Mycobacterium marinum is a non-tuberculous mycobacterium (NTM) that can infect both fish and humans (1). Multiple presentations are described in fish, for which it is considered the most important pathogen (1). Human infections typically occur several weeks after exposure of wounded skin to contaminated water, or after handling fish (2–6). Lesions can be solitary or multiple, and a linear distribution of papulonodular lesions along lymphatic channels, resembling sporotrichosis, can occur in up to one-third of cases, due to lymphatic spread (7).

We report here a case of *M. marinum* infection with sporotrichoid pattern, in which, after several inconclusive biopsies, diagnosis was made by aquarium water culture.

**CASE REPORT**

An immunocompetent 60-year-old woman presented to an outpatient clinic with nodular lesions on her right arm after getting stung in the 5th finger by a plant thorn while gardening. Inflammation appeared at the laceration site, and in the following weeks several subcutaneous nodules spread along a lymphatic trajectory, despite initial empirical treatment with amoxicillin followed by flucloxacillin. Her medical history was unremarkable. She owned a tropical fish tank, which she cleaned without any protection, and also owned 2 cats. There was no recent history of travel. She reported no fever or systemic symptoms. On examination, erythematous papulonodules (Fig. 1A) spanned from her 5th finger to her elbow in a sporotrichoid pattern. The rest of the clinical examination was unremarkable. All laboratory tests were within normal ranges or negative. Soft-tissue ultrasound revealed a 2-mm foreign object at the injury site and several hypoechogenic and hypervascular nodules in the dermis, but no deep tissue involvement, abscesses or tenosynovitis. Histopathology of a nodule demonstrated dermal granulomatous inflammation with multinucleate giant cells, lymphocytes, histiocytes and neutrophils. Periodic acid-Schiff (PAS) and Fite stains were negative. No acid-fast bacilli were detected on direct Ziehl-Neelsen (ZN) staining. Bacteriological cultures, incubated at 30°C and 37°C, were negative. Based on the hobby-related exposure, *M. marinum* infection was evoked. However, diagnostic tests remained inconclusive and new lesions appeared, therefore the foreign object (a fragment of a thorn) was removed and another nodule was biopsied. The biopsy revealed massive granulomatous infiltration of the dermis with multinucleate giant cells and histiocytes, but no neutrophils (Fig. 1B). Some granulomas had central fibrinoid necrosis. PAS, Grocott and ZN stains and an immunohistochemical search for *Mycobacterium tuberculosis* were negative. Tissue cultures, incubated on blood agar plates, Sabouraud medium and
mycobacterium-specific media at 30°C and 37°C, were negative after 3 months. As the diagnosis remained elusive, the patient underwent a third biopsy. In addition to previous tests, PCR for 16S rRNA, *M. tuberculosis*, *Aspergillus* and panfungal were performed. Although no antibiotics had been prescribed during the diagnostic process, all tests were negative. The patient reported that her fish had developed nodules on their skin and several had died; therefore, the aquarium water was cultured. One sample grew *M. marinum*, and another sample *Mycobacteriumalsense*, another slow-growing NTM. *M. al Dense* has been isolated from human respiratory samples (8), but no skin or soft-tissue involvement has been reported. Given the typical presentation in both patient and fish, *M. marinum* was considered responsible. Oral clarithromycin, 1 g/day, was started 173 days after the first consultation, and continued for 14 months due to slow remission of the lesions, typical of *M. marinum* infections with sporotrichoid pattern (Fig. 1C, D). The therapy was discontinued 1 month after the lesions subsided. A follow-up 1 month later showed no lesions. Another visit is scheduled 6 months after treatment discontinuation, according to experts’ recommendations (9).

**DISCUSSION**

This case highlights the diagnostic challenges that *M. marinum* poses, even when suspicion is high. In this case, adequate treatment was initiated more than 6 months after the first nodule appeared. In Holden’s series, the median time from presentation of symptoms to diagnosis was 194 days (maximum 548 days) (10), while Johnson reported a median time of 3.5 months, with extremes of up to 24 months (6). This case also illustrates how a broad diagnostic approach enabled *M. marinum* to be singled out from other infectious (11) and non-infectious diseases, such as neoplasms or reactive dermatoses, which can also present with nodular lesions distributed in a sporotrichoid pattern. A history of exposure to fish can raise suspicion of *M. marinum* infection, but histology and cultures or positive PCR are needed to confirm the diagnosis. In *M. marinum* infection, histopathology reveals granulomatous inflammation without caseation. Fibrinoid necrosis may be present in the granulomas, whilst lymphohistocytic infiltrates and Langhans giant cells are present in the dermis (5, 12, 13). Early lesions usually present as conglomerates of polymorphonuclear cells surrounded by histiocytes (12). *M. marinum* may be identified on ZN or Fite stains, but its absence does not exclude diagnosis, as few bacilli are present in the lesions, except in immunocompromised patients (5, 13, 14). The current gold standard for diagnosis is mycobacterial culture. However, cultures are positive in only 40–60% of cases (5, 15). Clinicians suspecting *M. marinum* infection should therefore notify the laboratory of the suspicion. Specimens should be delivered on ice if the transport time to the laboratory is longer than 2 h and then be cultured on selective media. *M. marinum* grows optimally at 30°C, but poorly at 37°C (1). Cultures should be kept for 6 weeks before being reported negative (2). Failure to observe these conditions may result in false-negative cultures. PCR can improve the diagnostic yield of cultures, but is not 100% sensitive. In Sia’s series, *Mycobacterium* 16S rRNA PCR was positive in only 46% of cases, but 60% of the culture-negative specimens were positive by PCR, which highlights the usefulness of molecular techniques (5).

In selected challenging cases when *M. marinum* is suspected, the diagnostic approach should include aquarium water and fish cultures, alongside histological investigations, mycobacterial culture, and 16S rRNA PCR. At times, this may be the only method that can be used to reach a diagnosis.

**ACKNOWLEDGEMENTS**

The patient provided written consent for publication of this case report and the accompanying images. A copy of the written consent is available for review.

_The authors have no conflicts of interest to declare._

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