

# Prompt identification of primary cutaneous nocardiosis with immunohistochemical staining



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## INTRODUCTION

*Nocardia* are aerobic bacteria that exist ubiquitously in the environment (eg, soil) and typically cause disease in immunocompromised individuals (the occurrence rate may be as high as 2.65% in solid organ transplant recipients, with an associated overall mortality of approximately 17%).<sup>1</sup> Nocardiosis typically presents as pneumonia in organ transplant recipients but may also cause primary cutaneous infections after traumatic inoculation and disseminate to multiple organs, including the brain, liver, kidney, bones, and pericardium.<sup>2,3</sup> Microscopically, *Nocardia* have gram-positive, branched filaments that may fragment into coccal or bacillary elements.<sup>4</sup> Both *Actinomyces* and *Nocardia* demonstrate similar gram-positive filaments but can be distinguished because *Actinomyces* stain negative on acid-fast staining, whereas *Nocardia* typically stain at least partially with the modified Kinyoun method.<sup>3</sup> Rapidly growing *Mycobacterium* and *Nocardia* grow on the same media (eg, Löwenstein-Jensen) but may be distinguished by the fine filamentous morphology of *Nocardia* (rapidly growing *Mycobacterium* typically exhibit coccobacillary morphology).<sup>5,6</sup> However, confirming the identity of *Nocardia* in culture according to the growth of aerial hyphae typically requires at least 3 to 5 days and can be obfuscated by more rapidly growing bacteria.<sup>3,5,7</sup>

## CASE REPORT

A 72-year-old man with a complex medical history (factor V Leiden deficiency, ischemic cardiomyopathy, treated pulmonary cryptococcosis, thin basement membrane disease, and renal transplantation

after nephrectomy for clear cell renal cell carcinoma) sustained a laceration to the right forearm after a fall onto gravel. A tetanus vaccine booster was administered, the wound was irrigated, and bacitracin was applied daily. He received maintenance mycophenolate, tacrolimus, and prednisone for immunosuppression without trimethoprim-sulfamethoxazole prophylaxis.

Three weeks after sustaining the laceration, he reported onset of a painful, pustular rash on the extensor surface of the right arm that did not respond to outpatient clindamycin or ceftriaxone (Fig 1). On admission, computed tomographic imaging of the arm showed no organized fluid, gas, or acute osseous abnormality. He began receiving cefepime, metronidazole, vancomycin, and acyclovir empirically, and skin biopsies for hematoxylin-eosin staining and tissue culture were obtained. Acyclovir was discontinued after herpes simplex virus and varicella zoster virus polymerase chain reaction swab results were negative. One day later, he had proximal extension of tender erythema. Empirical therapy was switched to amphotericin B for fungal coverage, ciprofloxacin for atypical water/environmental bacteria, and meropenem and linezolid for empirical *Nocardia* coverage. Typical processing methods were used for gram, modified Kinyoun, Fite, fungal, and auramine-rhodamine staining. Microbiology laboratory analysis showed no organisms on gram or calcofluor stain. Auramine rhodamine acid-fast bacilli screening showed greater than 2 acid-fast bacilli; thus, empirical therapy was modified to azithromycin, imipenem, tigecycline, ciprofloxacin, and linezolid to cover rapidly growing *Mycobacterium* (eg, *M abscessus*, *M chelonae*,

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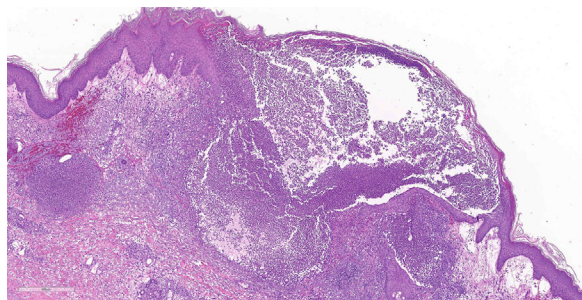


**Fig 1.** Clustered pustules on a diffuse erythematous, edematous plaque of the elbow.

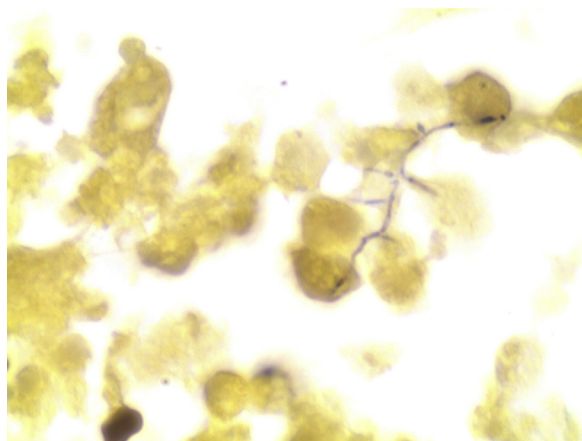
*M fortuitum*). Results for 2 series of blood cultures were negative. An initial surface bacterial swab grew only coagulase-negative staphylococcus.

On microscopic examination, hematoxylin-eosin staining demonstrated extensive neutrophilic dermal infiltration concerning for infection, but no organisms were observed (Fig 2). Fine, filamentous bacteria weakly stained with tissue gram stain (Fig 3) and acid-fast bacilli stain (Fig 4) but were rare and difficult to visualize. Because of the initial difficulty in finding these structures on tissue gram and acid-fast bacilli stains, an immunohistochemistry stain for *M tuberculosis* was used. The staining clearly highlighted the filamentous bacteria and showed long, branching forms (Fig 5) characteristic of *Nocardia* infection. The immunohistochemistry staining consisted of polyclonal rabbit IgG to *M tuberculosis* and was used according to manufacturer specifications (902-140-062713, Biocare Medical, Concord, CA). Furthermore, the filamentous morphology of the *Nocardia* organisms was better appreciated in the immunohistochemistry *M tuberculosis* staining in tissue sections than in the previous auramine-rhodamine–stained smear. Based on the morphologic findings in the histologic slides, the antibiotic therapy was modified to cover nocardiosis with linezolid, trimethoprim-sulfamethoxazole, and renally dosed amoxicillin/clavulanate.

Three days after biopsy, white chalky colonies typical of *Nocardia* spp were observed on chocolate and colistin nalidixic acid agars of the routine culture of the biopsy. The same organism also grew on the fungal culture, but the acid-fast bacilli culture remained without growth. The organism was identified as *N brasiliensis* via matrix-assisted laser desorption ionization–time of flight mass spectrometry using the VITEK MS (bioMérieux, Durham, NC) with knowledge base v3.2 according to the manufacturer’s recommended protocol for *Nocardia* and mycobacteria. Antibody results for blastomycosis,



**Fig 2.** Extensive papillary dermal edema with sheets of neutrophils in the upper and mid reticular dermis, accompanying hemorrhage, and rare multinucleated giant cells (well-formed granulomas were not found). The deeper dermis and subcutaneous fat show edema with patent blood vessels and few inflammatory cells. The epidermis shows acanthosis, spongiosis, focal parakeratosis, and foci of neutrophils. No bacteria were noted. No herpetic viral cytopathic changes were noted. (Hematoxylin-eosin stain; original magnification:  $\times 5$ .)

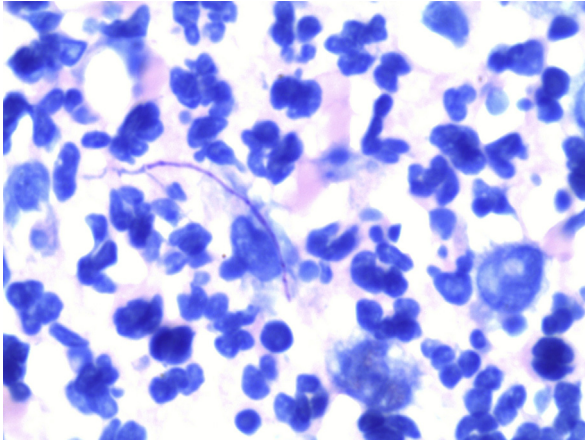


**Fig 3.** Weakly stained filamentous structures suggestive of *Nocardia* infection. (Gram stain; original magnification:  $\times 100$ .)

aspergillus, histoplasmosis, and coccidioidomycosis were negative. On postbiopsy day 4, trimethoprim-sulfamethoxazole was discontinued because of acute renal dysfunction, and the patient continued receiving linezolid and amoxicillin/clavulanate for nocardiosis (sensitivity testing confirmed organism susceptibility). The patient declined recommended brain magnetic resonance imaging and dedicated chest computed tomography to assess for occult dissemination. After 14 days of therapy, his right-arm wounds were markedly improved. However, 25 days after skin biopsy, he died because of cardiac failure.

## DISCUSSION

In the current case, misidentification of *Nocardia* as rapidly growing *Mycobacterium* led to a delay in

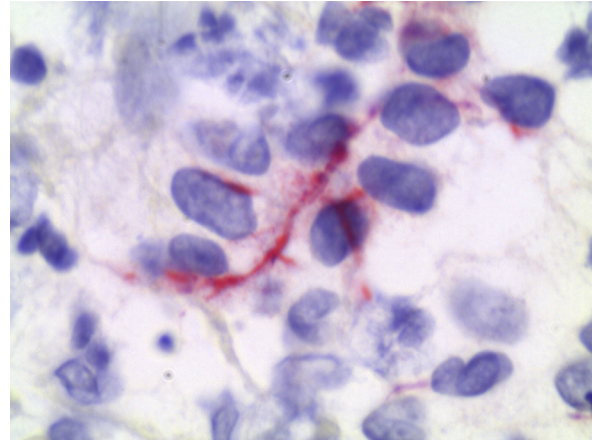


**Fig 4.** Weakly stained filamentous structures suggestive of *Nocardia* infection. (Acid-fast bacilli stain; original magnification:  $\times 100$ .)

pathogen-directed anti-infective therapy by 1 day. Because *Nocardia* can take days to weeks to grow in culture, it is important to use reliable staining techniques to promptly obtain a more narrow presumptive diagnosis and to avoid prematurely discarding cultures with falsely negative results.<sup>7</sup>

Although auramine-rhodamine staining allows greater sensitivity and ease of slide review, there is decreased specificity and the potential for false-positive *Mycobacterium* results, as in our case.<sup>8</sup> *Nocardia* may also be misidentified as mycobacteria when acid-fast staining is used, particularly in older cultures in which partially acid-fast branched long filaments fragment into bacilli and cocci that may be misinterpreted as mycobacteria.<sup>4,5</sup> In contrast, younger cultures demonstrate the branched filaments characteristic of *Nocardia*.<sup>5</sup> Although the use of 1% acid in the modified Kinyoun protocol allows distinction between partially acid-fast *Nocardia* and nonstaining *Actinomyces*, there is a greater potential for misdiagnosis because of the retention of the stain by a wider range of organisms than just *Mycobacterium*.<sup>3,7</sup> In this case, although auramine-rhodamine confirmed the presence of acid-fast organisms, rapidly growing *Mycobacterium* rather than *Nocardia* was initially suspected.

Because of equivocal acid-fast bacilli staining showing filamentous structures and difficulty identifying organisms on tissue gram stain, we used an immunohistochemistry *M tuberculosis* stain to confirm our suspicion of nocardiosis before verification with culture and matrix-assisted laser desorption ionization–time of flight analysis. This method allowed pathogen-directed narrowing of antimicrobial therapy 24 hours before culture-based identification. The immunohistochemistry *M tuberculosis*



**Fig 5.** Staining using *M tuberculosis* antibody, showing rare filamentous structures suggestive of *Nocardia* infection. (Immunohistochemistry stain; original magnification:  $\times 100$ .)

stain we used has previously demonstrated cross-reactivity with *Nocardia* species, a feature that is likely due to shared cell wall mycolic acids and polysaccharides (arabinomannan and arabinogalactan) among the Corynebacteriaceae suborder (eg, *Nocardia* spp and *Mycobacterium* spp).<sup>2,9</sup> Although the immunohistochemistry stain for *M tuberculosis* antibody we used reacts with other atypical organisms (eg, *M avium*, *M. phlei*, and *M parafortuitum*), atypical mycobacteria tend to appear shorter, thicker, more clustered, and sometimes beaded, and have less branching than *Nocardia* spp. Thus, on reexamination of the skin biopsy with immunohistochemistry stain for *M tuberculosis*, the branching filamentous appearance of the bacteria in concert with weak staining on tissue gram staining and acid-fast bacilli staining facilitated the diagnosis of nocardiosis. Nocardiosis was subsequently confirmed by culture and matrix-assisted laser desorption ionization–time of flight results.

Despite the delay in pathogen-directed therapy, our patient improved clinically with linezolid and amoxicillin/clavulanate. Given a high rate of susceptibility, trimethoprim-sulfamethoxazole and linezolid are considered the treatment of choice, with a treatment duration of at least 3 months up to 1 year.<sup>4,10</sup> Regardless, prompt initiation of appropriate antimicrobials is crucial to mitigating morbidity and mortality, especially in immunosuppressed patients.

In summary, careful evaluation of bacteria morphology in skin biopsy sections with gram, acid-fast, and immunohistochemistry *M tuberculosis* stains may allow prompt distinction between

nocardiosis and other types of acid-fast bacterial infections before organism confirmation by culture analysis.

We would like to honor the memory of our wonderful patient and thank his gracious spouse for encouraging us to report his case, which we hope will benefit the medical community and other patients. We would also like to thank Dr Kevin Alby for his expertise and guidance on the use of matrix-assisted laser desorption ionization–time of flight and cultures for this case.

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