Skin and Soft Tissue Infections Caused by *Mycobacterium chelonae*: More Common Than Expected?

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Mycobacterium chelonae is a rapidly growing nontuberculous mycobacterium, which causes infections of the human skin and soft tissue. Despite an increasing incidence of such infections, patients are often misdiagnosed. We report here 5 patients with cutaneous and/ or soft tissue infection due to M. chelonae who were diagnosed and treated at our centre. Two of the 5 patients were on immunosuppressive treatment. While clinical presentations differed in each patient, all had a long history of skin lesions. In addition to careful history-taking, tissue biopsies were obtained for mycobacterial culture and histopathological examination. Culturedirected antibiotic therapy was initiated, which resulted in a slow, but continuous, healing of the lesions. In summary, M. chelonae infections are still relatively rare, but should be considered in both immunocompromised and immunocompetent patients with prolonged skin lesions resistant to standard antibiotic treatment. For diagnosis, tissue analysis for mycobacterial culture and histopathological examination, and once diagnosed, adequate antibiotic treatment, is needed.

Key words: non-tuberculous mycobacteria; NTM; rapidly growing mycobacterium; RGM; skin infection; soft tissue infection.

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Mycobacterium chelonae is classified as a rapidly growing non-tuberculous mycobacterium (NTM) (1–3). While other mycobacteria may take several weeks to grow when subcultured in the laboratory, rapidly growing NTMs are usually characterized by growth within 7 days (1–3). In general, NTM are considered less pathogenic than slow-growing species (4). *M. chelonae*, in particular, was first isolated by Freidmann from a sea turtle (Greek: chelōnē) in 1903 and is commonly associated with human skin and soft-tissue infections, mainly of the extremities (1–3, 5, 6). *M. chelonae* shows optimal growth at 28–32°C and usually causes disseminated rather than localized skin infection (1–3, 5, 6).

Infections with *M. chelonae* may affect immunocompromised patients, e.g. HIV-positive and cancer patients, as well as patients on immunosuppressive or biologic therapy due to autoimmune disorders or after organ transplantation (7–10). *M. chelonae* is frequently found in the

SIGNIFICANCE

Patients can present with infections of the skin and soft tissues due to the pathogen *Mycobacterium chelonae*. Clinical presentation of this relatively rare infectious disease may differ from individual to individual. Despite an increasing incidence, affected patients are often misdiagnosed. Thus, in addition to careful history-taking, tissue biopsies for mycobacterial culture and histopathological examination are needed to reach a correct diagnosis. Once diagnosed, culture-directed antibiotic therapy is required. We report here 5 patients with cutaneous and/or soft-tissue infections due to *M. chelonae*, who were diagnosed and treated appropriately at our centre.

natural environment, e.g. in soil, natural or treated water (11-13), and in association with plants or fish (14). M. *chelonae* has also been found within free-living amoebae (15, 16). Reports of infections of immunocompetent patients with *M. chelonae* have increased in recent decades. especially following medical surgical procedures (17, 18), mesotherapy (19), or cosmetic procedures, e.g. cosmetic face surgery, breast surgery, or liposuction (20-23). These postsurgical infections are thought to be due to insufficient sterilization of used surgical instruments (24, 25). In addition, M. chelonae infections have been reported after visits to tattoo studios or nail salons, due to contaminated pre-mixed ink or water (26, 27). More severe and disseminated cases of infection with *M. chelonae*, however, have been reported in immunocompromised rather than immunocompetent patients (7–10).

These studies show that exposure to, and infections with, *M. chelonae* is possible in different areas of daily life, including nosocomial surroundings. Although an increase in incidence has been seen over recent years (1, 28), infections are often misdiagnosed, since clinical presentations may vary in each patient. In addition, optimal therapy is not well-established to date, and calculated antibiotic therapy based on *in vitro* sensitivity testing is needed in each case. The use of standard antibiotics is generally not adequate and often results on a prolongation of symptoms and/or complications.

We report here 5 patients with a diagnosis of cutaneous and/or soft tissue infection due to the rapidly growing NTM, *M. chelonae*. In addition to a detailed description of these cases, a summary of the disease characteristics, diagnostic procedures, and adequate treatment regimens is given for this relatively rare disorder.

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Fable I. Clinical characteristics of	f patients presentin	g with Mycobacterium of	chelonae infection at o	our department
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	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age, years, sex	87, F	39, F	85, F	85, F	77, F
Localization of skin lesions	Left lateral malleolus	Lower extremities	Left dorsal hand and arm	Second toe of the left foot	Distal left leg
Clinical presentation	Ulceration, abscesses	Ulcerated papules, plaques, abscesses	Ulcerated nodules	Ulceration	Nodules, papules
Associated pain	Yes	No	Yes	Yes	Yes
Immunosuppressive treatment	None	TNF-blockers, oral prednisone	None	None	Methotrexate oral prednisone
Bacterial culture, acid-fast staining	Culture +, acid-fast +	Culture +, acid-fast +	Culture +, acid-fast +	Culture +, acid-fast +	Culture +, acid-fast +
Therapy (based on sensitivity in bacterial culture)	Clarithromycin, imipenem, tobramycin	Clarithromycin, ciprofloxacin, meropenem, tobramycin	Clarithromycin, imipenem, tobramycin	Clarithromycin, imipenem, tobramycin	Clarithromycin, imipenem, tobramycin
Time for lesions to heal	2 years	6 months	4 months	6 months	1 year

TNF: tumour necrosis factor.

PATIENTS AND RESULTS

All patients presented at the Department of Dermatology of Erlangen University Hospital, Germany between 2010 and 2014. The clinical characteristics of the patients are listed in **Table I**.

Patient 1. An 87-year-old woman presented with a 6-month-history of painful ulcerations, erythema and oedematous swelling of the left lateral malleolus. Since erysipelas was initially suspected, treatment with different oral antibiotics, including cefuroxime, clindamycin and ofloxacin, was initiated by the treating family physician. However, further progression occurred with abscess formation. The patient denied any recent exposure to swimming pools or gardening, or any fever, night sweats, or other constitutional symptoms. Secondary diagnoses included arterial hypertension, heart failure, coronary heart disease, bronchial asthma, and chronic venous insufficiency, which were adequately treated with no recently changed agents.

Clinically, 2 ulcerations of the left lateral malleolus, approximately 2.0 and 0.5 cm in diameter, with surrounding erythema, oedema, and abscess formation, in addition to pronounced varicosis of both legs, were seen on first presentation (**Fig. 1**A). Skin biopsy from the lesion was performed for histopathological examination as well as standard bacterial and mycobacterial culture. Histologically, lymphocytic inflammatory infiltrates and reactive vasculopathy were seen. Fite's acid-fast stain showed bacilli in the dermis, consistent with a mycobacterial infection. Culture of the skin biopsy further confirmed the rapid-grower *M. chelonae*.

Based on the patient's history, clinical presentation, positive bacterial stain and culture, a diagnosis of cutaneous NTM-infection due to *M. chelonae* was made. Surgical debridement of the ulcerations was performed and culture-directed systemic treatment with clarithromycin, imipenem and tobramycin was started. Slow, but continuous, improvement and subsequent healing of the ulceration occurred over a period of 2 years (Fig. 1). There have been no recurrences to date.

Patient 2. A 39-year-old woman presented with a 2-year history of ulcerations of her proximal lower extremities. Her medical history included rheumatoid arthritis of the small joints of the hands and

feet, which was first diagnosed 6 years ago. The patient reported relapsing symptoms, but was currently well treated with oral prednisone. In addition, she had been diagnosed with granulomatous leukocytoclastic vasculitis approximately 4 months prior to presentation and therapy with tumour necrosis factor (TNF)-blockers was started. The patient denied recent exposure to swimming pools, tattoo needles or gardening. However, after starting the treatment with TNF-blockers, a worsening of pre-existing skin lesions and ulcerations occurred. She denied any current pain, fever, night sweats, or other constitutional symptoms. No further diagnoses, and no new or additional medications, besides concomitant oral treatment with pantoprazole, vitamins, and calcium, based on her immunosuppressive therapy, were known.

Clinically, multiple erythematous-violaceous, ulcerated papules, plaques, and abscesses on both proximal and distal lower extremities were seen, each approximately 0.5–3.0 cm in diameter. Clinical and sonographic examination of the veins and arteries of the lower extremities were unremarkable. After incision of abscesses, draining fluid was sent for smear testing and culture. In addition, a lesional skin biopsy for histopathological examination and standard bacterial, as well as mycobacterial, culture was performed. Histologically, lymphocytic infiltrates and signs of granulomatous inflammation with no evidence of malignancy were seen. Fite's acid-fast stain showed bacilli in the dermis, consistent with a mycobacterial infection. Bacterial culture of the obtained tissue identified rapid-growing *M. chelonae*.

In consultation with the treating rheumatologists, TNF-blockers were stopped and calculated systemic treatment with ciprofloxacin, clarithromycin, meropenem und tobramycin was initiated and continued over a period of 6 months. Slow, but continuous, improvement of the skin lesions occurred subsequently. Minor recurrences of papular skin lesions at the lower extremities were interpreted as manifestations of the already known granulomatous leukocytoclastic vasculitis, as both histological and culture examination of repetitive skin biopsies could not show NTMs. After 6 months of antibiotic treatment and successive improvement and, finally, healing of the skin lesions, immunosuppressive therapy with azathioprine was started for systemic vasculitis and rheumatoid arthritis.





Patient 3. An 85-year-old woman presented with a 5-month history of painful nodules and ulcerations of her left dorsal hand and proximal arm. While doing regular and intensive gardening she frequently washed her garden tools with tap water. In addition, approximately 2 months prior to her presentation, she was treated for urosepsis with antibiotics at a local hospital. The biopsy of a skin nodule at that occasion showed only unspecific results in histopathological analysis. However, no bacterial culture was performed. Secondary diagnosis included arterial hypertension and atrial fibrillation, which were adequately treated with oral medication. She was not taking any new or immunosuppressive medications. Approximately 10 years previously, a chronic B-cell leukaemia was diagnosed and treated repetitively with rituximab, bendamustine, and prednisone several years ago. The patient is currently in complete remission of medication and receives regular follow-up examination only.

Clinically, multiple erythematous-violaceous and ulcerated nodules were seen on her left dorsal hand and distal arm, each approximately 0.5-3.0 cm in diameter (Fig. 2A). In a lesional skin biopsy, infiltrates of inflammatory lymphocytes were histologically seen and Fite's acid-fast stains showed bacilli in the dermis, consistent with a mycobacterial infection. Culture of the skin tissue further confirmed the rapid-grower M. chelonae.

Adequate culture-directed systemic treatment with clarithromycin, imipenem and tobramycin was started, in addition to surgical debridement of the ulcerations. Due to nausea, imipenem was changed to meropenem, which was well tolerated. Continuous and relatively rapid improvement (Fig. 2) and subsequent healing of the ulceration occurred within 4 months, with no recurrences to date over the course of 4 years.

Patient 4. An 85-year-old woman presented with a 6-month history of ulceration of the second toe of her left foot. The lesion started following a tick bite with mild erythema and a papule. Despite negative serological examination for Borrelia infection performed by the family physician, she received antibiotic therapy with doxycycline over a period of 3 weeks with no improvement in the skin lesion. On the contrary, progression occurred with painful ulcerations and local swelling. The patient denied any recent exposure to swimming pools or gardening or fever, night sweats, or other constitutional symptoms. Arterial hypertension, heart failure, coronary heart disease, and atrial fibrillation were treated adequately with no new, or other, immunosuppressive medication.

Clinically, a well-demarcated ulceration was seen on the dorsal second toe of the patient's left foot, approximately 1.0 cm in diameter with mild surrounding erythema and swelling. Clinical and sonographic examinations of the veins and arteries of the



lower extremity were unremarkable. In a lesional skin biopsy, histopathologically inflammatory lymphocytes and reactive vasculopathy were seen. Fite's acid-fast stain showed bacilli in the dermis, consistent with a mycobacterial infection. Culture of the skin biopsy confirmed the rapid-grower M. chelonae.

Skin infections caused by mycobacterium chelonae

891

Based on the results of antibiotic susceptibility testing, systemic treatment with clarithromycin, imipenem and tobramycin was started. In addition, repetitive surgical debridement of the ulcerations was performed. Slow, but continuous, improvement and subsequent healing of the ulceration occurred after 6 months, with no recurrences over 2 years.

Patient 5. A 77-year-old woman with a 1-year history of giant-cell arteritis on immunosuppressive therapy with subcutaneous methotrexate and oral prednisone presented with a 3-month history of painful skin lesions of her proximal left leg. She denied any recent exposure to swimming pools, but reported that she was doing a lot of gardening. The skin lesions developed after a minor local injury while gardening and washing with tap water. The patient denied any fever, night sweats, or other constitutional symptoms. Oral medications included pantoprazole, vitamins, folate, and calcium, based on her immunosuppressive therapy. In addition, atrial fibrillation was adequately treated.

Clinically, multiple violaceous and excoriated nodules and papules were seen on the left distal leg, each approximately 0.5-2.0cm in diameter (Fig. 3A). A lesional skin biopsy histologically showed infiltrates of inflammatory lymphocytes and inflammation of the small arterioles and Fite's acid-fast stains further revealed bacilli in the dermis, consistent with a mycobacterial infection. Culture of the skin biopsy confirmed the rapid-grower M. chelonae.

Culture-directed systemic treatment with clarithromycin, imipenem and tobramycin was started with continuous improvement (Fig. 3) and subsequent healing of the ulceration. To date, after approximately 5 years, no recurrences have been observed in follow-up.



Fig. 3. Clinical presentation of patient 5. (A) Skin lesions prior to adequate antibiotic treatment, and (B) approximately 1 month after initiation of therapy.



Fig. 2. Clinical presentation of patient 3. (A) Skin lesions prior to adequate antibiotic treatment, and approximately (B) 1 month and (C) 2 months after initiation of therapy.

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DISCUSSION

We report here 5 cases of non-tuberculous mycobacterial (NTM) infections of the skin and soft tissue due to *M. chelonae*. While 2 patients developed their infection during ongoing immunosuppressive therapy (patients 2 and 5), the other 3 patients were not taking any immuno-suppressive medication and did not have any other known immunosuppressive state. Two patients reported doing gardening (patients 3 and 5), one of these with a definite injury prior to symptom development at the same site (patient 5). Immunosuppression, as well as skin injury while gardening, might have caused the infection. The detailed history of these 5 patients confirms that exposure to *M. chelonae* can occur in different areas of life.

Cases of *M. chelonae* infections have been reported globally, indicating that there is no specific geographical distribution, and perennially, with no seasonal trend (1-3, 1)5, 6). In addition, no associations with sex or race can be found (1-3, 5, 6). Although no obvious association with age has been reported so far (1-3, 5, 6), older age may present with risk factors and events, such as waning immune competence, malignancies or autoimmune disorders, surgical procedures and intake of immunosuppressive agents. The incidence of infections caused by NTMs varies between 0.9 and 7.2 cases per 100,000 persons per year (1-3, 5, 6, 28, 29). As these infections are not notifiable, the true prevalence is thought to be higher (28-31). This may contribute to a low awareness of the disorder in the lay population, as well as in the medical population, even though studies showed a worldwide increasing trend of NTM infections (28-31). A recent retrospective study from a medical centre in the USA reported an incidence of skin NTM infections of 0.7 per 100,000 person-years in 1980 to 1999 and 2.0 per 100,000 person-years in 2000 to 2009 (28). In particular, infections caused by M. chelonae showed an increase from 7% to 46% of cases (28). Regarding this increase in infections, appropriate and timely diagnosis remains a challenge for treating physicians.

The clinical manifestation of the disease may vary over a wide range, as seen in the cases reported here, which makes correct diagnosis demanding. Skin lesions may appear as erythematous or violaceous papules or plaques, pustules, folliculitis, or panniculitis (1-3, 5, 6). In rare cases, the infection may also present with a "sporotrichoid" pattern, spreading along subcutaneous lymphatics from the site of entry (32). Later on, skin lesions can become erosive or ulcerated (1-3, 5, 6). Lesions may be painful, but asymptomatic cases, as observed in one of our patients, have also been reported (1-3, 5, 6). In rare cases, fever or other constitutional symptoms may be associated with the disease (1-3, 5, 6).

In addition to detailed patients' history, skin biopsies from representative lesions are required for histological and microbacterial culture (1–3, 5, 6). Histopathologically, unspecific rather than pathognomonic signs, such

as acute inflammation, microabscesses, granulomas (with or without caseation), and/or reactive vasculopathy, are seen using routine staining, such as haematoxylin and eosin (1–3, 5, 6). Acid-fast staining with, for example, Ziehl-Neelsen, will be needed to identify bacilli (33, 34). Fites' acid-fast stain, as performed at our centre, proved to be an effective method, which uses lower alcohol concentrations and a milder destaining acid compared with classical acid-fast bacilli staining (33, 34). In addition, appropriate bacterial culture determines the correct mycobacteria species (1-3, 5, 6). Basic bacterial cultures do not include the detection of mycobacteria and thus, mycobacterial culture with prolonged culture should be performed (1-3, 5, 6). Molecular biological techniques, such as PCR and restriction fragment length polymorphism (RFLP), may be used to identify NTMs, but cannot distinguish M. chelonae from other species (1-3, 5, 6). Similarly, high-performance liquid chromatography (HPLC) alone is not able to separate M. chelonae from *M. abscessus* (1-3, 5, 6). However, recently the PCR-restriction fragment length polymorphism analysis (PRA), which combines the PCR of a NTM-related heat shock protein with restriction fragment length identification, was shown to efficiently detect and differentiate NTM (35). In addition, it was possible to distinguish M. chelonae from other species (35), showing potential for its use in detecting the causative agent.

On diagnosis, adequate treatment with systemic antibiotic agents is needed (30). As M. chelonae might show an unpredictable resistance pattern, susceptibility testing should be performed in each patient (1-3, 5, 6). While clarithromycin monotherapy can be sufficient for localized skin infections, combination therapy with at least 2 antibiotic agents is recommended due to potential development of resistance during the often prolonged therapy (30). The American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA), for instance, recommends an oral macrolide initially combined with cefoxitin, amikacin, or imipenem-cilastatin (30) for the treatment of cutaneous rapidly growing mycobacteria (RGM) infections. In case of disseminated or invasive disease with bone and/or soft tissue involvement, 4-6 months of systemic therapy is recommended (30). In addition to systemic antibiotic treatment, local antiseptic and antibacterial therapy, repetitive surgical debridement in case of ulcerations, as well as incising and draining abscesses, are important and necessary tools for appropriate treatment (1-3, 5, 6, 30).

In summary, despite increasing prevalence, infections of the skin and soft tissue due to the rapidly growing NTM *M. chelonae* is still a rare dermatological phenomenon and further studies and cases should be reported to increase awareness of this disorder. In particular, appropriate and timely diagnosis remains a challenge and, thus, once suspected, biopsy of a skin lesion to obtain tissue for histopathological examination, including acidfast staining, routine bacterial culture and mycobacterial culture with prolonged culture periods, should be performed to detect the bacilli and make a correct diagnosis. In particular, NTM infections should be considered in the differential diagnoses of long-lasting and therapyrefractory skin lesions and/or ulcerations, primarily after trauma, surgery, and cosmetic procedures in both immunocompromised and immunocompetent patients. Upon diagnosis, adequate culture-directed treatment is needed.

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