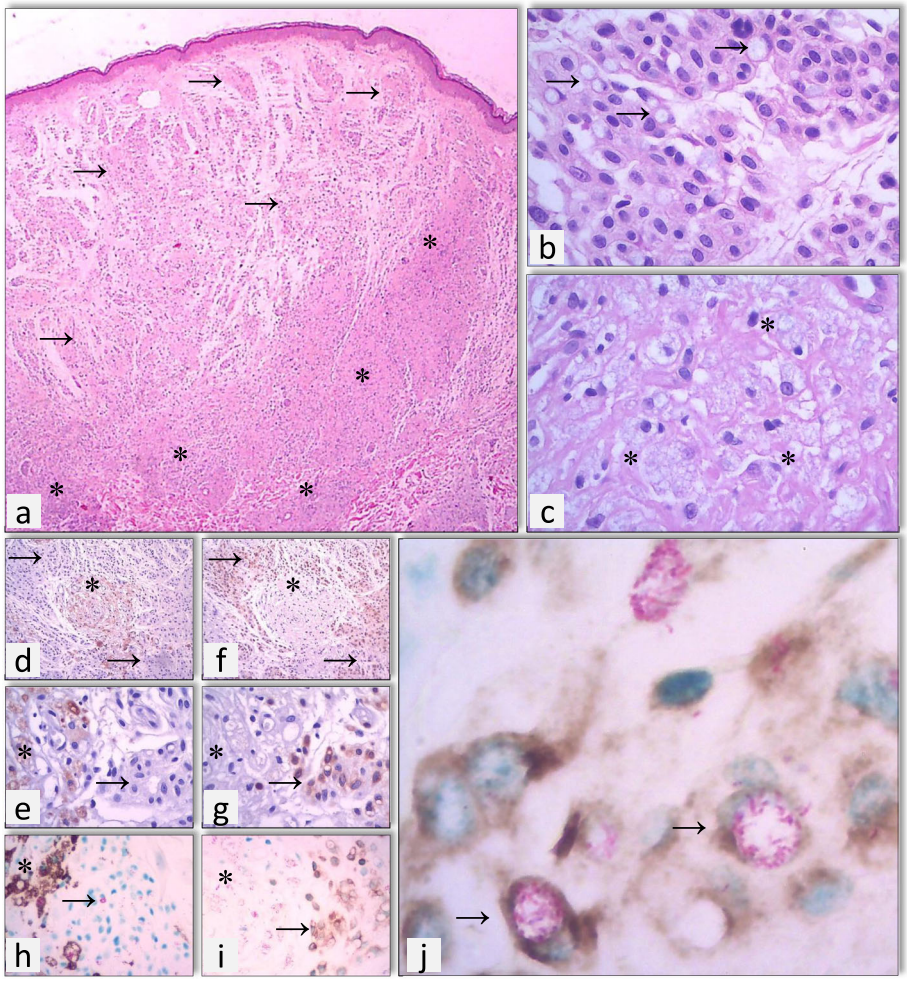

Fig. 2 Parasitism of melanocytic nevus by *M. leprae* in case 2. **a** Histological sections showing intradermal melanocytic nevus (→) and leprosy lesion (*) (HE). **b** Melanocytic nevus cells characteristically have intracytoplasmic vacuoles that are filled by *M. leprae* (→) (HE). **c** Leprosy lesion adjacent to nevus cells with LL characteristics (*). **d** and **e** Immunohistochemistry (IHC) showing CD68-positive macrophages (*) and adjacent negative nevus cells (→). **f** and **g** IHC showing melanocyte-positive cells for Melan A (→) and negative macrophages in adjacent tissues (*). **h** Double staining (IHC-CD68 and FF) showing leprosy lesion macrophages immunostained (*) and non-immunostained nevus cells (→) containing bacilli in the cytoplasm. **i** Double staining (IHC-Melan A and FF) showing non-immunostained leprosy lesion (*) and immunostained nevus cells (→), both containing bacilli in the cytoplasm. **j** Double staining (IHC-Melan A and FF) showing immunostaining nevus cells (→) containing bacilli inside the intracytoplasmic vacuole

Case 3

A 69-year-old man was referred to our Dermatology unit with subacute eczema in the lower limbs. Clinical examination showed exudative plaque-like lesions on the lower limbs associated with edema, erythematous-scaly plaques on the upper limbs, truncated limb papules, and diffuse xerosis. Several erythematous plaques and small papules distributed throughout the body showed decreased sensitivity associated with diffuse cutaneous infiltration and leprosy-compatible peripheral nerve thickening (BL-LL) (Fig. 3a).

Histopathological examination of an abdominal skin biopsy specimen showed diffuse infiltration by multivacuolated macrophages, accompanied by rare lymphocytes and plasma cells. The bacilloscopy of the histological sections (FF) showed a large number of intact and fragmented (6+) bacilli inside macrophages, neural branches, erector muscle of the hair, vessel walls, endothelium, and some in the adnexal glands. Accordingly, a diagnosis of BL leprosy was made (Fig. 3b and c). A lesion was found in the patient's left infraorbital lesion; it had pearlaceous borders, telangiectasia, and an ulcerated center compatible with basal cell carcinoma (Fig. 3d and e).

The infraorbital lesion was excised, and histological examination found proliferation of basaloid cells infiltrating the dermis with features of basal cell carcinoma and leprosy infiltrate (BL) in the adjacent dermis (Fig. 4a). FF staining and double staining (IHC and FF) showed numerous bacilli inside macrophages, other adjacent cells, and also within vacuoles in the cytoplasm of basal cell carcinoma cells (Fig. 4b-h).

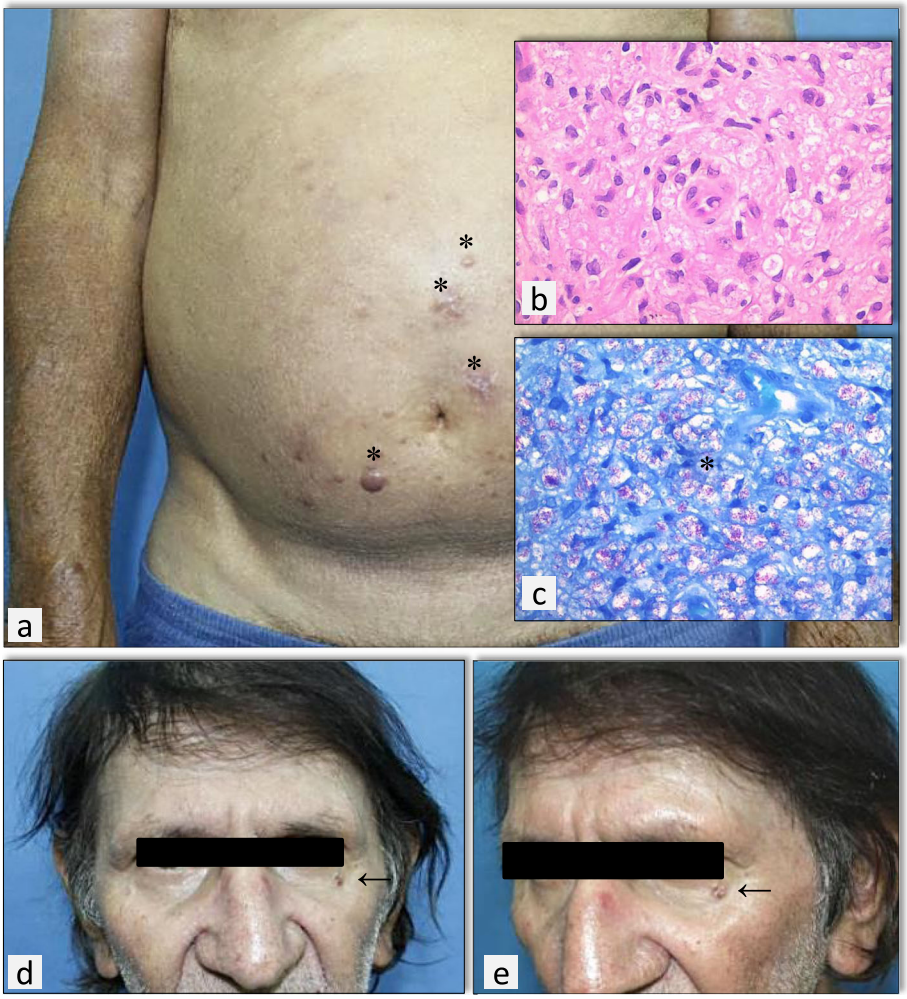

Fig. 3 Clinical characteristics of leprosy lesions and basal cell carcinoma in case 3. **a** Patient with leprosy in the borderline Virchowian form (BL) presenting papule-nodular lesions (hansenomas) in the abdomen (*). **b** Histological sections (HE) of abdominal lesion showing leprosy lesions compatible with BL and characterized by multivacuolated histiocytes. **c** Bacilloscopy of histological sections (FF) shows numerous bacilli in the cytoplasm of macrophages (*). **d** and **e** On the face, the patient presents madarosis associated with a pigmented lesion with features of basal cell carcinoma, located in the lower left peripalpebral region (←)

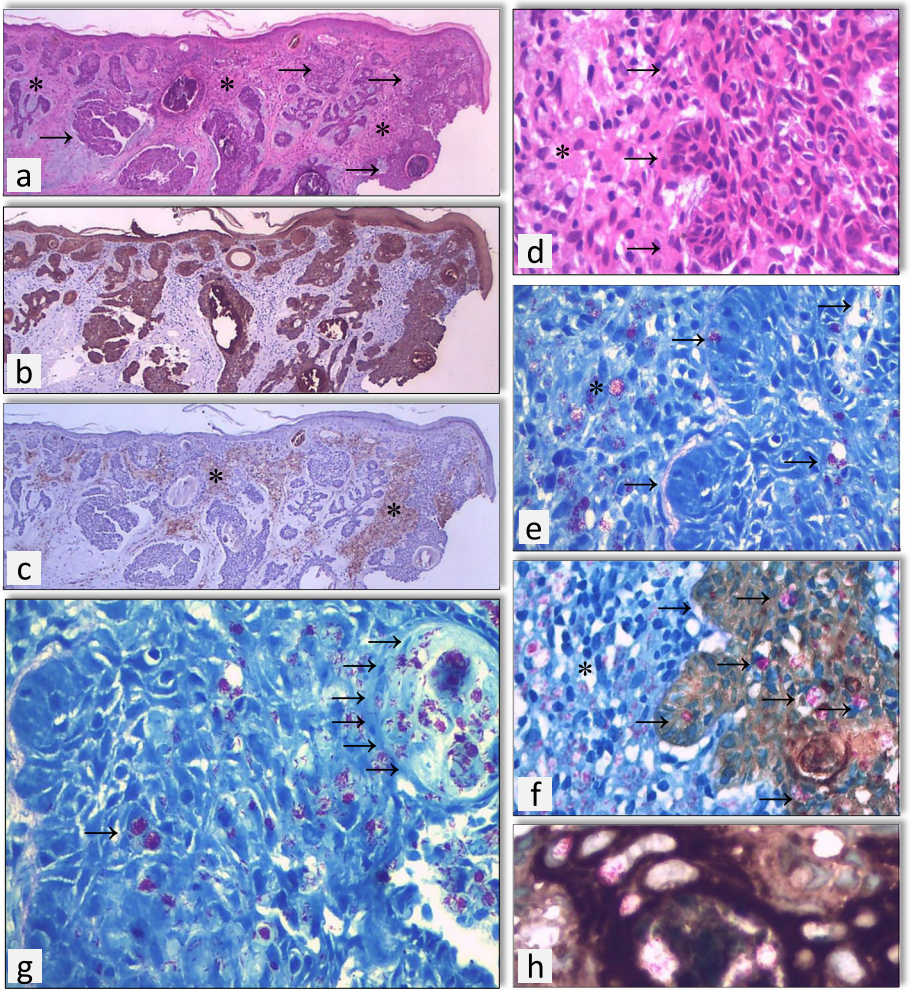

Fig. 4 Parasitism of basal cell carcinoma by *M. leprae* in case 3. Sequential sections stained with hematoxylin-eosin (HE) (**a**) and immunohistochemistry (IHC) (**b** and **c**). **a** Basal cell carcinoma (→) and adjacent leprosy lesion (*) are observed. **b** IHC anti-cytokeratin (34ßE12) immunostaining highlighting basal cell carcinoma (→) and negative leprosy lesion (*). Sequential sections stained by HE (**d**), Fite-Faraco (FF) (**e**), and IHC (**f**). **d** Left: lepromatous lesion (*); right: basal cell carcinoma (→). **e** Bacilloscopy of the histological sections (FF) shows numerous bacilli inside the macrophages (*) and in vacuoles of basal cell carcinoma cells (→). **f** and **h** Positive double staining (IHC-34ßE12 and FF) for basal cell carcinoma (→) and negative for the leprosy lesion macrophages (*), both containing bacilli in the cytoplasm. **g** FF staining showing large numbers of bacilli in intracytoplasmic vacuoles of basaloid cells and also in areas of formation of horny pseudocysts (→)

Special techniques performed

All skin samples were submitted to a routine histopathological analysis with histological sections stained with hematoxylin and eosin (HE) and FF. Both stains served to define the diagnosis of neoplasms (melanocytic nevus and basal cell carcinoma) and associated leprosy.

To confirm that the neoplastic cells were parasitized by *M. leprae*, a double staining technique (IHC with FF) was performed. The IHC indirect technique was used and performed following the manufacturer's instruction (DAKO EnVision™ + System). Immediately after IHC, the immunostained slides were subjected to FF staining. For nevus lesions, sequential biopsy sections were submitted to IHC analysis with cell marker for nevus (Melan A, clone A-103, 1:400, Dako, Denmark), and for macrophages (CD68, clone PG-M1, 1:400, Biogenex, Fremont-USA). For basal cell carcinoma, biopsy sequential sections were subjected to IHC analysis with cell markers for epithelial cells by anti-cytokeratin, high molecular weight (clone 34ßE12, 1: 200, Dako, Carpinteria-USA) and pan-cytokeratin (clone AE1). / AE3, 1; 500, Dako, Carpinteria-USA), for macrophages (CD68, clone PG-M1, 1: 400, Biogenex, Fremont-USA and CD163, clone 10D6, 1: 400, Novocastra, New Castle - UK) and for dendritic cells (CD1a, clone O10, 1: 200, Neomarkers, Fremont-USA).

Histological analysis after the double staining demonstrated the presence of bacilli in the cytoplasm of the macrophages [(CD68 (+), CD163(+), Melan A (–) and

FF (+)], in the leprosy inflammatory infiltrate, and in melanocytic cells [(CD68 (−), Melan A (+) and FF (+)] that comprised melanocytic nevus (Figs. 1h-l and 2d-j) and basal cell carcinoma epithelial cells [(CD68 (−), CD163(−), 34ßE12 (+), AE1/AE3(+), CD1a(−) and FF (+)] (Fig. 4b, c, f, and h and Additional files 1, 2, 3, 4 and 5). *The work was submitted to the ethics committee of the Lauro de Souza Lima Institute and approved, under the process number CAAE* 18115819.1.0000.5475.

Discussion and conclusions

The clinical and histological characteristics of leprosy differ according to the patient's cellular immunity reaction to the *M. leprae* infection. Bacillus-host interaction is peculiar in leprosy, and the response to this interaction results in an enduring spectral disease that presents as different clinical forms and histopathological patterns. In skin biopsies of tuberculoid spectrum patients (TT and BT), parasitism is limited to neural branches and macrophages. Meanwhile, in those of the lepromatous spectrum (BL and LL), bacilli may be observed in the endothelium, smooth muscle cells of the vessel wall and the piloerector muscles, myoepithelial and epithelial cells of adjoining glands, and in the squamous epithelial cells of the epidermis and the pilosebaceous follicle.

An evolved leprosy infection is sine qua non to bacilli parasitizing different cell or tissue types. LL patients may eventually have parasitism of the skin throughout the body (Fleury 1998). Histological sections can demonstrate the presence of leprosy lesion even in areas without apparent clinical signs of injury (El-Darouti et al. 2006; Rastogi et al. 2011).

All three reported cases were diagnosed as leprosy with Virchowian characteristics (two BL and one LL). It is probable that the close coexistence of the melanocytic nevi and basal cell carcinoma with leprosy infiltrates and the continuous progression of these structures provided the *M. leprae* bacterium the necessary conditions to parasite the nevus cells and squamous basal cells. Histological and bacilloscopic analysis showed the presence of bacilli within the intracytoplasmic vacuoles of neoplastic cells (Fig. 1g and l, Fig. 2b and j, and Fig. 4f and h). This pattern, also known as *globi*, is similar to that observed in macrophages and other cells or tissues parasitized by *M. leprae* in cutaneous lesions and internal organs (Fleury 1998).

A facial nevus in a leprosy case classified as BB-BL had cells parasitized by *M. leprae* (Barr and Herzlinger 1977). However, parasitism in nevus cells occurs not in the early stages of the disease, but rather in later stages, when the bacillus can eventually parasitize almost any type of tissue (Barr and Herzlinger 1977). A previous case report described a leprosy patient with concomitant basal cell carcinoma. Excision of the skin with the basal cell carcinoma showed parasitism of macrophages in adjacent tissues (Ratoosh et al. 1994). To the best of our knowledge, no previous cases of parasitism of basal cell carcinoma by *M. leprae* have been reported.

In leprosy-endemic areas, pathologists should be aware of the possibility of finding associated leprosy lesions in biopsy specimens collected for the diagnosis of different types of skin lesions. Patients in the lepromatous spectrum (BL and LL) present extensive and widespread involvement of the skin by *M. leprae* infection, but there is often no clinical evidence of significant cutaneous lesion. In this context, skin biopsy specimens for the diagnosis of nevus lesions, basal cell carcinoma, or different types of neoplastic and non-neoplastic lesions may show histological characteristics of leprosy, particularly when a lymphohistiocytic infiltrate compromising neural branches is observed. To prevent delayed or misdiagnosis of leprosy, FF staining should be performed for diagnostic confirmation, with a positive staining indicating the presence of bacilli in parasitized tissues.

In summary, *M. leprae* has the ability to parasitize different types of cells or tissues in the human body. We report herein the parasitism of nevus neoplastic cells (melanocytic nevus) and squamous cells (basal cell carcinoma) diagnosed via double staining (IHC + FF). Because leprosy can be a comorbidity to other lesions, pathologists, particularly those working in leprosy-endemic countries, such as India and Brazil and countries in Africa, the Middle East, Asia, and South America, should be aware of the histopathological features of leprosy that may be present in tissue biopsy samples collected for the routine diagnosis of neoplastic or non-neoplastic lesions.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s42047-019-0051-x.

Additional file 1. Figures "a" and "b" for case 1. Double staining (Melan A and FF) showing positive Melan A nevus cells containing bacilli in the cytoplasm (→).

Additional file 2. Figures for case 2. (a) Double staining (CD68 and FF) showing negative nevus cells containing bacilli in the cytoplasm (→). (b and c) Double staining (Melan A and FF) showing positive nevus cells containing bacilli in the cytoplasm (→).

Additional file 3. Figures related to case 3. (a) HE staining showing leprosy lesion adjacent to basal cell carcinoma (→). (b) FF staining showing bacilli within basal cell carcinoma epithelial cells (→).

Additional file 4. Figures related to case 3. (a) Double staining by (CD163 and FF) showing positive leprosy lesion (CD163 + macrophages) adjacent to basal cell carcinoma with bacilli inside neoplastic cells (→). (b) Double staining by (CD1a and FF) showing that leprosy injury and basal cell carcinoma are negative for CD1a (absence of dendritic cells containing bacilli). Neoplastic cells of basal cell carcinoma are CD1a negative containing bacilli (→).

Additional file 5. Figures related to case 3. (a and b) Double staining (AE1 / AE3 and FF) showing basal cell carcinoma (AE1 / AE3 +) cells containing bacilli (FF +) inside the neoplastic cells (→).

Abbreviations
BB: mid-borderline; BL: Borderline-lepromatous; BT: Borderline-tuberculoid; FF: Fite-Faraco; IHC: Immunohistochemistry; LL: Lepromatous leprosy; *M. leprae*: *Mycobacterium leprae*; TT: Tuberculoid

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Authors' contributions
CTS conceived and designed the study; acquired and interpreted the data; contributed to histopathological and immunohistochemistry analysis; and drafted the manuscript and revised it critically for important intellectual content. ITO and, PPM acquired analyzed, and interpreted the data. PAW drafted and critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials
The dataset supporting the conclusions of this article is included within the article.

Ethics approval and consent to participate
This study was reviewed by the local ethics committee of Instituto Lauro de Souza Lima (CAAE 18115819.1.0000.5475), and the patients provided informed consent. Written informed consent to publish the case was obtained from all patients.

Consent for publication
Consent for publication was obtained using institutional consent form, and submitted as Additional file.

Competing interests
The authors declare that they have no competing interests.

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References
Barr RJ, Herzlinger DC (1977) Nevi in leprosy. Arch Dermatol 113:1131–1132
El-Darouti MA, Hussein S, Marzouk SA, Nabil N, Hunter NS, Mahgoub D et al (2006) Histopathological study of apparently normal skin of patients with leprosy. Int J Dermatol 45:292–296
Fachin LR, Soares CT, Belone AF, Trombone AP, Rosa PS, Guidella CC et al (2017) Immunohistochemical assessment of cell populations in leprosy-spectrum lesions and reactional forms. Histol Histopathol 32:385–396
Fleury RN (1998) Visceral leprosy - pathogenesis. Hansen Int 23(1):67–71
Massone C, Belachew WA, Schettini A (2015) Histopathology of the lepromatous skin biopsy. Clin Dermatol 33:38–45
Rao PN (2017) Global leprosy strategy 2016-2020: issues and concerns. Indian J Dermatol Venereol Leprol 83(1):4–6
Rastogi R, Budhiraja V, Babu CS, Madan M, Krishna A, Asthana AK (2011) Micropathological changes in the sub-epidermal zone of normal appearing skin in leprosy. Romanian J Morphol Embryol 52:165–169
Ratoosh SL, Cohen PR, Troncoso P (1994) Cutaneous-malignancy and leprosy. Report of a patient with Mycobacterium leprae and basal cell carcinoma concurrently present in the same lesion. J Dermatol Surg Oncol 20:613–618
Ridley DS, Jopling WH (1966) Classification of leprosy according to immunity. A five-group system. Int J Lepr Other Mycobact Dis 34:255–273
Talhari C, Talhari S, Penna GO (2015) Clinical aspects of leprosy. Clin Dermatol 33: 26–37

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