Original Article

Nontuberculous mycobacterial infection of the musculoskeletal system in immunocompetent hosts

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Abstract

Background: Nontuberculous mycobacteria (NTM) were considered saprophytic organisms for many years but now are recognized as human pathogens. Although humans are routinely exposed to NTM, the rate of clinical infection is low. Such infections usually occur in the elderly and in patients who are immunocompromised. However, there has been an increasing incidence in recent years of infections in immunocompetent hosts. NTM infections in immunocompetent individuals are secondary to direct inoculation either contamination from surgical procedures or penetrating injuries rather than hematogenous dissemination. Clinically and on histopathology, musculoskeletal infections caused by NTM resemble those caused by *Mycobacterium tuberculosis* but are mostly resistant to routine antituberculosis medicines.

Materials and Methods: Six cases of NTM infection in immunocompetent hosts presenting to the department from 2004 to 2015 were included in study. Of which two cases (one patella and one humerus) of infection were following an open wound due to trauma while two cases (one hip and one shoulder) of infection were by inoculation following an intraarticular injection for arthrogram of the joint, one case was infection following arthroscopy of knee joint and one case (calcaneum) was infection following local injection for the treatment of plantar fasciitis. All patients underwent inaging and tissue diagnosis with samples being sent for culture, staining, and histopathology.

Results: Clinical suspicion of NTM inoculation led to the correct diagnosis (four cases with culture positive and two cases with histopathological diagnosis). There treatment protocol for extrapulmonary NTM infection was radical surgical debridement and medical management based on drug sensitivity testing in culture positive cases. At a mean follow up of 3 years (range1–9 years) all patients had total remission and excellent results.

Conclusions: Whenever a case of chronic granulomatous infection is encountered that does not respond to standard anti-tuberculous treatment, with a history of open trauma, surgical intervention, or injection as shown in this study, a possible NTM infection should be considered and managed appropriately.

Key words: Atypical mycobacteria, chronic granulomatous inflammation, immunocompetent hosts, infection by inoculation, musculoskeletal system

MeSH terms: Musculoskeletal system, immunocompetence, mycobacterium infections, atypical, chronic, granulate disease

Introduction

The nontuberculous mycobacteria (NTM) are a group of *Mycobacterium* species other than the obligate pathogens *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*. The American Thoracic Society in their statement endorsed the name NTM.[1,2] These organisms were typically regarded as nonpathogenic because of their low virulence until 1950, but they are now recognized as opportunistic pathogens and important causes of human disease.[3,4] NTM are ubiquitous in nature and widely distributed in water and soil. These are the main sources of infection in humans.[5] Clinically and histopathologically, musculoskeletal infections caused by

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NTM resemble those caused by *M. tuberculosis*, i.e., chronic granulomatous infection, although the overall course of NTM disease is often milder than that of tuberculosis.[6] The clinical significance of diagnosing NTM infection is that they are mostly resistant to routine antituberculosis drugs.[1] Although immunocompromised patients are usually more susceptible to these infection,[7] there has been an increasing incidence of infections caused by NTM in recent years in both immunocompromised and normal hosts,[3] leading to significant confusion as regards diagnosis, leading to delay in treatment. NTM infection in nonimmunocompromised individuals is secondary to direct inoculation either contamination from surgical procedures or penetrating injuries rather than hematogenous dissemination (that is common in immunocompromised individuals).[6,8]

We report six cases of NTM infection treated in our institute since March 2004 to 2015 to create awareness about the diagnosis of NTM infections in the musculoskeletal system.

Materials and Methods

All cases with a history of open trauma or invasive intervention that developed chronic infection not responding to empirical chemotherapy were evaluated for the possibility of NTM infection. Six patients (5:1 male:female) averaging 33 years in age (range 15–44 years) meeting the above criteria were included in the study. All patients underwent radiological (X-rays and magnetic resonance imaging [MRI]) and tissue diagnosis with samples being sent for culture, staining, and histopathology. Accompanied clinical data and good communication between the clinician and microbiologists were essential to optimize culture conditions to increase the sensitivity of culture and laboratory diagnosis of NTM disease. Samples were inoculated onto at least one solid medium for quantitative as well as species diagnosis. Susceptibility of rapid growing mycobacteria for eight antimicrobial agents[2] [Table 1] was advocated in culture-positive cases.

As there is no available guideline or treatment protocol for extrapulmonary NTM infection[2,7] except for radical debridement, which was performed in all cases and appropriate medical therapy was decided and monitored by the infectious disease specialists at our institution.

Table 1: Susceptibility testing of antimicrobial agents and therapeutic doses

Antimicrobial agent	Therapeutic dose for NTM infection
Amikacin	6-7.5 mg/kg/day
Cefoxitin	2 g IV QDS
Clarithromycin	500 mg B.D.
Ciprofloxacin	750 mg B.D.
Doxycycline	200 mg B.D.
Linezolid	600 mg O.D.
SMX (TMP-SMX)	1-1.5 g B.D.
Tobramycin	3-5 mg/kg/day

TPM=Trimethoprim, SMX=Sulfamethoxazole, NTM=Nontuberculous mycobacteria, IV=Intravenous

Cases

Six cases of chronic infection postintervention/open trauma were diagnosed and treated at musculoskeletal NTM infections [Table 2].

Cases were divided into two groups: Group A: NTM infection following open trauma, Case A1: Open facture, humerus [Figure 1], Case A2: Open fracture, patella [Figure 2]; Group B: NTM infection following invasive intervention, Case B1: Intraarticular injection for arthrogram, hip [Figure 3], Case B2: Intraarticular injection for arthrogram, shoulder, Case B3: Local injection for plantar fasciitis, calcaneum [Figure 4], Case B4: Arthroscopy, knee [Figure 5].

Clinical description of 1 case from each group is described below:

Case A1: The open fracture with wound contamination

A 32-year-old male sustained an open comminuted fracture of the left middle 1/3rd humerus after a road traffic accident in March 2004. No surgical treatment had been given until arrival to our hospital six days after the injury, with obvious signs of infection. Radical debridement was performed, infected material and several foreign bodies were removed and external fixator applied [Figure 1a and b]. Initial

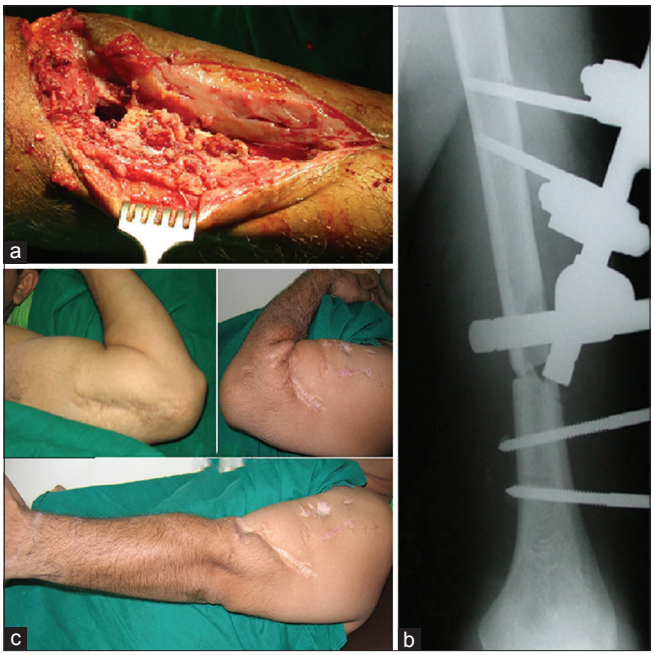

Figure 1: (Case A1) (a) Peroperative photograph showing, open wound, extensive contamination of open fracture wound taken up for debridement. (b) Radiograph of humerus anteroposterior view showing external fixator *in situ* (c) Clinical photograph showing final range of motion

Table 2: Case summary and clinical implications

Case	A1	A2	B1	B2	B3	B4
Age, (years)	32	15	42	44	36	30
Sex	Male	Male	Male	Male	Female	Male
Local complaints	March 2004: Open fracture of the left humerus with wrist drop	December 2012: Open fracture of the right patella following RTA	Right hip pain on exertion since 2007	Pain in the left shoulder since 2008	Pain in right heel with localised swelling since 2009	February 2014: Twisting injury of right knee with complete ACL tear
NTM inoculation	Wound contamination	Wound contamination	January 2011: MR arthrogram	October 2010: MR arthrogram and diagnostic arthroscopy	January 2010 : Local steroid injection thrice over a period of 6 months	February 2014: Arthroscopic ACL reconstruction with semitendinosus graft
Mean duration time from first event to diagnosis	6 weeks	2 months	7 months	2 months	8 months	6 months
Presentation of NTM infection	Chronic discharging sinus with foul smelling discharge	3 discharging sinuses around knee with knee swelling and painful range of motion	Chronic discharging sinus following arthrogram	Painful active range of motion of the shoulder with swelling and warmth	Discharging sinus with serosanguinous discharge	Dull aching knee pain and thigh abscess
Involved bone/ joint	Humerus	Patella	Hip	Shoulder	Calcaneum	Knee
Acid-fast smear	Positive	-	-	-	Positive	Positive
Culture positive	Atypical mycobacteria	-	Atypical mycobacteria	-	Atypical mycobacteria	Atypical mycobacteria
Pathology	CGI with AFB	CGI without AFB	CGI without AFB	CGI without AFB	CGI with AFB	CGI with AFB
Mycobacteria	*Mycobacterium fortuitum*	-	*Mycobacterium chelonae*	-	Rapid growing *Mycobacterium* (*chelonae/fortuitum/ abscessus*)	Rapid growing *Mycobacterium* (*chelonae/fortuitum/ abscessus*)
Surgery	Application of external fixator Debridement four to five times Radial nerve exploration and release Drainage of loculated fluid from arm	Exploration and debridement Insertion of biodegradable vancomycin impregnated cement beads	Debridement and hip excision arthroplasty Tobramycin antibiotic cement beads insertion	Open exploration and synovectomy	Excision of lower 1/3 right calcaneum	Right thigh abscess drainage, excision of ACL graft and interference screw Debridement of femoral tunnel
Chemotherapy	Amikacin 1 g Clarithromycin 500 mg B.D. Linezolid 600 mg O.D.	Amikacin 1 g Clarithromycin 500 mg B.D. Linezolid 600 mg O.D.	Amikacin 1 g Clarithromycin 500 mg B.D. Linezolid 600 mg O.D. Clofazimine 100 mg O.D.	Clarithromycin 500 mg B.D. Clofazimine 100 mg O.D.	Amikacin 1 g Clarithromycin 500 mg B.D. Linezolid 600 mg O.D.	Amikacin 1 g TPM+SMX - 800+160 Doxycycline 100 mg
Followup	9 years	9 months	15 months	3 years	3 years	1 year

Acid-fast smear and mycobacterial culture for specimens from large joints. AFB=Acid-fast *Bacilli*, CGI=Caseating granulomatous inflammation, GI=Granulomatous inflammation, NTM=Nontuberculous mycobacteria, , ACL=Anterior cruciate ligament, MR=Magnetic resonance, TPM=Trimethoprim, SMX=Sulfamethoxazole, RTA=Road Traffiic Accident

cultures were suggestive of acute infection by *Enterococci* and Gram-negative bacilli and hence treatment started with susceptible antibiotics (ciprofloxacin 750 mg B.D., and co-amoxiclav 1.2 g B.D.). Over the next 2 weeks, the wound was debrided twice as the infection was not coming under control. Histopathology done at two weeks revealed a dense inflammatory exudate with granulomas. The intraoperative tissue cultures grew the NTM (*Mycobacterium fortuitum*, rapid-growing *Mycobacterium*). The patient was started on combination therapy of amikacin 1 g intravenous. daily for 6 weeks, linezolid 600 mg O.D., and clarithromycin 500 mg B.D., for 6 months based on the antibiotic sensitivity report. Renal profile was periodically monitored while on injectable amikacin therapy. The wound improved immediately and secondary suturing was done 2 weeks after starting the treatment. The external fixator was removed after 3 months and "U" slab was given for support for 4 weeks. The fracture united uneventfully with a full range of movements at the elbow and shoulder [Figure 1c].

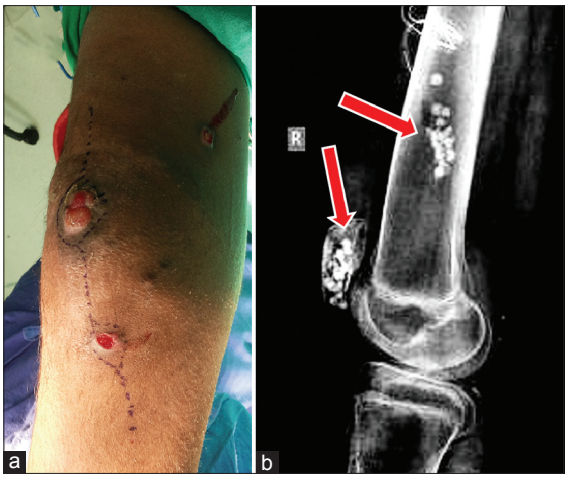

Figure 2: (Case A2) (a) Clinical photograph showing multiple discharging sinuses around the knee. (b) postoperative radiograph of the knee joint lateral view showing bio-degradable cement beads (arrows)

Nearing 10 years postcompletion of treatment, he has no evidence of recurrence/re-activation of disease and excellent function.

Case B1: Inoculation by intraarticular injection

A 42-year-old male, presented with intermittent dull aching pain in the right hip since 2007, which was aggravated on exertion. Since there was no relief of symptoms with medications and physiotherapy, an MRI arthrogram was performed. One month following arthrogram, the pain in the right hip exacerbated and was associated with swelling, induration, and intermittent fever. Empirical antibiotics were tried before a computed tomography-guided aspiration for obtaining material was performed at another hospital prior to presentation at our institute which revealed granulomatous infection on histopathology, while there was no growth on culture. The patient was started on four drug

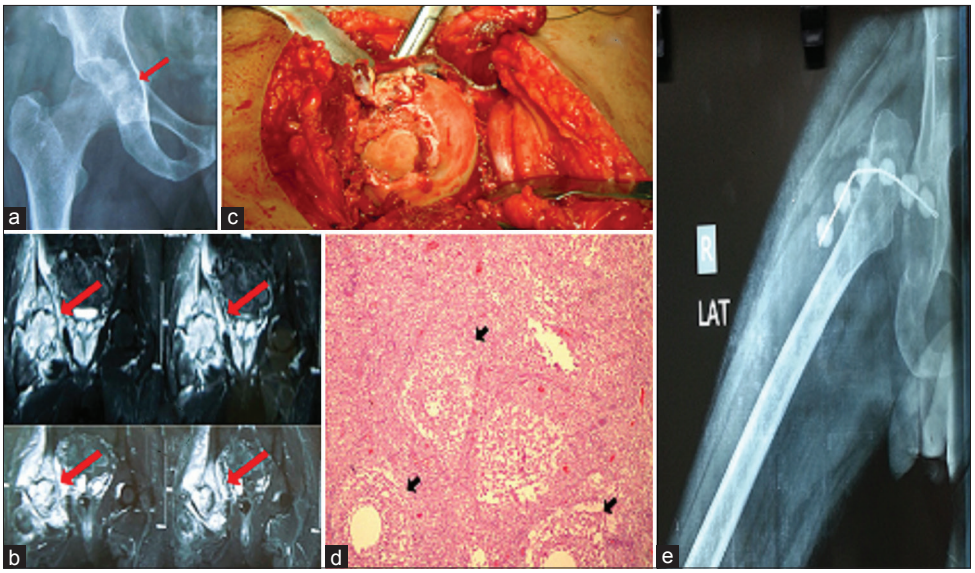

Figure 3: (Case B1) (a) Radiograph of the right hip at presentation, showing decreased joint space and destructive changes in the right hip (arrow) (b) Magnetic resonance imaging with gadolinium contrast of the right hip showing periarticular soft tissue and intraarticular involvement with destruction of the femoral head (c) Intraoperative photograph showing destroyed and irregular femoral head (d) Histopathology: microphotograph showing granulomatous inflammation with the presence of acid-fast *Bacilli* (arrow). (e) postoperative radiograph hip with thigh lateral view showing excision of damaged femoral head and insertion of antibiotic cement beads

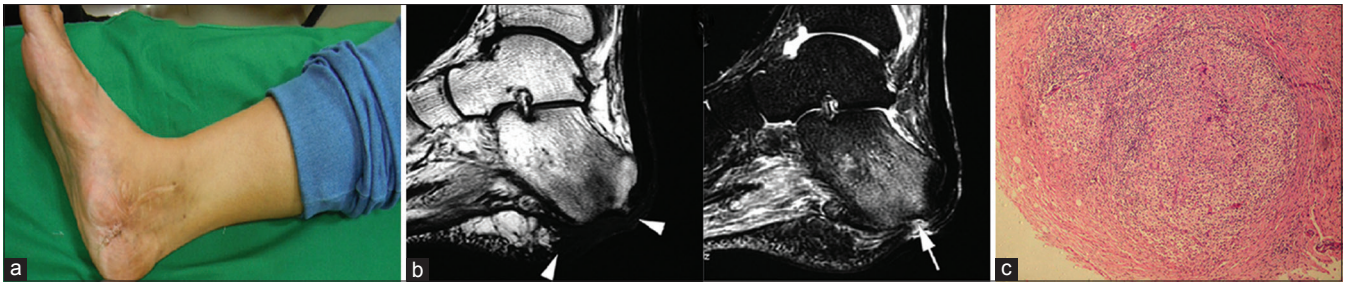

Figure 4: (Case B3) (a) Clinical photograph showing, discharging sinus and old surgical scar in the inner aspect of heel. (b) Magnetic resonance imaging of calcaneum with gadolinium contrast showing marrow edema and soft tissue involvement along the plantar fascia. (c) Histopathology microphotograph showing granulomatous inflammation with the absence of caseating necrosis

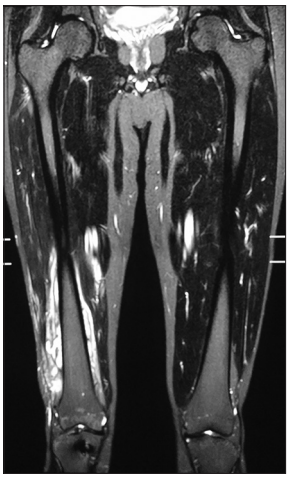

Figure 5: (Case B4) Magnetic resonance imaging showing thigh abscess

antitubercular treatment (ATT). In spite of 3 months of ATT, the pain worsened significantly and the patient presented to our institute with systemic symptoms, toxic appearance, leukocytosis and raised erythrocyte sedimentation rate. The radiograph [Figure 3a] at presentation showed decreased joint space and destructive changes in the right hip, while MRI [Figure 3b] with contrast suggested significant soft tissue and joint involvement and destruction of the femoral head and joint effusion. In view of the presentation of severe infection and extensive bone and cartilage destruction [Figure 3c], an excision arthroplasty was performed. The tissue samples were cultured specifically for *M. tuberculosis* and tissue was also sent for histopathology. Histopathology [Figure 3d] indicated granulomatous inflammation with the presence of acid-fast *Bacilli* (AFB) and cultures grew NTM (*Mycobacterium chelonae*; rapid-growing *Mycobacterium*) sensitive to linezolid, amikacin, and clarithromycin. In consultation with a pulmonologist in view of mycobacterial infection, appropriate antibiotics were started based on the sensitivity pattern with amikacin 750 mg injected 3 times a week, clarithromycin 500 mg, B.D., and linezolid 600 mg, O.D. In spite of starting the treatment, the wound did not completely heal and a chronic discharging sinus from the surgical site persisted. The sinus was traced by performing a sinogram and excised but the infection persisted.

An infectious disease specialist was consulted who adjusted the dose and frequency of amikacin – 1 g intravenously daily instead of thrice a week for 1 month followed by 5 times a week for the next 5 months. Clofazimine (100 mg tablet, O.D.) was added while continuing clarithromycin and linezolid as before, also a reexploration and extensive debridement with obtaining sample for culture and histopathology along with insertion of tobramycin antibiotic cement beads (based on the antibiotic sensitivity report) were performed [Figure 3e]. Renal profile was periodically monitored while on injectable amikacin therapy. The wound healed almost immediately following appropriate antibiotic therapy and insertion of cement beads. One year since the first visit and 5 months since the last surgery, the patient was admitted for removal of the antibiotic cement beads, as the wound had healed well and with no fresh complaints. An intraoperative sample was sent for culture but showed no growth. Two years after completion of the treatment, his total hip arthroplasty (THA) was done and he is totally asymptomatic at 6 months post-THA.

Results

Six (5:1 male:female) immunocompetent patients with a history of invasive procedures in four cases and open trauma in two cases presenting with chronic infections not responding to routine chemotherapy were evaluated radiologically and by tissue diagnosis. Average time to diagnosis from inoculation: 4 months (6 weeks to 8 months), Histopathological diagnosis based on chronic granulomatous inflammation: 100% cases (6/6 cases), Culture growth with mycobacterial species identification: 66.6% (4/6 cases), AFB positive on smear: 50% (3/6 cases).

Based on clinical profile, histopathology, drug sensitivity reports and opinion of infectious disease consultant, chemotherapy consisted of a combination of (A) Amikacin + clarithromycin + linezolid was administered in three patients (B) Amikacin + clarithromycin + linezolid + clofazamine in one patient who had not responded to ATT as well as treatment for NTM (C) Clarithromycin + clofazimine in one patient (patient had been treated empirically by ATT prior to presentation to our institute) (D) Amikacin + trimethoprim-sulfamethoxazole + doxycycline was administered in one patient.

Injection amikacin was administered for a maximum duration of 6 weeks while oral therapy for a minimum duration of 6 months in all patients while monitoring blood, renal and liver profiles for toxicity.

At a mean follow up of 3 years (range1–9 years) all patients (6/6) had total remission and good function.

Discussion

NTM have been recognized as saprophytic organisms for many years until the 1950s when they were recognized as human pathogens.[9] To date, more than 125 species have been identified and about sixty are known to cause clinically significant disease.[4,10] Traditionally, NTM have been grouped into four broad categories according to the Runyon system. In this classification, NTM are divided by growth rates and

pigment production. Groups I, II, and III are slow-growing NTM, and Group IV are fast growers (i.e., detectable in culture within 7 days). The slow-growing NTM are subdivided into Group I photochromogens (pigment producers in the presence of light), Group II scotochromogens (pigment producers in the absence of light), and Group III nonchromogens (not producing pigments).[3,10]

NTM are ubiquitous in nature and widely distributed in water, soil, and animals. Water (natural as well as reservoirs) and soil are the main sources of infection in human.[11,12] They can also be found as colonizers of medical equipment such as endoscopes and surgical solutions.[13] Human-to-human transmission has not yet been reported.[14,15] Biofilm formation is a successful survival strategy for these very hydrophobic organisms. Dispersal from biofilms may be a mechanism of shedding from a device or water pipe to infect the patient.[16-18] They are difficult to eradicate with common decontamination practices and are relatively resistant to standard disinfectants such as chlorine, organomercurials, and alkaline glutaraldehydes.[19] There is a wide spectrum of clinical diseases caused by NTM, which can be divided into chronic pulmonary infections, superficial lymphadenitis, skin and skeletal infections, and disseminated disease.[3,4] As culture with strict criteria is still not routinely done in most parts of India, there is a tendency to ignore such isolates as contaminants, so it is difficult to comment on the exact magnitude of the problem.[20] Clinically important species by group include *Mycobacterium kansasii* and *Mycobacterium marinum* (Group I); *Mycobacterium gordonae* and *Mycobacterium scrofulaceum* (Group II); *Mycobacterium avium* intracellulare and *Mycobacterium ulcerans* (Group III); and *M. fortuitum, M. chelonae, Mycobacterium abscessus* (Group IV).[3,11] Out of these, NTM strains often acquired by trauma are *M. fortuitum, M. chelonae,* and *M. marinum*.[2,21]

Clinically, musculoskeletal infections caused by atypical mycobacteria often resemble those caused by *M. tuberculosis*, i.e., chronic granulomatous infection,[22] although the overall course of atypical mycobacterial disease is often milder than that of tuberculous infection.[6] The histopathology is often suggestive of a granulomatous lesion.[23] Thus, these cases are started on ATT either empirically or after histopathology. The microbiological diagnosis of rapidly growing mycobacteria infections includes direct microscopic observation of the microorganism in the samples,[10,24] culture in selective media, and identification of the isolated species by phenotypic, biochemical, molecular, and chromatographic techniques.[25] The finding of AFB in stained smears by the Ziehl–Neelsen or auramine techniques examined under a microscope is the first evidence of the presence of mycobacteria in a clinical specimen. On histopathological examination, a spectrum of inflammatory changes has been reported including granulomatous lesions with or without caseation.[26-28] Accompanied by clinical data, i.e., history of open wound or surgical procedure or injections, it can help to establish the presumptive diagnosis of NTM infection. However, the gold standard in diagnosis is the identification of *Mycobacterium* species in culture.[7,10] Besides confirming diagnosis, it allows us to do drug sensitivity so that appropriate drug regimen can be selected, as NTM are usually resistant to routine antitubercular medicines and there is variability in susceptibility among species.[1,24] However, it is important to have a good communication between the clinicians and microbiologists to optimize culture conditions and to increase the sensitivity of culture and laboratory diagnosis of NTM disease. Samples should be inoculated onto at least one solid medium (Lowenstein–Jensen or Middlebrook 7H10 and 7H11) and into a liquid culture system (BACTEC 460, MGIT, MB9000, MB BacT, ESP). The latter systems permit more rapid culture and isolation of a greater range of species than the use of solid media alone, but solid culture permits quantification of the isolated *Mycobacterium*.[29,30] The optimal temperature for most cultures for NTM is between 28°C and 37°C.[23] All skin specimens should be cultured at 28°C–30°C and at 35°C–37°C, both are essential for optimal recovery.[31] Many NTM grow within 2–3 weeks on subculture, but *M. ulcerans* or *Mycobacterium genavense* cultures should be incubated for at least 8–12 weeks. Rapidly growing mycobacteria usually grow within 7 days of subculture.[32] When NTM infection is suspected, it is important to inform the microbiologist accordingly.

As far as treatment is concerned, there is no firmly established standardized treatment regimen.[33] Treatment is usually guided by the drug sensitivity report. If there is no growth on culture but diagnosis of NTM is suspected based on clinical history and histopathology findings (as in cases A2 and B2), treatment has to be started on the basis of the susceptibility results published in the literature.[31] Agents which can be used for treating NTM infections are macrolides (clarithromycin, azithromycin); rifampin or rifabutin; ethambutol; doxycycline; quinolones (ciprofloxacin, moxifloxacin, and gatifloxacin); sulfonamides; amikacin; streptomycin; isoniazid; ethionamide; cefmetazole; and imipenem.[34] The number of agents required for effective treatment is not clear, although three drug regimens are often adopted.[4] For most NTM infections, macrolide-based drug regimens are an effective option.[4] Furthermore, the optimal duration of therapy is unknown, although courses of 6–12 months are generally used guided by clinical and radiologic improvement on therapy.[31] All patients have to be monitored for possible adverse drug effects of individual and combination antibiotic therapies.

Conclusions

NTM are now recognized as opportunistic but true pathogens and are an important cause of human disease.[3,4,7] There has been an increasing incidence of infections caused by NTM in recent years in both immunocompromised and normal hosts.[3] Whenever a case of chronic granulomatous infection is encountered, that does not respond to standard anti-tuberculous treatment, with a history of open trauma, and surgical intervention or injection, there should be clinical suspicion of a possible NTM infection.[2,23] It is important to have a good communication between clinicians and microbiologists so as to optimize culture conditions. This will increase the sensitivity of culture so that antimicrobial susceptibility testing can be performed. In case there is no growth on culture and still diagnosis of NTM is suspected based on clinical history and histopathology findings (as in cases A2 and B2), treatment has to be started on the basis of the susceptibility results published in the literature.[12,17,22]

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Conflicts of interest
There are no conflicts of interest.

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