Case Report

When biopsy fails, DNA speaks: a difficult diagnosis of cutaneous tuberculosis

Quando a biópsia falha, o DNA fala: diagnóstico difícil de tuberculose cutânea

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ABSTRACT

Cutaneous tuberculosis presents a diagnostic challenge. This report describes the case of an adolescent with a chronic, single verrucous lesion that persisted for years without resolution. Despite an epidemiological link to tuberculosis, the absence of corroborating evidence hindered a definitive diagnosis (multiple inconclusive microbiological, histological, and molecular examinations had been performed). The elucidation occurred through a new biopsy in which quantitative polymerase chain reaction (qPCR) was used to detect *Mycobacterium tuberculosis* DNA in fresh tissue. Once the diagnosis was confirmed, the patient received standard tuberculosis treatment and experienced complete resolution of the lesion.

Headings: Tuberculosis, Cutaneous; Polymerase Chain Reaction; *Mycobacterium tuberculosis*; Case Report.

RESUMO

A tuberculose cutânea representa um desafio diagnóstico. Este relato descreve o caso de um adolescente com lesão única verrucosa crônica, sem resolução ao longo de anos. Apesar de haver nexo epidemiológico com a tuberculose, a ausência de evidências comprobatórias dificultava sua definição diagnóstica (vários exames microbiológicos, histológicos e moleculares já haviam sido realizados, sendo todos inconclusivos). A elucidação ocorreu por meio de uma nova biópsia onde a reação em cadeia da polimerase quantitativa (qPCR) para detecção de DNA de *Mycobacterium tuberculosis* foi realizada em tecido fresco. Uma vez confirmado o diagnóstico, o paciente recebeu o tratamento padrão para tuberculose e evoluiu com resolução completa da lesão.

Descritores: Tuberculose Cutânea; Reação em Cadeia da Polimerase; *Mycobacterium tuberculosis*; Relato de Caso.

INTRODUCTION

Cutaneous tuberculosis is an uncommon manifestation of extrapulmonary tuberculosis that requires special attention due to its diagnostic complexity, often leading to treatment delays. This condition can manifest in various forms (from ulcerative lesions to indurated nodules) and thus mimics other diseases, challenging clinical practice[1]. Despite being a treatable infection, it can cause significant complications and unsightly scars, with consequent social stigmatization for those affected.

This case illustrates this context by reporting on na adolescent whose lesion had persisted for more than seven years and, despite undergoing various treatments and diagnostic methods during this period (including biopsies and molecular biology tests), only achieved a definitive diagnosis of tuberculosis through insistence

on epidemiological correlation and a methodological peculiarity used in a new search for DNA of the most probable etiological agent.

CASE REPORT

A 14-year-old adolescent, white, born and residing in São Paulo, reported that about seven years ago he began to present with a chronic cutaneous lesion on his left calf, with no apparent triggering factor. The condition began as an erythematous nodule that became raised after two weeks, with subsequent formation of an initially painless ulcer. The ulcerated lesion acquired a hypertrophic scar-like appearance with the formation of central crusts. During this period, he used oral antibiotics (several cycles) and underwent various local therapies such as moisturizers, liquid nitrogen, trichloroacetic acid, corticosteroids, and topical antibiotics, with some moments of improvement and even a reduction in the diameter of the lesion. As a consequence of delays in medical follow-up due to the COVID-19 pandemic, however, the lesion grew again and became a painful, pruritic plaque with occasional bleeding from minor trauma (Figure 1, image provided by the patient). Over these seven years, lesion biopsies were performed repeatedly, always with nonspecific findings and without a definitive diagnosis. Although we were unable to access all the reports, the available documents described findings such as “lichenoid dermatitis and focal granulomatous component over scar fibrosis” or “chronic cutaneous inflammatory process with dense fibrosis” without identification of etiological agents, including mycobacteria or fungi, even after specific stains. Of important history, the patient’s father was treated for pulmonary tuberculosis (cured) when his son was five years old. The child’s complete vaccination schedule, including vaccination with Bacillus Calmette-Guérin (BCG) administered in the first month of life, was confirmed.

Figure 2 corresponds to the current physical examination: on the upper posterior aspect of the left calf, there was a well-defined, violaceous erythematous vegetative plaque of 5.0 x 4.0 cm², with regular borders, of infiltrative appearance, covered in its central region by a dense, white, and well-adhered layer, with some blackened points corresponding to blood crusts. There was no thickening of lymphatic pathways or palpable lymphadenopathy in the region. Considering the relevant epidemiological link, a tuberculin test (TT) was performed, which resulted as “strongly reactive” (22 mm). A chest tomography did not reveal alterations suggestive of pulmonary tuberculosis or mediastinal lymphadenopathy. Histological review of previously biopsied samples (stored in paraffin blocks) was then performed, maintaining the nonspecific findings of “chronic cutaneous inflammatory process with epidermal hyperplasia, dermal fibrosis, and perivascular mononuclear infiltrate, with focal granulomatous outline”; the search for fungi and acid-fast bacilli (AFB) with specific stains (Ziehl-Neelsen and Grocott), as well as immunohistochemical tests for BCG, fungi, and leishmania were again all negative. This same material was also sent for detection and quantification of *Mycobacterium tuberculosis* DNA by quantitative polymerase chain reaction (qPCR), whose result was also negative.

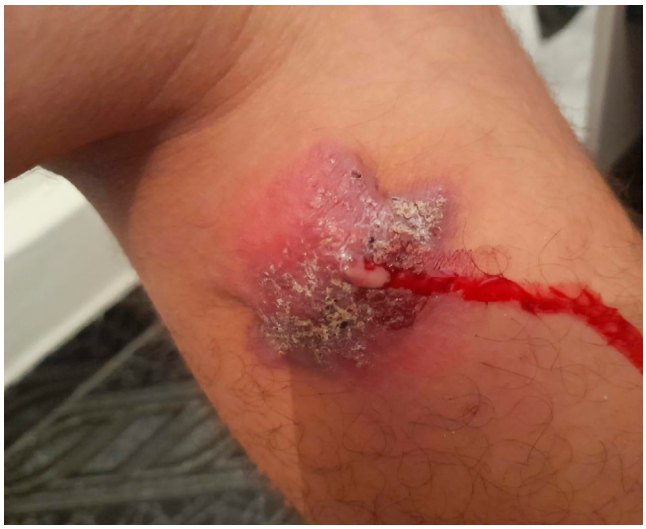

Figure 1. Vegetating, erythemato-crusted plaque, with thick, whitish crusts and numerous black dots on the leg, with fissure and active bleeding after local trauma.

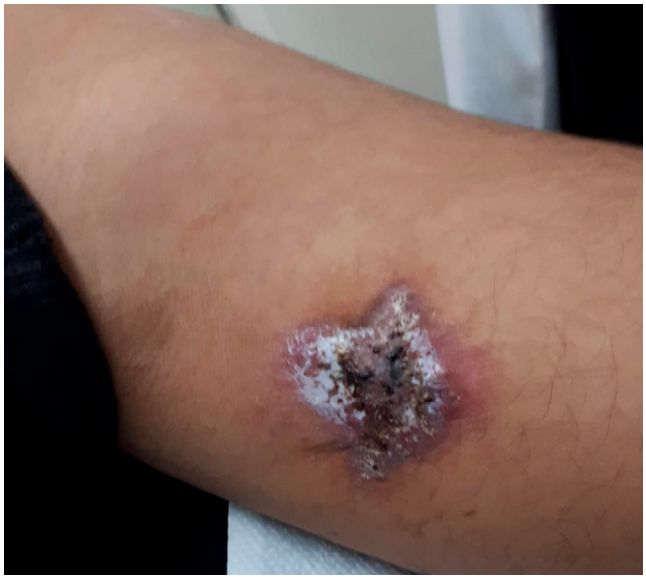

Figure 2. Vegetating, erythemato-crusted plaque with reduction in diameter, reduction of crusts, and black dots.

In common agreement with the family and the patient, it was finally decided to perform new biopsies, however with an additional collection of fresh samples for direct microbiological examinations, culture, and molecular research. The histological study of the sample fixed in formalin and stained with hematoxylin and eosin showed “chronic granulomatous dermatitis with suppurative foci and fibrosis” as demonstrated in Figure 3. The search for fungi and AFB with specific stains and immunohistochemical tests again did not identify any etiological agent. In the fresh samples, baciloscopy and the late result of the culture (Löwenstein-Jensen) were also negative, but this time the qPCR detected DNA of *Mycobacterium* spp “whose sequencing showed 100% nucleotide similarity with *Mycobacterium tuberculosis*”.

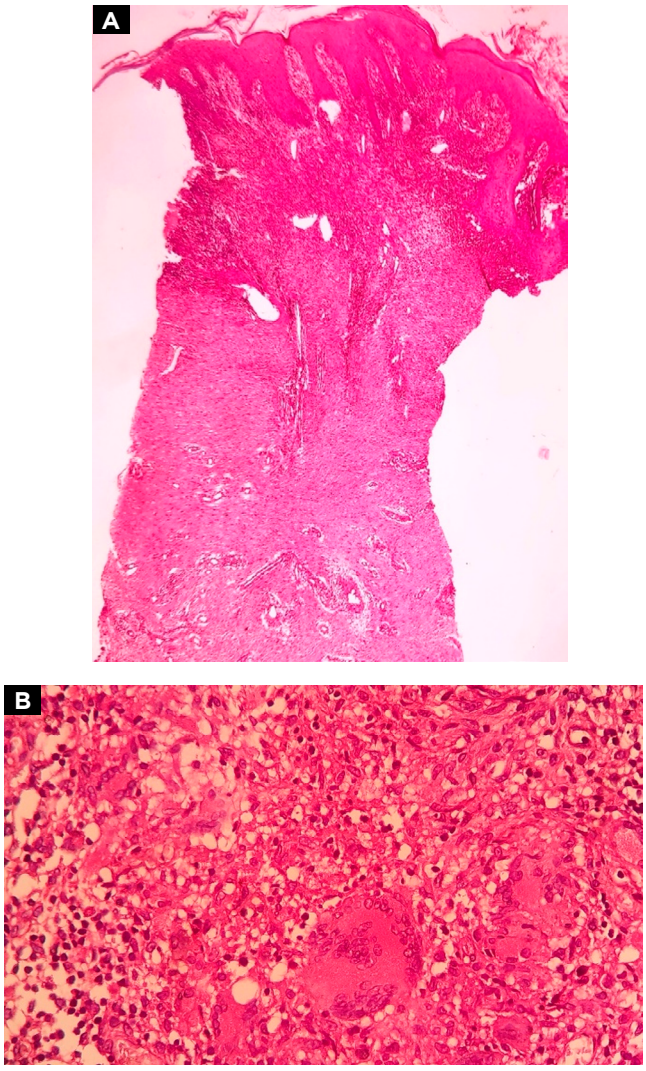

Figure 3. Histological sections of skin “punch” biopsy. **A -** Epidermal hyperplasia, chronic inflammation and fibrosis with extension to superficial, medium, and deep dermis (H&E 40X). **B -** Granulomas with multinucleated giant cells (H&E 400X).

With the definitive diagnosis of cutaneous tuberculosis, the patient was medicated with the standard supervised regimen of rifampicin, isoniazid, pyrazinamide, and ethambutol, without intercurrences and with good adherence. At the end of the sixth and final month of treatment, there was still a whitish layer over the scarred lesion now painless and no longer bleeding (Figure 4A), with total resolution only observed after another six months of observation (Figure 4B), at which point the discharge by cure was documented.

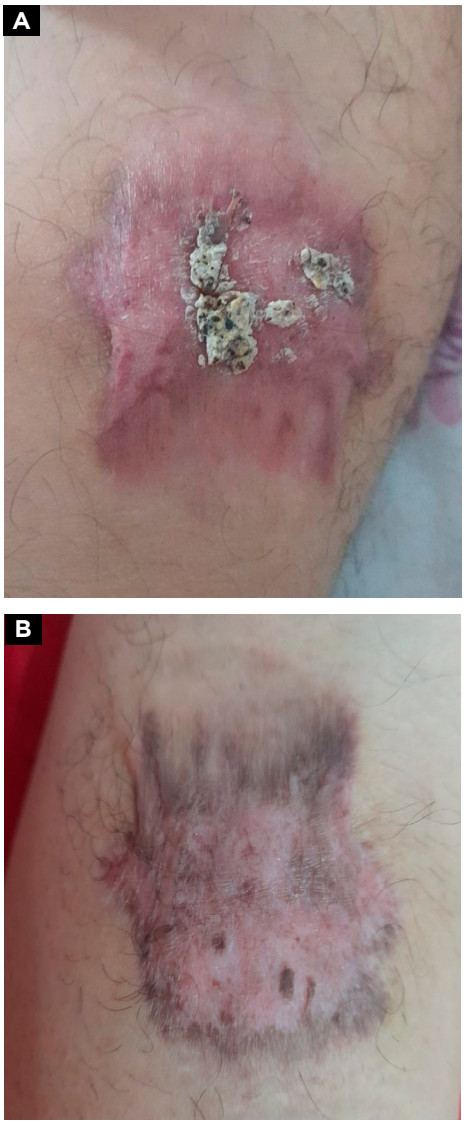

Figure 4. Appearance of the skin lesion after tuberculosis treatment. **A -** In the last (sixth) month of treatment, an erythemato-crusted plaque with scar-like edges and a decrease in the overall size of the lesion is observed in relation to the initial condition. **B -** Scarred appearance of the lesion six months after completing pharmacological treatment.

DISCUSSION

Even in children, the most frequent clinical form of tuberculosis is pulmonary, while extrapulmonary presentations are more insidious, generally without signs and constitutional symptoms. According to the Epidemiological Bulletin of the Ministry of Health of Brazil[2], between 2015 and 2022, 35.5% of the total of new cases of tuberculosis in children aged 5 to 9 years manifested in an extrapulmonary form, while cutaneous involvement usually occurs in no more than 2% of these cases[1,3].

The report presented here corresponds to verrucous cutaneous tuberculosis, resulting from direct post-primary inoculation of *Mycobacterium tuberculosis* in an area of previous microtrauma that probably went unnoticed. In this form of presentation of cutaneous tuberculosis, the lesion is generally single, restricted to the topography of the bacillus entry, with a low bacillary load, in addition to characteristically affecting immunocompetent, previously sensitized hosts, with a well-established cellular immune response - this causes the TT (which evaluates the cellular immune response by delayed hypersensitivity) to be “strongly reactive”, precisely because it reflects the immunological memory of the host previously exposed to the bacillus[3,4]. The low bacillary load implies that direct examinations of scrapings, punctures, and biopsies frequently and successively present negative baciloscopy, despite the ease of obtaining generous samples from skin lesions. In a series of more than 100 cases of cutaneous tuberculosis in children, only 18.4% had positive baciloscopy and 10.7% had growth of mycobacteria in culture, even when there was a highly suggestive clinical-histopathological correlation[1]. It is important to remember that positivity for AFB is not exclusive to tuberculosis, since other pathogens such as non-tuberculous mycobacteria, *Nocardia*, and *Corynebacterium* can also manifest through cutaneous lesions and have positive baciloscopy[4]. In clinical practice, it is common for skin biopsies not to be sent for culture - in our case, however, they were, but there was no growth of either mycobacteria or fungi.

The histological review of previous biopsies ratified the nonspecific findings of chronic inflammatory process with outlines of granulomas (without caseous necrosis), epidermal hyperplasia, and absence of identifiable microorganisms. Although compatible with paucibacillary forms of cutaneous tuberculosis, this pattern can also be observed in other dermatoses such as leishmaniasis, hanseniasis, cryptococcosis, superficial granulomatous pyoderma or even in non-infectious diseases such as sarcoidosis and miliary lúpus[5]. In order to deepen the investigation, molecular biology techniques were then applied to these samples, in addition to specific stains and immunohistochemical tests. The sensitivity of specific amplification of mycobacterial DNA fragments (such as the IS6110, MPB64, 65 kDa, hsp65 genes) varies between 24% and 100%[4,6], and here the result was also negative. It is well known that real-time reactions and protocols based on two-step amplification (“nested” PCR) are capable of detecting genetic material even in paraffin blocks, although variables such as the type of sample, storage time, fixatives used, or inadequate conservation can negatively impact DNA integrity and, consequently, the sensitivity of the methods[6,7].

In any case, the outlines of granulomas added to the strong family epidemiological link and the highly reactive TT to support tuberculosis as the main diagnostic hypothesis of the present case, even more after confirming that the child was neither investigated nor received treatment for latent tuberculosis at the time of his father’s illness. In this context, initiating empirical treatment even without proper etiological confirmation was considered. However, the guidelines of the Ministry of Health for the management of tuberculosis in children[8] are based on clinical-radiological scores aimed at those under 10 years of age with suspected pulmonary tuberculosis and, consequently, do not cover or apply to extrapulmonary forms. Thus, obtaining new samples was conditioned to the addition of some previously unused laboratory technical particularity: the successful performance of qPCR in a fresh tissue sample. This better performance in fresh material (unfixed) had already been evidenced in the literature, for example, in a Brazilian study that achieved 100% sensitivity and specificity in PCR directed at the IS6110 gene of decontaminated, neutralized, and stored at -20 °C samples[9], while other contemporary researchers reported significantly lower sensitivity with the same methodology applied to paraffinized tissues[10].

CONCLUSION

Cutaneous tuberculosis remains a challenge, and clinical suspicion should be maintained in cases of chronic lesions unresponsive to usual treatments. The histopathological findings and the appreciation of the epidemiological context of this case, even in the absence of a specific confirmatory examination, were considered priorities to support the diagnostic hypothesis, such that the performance of new biopsies for the application of qPCR in fresh samples

proved decisive for the etiological diagnosis in this paucibacillary form of the disease.

"This case report deserved an official declaration of acknowledgement and ethical approval by its institution of origin and was peer-reviewed before publication, whilst the authors declare no fundings nor any conflicts of interest concerning this paper. It is noteworthy that case reports provide a valuable learning resource for the scientific community but should not be used in isolation to guide diagnostic or treatment choices in practical care or health policies.

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