

Antiretroviral therapy-induced paradoxical worsening of previously healed *Mycobacterium haemophilum* cutaneous lesions in advanced HIV infection

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ABSTRACT

Mycobacterium haemophilum is a nontuberculous mycobacterium that causes localized or disseminated disease, mainly in immunocompromised hosts. We report the case of a 35-year-old HIV-infected woman who presented with several enlarging cutaneous lesions over the arms and legs. Histopathological examination revealed the diagnosis of a cutaneous mycobacterial disease. Mycobacterial analyses unveiled *M. haemophilum* infection. Six months after completion of a successful antimycobacterial treatment, she developed an immune reconstitution inflammatory syndrome (IRIS). This paradoxical relapse presented as tenderness, redness and swelling at the precise sites of the healed lesions and took place in the setting of significant recovery of the CD4 cell count (from 05 to 318 cells/mm³). Microbiological analyses of these worsening lesions were negative, and they spontaneously remitted without the initiation of a novel antimycobacterial treatment cycle. *M. haemophilum* infection should always be considered as a cause of skin lesions in immunocompromised subjects. Physicians should be aware of the possibility of IRIS as a complication of successful antiretroviral therapy in HIV-infected patients with *M. haemophilum* infection.

KEYWORDS: AIDS. HIV infection. Immune Reconstitution Inflammatory Syndrome. *Mycobacterium haemophilum*. Nontuberculous mycobacterium.

INTRODUCTION

Mycobacterium haemophilum is a slow-growing nontuberculous mycobacterium that causes localized or disseminated disease, mainly in immunocompromised hosts¹. It was first described and named in 1978 by Sompolinsky *et al.*² who recovered the organism from subcutaneous lesions of a woman with Hodgkin's disease. *M. haemophilum* exhibits unique requirements of iron supplementation to grow *in vitro* and shares close phylogenetic relatedness with *M. leprae*³. As it grows better at 30 °C, disease is usually located in cooler places, mainly the lower and upper limbs of immunocompromised subjects. However, the organism may also cause a variety of other clinical presentations⁴. Due to its unique growth and temperature requirements, *M. haemophilum* infection may be underdiagnosed. We report a case of an antiretroviral therapy-induced immune reconstitution inflammatory syndrome (IRIS) on previously healed *M. haemophilus* cutaneous lesions in an

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HIV-infected patient whose antimycobacterial agents had been interrupted some months earlier.

CASE REPORT

A 35-year-old HIV-infected woman presented with a three-month history of enlarging cutaneous lesions over the arms and legs. The diagnosis of HIV infection was made 12 years earlier and she had a great difficulty adhering to the treatment. Previous complications of HIV infection included oropharyngeal candidiasis, *Pneumocystis jirovecii* pneumonia and *Toxoplasma* encephalitis. On clinical examination, there were around 20 brownish-red papules, plaques, nodules and ulcerative nodular lesions of variable sizes, some of which drained a seropurulent discharge, while others were covered with a hemorrhagic crust (Figure 1). The larger lesions were painful.

Laboratory evaluations were remarkable showing a normochromic, normocytic anemia with a hemoglobin of 9.1g/dL (reference values 13.8-17.2g/dL), CD4 cell count of 04 cells/mm³ and plasma HIV viral load of 329,642 copies/mL (5.5 log/mL). An HIV genotyping study showed no mutations associated with reduced drug susceptibility. Serological tests for hepatitis B, hepatitis C and syphilis were negative. There was no evidence of abdominal or thoracic disease. Microbiological analyses of an aspirate of

seropurulent material, as well as a cutaneous biopsy, were performed as diagnostic procedures. Acid-fast staining of the seropurulent material showed mycobacterial structures. Histopathological examination of skin biopsy samples revealed diffuse inflammatory infiltrate in the reticular dermis, composed of histiocytes with abundant cytoplasm, as well as lymphocytes, plasma cells and neutrophils. An acid-fast staining of skin biopsies unveiled countless mycobacterial organisms, thus confirming the diagnosis of a cutaneous mycobacterial infection (Figure 2).

Therefore, seropurulent aspirate samples were used for species identification in a research laboratory specialized in mycobacteria. Due to unavailability of important laboratory supplies, these analyses took several months until a final result could be issued. While results were pending, antimycobacterial treatment was initiated with rifampin, ethambutol and clarithromycin. An antiretroviral combination consisting of tenofovir, lamivudine and raltegravir was also started. The skin lesions gradually improved over the ensuing weeks. One year later, the antimycobacterial combination was interrupted. The skin lesions had completely remitted, leaving hyperchromic scars (Figure 3A). However, the patient was on virological treatment failure: CD4 cell count was 05/mm³ and HIV plasma viral load was 141,227 copies/mL (5.1 log/mL). A novel HIV genotyping study showed the emergence of

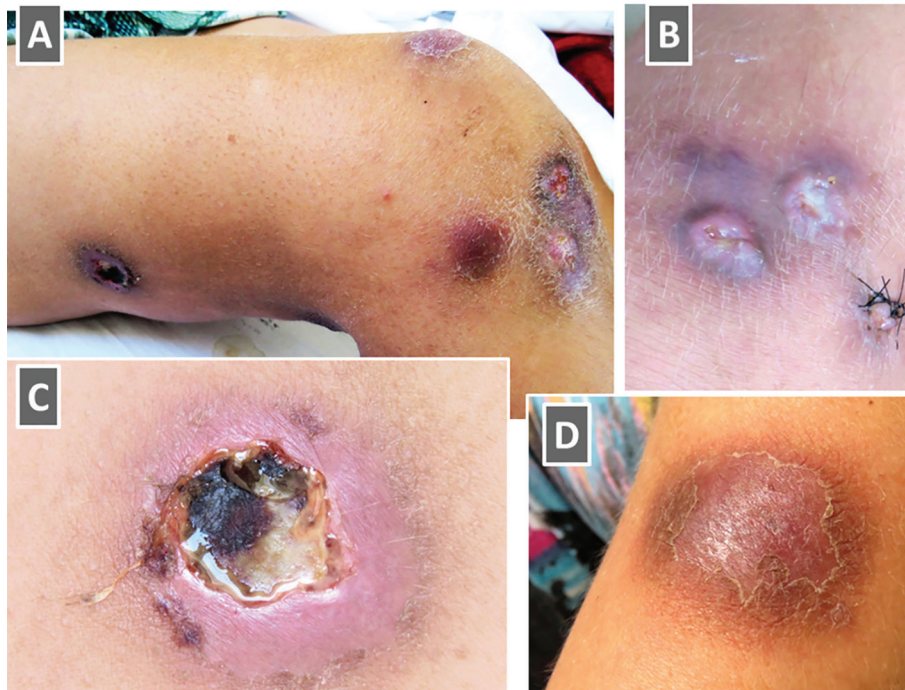


Figure 1 - Clinical images of a 35-year-old female patient who presented with a three-month history of growing cutaneous lesions on the arms and legs: A) brownish-red oval nodular papules and ulcerative nodular lesions of variable sizes on the right leg. Some lesions are confluent; B) Growing nodules covered by a purulent ulcer; C) A large ulcerative lesion draining a seropurulent discharge, with irregular undermined borders and surrounded by a thick rim of erythema. A central area of a hemorrhagic crust is seen; D) A large rounded plaque covered by a scaling surface, on the right arm.

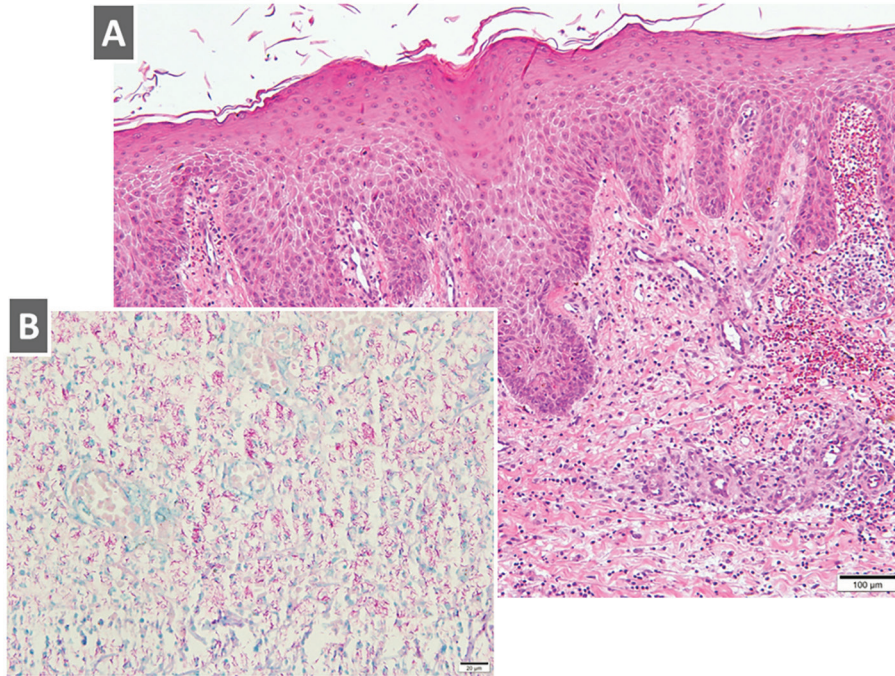


Figure 2 - Histopathological analyses of skin biopsy samples: A) Hematoxylin and eosin staining reveals diffuse inflammatory infiltrate in the reticular dermis, composed of histiocytes with abundant cytoplasm, as well as lymphocytes, plasma cells and neutrophils (original magnification x 40); B) Acid-fast staining unveils countless mycobacterial organisms (original magnification x 400).

mutations associated with reduced susceptibility to reverse transcriptase inhibitors (M184V) and integrase inhibitors (G140S, Q148R, G163R). Then, a new antiretroviral therapy combining tenofovir, lamivudine, ritonavir-boosted darunavir and etravirine was initiated. She was also kept

on azithromycin and sulfamethoxazole-trimethoprim prophylaxis.

Six months after the antimycobacterial treatment interruption, the patient noted tenderness, redness and swelling at the precise sites of the previously healed

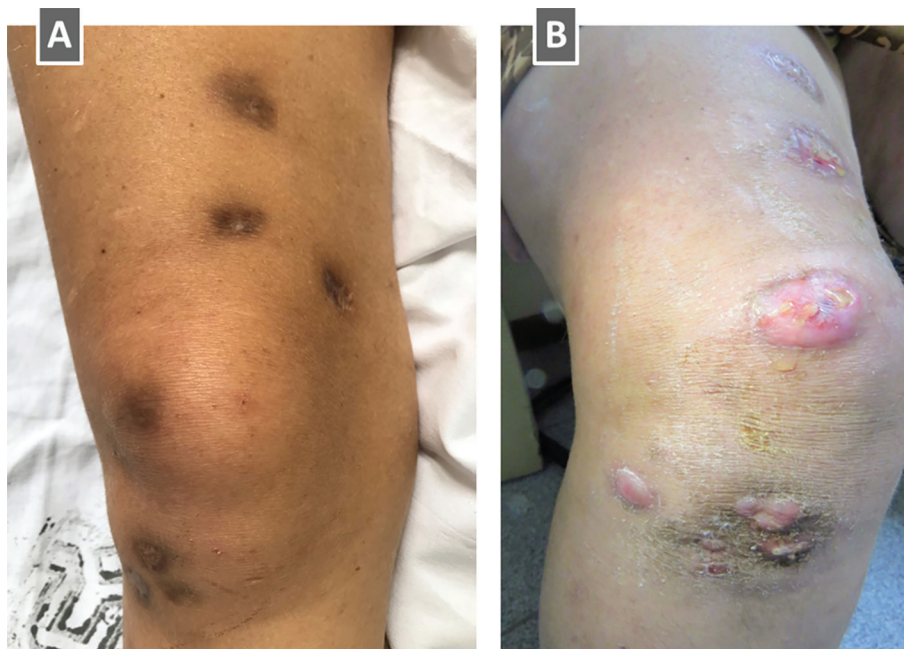


Figure 3 - A) Completely healed lesions with a hyperchromic scar; B) Immune reconstitution inflammatory syndrome six months after antimycobacterial treatment interruption. Redness and swelling at the precise locations of the previously healed lesions. Molecular and histopathological analyses failed to reveal mycobacteria. The lesions eventually improved spontaneously, while the patient was not on antimycobacterial treatment.

M. haemophilum cutaneous lesions (Figure 3B). At this point, the CD4 cell count had significantly increased to 318/mm³ and the HIV plasma viral load had dropped to 331 copies/mL (2.5 log/mL). A novel cutaneous biopsy was performed and samples were sent again to the same laboratory specialized in mycobacteria. Histopathological analyses of the new biopsies revealed a necrotizing dermatitis with fibrinoid necrosis of the vessel walls and absence of mycobacterial structures. A novel cycle of antimycobacterial therapy was prescribed, but was only initiated five months later due to unavailability of rifabutin. During this five-month period, the lesions spontaneously and completely remitted, while the CD4 cell count kept increasing to 485/mm³ and the plasma HIV viral load fell to 116 copies/mL (2.0 log/mL). The paradoxical worsening of the previously healed lesions was consistent with IRIS. The patient has also developed herpes zoster in the right T6 dermatome. Even though, the lesions had spontaneously improved, an additional five months of rifabutin, ethambutol and clarithromycin was prescribed.

The results of mycobacterial species identification only became available by the end of this second round of antimycobacterial therapy. DNA extracted from the aspirate specimens was amplified by polymerase chain reaction (PCR) to detect bacterial DNA using primer sets targeting the *16S* rRNA, the β subunit of bacterial RNA polymerase (*rpoB*) and the 65 kDa heat-shock protein (*hsp65*) gene (Supplementary Material). The *16S* rRNA, *rpoB* and *hsp65* amplified DNA fragments with 99.89%, 99% and 99% similarity with the corresponding regions of *M. haemophilum*, respectively. PCR results from the samples collected during the IRIS-related relapse were negative for the three target genes. Mycobacterial culture was not performed.

DISCUSSION

Cutaneous lesions in immunocompromised subjects are the most common presentation of *M. haemophilum* disease. These lesions usually present on the extremities, particularly the joints, and can be painful. The organism may also cause arthritis, osteomyelitis, dactylitis, tenosynovitis and lung disease^{5,6}. Most reported cases occur in advanced HIV infection, but the disease also occurs in other immunosuppressed individuals, such as transplant recipients and patients who receiving immunosuppressants⁷⁻⁹. However, *M. haemophilum* may also cause cervical lymphadenitis in otherwise healthy children^{10,11}, and has been reported as a cause of chronic skin lesions after contact with marine corals¹², of a pulmonary nodule in an immunocompetent host¹³ and

cutaneous lesions in freshly tattooed healthy adults¹⁴. There are currently no established guidelines on the ideal combination and duration of *M. haemophilum* treatment. Isolates are generally susceptible to clarithromycin, rifampin, rifabutin and ciprofloxacin^{15,16}. However, all the isolates are resistant to ethambutol¹⁶. Our patient responded favorably to a combination of clarithromycin, rifabutin and ethambutol. The latter, was only prescribed because the species identification results were not yet available. Duration of therapy is in general guided by the underlying syndrome and the patient's immunological status¹⁷. Surgical excision may be considered for localized disease¹⁸.

IRIS is a well-known complication of tuberculous and nontuberculous mycobacterial infections. Most reported patients with nontuberculous mycobacterial IRIS had *Mycobacterium avium* complex infections. The three major clinical presentations are peripheral lymphadenitis, pulmonary-thoracic disease and intra-abdominal disease¹⁹. IRIS has already been described in the setting of *M. haemophilum* disease. Tyner *et al.*¹⁷ reported a case series of ten HIV-negative adult patients diagnosed between 2000 and 2015 with diseases caused by *M. haemophilum*. Seven of ten presented with skin lesions on the extremities and all but one had immunosuppressive conditions. Even though all the patients responded favorably to treatment, three developed worsening of previous lesions or emergence of new ones while on antimycobacterial treatment. Microbiological analyses of these worsened or new lesions were negative and all the patients eventually improved. Geisler *et al.*²⁰ reported a similar case of an HIV-infected man with worsened *M. haemophilum* lesions while on antimycobacterial treatment. Two cases of *M. haemophilum* disease in HIV-infected subjects soon after initiation of antiretroviral therapy have also been described^{5,6}. It remains to be determined whether *M. haemophilum* disease is more prone to the development of IRIS, when compared to disease caused by other mycobacterial species.

In summary, we present the case of an HIV-infected woman with advanced HIV-related immunosuppression in whom a paradoxical worsening of previously healed cutaneous *M. haemophilum* lesions occurred as a manifestation of IRIS. This relapse presented as tenderness, redness and swelling at the precise sites of the healed lesions and took place in the setting of significant recovery of CD4 cell count (from 05 to 318 cells/mm³). Interestingly, the present report seems to be the only one in which paradoxical worsening and spontaneous remission of *M. haemophilum* cutaneous disease occurred while the patient was not on antimycobacterial treatment. Moreover, the patient has also developed herpes zoster, which is a well-recognized manifestation of IRIS. *M. haemophilum*

infection should always be considered as a cause of skin lesions in immunocompromised subjects. Careful study of individual cases is of utmost importance to shed light on the full spectrum of its clinical presentations. *M. haemophilum* diagnosis and successful treatment require a concerted cooperation between clinicians, dermatologists, pathologists and mycobacteriologists.

CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

INFORMED CONSENT

A signed informed consent of the patient was obtained from the patient for publishing the case report and accompanying images.

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SUPPLEMENTARY MATERIAL

The following molecular biology methods were used. Bacterial DNA was extracted from skin biopsy and aspirate specimens by using the DNeasy Blood & Tissue kit (Qiagen Biotecnologia) following the manufacturer's guidelines. Amplifications were performed using primer sets targeting the *16S* rRNA (*16S* P1-F/5-TGCTTAACACATGCAAGTCCG-3 and *16S* P2new/ 5- TCTCTAGACGCGTCCTGTGC-3), the β subunit of bacterial RNA polymerase (*rpoBF*/5-GGCAAGTC ACCCCGAAGGG-3 and *rpoBR*/5-AGCGGCTGCTGGGTGATCATC-3), and the 65 kDa heat-shock protein *hsp65F*/5-ATCGCCAAGGAGATCGAGCT3) and *hsp65R*/5-AAGGTGCCGCGGATCTTGTT-3). PCR master mix contained 5X KAPA2G Robust HotStart ReadyMix, 10 μ M each of the primers, 5% DMSO (100%), PCR-grade water and 5 μ L of target DNA for the 25 μ L of final of reaction volume. Amplifications were performed in a Veriti Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, Massachusetts, USA), performed using the following cycling conditions. For *16S* rRNA: an initial denaturation step of 3 min at 95 °C, followed by 40 cycles of 15 s at 94 °C, 15 s at 60 °C, and 15 s at 72 °C, and ending

with a final elongation step for 1 min and 30 s at 72 °C; *rpoB*: an initial denaturation step of 3 min at 95 °C, followed by 35 cycles of 15 s at 94 °C, 15 s at 60 °C, and 15 s at 72 °C, and ending with a final elongation step for 1 min at 7 °C; and *hsp65*: an initial denaturation step of 3 min at 95 °C, followed by 35 cycles of 15 s at 94 °C, 15 s at 64 °C, and 30 s at 72 °C, and ending with a final elongation step for 1 min and 30 s at 72 °C. PCR assays included samples containing DNA of *M. tuberculosis* strain H37Rv (ATCC 27294T) and *M. leprae* NHDP-55 when appropriate, as positives controls. All the controls consistently provided the expected results for each of the screened genes. Negative control PCRs containing no template DNA, were also included to control DNA contamination. The amplicon sequencing was performed using the BigDye Terminator kit (PE Applied Biosystems) on an ABI 3730 DNA Analyzer, at the Oswaldo Cruz Foundation (PDTIS DNA Sequencing Platform/FIOCRUZ, Rio de Janeiro, Brazil). The analysis of gene sequences was performed using SeqScape® and Chromas® softwares, the sequences were aligned and compared with database using Blast (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).