



Nontuberculous mycobacterial infections of the lower extremities: A 15-year experience

Mark Anthony A. Diaz^a, Tamara N. Huff^b, Claudia R. Libertin^{a,*}

^a Division of Infectious Diseases, Mayo Clinic, 4500 San Pablo Rd, Jacksonville, FL, United States

^b St. Francis Orthopaedic Institute, St. Francis Medical Group, Columbus, GA, United States

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ABSTRACT

Objectives: Nontuberculous mycobacterial infection (NTMI), which is increasing in prevalence, is challenging to diagnose and manage despite the availability of capable laboratories because of subtle and nonspecific clinical findings and nonstandardized treatment guidelines. We aimed to present our experience with lower-extremity NTMI and to compare clinical characteristics and treatment outcomes between immunocompetent and immunocompromised patients.

Methods: To determine clinical presentations and outcomes, we reviewed electronic health records of all patients with lower-extremity NTMI treated and followed up at our institution from January 2002 through December 2017.

Results: Twenty-four patients were included in this study. Mean (SD) age was 58 (19) years. Eighteen patients (75%) were men; 13 (54%) were immunocompetent; and 9 (37%) had bone and joint involvement. No significant differences existed between immunocompetent and immunocompromised patients, except immunocompetent patients had significantly more infections at the hip, thigh, and toe. Bone and joint infection required significantly longer treatment time than skin and soft-tissue infection.

Conclusions: Regardless of immune status, patients with lower-extremity NTMI had similar characteristics, treatments, and outcomes. However, immunosuppression can be a major risk factor in the development of disseminated NTMI and associated complications. Acid-fast bacilli culture is strongly recommended for evaluation of delayed or nonhealing lesions. Aggressive medical and surgical management can be associated with good clinical outcomes.

1. Introduction

Nontuberculous mycobacteria (NTM) species are ubiquitous environmental organisms and can be cultured from samples obtained from soil, tap water, animals, and food [1,2]. In the human body, NTM can reside as commensal organisms or cause devastating infection. Nontuberculous mycobacterial infection (NTMI) can present as a progressive, nodular pulmonary infiltrate or a mild cutaneous infection, both of which can progress to multiorgan infection. Infection can also occur by direct inoculation. Typically, these infections require prolonged duration of antimicrobial therapy, alone or in combination with surgical intervention [3]. Diagnosis is limited by clinicians' lack of awareness of the presentation of NTMI outside the lungs.

This study describes our population-based, 15-year experience

treating culture-positive, lower-extremity NTMI at our tertiary center. To our knowledge, this is the first study to describe the clinical presentations of lower-extremity NTMI and to compare clinical findings and treatment outcomes between immunocompromised and immunocompetent patients.

2. Methods

2.1. Study patients

We reviewed electronic health records of all patients with NTMI of the lower extremity (from hip to toes) treated at Mayo Clinic, Jacksonville, Florida, from January 1, 2002, through December 31, 2017. We queried the clinical microbiology database to identify

Abbreviations: AFB, acid-fast bacilli; MRI, magnetic resonance imaging; NTM, nontuberculous mycobacteria; NTMI, nontuberculous mycobacterial infection; SSTI, skin and soft-tissue infection

* Corresponding author.

E-mail address: libertin.claudia@mayo.edu (C.R. Libertin).

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patients with NTM isolates from all sites. We excluded patients with isolates from any anatomical site except the lower extremities. Isolates had to be cultured and the species documented on the laboratory report. If the NTM isolate was deemed to represent colonization and not infection, then that patient was excluded. If the patient did not receive a follow-up evaluation, then that patient was excluded. We performed a retrospective analysis of the patients' baseline characteristics (age, sex, and immune status), risk factors for infection, clinical presentation (site and symptoms), results of diagnostic testing (culture results, histopathologic findings, and radiologic evidence of infection), treatment characteristics (antimicrobial therapy, duration of treatment, and surgical intervention), and outcomes (cure, failure, or death).

We classified patients as immunocompetent if they did not have a known predisposing condition that impaired the immune response. As classified by Sotello et al. [4] in their study of upper-extremity NTMI, we also classified patients as immunocompromised if they had any of the following characteristics: received 15 mg prednisone or more daily for at least 3 weeks; receiving active chemotherapy or immunosuppressive monoclonal antibodies; solid organ-transplant or bone marrow-transplant recipient; or diagnosis of an active oncologic process, diabetes mellitus ($\geq 6.5\%$ hemoglobin A_{1c}), end-stage renal or liver disease, or uncontrolled AIDS.

2.2. Microbiologic analysis

Our microbiologic analysis was similar to the analysis of upper-extremity NTMI performed by Sotello et al. [4], which was performed at the same hospital and in-house laboratory. Cultured samples were obtained from skin nodules, ulcers, abscesses, fistulas, bursae, synovial membranes, and synovial fluid of tendon sheaths, joints, or bone. Mycobacterial samples were cultured by using a mycobacterial growth indicator tube (BACTEC MGIT 960 system; Becton, Dickinson and Co) and Middlebrook 7H11//7H11 Selective Agar biplates (Becton, Dickinson and Co). The mycobacterial growth indicator tubes were incubated for 6 weeks in the BACTEC MGIT 960 system. Culture plates were incubated for 8 weeks at 37 °C in an 8% CO₂ atmosphere. Cultures of suspected *Mycobacterium marinum* or *Mycobacterium ulcerans* samples were inoculated on a separate biplate and incubated at 30 °C for weeks. Organisms were identified according to the type of species by using standard criteria, such as growth rate, morphologic structure, and results of mycolic acid analyses and biochemical tests (i.e., nitrate reduction and arylsulfatase). Since November 1999, our institution's laboratory has used 16S ribosomal RNA gene sequencing to identify NTM. Because *Mycobacterium abscessus* and *Mycobacterium chelonae* are indistinguishable by this method, they were reported together (*M abscessus/chelonae*) [5]. Now, definitive identification is performed with standard biochemical and DNA-sequencing methods by the mycobacteriology laboratory at Mayo Clinic in Rochester, Minnesota [6].

2.3. Statistical analysis

Continuous variables are summarized as mean (SD) and median (range), and categorical variables are reported as frequency (percentage). The Wilcoxon rank sum test was used to compare continuous variables between immunocompetent and immunocompromised patients, and the Fisher exact test was used to compare categorical variables. All tests were 2-sided, and the α level was set to 0.05 to indicate statistical significance.

3. Results

A total of 24 patients with lower-extremity NTMI were treated and received follow-up at our institution. The mean (SD) age of the patients with lower-extremity NTMI was 58 (19) years. Eighteen patients (75%) were men, and 13 patients (54%) were immunocompetent. The mean (SD) age was 53 (23) years for immunocompetent patients and 62 (11)

Table 1
Clinical characteristics of patients with lower-extremity NTM infections.

Characteristic	No. (%) (N = 24)
NTM species	
<i>Mycobacterium abscessus/chelonae</i>	5 (21)
<i>Mycobacterium chelonae</i>	5 (21)
<i>Mycobacterium marinum</i>	5 (21)
<i>Mycobacterium abscessus</i>	4 (17)
<i>Mycobacterium fortuitum</i>	3 (13)
<i>Mycobacterium terrae</i>	1 (4)
<i>Mycobacterium avium</i> complex	1 (4)
Cause of exposure	
Wound or soft-tissue injury	17 (71)
Previous surgery	5 (21)
Bacteremia or disseminated infection	2 (8)
Result of AFB smear	
Negative	17 (71)
Positive	7 (29)
Histopathologic study	
No	8 (33)
Yes	16 (67)
Presence of granulomatous inflammation	11 (69) ^a
Imaging study ^{b,c}	
None	12 (50)
MRI	7 (29)
Radiography	10 (42)
Antimycobacterial therapy	
No	1 (4)
Yes	23 (96)
Required surgical intervention	13 (54)

Abbreviation: AFB, acid-fast bacilli; MRI, magnetic resonance imaging; NTM, nontuberculous mycobacteria.

^a Percentage determined according to the number of patients who underwent a histopathologic study.

^b Results of imaging studies suggested skin and soft-tissue or bone and joint infection.

^c Five patients underwent MRI and radiography.

years for immunocompromised patients.

Skin and soft-tissue infection (SSTI) (15 patients [63%]) was more common than bone and joint infection (9 patients [37%]) among patients with lower-extremity NTMI. SSTI was diagnosed in similar numbers of immunocompetent (8 of 13 patients [62%]) and immunocompromised patients (7 of 11 patients [64%]). Immunocompetent patients had significantly ($P < .05$) more infections at the hip and thigh (proximal lower extremity) than immunocompromised patients.

The most common route of infection was a wound or soft-tissue injury (17 patients [71%]) (Table 1). Previous surgery (5 patients [21%]) and disseminated infection due to bacteremia (2 patients [8%]) were less common causes of infection. Among immunocompetent patients, the prevalence of infection due to wound injury was 62% (vs 82% for immunocompromised patients), and prevalence of infection due to a previous surgical procedure was 38% (vs 0% for immunocompromised patients). No immunocompetent patient had a lower-extremity infection due to disseminated infection or bacteremia (vs 2 of the immunocompromised patients [18%]). These differences did not reach statistical significance (all $P > .05$).

Pain was the most common symptom among patients, especially among immunocompetent patients (9 patients [69.2%]) vs 4 immunocompromised patients [36.4%]), but the difference was not significant ($P = .22$). Pustulonodular rash was less common among immunocompetent patients (3 patients [23%]) than immunocompromised patients (5 patients [45%]) ($P = .39$). No significant difference in time from symptom onset to diagnosis was noted between immunocompetent and immunocompromised patients (median, 2.0 vs 2.0 months; $P = .41$).

The isolated NTM species are reported (Table 1). They included (in order of decreasing frequency) *M marinum* (21%), *M abscessus/chelonae*

Table 2
Summary of 24 patients with lower-extremity nontuberculous mycobacterial infection treated at Mayo Clinic, Jacksonville, Florida, from 2002 through 2017.

Age/Sex	Site	Presenting symptom	Involvement	Known cause of exposure	Duration of symptoms, mo	Immune status	Mycobacterial species	Antibiotics administered	Duration of therapy, mo	Surgery	Outcome
30/F	L hip	Pain	Bone and joint	Hip and femoral operations	9	Immunocompetent	<i>Mycobacterium abscessus</i>	Cefoxitin, amikacin, and clarithromycin	6	Y	Cure
67/M	L knee	Pain	Bone and joint	R elbow injury	4	Immunocompromised due to HIV infection	<i>Mycobacterium marinum</i>	Clarithromycin, sulfamethoxazole and trimethoprim, and minocycline	5	Y	Cure
21/M	L knee	Pain	Bone and joint	L lower-extremity fracture	31	Immunocompetent	<i>Mycobacterium terrae</i>	Clarithromycin, sulfamethoxazole and trimethoprim, rifampin, and ethambutol	12	Y	Cure
47/M	R knee	Swelling	Bone and joint	Disseminated mycobacterial infection	1	Immunocompromised due to lung transplant	<i>M abscessus</i>	Amikacin, azithromycin, and doxycycline	20	Y	Cure
40/F	L knee	Swelling	Bone and joint	Exposed to soil without personal protective equipment	9	Immunocompromised due to rheumatoid arthritis treated with immunotherapy	<i>Mycobacterium avium</i> complex	Clarithromycin, ethambutol, and rifampin	12	Y	Cure
55/M	L thigh	Pain	SSTI	Inguinal hernia operations	24	Immunocompetent	<i>Mycobacterium fortuitum</i>	Clarithromycin and sulfamethoxazole and trimethoprim	3	Y	Cure
52/M	R toe	Pain	Bone and joint	Puncture wound with osteomyelitis	2	Immunocompetent	<i>Mycobacterium abscessus/chelonae</i>	None	None	Y	Cure
83/M	L toe	Cellulitis	SSTI	Puncture wound	1	Immunocompetent	<i>M fortuitum</i>	Clarithromycin and sulfamethoxazole and trimethoprim	6	Y	Cure
68/M	B hip	Pain	Bone and joint	Hip surgery	4	Immunocompetent	<i>M abscessus</i>	Clarithromycin	6	N	Cure
84/M	B toe	Pain	Bone and joint	Multiple vascular lower-extremity operations	1	Immunocompetent	<i>M abscessus/chelonae</i>	Clarithromycin	12	Y	Treatment failure
77/M	R leg	Pustulonodular rash	SSTI	Disseminated mycobacterial infection	1	Immunocompromised due to lung transplant	<i>M abscessus/chelonae</i>	Amikacin, clarithromycin, and ciprofloxacin	6	N	Death
67/M	R toe	Pain	Bone and joint	Nonhealing wound and osteomyelitis	4	Immunocompromised due to uncontrolled diabetes mellitus	<i>Mycobacterium chelonae</i>	None	None	Y	Cure
58/F	R ankle	Pustulonodular rash	SSTI	Wound exposed to fish and river water	1	Immunocompetent	<i>M marinum</i>	Doxycycline and clarithromycin	3	N	Cure
70/F	L ankle	Pustulonodular rash	SSTI	Possible insect bite	1	Immunocompromised due to stem cell transplant	<i>M chelonae</i>	Clarithromycin	3	N	Cure
76/M	B buttock	Pustulonodular rash	SSTI	Testosterone injections	3	Immunocompromised due to uncontrolled diabetes mellitus	<i>M abscessus/chelonae</i>	Clarithromycin	3	Y	Cure
61/M	R leg	Pustulonodular rash	SSTI	Pierced nodule	36	Immunocompromised due to Crohn disease treated with immunotherapy	<i>M marinum</i>	Minocycline	3	N	Cure
67/M	L thigh	Pustulonodular rash	SSTI	Insulin injection	2	Immunocompromised due to kidney transplant	<i>M fortuitum</i>	Ciprofloxacin and azithromycin	3	N	Cure
80/M	R thigh	Pustulonodular rash	SSTI	Puncture wound	2	Immunocompetent	<i>M chelonae</i>	Clarithromycin	6	N	Cure
18/M	R foot	Pain	SSTI	Open wound exposed to ocean water while surfing	12	Immunocompetent	<i>M marinum</i>	Minocycline	3	N	Cure
62/M	L leg	Pain	SSTI	Nonhealing ulcer	1	Immunocompromised due to kidney transplant	<i>M chelonae</i>	Azithromycin	4	N	Cure
32/F	R thigh	Pain	SSTI	Saphenous vein removal	2	Immunocompetent	<i>M abscessus</i>	Clarithromycin, amikacin, ceftioxin, and tigecycline	6	Y	Cure
55/F	L thigh	Pain	SSTI	Vitamin B12 injections	6	Immunocompetent	<i>M chelonae</i>	Clarithromycin and linezolid	2	Y	Cure
52/M	R thigh	Pustulonodular rash	SSTI	Wound exposed to roof debris	2	Immunocompetent	<i>M abscessus/chelonae</i>	Clarithromycin and linezolid	4	Y	Cure

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Table 2 (continued)

Age/Sex	Site	Presenting symptom	Involvement	Known cause of exposure	Duration of symptoms, mo	Immune status	Mycobacterial species	Antibiotics administered	Duration of therapy, mo	Surgery	Outcome
64/M	L leg	Pain	SSTI	Wound exposed to river water	1	Immunocompromised due to lung transplant	<i>M marinum</i>	Clarithromycin and ethambutol	6	N	Cure

Abbreviations: B, bilateral; F, female; L, left; M, male; N, no; R, right; SSTI, skin and soft-tissue infection; Y, yes.

(21%), *M chelonae* (21%), *M abscessus* (17%), *Mycobacterium fortuitum* (13%), *Mycobacterium avium* complex (4%), and *Mycobacterium terrae* (4%). *M abscessus* (23%) and *M abscessus/chelonae* (23%) were the most common species isolated from immunocompetent patients; *M marinum* (27%) and *M chelonae* (27%) were the most common species isolated from immunocompromised patients.

Many acid-fast bacilli (AFB) smears showed negative results (17 patients [71%]) but showed positive culture results (Table 1). Among the tissue samples analyzed with histopathologic studies (16 patients [67%]), granulomatous inflammation was seen in only 11 samples (69%).

Half the patients underwent an imaging study (radiography or magnetic resonance imaging [MRI]). Among those who did not have an imaging study, 11 patients (92%) had SSTI. Of those with suspected bone and joint infection, 8 of 9 patients (89%) underwent an imaging study (Table 1). Overall, 10 of 12 patients (83%) who underwent imaging studies showed radiographic signs of inflammation or infection. One patient with suspected osteomyelitis underwent computed tomography angiography to evaluate extensive peripheral vascular disease and a chronic nonhealing wound. He ultimately underwent below-knee amputation without follow-up antimycobacterial therapy (Table 2).

All patients were treated with antibiotics, except 2 patients (1 with extensive debridement of a toe and 1 with right below-knee amputation) (Table 2). The median number of antimicrobial agents administered was 2. The duration of antimicrobial treatment was similar for both groups of patients (median, 4.5 months for immunocompetent patients vs 6.0 months for immunocompromised patients; $P = .89$). The median (range) duration of treatment for patients with *M abscessus/chelonae* was 6 (3–12) months; *M abscessus*, 9.5 (6–20) months; *M chelonae*, 3.5 (2–6) months; and *M marinum*, 4 (3–6) months. Surgical intervention was involved in the treatment of 14 patients (58%). Patients with bone and joint infection received significantly longer treatment (median, 12.0 months) than patients with SSTI (median, 3.0 months; $P = .002$). Cure rates were similar regardless of immune status (92.3% of immunocompetent patients vs 100% of immunocompromised patients; $P = 1.0$).

4. Discussion

The exact incidence of lower-extremity NTMI is unknown [7]. NTMI was not a reportable infection in the United States until an electronic laboratory-based reporting system was recently instituted in Oregon [8]. However, reporting is currently limited to extrapulmonary NTMIs for the purposes of identifying outbreaks, trends, sources of transmission, information for public education campaigns, and predictors of infection [8]. Disseminated NTMIs are commonly observed in severely immunocompromised patients [9]. Worldwide, localized infections can be observed in immunocompromised or immunocompetent patients and are usually associated with skin trauma or injuries; in certain geographic regions, NTMI may be associated with other species such as *M. ulcerans* [10].

Previously, disease outbreaks were usually due to nosocomial infections, and pseudo-outbreaks were related to contaminated hospital equipment and water supplies [11]. More recently, additional NTMI cases and outbreaks have been reported owing to better awareness, diagnostic testing, and understanding of the importance of mycobacterial infections in the community [12]. In 2000, an outbreak of furunculosis due to *M. fortuitum* was associated with whirlpool footbaths in nail salons in California [13]. In 2015, the Florida Department of Health in Miami-Dade County reported an outbreak of NTM-associated SSTIs that affected 38 people [14]. Whole-genome sequencing and single-nucleotide polymorphism analysis were used to identify isolates of *M fortuitum*, *M abscessus*, and *M chelonae* in tap water and contaminated greywash tattoo ink, which was identified as the source of infection at a local tattoo studio. Similar cases of NTMIs in Scotland

and Brazil were traced to tattoo studios [15]. Although less common than SSTIs, reported cases of tenosynovitis due to NTM usually involve the hand, and *M. marinum* is the common culprit for hand injuries with aquatic exposure [16]. However, infection at the site of prosthetic knee joints commonly involves rapidly growing mycobacteria, such as *Mycobacterium smegmatis*, *M. chelonae*, and *M. fortuitum*, and most patients with these infections require resection arthroplasty [17].

In 2 population-based studies of cutaneous and musculoskeletal NTMI performed in the United States [3,4], the most commonly isolated species were *M. marinum* and *M. chelonae-abscessus* complex. The most commonly isolated NTM species were *M. marinum* (21%), *M. abscessus/chelonae* (21%), and *M. chelonae* (21%), which was similar to our experience. Of the 5 cases of *M. marinum*, 4 were SSTIs. Half were treated with a single oral antibiotic agent, and the others were treated with a dual antibiotic regimen for 3 to 6 months. The single case of a bone and joint infection due to *M. marinum* was treated with surgery followed by 3 oral antibiotics for 5 months. All cases of *M. marinum* resolved. *M. chelonae* and *M. abscessus* species were more commonly isolated from immunocompromised patients, with an overall cure rate of 80% (4 of 5 patients) in that specific cohort. No case of *M. ulcerans* was isolated.

Most reported SSTIs and bone and joint NTMIs involve the distal extremities through direct inoculation from the environment or a contiguous source of infection after penetrating trauma, other types of injury, or contaminated needles. However, hematogenous spread of infection from the lungs or gastrointestinal tract can also occur, where NTM species are common commensal organisms—this is typically seen in patients with AIDS [18]. Similar to our results, the most common source of infection was a wound or injury, regardless of immune status [3,4]. In the present study, hematogenous spread or multiorgan involvement was uncommon and noted in only 1 immunocompromised patient with HIV and 2 patients who underwent lung transplants.

Cutaneous NTMI can present as an indolent nodule with a sporotrichotic distribution and then progress to suppurative folliculitis and abscess [19]. Musculoskeletal NTMI can initially present with subacute septic arthritis [17] or tenosynovitis and then progress to suppurative necrosis with osteomyelitis. Such infections involving tendon sheaths, bursae, joints, and bones can be destructive and lead to amputation if antibiotics and débridement are ineffective.

In our population-based study, we had more patients with NTM-associated SSTI than NTM-associated bone and joint infection. A known wound or injury to the affected area was the most common source of infection. Pain was a common symptom, especially among immunocompetent patients. But, development of a pustulonodular rash was the most common initial presenting symptom among immunocompromised patients.

NTMI is diagnosed mainly on the basis of clinical features, microbiologic culture results, radiographic findings, and histopathologic results [20]. The estimated delay in the diagnosis of upper-extremity NTMI is about 36 months [4]. In the study by Park et al. [21], the estimated mean interval between symptom presentation and diagnosis of NTMI was 20.8 months, but this interval can be as long as 180 months. As reported in several studies [22–24] of NTM involving the musculoskeletal system, the main reasons for diagnostic delay were nonspecific characteristics of infection, lack of familiarity with NTMI, and lack of clinical suspicion of NTMI until the primary infection did not resolve after administration of the initial antimicrobial treatment regimen. In general, history of wound exposure to water, surgical procedure, or infection; lack of response to empiric antibiotic treatment; and negative results for routine bacterial culture should raise suspicion for NTMI [19]. In our study, no difference was noted in time from symptom onset to diagnosis between immunocompetent and immunocompromised patients. For both immunocompetent and immunocompromised patients, the median time to diagnosis was 2 months, which is substantially shorter than the results of previous studies. These data were likely affected by the inclusion criteria of the present study, which required diagnosis, treatment, and follow-up at

Mayo Clinic. Mayo Clinic, which is a tertiary referral center with extensive laboratory capabilities, can perform in-house laboratory examinations and anticipate rare diseases such as NTMI. Moreover, our orthopedic practice performs aerobic, anaerobic, fungal, and AFB culturing when specimens are submitted.

NTM species may be detected initially with AFB smear. At some institutions, DNA probes and high-performance liquid chromatography are used to rapidly identify cultured NTM species. However, rapidly growing NTM isolates (*M. fortuitum*, *M. abscessus*, and *M. chelonae*) may require other techniques for taxonomic identification, such as DNA sequencing, polymerase chain reaction and restriction endonuclease assay, or weeks of in vitro antibiotic susceptibility testing [25]. Hence, traditional cultures of specimens obtained with biopsy (skin, synovial membrane, bone, and cartilage), drainage, or aspiration of the affected area remain critical to confirm the diagnosis and to test for antimicrobial susceptibilities. Percutaneous or operative biopsy offers the best chance of species isolation and identification. In our study, only about 30% of AFB smears had positive results. Of note, care and attention during specimen collection is very important given the ubiquity of NTM species. Contamination during specimen handling (e.g., collection, transportation, processing) can result in incorrect identification of organisms or colonization [26].

Originally, *M. abscessus* and *M. chelonae* were considered a single **specie**, but *M. abscessus* was reclassified as an individual **specie** in 2002 [27]. Serial changes were made to the classification and nomenclature of the *M. abscessus* complex between 1992 and 2013. Previously, the distinction between closely related species such as *M. chelonae* and *M. abscessus* complex relied on phenotypic differences, which were few. In addition, the limited differences between *M. abscessus* complex subspecies made further identification difficult. However, despite the controversy regarding the taxonomy of *M. abscessus* complex, whole-genome sequencing data strongly support the presence of 3 subspecies: *M. abscessus* subsp *abscessus*, *M. abscessus* subsp *bolletii*, and *M. abscessus* subsp *massiliense* [28]. To aid in speciation of NTM species, laboratories use multilocus sequence typing, *erm* gene sequencing, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and heat-shock protein and line probe assays. Kim et al. [29] were able to identify all NTMs by using a nucleotide sequence (604 base pairs) of the partial heat-shock protein gene (*hsp65*). This targeted sequence is useful for phylogenetic analysis and identification of mycobacterial species. Therefore, the molecular techniques used for identification of NTM isolates cultured from patient specimens include direct probe hybridization and sequence-based techniques. Line probe assays are available for certain NTM species, but sequence-based identification has become the primary method to identify mycobacteria rapidly [30].

Histopathologic analysis of mycobacterial infection typically shows necrotizing granulomatous inflammation. The presence of granulomas on a biopsy specimen should prompt specific cultures, polymerase chain reaction analysis, or both to look for evidence of mycobacterial infection. However, granuloma may be absent in some patients, especially immunosuppressed patients, because of tumor necrosis factor blockade [31]. As a result, these patients may only have chronic inflammatory changes with poor granulomatous formation. Therefore, the absence of necrotizing granulomatous formation does not exclude infection. In the present study, 69% of patients had results of histopathologic analysis that showed necrotizing granulomatous inflammation, whereas the other studies did not show any sign of granulomatous inflammatory changes.

Although no single imaging characteristic can distinguish NTMI, imaging studies may provide signs of disease extent or identify heavily affected areas, which clinicians can use to guide invasive diagnostic tests and source control [17]. MRI is considered the best available imaging modality because of its high sensitivity for identifying early osteomyelitis and excellent soft-tissue resolution and anatomical detail [32]. Compared with pyogenic infection, NTMI is characterized by slower progression of osteoarticular infection. Compared with

tuberculous osteomyelitis, NTMI does not typically involve the metaphysis and diaphysis, cause marginal sclerosis with discrete lytic areas, or involve multiple sites [33]. In the present study, most patients who had suspected bone and joint involvement underwent MRI or radiography. The imaging studies showed signs of inflammation or infection that aided in diagnosis, evaluation, or treatment, or a combination of these. However, most patients with localized SSTIs did not require imaging studies for further evaluation and management.

Treatment of NTMI is guided by the antimicrobial susceptibility report. This is critical because of NTM's usual multidrug-resistant pattern, which may be associated with poor outcomes. The initial combination antimicrobial regimens are recommended in the guidelines of the American Thoracic Society and the Infectious Disease Society of America [25]. Recommendations depend on the species and include clarithromycin, rifampin, and ethambutol for *M. avium* complex and *M. marinum*; clarithromycin, amikacin, and cefoxitin or imipenem for *M. abscessus*; and clarithromycin, tobramycin, or imipenem for *M. chelonae*. Newer macrolides (i.e., clarithromycin or azithromycin) are the backbone of the treatment regimen because of their activities against several NTMIs [34,35]. Azithromycin has some advantages over clarithromycin, such as a pharmacokinetic profile suitable for daily dosing, better tolerability, less severe drug interactions, and less potential for antimicrobial resistance; however, notably, clarithromycin was the macrolide used in many studies that showed the effectiveness of macrolides, especially against *M. avium* complex [36,37].

Rifabutin can be substituted for rifampin, especially for patients receiving immunosuppressants because rifabutin is a less potent inducer of CYP450 and minimizes drug-drug interactions. Moreover, rapidly growing mycobacteria (often *M. abscessus*, *M. chelonae*, and *M. fortuitum*) should be assessed for inducible macrolide resistance because detection of the presence of an *erm* gene requires 14 days of incubation—the exception is *M. chelonae*, which does not have the *erm* gene. The choice of antimicrobial therapy is mainly influenced by antimicrobial susceptibility and patient tolerance, especially after consideration of the amounts, treatment durations, and adverse effects of the medications involved [38]. However, caution against reliance on in vitro antibiotic susceptibility is strongly warranted because of its limitations and pitfalls.

The Clinical and Laboratory Standard Institute and European Committee on Antimicrobial Susceptibility Testing methodologies may provide some level of standardization for establishment of break-point concentrations. However, no consensus has been reached regarding a standardized method for drug susceptibility testing and established clinical break points for many drugs. Currently, recommended break-point concentrations for most drugs have limited clinical validation or evidence, which makes interpretation and application of minimum inhibitory concentrations more difficult. In vitro susceptibility may not predictably correlate with in vivo susceptibility owing to the naturally occurring antibiotic resistance inherent to NTM [39]. Combination drug testing for selected drugs may be useful to provide synergistic activities and to help overcome natural resistance. However, quality evidence that shows the clinical efficacy of this strategy is lacking.

Interestingly, several individuals in our cohort were treated with only a single antimicrobial therapy. Isolated organisms treated with a single drug included *M. chelonae* (3 patients), *M. abscessus/chelonae* (2 patients), *M. marinum* (2 patients), and *M. abscessus* (1 patient). Patients with *M. chelonae* and *M. abscessus/chelonae* were usually treated with newer macrolides (azithromycin or clarithromycin), but patients with *M. marinum* were usually treated with minocycline. Most patients with SSTI were cured (7 of 8), except for 1 patient who had osteomyelitis of the toe due to *M. abscessus/chelonae*, which was intolerant to multiple antibiotics; this patient was eventually lost to follow-up. These data suggest that uncomplicated cutaneous NTMIs can probably be treated with a single drug, as also indicated by the previous NTM guidelines by the American Thoracic Society [40] but not recommended in the current guidelines [25].

The recommended duration of therapy for osteoarticular NTMI is 6 to 12 months [25], but duration of therapy for NTM-associated SSTI can be 6 to 12 weeks (or longer, if needed) if the infection is localized [41]. The duration of treatment ultimately depends on multiple factors, including the NTM species, extent of infection, treatment response, and immune status of the patient. Treatment may require a combination of medical and surgical therapies. Abscess drainage, debridement, removal of foreign bodies, and, in extreme cases, amputation may be necessary to achieve a cure. In our cohort, the overall rate of treatment success was approximately 92%, and the median duration of therapy was 3 months for SSTIs and 12 months for bone and joint infections. This treatment rate is slightly higher than the rate reported by reviews of NTMI of the extremities (range, 70%–88%) [4,21,42,43]. Our study did not show any association between treatment duration and identified NTM species. Duration of therapy was determined mostly on the basis of extent of body involvement and response to therapy. Two patients who were not treated with antibiotics were cured by surgery (extensive debridement and right below-knee amputation). Patients with cutaneous and superficial NTMIs also have consistently shown excellent prognosis compared with those with deep-seated infection [3,21].

In the present study, no significant differences were noted in characteristics, treatments, or outcomes between immunocompetent and immunocompromised patients with lower-extremity NTMI. A study by Sotello et al. [4] evaluated a similar group of patients with upper-extremity NTMI and reported no significant differences in clinical presentation, diagnostic delay, or outcome among patients with upper-extremity NTMI. Also, results in our cohort did not support findings reported in other studies of NTM SSTI and bone and joint infection with lower cure rates (range, 40%–60%), especially in patients with deep-seated infections [1,4,21]. Only 1 patient had presumed treatment failure due to multidrug intolerance and was lost to follow-up after initial toe debridement. Only 1 patient died, and this patient had undergone a lung transplant and had other medical complications and *M. abscessus/chelonae* SSTI due to hematogenous dissemination. Interestingly, disseminated mycobacterial infection was observed in 2 immunocompromised patients and no immunocompetent patients. Hence, the risk of complications is greater in patients with disseminated or hematogenous mycobacterial infection and should always be an important consideration in immunosuppressed patients. Given the high cure rate in our cohort, ascertaining whether the shorter interval from symptom presentation to diagnosis was related to outcome may be difficult. In a retrospective review of 31 cases of atypical mycobacterial infections of the upper extremity (mean diagnostic delay, 10 months), delays and inappropriate management resulted in a higher risk of treatment failure of up to 68%, especially in patients with *M. avium* and *M. fortuitum*, compared with patients with *M. marinum* [44]. Follow-up ranged from 1 month to 9 years for those who had medical treatment alone or medical treatment combined with surgery. Those who did not have follow-up moved to a different state, refused recommended surgery, or died. One patient relapsed. This patient had documented clearance of an *M. abscessus* infection at the site of a prosthetic hip and was initially treated with a combination of amikacin, clarithromycin, ethambutol, and doxycycline. On recurrence, hemipelvectomy was recommended for source control of the infection, but the patient declined to proceed with surgery. She started to receive long-term therapy with azithromycin and was lost to follow-up.

The strengths of this study include the long observation period (15 years), which identified a large number of cases and reflects our patient population. We reported one of the largest sample sizes and focused on the lower extremities, which is a rare site of NTMI. Underlying immunosuppressive conditions were included in the analysis, and we directly compared characteristics, risks, and outcomes between immunocompetent and immunocompromised patients.

The limitations of the study include the single-center study design and small sample size. The small sample size can be attributed to the rarity of NTMI, despite the recognition of our hospital as a referral

center for complex disease with sophisticated laboratory capabilities. As a result, the reported time from symptom onset to diagnosis may be shorter than in community settings, and the prevalence of NTMI may be greater. The relatively small sample size may have resulted in limited power to detect differences between immunocompromised and immunocompetent patients. In addition, the descriptive nature of the study hindered our ability to use predictive models or to perform a multivariate analysis to identify independent risk factors for treatment outcomes. Lastly, inability to distinguish *M. chelonae* from *M. abscessus* in cases with *M. abscessus/chelonae* limited a clearer delineation of infection between immunocompetent and immunocompromised patients. Therefore, it is also important to clarify that our results require validation in larger cohorts. A meta-analysis of small studies may elucidate these uncertainties and provide a better understanding of NTMI. Moreover, development of a patient registry and an electronic laboratory-based reporting system (similar to the system developed by the Oregon Health Authority) [45] may help pool relevant data from patients and clinicians and help to develop a more systematic protocol for evaluation, treatment, and follow-up of patients with lower-extremity NTMI.

In conclusion, regardless of immune status, patients with lower-extremity NTMI have similar characteristics, treatments, and outcomes. However, identification of immunosuppressed individuals is important because they are at substantial risk for the development of disseminated NTMI and associated complications. A nonhealing and indolent infection should trigger a high index of suspicion for NTMI. Results of cultures, biopsies, and imaging studies are critical to diagnosis. No standard antimicrobial therapy exists, and the treatment regimen depends on a number of factors, including NTM species, antimicrobial susceptibility, and severity of infection. Surgery may be needed, especially to eradicate deep-seated infections. Bone and joint infection requires longer treatment time than SSTI, and clinical and radiologic improvement can guide treatment.

Conflict of interest

None.

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References

- Bi S, Hu FS, Yu HY, Xu KJ, Zheng BW, Ji ZK, et al. Nontuberculous mycobacterial osteomyelitis. *Infect Dis (Lond)* 2015;47(10):673–85.
- Falkinham 3rd. JO. Nontuberculous mycobacteria in the environment. *Clin Chest Med* 2002;23(3):529–51.
- Wentworth AB, Drage LA, Wengenack NL, Wilson JW, Lohse CM. Increased incidence of cutaneous nontuberculous mycobacterial infection, 1980 to 2009: a population-based study. *Mayo Clin Proc.* 2013;88(1):38–45.
- Sotello D, Garner HW, Heckman MG, Diehl NN, Murray PM, Alvarez S. Nontuberculous mycobacterial infections of the upper extremity: 15-year experience at a tertiary care medical center. *J Hand Surg Am* 2018;43(4):387. e1–e8.
- Hall L, Doerr KA, Wohlfel SL, Roberts GD. Evaluation of the MicroSeq system for identification of mycobacteria by 16S ribosomal DNA sequencing and its integration into a routine clinical mycobacteriology laboratory. *J Clin Microbiol* 2003;41(4):1447–53.
- Clinical and Laboratory Standards Institute. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing: approved guideline. Wayne, PA: Clinical and Laboratory Standards Institute; 2008. 71s p.
- Gonzalez-Santiago TM, Drage LA. Nontuberculous mycobacteria: skin and soft tissue infections. *Dermatol Clin* 2015;33(3):563–77.
- Winthrop KL, Henkle E, Walker A, Cassidy M, Hedberg K, Schafer S. On the reportability of nontuberculous mycobacterial disease to public health authorities. *Ann Am Thorac Soc* 2017;14(3):314–7.
- Al-Anazi KA, Al-Jasser AM, Al-Anazi WK. Infections caused by non-tuberculous mycobacteria in recipients of hematopoietic stem cell transplantation. *Front Oncol* 2014;4:311.
- Piersimoni C, Scarparo C. Extrapulmonary infections associated with nontuberculous mycobacteria in immunocompetent persons. *Emerg Infect Dis* 2009;15(9):1351–8. quiz 544.
- Wallace Jr. RJ, Brown BA, Griffith DE. Nosocomial outbreaks/pseudo-outbreaks caused by nontuberculous mycobacteria. *Annu Rev Microbiol* 1998;52:453–90.
- Sood G, Parrish N. Outbreaks of nontuberculous mycobacteria. *Curr Opin Infect Dis* 2017;30(4):404–9.
- Vugia DJ, Jang Y, Zizek C, Ely J, Winthrop KL, Desmond E. Mycobacteria in nail salon whirlpool footbaths, California. *Emerg Infect Dis* 2005;11(4):616–8.
- Griffin I, Schmitz A, Oliver C, Pritchard S, Zhang G, Rico E, et al. Outbreak of tattoo-associated nontuberculous mycobacterial skin infections. *Clin Infect Dis* 2018.
- Sousa PP, Cruz RC, Schettini AP, Westphal DC. Mycobacterium abscessus skin infection after tattooing—case report. *An Bras Dermatol* 2015;90(5):741–3.
- Hsiao CH, Cheng A, Huang YT, Liao CH, Hsueh PR. Clinical and pathological characteristics of mycobacterial tenosynovitis and arthritis. *Infection* 2013;41(2):457–64.
- Hogan JI, Hurtado RM, Nelson SB. Mycobacterial musculoskeletal infections. *Infect Dis Clin North Am.* 2017;31(2):369–82.
- Flegg PJ, Laing RB, Lee C, Harris G, Watt B, Leen CL, et al. Disseminated disease due to Mycobacterium avium complex in AIDS. *QJM* 1995;88(9):617–26.
- Elston D. Nontuberculous mycobacterial skin infections: recognition and management. *Am J Clin Dermatol* 2009;10(5):281–5.
- Jarzembowski JA, Young MB. Nontuberculous mycobacterial infections. *Arch Pathol Lab Med* 2008;132(8):1333–41.
- Park JW, Kim YS, Yoon JO, Kim JS, Chang JS, Kim JM, et al. Non-tuberculous mycobacterial infection of the musculoskeletal system: pattern of infection and efficacy of combined surgical/antimicrobial treatment. *Bone Joint J* 2014;96-B(11):1561–5.
- Astagneau P, Desplaces N, Vincent V, Chicheportiche V, Botharel A, Maugat S, et al. Mycobacterium xenopi spinal infections after discoveterebral surgery: investigation and screening of a large outbreak. *Lancet* 2001;358(9283):747–51.
- Dolenc-Voljc M, Zolnir-Dovc M. Delayed diagnosis of Mycobacterium marinum infection: a case report and review of the literature. *Acta Dermatovenerol Alp Pannonica Adriat* 2010;19(2):35–9.
- Sparks R, Khatami A. Mycobacterium fortuitum complex skin infection in a healthy adolescent. *Infect Disord Drug Targets* 2014;14(3):168–71.
- Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;175(4):367–416.
- Phillips MS, von Reyn CF. Nosocomial infections due to nontuberculous mycobacteria. *Clin Infect Dis* 2001;33(8):1363–74.
- Brown-Elliott BA, Wallace Jr. RJ. Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. *Clin Microbiol Rev* 2002;15(4):716–46.
- Tortoli E, Kohl TA, Brown-Elliott BA, Trovato A, Leao SC, Garcia MJ, et al. Emended description of Mycobacterium abscessus, Mycobacterium abscessus subsp. abscessus and Mycobacterium abscessus subsp. bolletii and designation of Mycobacterium abscessus subsp. massiliense comb. nov. *Int J Syst Evol Microbiol.* 2016;66(11):4471–9.
- Kim H, Kim SH, Shim TS, Kim MN, Bai GH, Park YG, et al. Differentiation of Mycobacterium species by analysis of the heat-shock protein 65 gene (hsp65). *Int J Syst Evol Microbiol.* 2005;55(Pt 4):1649–56.
- Forbes BA, Hall GS, Miller MB, Novak SM, Rowlinson MC, Salfinger M, et al. Practice guidelines for clinical microbiology laboratories: mycobacteria. *Clin Microbiol Rev* 2018;31(2).
- Hamilton CD. Immunosuppression related to collagen-vascular disease or its treatment. *Proc Am Thorac Soc* 2005;2(5):456–60.
- Lee YJ, Sadigh S, Mankad K, Kapse N, Rajeswaran G. The imaging of osteomyelitis. *Quant Imaging Med Surg* 2016;6(2):184–98.
- Theodorou DJ, Theodorou SJ, Kakitsubata Y, Sartoris DJ, Resnick D. Imaging characteristics and epidemiologic features of atypical mycobacterial infections involving the musculoskeletal system. *AJR Am J Roentgenol* 2001;176(2):341–9.
- Tartaglione T. Treatment of nontuberculous mycobacterial infections: role of clarithromycin and azithromycin. *Clin Ther* 1997;19(4):626–38. discussion 03.
- Chaisson RE, Benson CA, Dube MP, Heifets LB, Korvick JA, Elkin S, et al. Clarithromycin therapy for bacteremic Mycobacterium avium complex disease. A randomized, double-blind, dose-ranging study in patients with AIDS. *AIDS Clinical Trials Group Protocol 157 Study Team. Ann Intern Med* 1994;121(12):905–11.
- Koletar SL, Berry AJ, Cynamon MH, Jacobson J, Currier JS, MacGregor RR, et al. Azithromycin as treatment for disseminated Mycobacterium avium complex in AIDS patients. *Antimicrob Agents Chemother* 1999;43(12):2869–72.
- Alvarez-Elcoro S, Enzler MJ. The macrolides: erythromycin, clarithromycin, and azithromycin. *Mayo Clin Proc* 1999;74(6):613–34.
- Stout JE, Floto RA. Treatment of Mycobacterium abscessus: all macrolides are equal, but perhaps some are more equal than others. *Am J Respir Crit Care Med* 2012;186(9):822–3.
- van Ingen J, Boeree MJ, van Soolingen D, Mouton JW. Resistance mechanisms and drug susceptibility testing of nontuberculous mycobacteria. *Drug Resist Updat* 2012;15(3):149–61.
- Diagnosis and treatment of disease caused by nontuberculous mycobacteria. This official statement of the American Thoracic Society was approved by the Board of Directors, March 1997. Medical Section of the American Lung Association. *Am J Respir Crit Care Med.* 1997;156(2 Pt 2):S1–25.
- Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL, et al. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2014;59(2):e10–52.

- [42] Liao CH, Lai CC, Ding LW, Hou SM, Chiu HC, Chang SC, et al. Skin and soft tissue infection caused by non-tuberculous mycobacteria. *Int J Tuberc Lung Dis* 2007;11(1):96–102.
- [43] Kozin SH, Bishop AT. Atypical mycobacterium infections of the upper extremity. *J Hand Surg Am* 1994;19(3):480–7.
- [44] Smidt KP, Stern PJ, Kiefhaber TR. Atypical mycobacterial infections of the upper extremity. *Orthopedics* 2018;41(3):e383–e8.
- [45] Shih DC, Cassidy PM, Perkins KM, Crist MB, Cieslak PR, Leman RL. Extrapulmonary nontuberculous mycobacterial disease surveillance - Oregon, 2014–2016. *MMWR Morb Mortal Wkly Rep* 2018;67(31):854–7.