Original Article

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Diagnosis and therapy of *Mycobacterium marinum*: a single-center 21-year retrospective analysis

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Summary

Background and Objectives: In Europe, infections with *Mycobacterium (M.) marinum* are rare. We conducted a retrospective single-center study to assess the clinical spectrum of *M. marinum* infection and its diagnosis, treatment and outcome under real-world conditions.

Patients and Methods: Eighteen patients presenting with *M. marinum* infections between 1998 and 2018 were identified in the data warehouse of the University Hospital Würzburg and considered for detailed analysis.

Results: Twelve patients reported aquatic exposure. In 16/18 cases the upper extremities were affected. No invasive infections were detected. Mean time to diagnosis was 15 weeks. Histology revealed granulomatous inflammation in 14 patients while mycobacterial cultures were positive for *M. marinum* in 16 cases. Most patients received antibiotic monotherapy (14/18) while combination therapy was administered in four cases. Treatment (with a median duration of 10 weeks) was successful in 13 patients. Five patients were lost to follow-up.

Conclusions: Our retrospective analysis of *M. marinum* infections at a German tertiary referral center revealed a considerable diagnostic delay and the relevance of microbiological culture, PCR and histology for diagnosis. Monotherapy with clarithromycin (rather than doxycycline) appeared as a reasonable treatment option while immunosuppressed or -compromised patients and those with extended disease received combination therapy.

Introduction

Mycobacterium (M.) marinum is one out of more than 125 recognized species of non-tuberculous mycobacteria (NTM), which occur ubiquitously in the human environment as opportunistic pathogens. Non-tuberculous mycobacteria are aerobic, with the notable exception of *M. marinum* non-spore-forming, non-motile, mycolic acid-containing rod-shaped bacteria [1–3] that may affect any human organ with pulmonary, skin and soft tissue infections being the most common [4].

In immunocompetent individuals, the majority of cutaneous NTM infections is caused by *M. marinum*. Infection occurs after traumatic injury and concurrent or subsequent exposure to contaminated water, predominantly aquaria, or infected fish. Due to chlorination, swimming pools are less important as a source of infection today. *M. marinum* mainly affects the upper extremities predominantly at acral sites which is favored by their preferred growth conditions at temperatures between 30–32°C. Disseminated infection is almost exclusively seen in immunosuppressed individuals [5, 6]. The incubation period is relatively variable and ranges from three weeks to nine months [7, 8].

Upon infection, mainly solitary papulonodular lesions evolve on fingers and hands. In some cases, a sporotrichoid distribution pattern along the lymphatic drainage pathways is observed. Occasionally, pustular, nodular-ulcerative, granulomatous or verrucous plaques occur. Affection of deeper structures may result in tendosynovitis, osteomyelitis, arthritis or bursitis [5, 9].

Since clinical presentation is not always indicative making the diagnosis may occasionally be difficult. Taking a thorough patient's history is important particularly in such cases. To confirm a suspected diagnosis of *M. marinum*

infection lesional tissue samples should be obtained for histopathological analysis, microbiological culture and PCR [10, 11]. Histological presentation depends on the age of the lesion and may vary from non-specific inflammatory infiltrates to granulomas with multinucleated giant cells and fibrinoid necrosis. Histological detection of mycobacteria by acid-fastness special stain methods such as Ziehl-Neelsen staining often succeeds only in a limited number of cases. Detection by microbiological culture is more successful and allows verification of *M. marinum* in 70–80 % of cases. The polymerase chain reaction (PCR) can be used to detect *M. marinum* but cannot securely distinguish between *M. marinum* and *M. ulcerans*. [2, 7]. It should be noted that both the tuberculin test as well as the interferon-gamma release assay may be positive due to cross-reactivity with *M. tuberculosis* [11].

The treatment of cutaneous *M. marinum* infections is not yet standardized and includes antibiotic mono- and combination therapies that often require several months of therapy [12]. Spontaneous cure has been reported as well [10].

In light of the limited data on *M. marinum* infection available we performed a retrospective study to better understand the spectrum of *M. marinum* infection including its clinical presentation, diagnostic measures, treatment and outcomes.

Material and Methods

Patients diagnosed with *M. marinum* infection at the University Hospital Würzburg in the years between 1998 and 2018 were identified in the SAP medical information system using Padavan software. In addition, the NEXUS histopathology databank of the Department of Dermatology was searched for mycobacterioses. Search terms included "Schwimmbadgranulom", "Mykobakteriose", "Mycobacteriose", "Mycobakteriose" and "Atypische Mykobakteriose". Moreover, the NEXUS SWISSLAB database of the Institute of Hygiene and Microbiology at the University of Würzburg was used to identify patients with microbiologically verified detection of *M. marinum*. The following data were extracted from clinical and laboratory records: sex, age, ethnic background, date of infection, beginning of symptoms, presumed cause of infection, symptoms, number and localization of lesions, organ involvement, immunosuppression (pre-existing immunocompromising conditions, use of immunosuppressive medication, other causes), methods used for pathogen detection (histology, culture, PCR), time from onset of symptoms to diagnosis (with the latter defined as time point of acquisition of tissue for laboratory diagnostics), therapy (medication, duration, treatment outcome), relapse/ resolution. Treatment outcomes were defined as *(1)* healing (complete healing of all sites/ lesions); *(2)* partial remission (regression of lesion(s) for at least 50 %); *(3)* stable disease (regression of lesion(s) less than 50 %); *(4)* progress (increase of lesions more than 50 % and/ or development of new lesions); *(5)* failed/ lost to follow-up (no documentation of treatment or outcome available).

Results

A total of 18 patients (13 males, 5 females) with a mean age of 52.2 ± 16.5 years (median 52 years) suffering from cutaneous *M. marinum* infection had been identified in the period between 1998 to 2018. All of them were of Caucasian background. Four patients (22 %) were immunocompromised of whom one suffered from breast cancer and recent treatment with epirubicin and cyclophosphamide. Another two had been treated with prednisolone due to pulmonary emphysema or polyneuropathy. The fourth patient was immunosuppressed due to hemodialysis and kidney transplantation.

Twelve (67 %) patients reported exposure to aquaria or ponds. One *M. marinum* infection occurred in temporal association with the visit of a thermal bath. In another patient an injury from a broken mirror was suspected as cause of infection. In four cases (22 %) information on the presumed portal of entry was missing. The mean time to diagnosis for all cases of *M. marinum* infection was 15 ± 13 weeks (median: 12 weeks).

In the vast majority of cases (16/18; 89 %) *M. marinum* infection occurred at the upper extremities. Three patients (19 %) showed skin lesions only on the forearm and in 13 cases (81 %) involvement of the dorsum of the hand was documented. Two cases (11 %) presented with involvement of other body sites (infraorbital region/nose and dorsum of the foot). Involved body sites and absolute frequencies of distribution are depicted in Figure 1.

Ten patients (56 %) presented with reddish-livid, partially scaling nodes, whereas three patients each (19 %) showed erythematous either firm and relocatable papules or plaques. In two cases (11 %) hyperkeratotic patches were observed. Figure 2 illustrates typical clinical presentations. Six individuals (33 %) reported pain on palpation, burning and/ or itching.

Histological examination revealed granulomatous inflammation with surrounding lymphohistiocytic infiltrate in 14 patients (78 %) as it is depicted in Figure 2c. Biopsies from three further patients (17 %) only showed lymphocytic or neutrophil-rich inflammatory infiltrates. In one case, no information on histology was available.

Mycobacterial cultures were performed for all patients and were positive for *M. marinum* in 16 cases (89 %). In four cases (22 %) PCR was conducted additionally. Acid-fast bacilli were identified using Ziehl-Neelsen stains in two (14 %) out of 14 patients.

Upon diagnosis, all patients received antibiotic treatment (Table 1). Fourteen patients (78 %) had exclusively

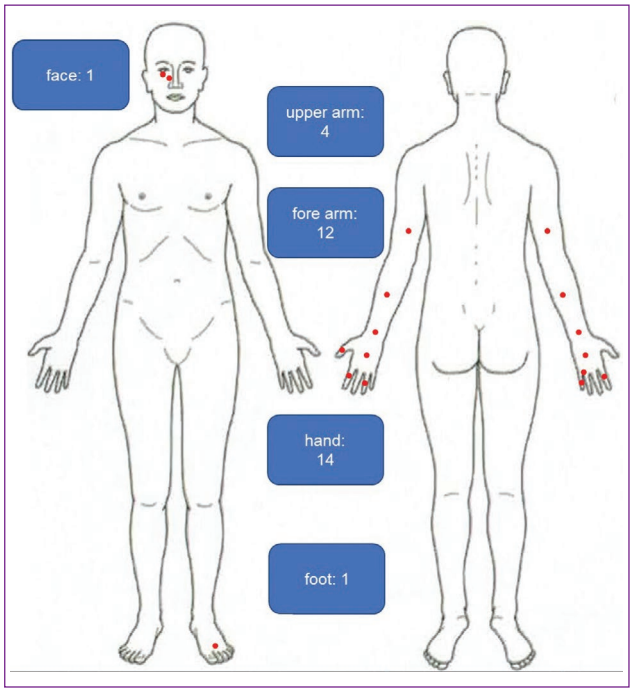

Figure 1 Localization of skin lesions due to *M. marinum* infection. Numbers of patients with affected body sites are shown.

been treated with a single antibiotic regime, of whom eleven patients (79 %) received clarithromycin (2 × 500 mg/d) and three (21 %) initially tetracyclines (doxycycline or minocycline, 2 × 100 mg/d each). In the latter group, one of the patients was later switched to clarithromycin. Due to extensive skin involvement or immunosuppression two patients (11 %) were administered from the beginning a combination therapy of three antibiotics: clarithromycin, ethambutol (15 mg/kg body weight [BW]) and rifampicin (10 mg/kg BW, max. 600 mg/d). Two other patients (11 %) received combination treatment during the further course of their infection. A total of five patients (28 %) were treated with doxycycline at least once, but in four of those it was terminated due to non-efficacy, relapse or side effects. The fifth patient on doxycycline was lost to follow-up.

Mean duration of monotherapy of *M. marinum* infections was 12 ± 6 weeks (median 10 weeks). The two patients with combination therapy were treated for a slightly longer period (mean 16 weeks). The treatment of the two patients who sequentially received both mono- and combination therapies lasted 31 and 41 weeks, respectively, of which the first 6 and twelve weeks were not guided by our department. In two patients, therapy with minocycline or doxycycline had to be switched because of side effects. The double combination of ethambutol and rifampicin had to be discontinued and switched in one patient due to side effects, as did the triple combination of ethambutol, rifampicin, and clarithromycin

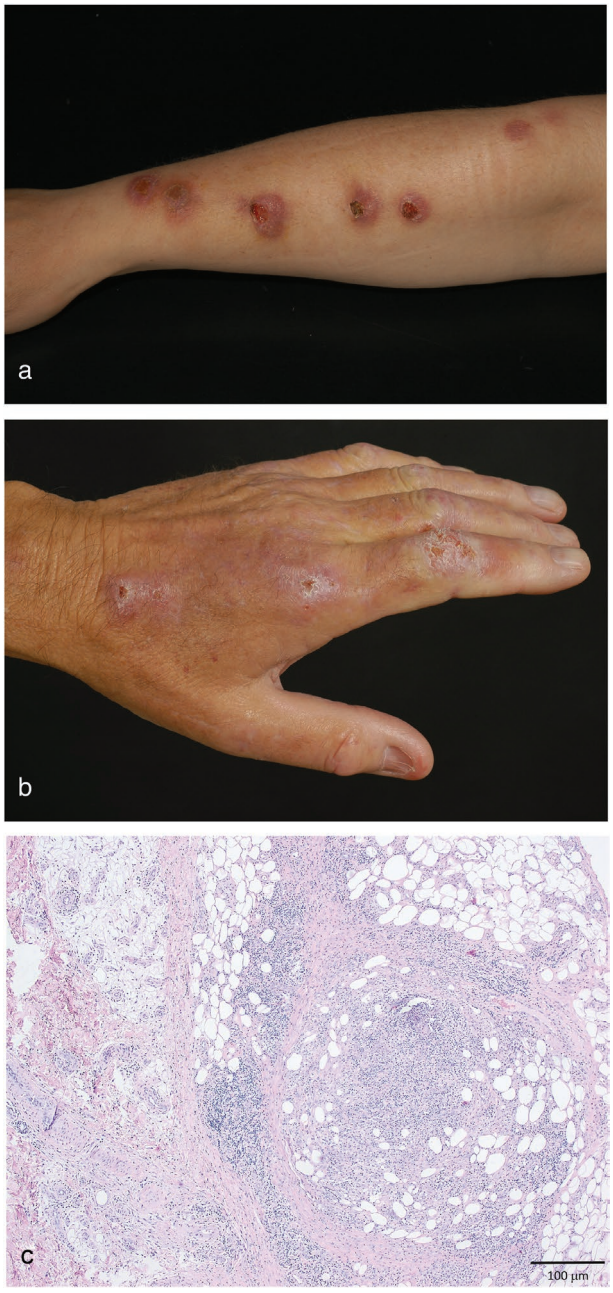

Figure 2 Characteristic clinical presentations of *M. marinum* infections. 45-year-old women (Patient-ID 8) with several reddish-livid, partially scaling plaques and nodes, extending from the wrist to the elbow in a sporotrichoid distribution (a). 55-year-old man (Patient-ID 4) with reddish, scaling plaques on the back of the hand and index finger (b). Histopathology of a skin lesion evolved after infection with *M. marinum*. Biopsy from the wrist showing dermal and subcutaneous nodular infiltrates with a partly granulomatous, partly abscessing inflammatory reaction without evidence of necrosis that is surrounded by a lymphoplasmacellular infiltrate (hematoxylin-eosin stain, bar: 100 µm) (c).

Table 1 Overview on clinical features, diagnostics, treatments and outcomes in patients with *M. marinum* infection.

Patient-ID	Age (Years), Sex (female/male)	Site of disease	Histological detection of Granulomatous inflammation (positive/negative)	Acid-fast staining (positive/negative)	Microbiological culture or PCR (positive/negative)	Treatment/antibiotic agent (duration of treatment in weeks)	Final outcome (healing/partial remission/stable disease/progress/lost to follow-up)
1	45, female	Left upper arm, left forearm, left hand	Negative	Positive	Positive (culture)	a) Minocycline (1) b) Doxycycline, (unknown)	Lost to follow-up
2	54, male	Left forearm, left and right hand	Positive	Negative	Positive (culture)	Doxycycline (2)	Healing
3	38, male	Right hand, right upper arm	Unknown	Unknown	Positive (culture)	a) Doxycycline (6) b) Ethambutol, Rifampicin (3) c) Clarithromycin (22)	Healing
4	55, male	Left forearm, left hand	Positive	Unknown	Positive (culture, PCR)	Clarithromycin, Rifampicin, EMB (12)	Healing
5	84, male	Right hand	Positive	Negative	Positive (culture)	Clarithromycin, (unknown)	Lost to follow-up
6	27, female	Right forearm, right hand	Positive	Unknown	Positive (culture)	a) Doxycycline (8) b) Clarithromycin (16)	Healing
7	55, female	Infraorbital, nose	Positive	Positive	Positive (PCR)	Clarithromycin (15)	Healing
8	45, female	Right forearm	Positive	Negative	Positive (culture, PCR)	Clarithromycin, Rifampicin, EMB (20)	Healing
9	59, male	Right upper arm, right forearm, right hand	Positive	Negative	Negative (culture)	Clarithromycin, (16)	Healing

Table 1 Continued.

Patient-ID	Age (Years), Sex (female/male)	Site of disease	Histological detection of Granulomatous inflammation (positive/negative)	Acid-fast staining (positive/negative)	Microbiological culture or PCR (positive/negative)	Treatment/antibiotic agent (duration of treatment in weeks)	Final outcome (healing/partial remission/stable disease/progress/lost to follow-up)
10	72, male	Right forearm	Positive	Positive	Positive (culture)	a) Doxycycline, (5) b) Clarithromycin, (5) c) Clarithromycin, Ethambutol, Rifampicin, (2) d) Clarithromycin, (6) e) Clarithromycin, Doxycycline, (15) f) Doxycycline, (8)	Healing
11	39, male	Left hand, left wrist	Positive	Negative	Positive (culture)	a) Doxycycline, (4) b) Clarithromycin (unknown)	Lost to follow-up
12	48, male	Left forearm, left wrist	Positive	Negative	Negative (culture)	Clarithromycin, (8)	Healing
13	79, male	Left forearm	Negative	Positive	Positive (culture)	a) Clarithromycin, (14) b) Clarithromycin (unknown)	Lost to follow-up
14	64, male	Right hand	Positive	Negative	Positive (culture)	Clarithromycin, (8)	Healing
15	50, female	Right forearm, right hand	Positive	Negative	Positive (culture)	Clarithromycin, (10)	Healing
16	58, male	Right upper arm, right forearm, right hand	Negative	Negative	Positive (culture)	Clarithromycin, (6)	Lost to follow-up
17	19, male	Left foot	Positive	Negative	Positive (culture)	Clarithromycin, (10)	Healing
18	48, male	Left forearm, left hand, left wrist	Positive	Negative	Positive (PCR)	Clarithromycin, (12)	Healing

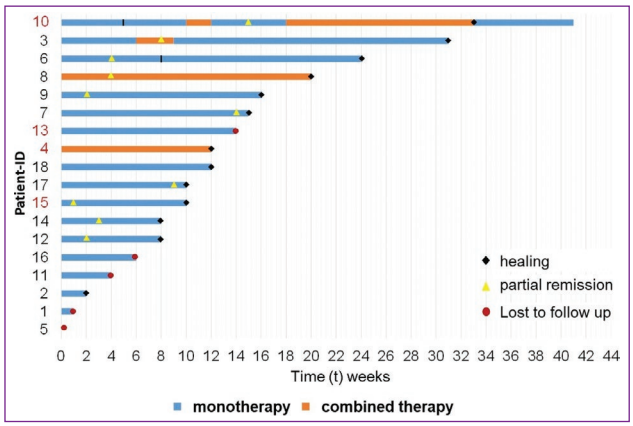

Figure 3 Swimmer plot illustrating treatments and outcomes in patients with *M. marinum* infection. Patient IDs in red font indicate immunosuppressed or immuno-compromised individuals. The black mark (patient ID 6 and 10) indicates a modification of monotherapy (change of antibiotic or dose).

in another patient. Monotherapy with clarithromycin, on the other hand, was well tolerated and did not need to be switched or prematurely discontinued in any patient.

In patients receiving monotherapy partial remission was observed after a mean of four weeks while healing was documented after a mean of twelve weeks. Patients receiving combination therapy from the beginning experienced healing within a mean of 16 weeks while those with sequential mono- and combination therapy showed complete resolution after a mean of 32 weeks. In 13 out of 18 patients (72 %) *M. marinum* skin lesions completely resolved while five patients (28 %) were lost to follow-up. Two patients (11 %) suffered a relapse, of whom one was immunosuppressed due to treatment with methylprednisolone. The second patient presented herself for the first time at our department already with a relapse. Duration of treatment was longer in immunosuppressed and -compromised than in immunocompetent patients. The latter were treated for a mean of 15 weeks, whereas the therapy of immunosuppressed individuals lasted 21 weeks on average. Figure 3 illustrates kind and duration of therapy and treatment response in patients with *M. marinum* infection.

Discussion

So far, only individual case reports, but no case series on *M. marinum* infections have been published from Germany. We here studied 18 patients who presented exclusively with cutaneous rather than invasive infections that in 89 % affected the upper extremities. In other studies, invasive infections had also been reported: In a North American case study 68 % showed invasive infections that mostly occurred after exposure during boating or fishing. An infection is classified as invasive if tenosynovitis, septic arthritis, or osteomyelitis are present [13]. In a national survey from France, 29 % of 63 patients showed an invasive infection and in 84 % an earlier exposure to fish tanks was documented [14]. In our case series fish tank exposure was most common (67 %) and infections presumably occurred as a consequence of rather superficial injuries due to minor trauma. In contrast, exposure during fishing and boating that is more often resulting in deeper wounds increases the risk for invasive infections.

Our results reveal a considerable delay between infection with *M. marinum* and its diagnosis. The period between the onset of first symptoms and diagnosis was 15 weeks on average which is well in accordance with published experiences [8, 14]. Delayed diagnosis may reflect an inadequate awareness of the disease and highlights the importance of precise anamnesis and, in case of suspicion, initiation of expedient diagnostic measures.

Histopathological examination suggested fish tank granuloma in 78 % of our cases. However, histopathology is not evidencing *M. marinum* infection and interpretation may be impaired by the age of the lesion at the time of sampling. Ziehl-Neelsen staining allowed identification of acid-fast bacilli in only 14 % of cases. Similarly low acid-fast bacilli detection rates were reported in other studies [13, 15, 16]. For this reason, other microbiological detection methods are obligatory for diagnostics and differentiation of mycobacterial skin infections [13, 17, 18]. In our study, detection of *M. marinum* by culture was successful in 16 cases but failed in two, which is in accordance with observations from Italy [17]. The latter situation illustrates the diagnostic importance of *M. marinum* detection by PCR [16]. Prospectively, molecular methods such as PCR techniques may play a more important role in the diagnosis of *M. marinum* infections. While surely not being 100 % sensitive PCR may be employed in parallel to microbiological cultures to increase the diagnostic yield. As far as detection by culture and PCR is not successful or available, diagnosis needs to be made – as in two of our patients – solely on the basis of anamnesis, clinical appearance and histology [9, 16, 19].

Antibiotic treatment of *M. marinum* infections is the therapy of choice. Due to the rarity of the disease, controlled studies on the effectiveness of antibiotic therapies are lacking to date [12]. In our cases series of exclusively cutaneous *M. marinum* infections 78 % of patients were treated by monotherapy with either clarithromycin or tetracyclines. While doxycycline treatment was terminated in 80 % of cases because of non-efficacy, relapse or side effects, clarithromycin monotherapy turned out to be effective in all immunocompetent patients with a good tolerability resulting in complete resolution of skin lesions. Our findings are thus in line

with observations by Dodiuk-Gad et al. [20] and Feng et al. [21] who observed complete remission of cutaneous *M. marinum* manifestations upon clarithromycin monotherapy in 50 to 83 % of patients. Our experiences of doxycycline failure parallel reports by Ljungberg et al. [22] and Cummins et al. [23] who described three patients with *M. marinum* infections whose therapies needed to be switched due to lack of efficacy of doxycycline.

Combination therapies might be advisable in two situations: *(1)*, in immunocompromised or immunosuppressed patients, and *(2)*, in cases of extensive disease including those with involvement of deeper structures such as joints, tendons and/ or bones [13, 14]. While the choice of the treatment regimen appears to more reflect personal experiences and preferences of individual authors/ health care professionals than proven efficacy [7], the combination of rifampicin, ethambutol and clarithromycin turned out as a reasonable treatment option for our immunocompromised patients.

In our case series, monotherapy was terminated after twelve weeks, whereas combination therapy lasted 16 weeks on average. The treatment duration of our patients was comparable to the results of other studies. Bonamonte et al. reported an efficacy of monotherapy in 13 of 15 patients within two to three months of treatment [17]. Ang et al. reported an average treatment duration of 15 weeks [9]. In the study by Johnson and Stout, which, however, also covered invasive *M. marinum* infections, patients were treated for an average of 20 weeks [13]. In a study from Thailand, the duration of combination therapy was also longer (18 weeks) than monotherapy (11 weeks). Here, patients receiving combination therapy suffered from extensive skin findings or chronic infection [24]. In our case series the analysis of immunosuppressed or -compromised patients revealed a longer duration of therapy as compared to immunocompetent patients (12 weeks longer) which is in accordance with Holden et al.'s observations from a nationwide retrospective analysis from Denmark [25].

Our study has several limitations. The data are from a single center and due to its retrospective approach incomplete records may undermine the accuracy of our estimates. For the same reason it has not been possible to determine the time period between resolution of skin lesions and treatment discontinuation. Nevertheless, since prospective controlled studies on *M. marinum* infections are barely possible due to their rarity, case series such as ours may help to increase physicians' awareness and to support their diagnostic approaches and treatment decisions.

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