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'TB or not TB?' Problems of differential diagnosis of cutaneous mycobacteriosis and tuberculosis – A Case Study and interdisciplinary discussion

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Abstract

The diagnosis of cutaneous tuberculosis poses a serious challenge due to many skin diseases of different etiology resembling the lesions caused by the TB (tuberculosis) bacillus, and difficulties in confirming the disease. The presented case concerns skin lesions in a hobby aquarist stung in the finger of the left hand by a fish. The resulting inflammatory infiltration was to be cutaneous tuberculosis or mycobacteriosis caused by MOTT (Mycobacterium other than tuberculosis). Laboratory, pathomorphologic, genetic and microbiologic tests of samples obtained from the patient, fish and water in the aquarium gave ambiguous results. A multidisciplinary discussion is presented on the difficulties in the differential diagnosis, problems with a clear interpretation of the results of various conducted tests, and possible ways of transmission of the infection, relevant to the described example.

Key words

cutaneous tuberculosis, mycobacteriosis, aquarist

INTRODUCTION

Diagnosis of cutaneous tuberculosis poses a serious challenge, not only due to numerous skin diseases of different etiology resembling the lesions caused by the TB bacillus, but also because of the difficulties in confirming the disease [1].

Cutaneous tuberculosis, one of the rarest localizations of the disease, makes up 1–4.4% of all forms of tuberculosis, according to different authors [2]. Data published by the Institute for Tuberculosis and Pulmonary Diseases (ITaPD) reports that 7.1% of 559 newly- diagnosed cases of tuberculosis were extrapulmonary (including cutaneous TB) [3]. Information on extrapulmonary tuberculosis in Poland in 1974–2010 was presented in a study by Rowińska-Zakrzewska in which a large amount clinical material was analyzed: over 60,000 cases of TB reported in the National Register of Tuberculosis Incidence kept by ITaPD during this period. In 9.9% of the analyzed cases, the extrapulmonary localization was the only one, and within this group, the cutaneous form made up 2.5% [4].

In addition to the national data concerning cutaneous tuberculosis, many research centres have recently suggested that the problem persists. Starzycki described a large group of patients affected with cutaneous tuberculosis (n=268) hospitalized in 1963–87 in the Dermatology Clinic in Kraków [5], while Serwin et al. described 7 cases of cutaneous tuberculosis diagnosed in 1994–2007 in the Dermatology Clinic in Białystok [6]. International literature is dominated by studies originating from Asian countries: Bangladesh [7], Hong-Kong [8], Nepal [9] and Pakistan [10]. Less research on cutaneous tuberculosis has been published by European authors, e.g. in one Spanish hospital, 30 new cases of skin TB were diagnosed in the last 30 years [11].

Another rare skin condition is caused by bacilli different than *M. tuberculosis* which is wide-spread in the natural human environment. Potentially pathogenic for humans are, among others: *Mycobacterium marinum, kansasii, xenopi, szulgai* and *ulcerans* classified as slow-growing bacilli, as well as some of the fast-growing species, such as *Mycobacterium fortuitum* complex, including *Mycobacterium peregrinum*, as in the presented case study below [12]. It was only in the second half of the 20th century that the first case study on cutaneous lesions in humans caused by *M. marinum* (bacillus previously isolated only in fish) paved the way for serious studies on nontuberculous mycobacteriosis, and since then reports about infections caused by the aforementioned pathogens in humans have been published in the international literature [13]. Skin lesions caused by nontuberculous bacilli very often resemble skin eruptions caused by cutaneous tuberculosis [14].

The presented case is an interesting one of skin lesions in a hobby aquarist stung in the finger of the left hand by a fish. The resulting inflammatory infiltration was suspected to be cutaneous tuberculosis or mycobacteriosis caused by MOTT.

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Laboratory, pathomorphologic, genetic and microbiologic tests of samples obtained from the patient, fish and water in the aquarium, gave ambiguous results. A multidisciplinary discussion is presented on the difficulties in the differential diagnosis, problems with a clear interpretation of the results of various conducted tests, and possible ways of transmission of the infection, relevant to the described example.

CASE DESCRIPTION

Clinical course. On 5 December 2009, while cleaning his aquarium, a hobby aquarist was stung on the skin of the proximal phalanx of his ring finger on the left hand by an iridescent shark, an exotic catfish. At the spot where the skin was pricked with the fin spike, he felt intense pain, and after about 3 weeks a nodular inflammatory infiltration with erythemal background appeared, which periodically evacuated purulent and serous. On 30 January 2010, anxious about the persisting skin lesion, the patient reported to the Emergency Department where the surgeon prescribed local antiseptic – to no avail. On 12 February 2010, the patient revisited the Emergency Department, describing increasing pain in the left hand, symptoms of progressing inflammation of the soft tissues of the injured finger, and inflammation of veins on the back of the left hand. An abscess, which had formed several days before, evacuated spontaneously. The surgeon opened and drained the lesion, prescribed a local antiseptic, Enoxaparin and oral Clinadmycin treatment. On 22 February 2010, the patient sought the surgeon again with new complaints: pain in his right hand, isolated nodules on the back of his right hand and wrist. The nodules measuring 2 centimeters in diameter were painful and tender when palpated, had an increased consistency and reddish-blue colour. Cotrimoxazole treatment was prescribed. Due to no improvement and local progression of the disease, the patient came back after 4 days. The surgeon asked for dermatologic and infectious disease consultation. Due to the lack of fluid in the nodules, no biologic samples were obtained; instead, Biseptol therapy was reinforced with 2 antibiotics: Azithromycin and Doxycycline. After 10 days and still no improvement, the patient was referred for a pulmonologic consultation, where diagnostics for tuberculosis and mycobacteriosis was carried out. On 4 March 2012, the largest fluctuant lesion was incised and the semi-fluid content was sent for microbiologic examination (staining, culture and genetic tests) for atypical pathogens, Mycobacterium tuberculosis and MOTT (Mycobacterium other than tuberculosis), independently to ITaPD in Warsaw and a local laboratory. Tissue samples were sent for a histopathological examination, blood samples were tested with QuantiFeron-TB and Mantoux screening test was performed. Other basic examinations were also carried out (complete blood count, C-reactive protein, biochemical blood tests, chest x-ray and CT-scan, EKG). The patient's general condition was very good. He had no complaints besides the local tenderness of the nodules. Physical examination had no pathological findings (peripheral lymphatic nodes not enlarged), nonfebrile, no weight loss. The patient had never suffered from a serious disease, had no history of infectious diseases in the family, and no contact with active, confirmed tuberculosis.

Because of the suspicion of cutaneous tuberculosis, on 30 March 2010, a trial treatment with bacteriostatic agents was implemented: Isoniazid, Rifampicin and Pyrazinamide in typical doses.

In parallel to the observation, diagnosis and treatment of the patient, the Department of Ichthyologic Diseases and Biology of the University of Life Sciences in Lublin observed and examined the suspected iridescent shark, as well as the other fish in the aquarium. The water from the tank and autopsy material of the fish were sent for pathomorphologic and microbiologic examination, including tuberculosis and atypical Mycobacteria screening.

After a month of tuberculostatic treatment, the patient reported moderate fatigue, concentration disturbances, light vertigo, feeling of fullness in the abdomen especially after dietary mistakes. Apart from a transient, moderate liver transaminases elevation, control laboratory tests were normal. The symptoms disappeared after completion of the tuberculostatic therapy, and liver enzymes normalized.

After a month and a half of therapy, local improvement was observed. Five nodules slowly and gradually became paler and resorbed. After another 2 months, during a medical check-up examination, further improvement was noted. New lesions did not appear. Finally, after 6 months of tuberculostatic therapy (2-month intensive phase: Isoniazid, Rifampicin, Pyrazinamide, afterwards followed-up with 2 drugs: Isoniazid, Rifampicin) the nodules disappeared, leaving scars on both hands.

As of now, several months after completion of treatment, the patient feels very well. No cutaneous lesions are visible except the scars on the patient's hands.

Results of the examinations. The Table below presents test results of the biological material obtained from the patient (fluid from the cutaneous nodules, tissue samples of the nodules, serum), water from the aquarium and the fish. The microbiologic examination results (culture, Bactec, staining) as well as genetic test results for tuberculosis and mycobacteriosis, are presented. The results of the histopathologic inspection of the nodules and immunological QuantiFeron TB test of serum are discussed below. The presented photographic material captures the dynamics of changes in the skin lesions during observation of the patient.

In the first biopsy of the cutaneous nodule, a genetic examination confirmed the presence of DNA of bacilli belonging to the *Mycobacterium tuberculosis* complex. However, the microbiologic Bactec culture did not yield the expected *Mycobacterium tuberculosis,* nor were they identified in the microscopic examination. Multiple tests of the other biologic samples harvested from the patient (second biopsy of the nodule, semi-fluid aspirated form the nodule, sputum and urine) did not confirm the presence of any potentials pathogens. The performed QuantiFeron TB blood serum test was positive (IFN: 0.68 IU/ml). The concentration of interferon gamma in this test indicated a *Mycobacterium tuberculosis* complex infection or certain MOTT strains, such as: *Mycobacterium kansasii*, *Mycobacterium szulgai* and *Mycobacterium marinum*. The histopathologic examination described the biopsy of the skin nodule on the hand as necrotic tissue, disseminated chronic lymphocytic inflammation and granulomas. The pathomorphologist suggested the differential diagnosis of aquarium granuloma and cutaneous tuberculosis.

The basic medical examinations, including a chest x-ray and EKG, were inconspicuous. The diameter of skin induration

Table 1. Results of tests

Date	Material	Test	Result
22.10.2010	Fish tissues	Conventional culture tbc	*Mycobacterium peregrinum*[1]
12.3.2010	Aquarium water	Bactec	MOTT[1]
12.3.2010	Patient – skin specimen	PCR	Mycobacterium complex[1]
11.5.2010	Patient – blood	QuantiFeron TB	Positive: infection with *Mycobacterium tuberculosis* or possible infection with *M. kansasii*, *szulgai* or *marinum*)
12.3.2010	Patient – throat swab	Bacterioscopy	Negative: no acid-resistant Mycobacteria found[1]
12.3.2010	Patient – skin specimen I	Bacterioscopy	Negative[1]
12.3.2010	Patient – skin specimen II	Bacterioscopy	Negative[1]
12.3.2010	Aquarium water	PCR	Negative – Mycobacterium complex[1]
12.3.2010	Patient – abscess swab	PCR	Negative – Mycobacterium complex[1]
12.3.2010	Patient – specimen II	PCR	Negative – Mycobacterium complex[1]
8.5.2010	Patient – specimen	BACTEC	Negative: no acid-resistant Mycobacteria found[1]
12.3.2010	Patient – skin specimen II	BACTEC	Negative – Mycobacterium complex[1]
8.5.2010	Patient – skin specimen I	BACTEC	Negative – Mycobacterium complex[1]
20.5.2010	Patient – abscess swab	Bacterioscopy	Negative: no acid-resistant Mycobacteria found – L-J culture[1]
20.5.2010	Patient – abscess swab	Automatic identification method – MB/BacT	Negative
5.5.2010	Patient – sputum	Conventional culture – Tbc	Negative
20.5.2010	Patient – abscess swab	Bacterioscopy	No acid-resistant Mycobacteria found
20.5.2010	Patient – abscess swab	Automatic identification method – MB/BacT	Automatic method: – negative, L-J culture – negative
5.5.2010	Patient – sputum	Conventional culture Tbc	Negative
6.5.2010	Patient – sputum	Conventional culture Tbc	Negative
6.5.2010	Patient – sputum	Conventional culture Tbc	Negative
19.5.2010	Patient – urine	Conventional culture Tbc	Negative
17.5.2010	Patient – urine	Conventional culture Tbc	Negative
13.5.2010	Patient – urine	Conventional culture Tbc	Negative
20.5.2010	Patient – urine	Conventional culture Tbc	Negative
18.5.2010	Patient – urine	Conventional culture Tbc	Negative
5.5.2010	Patient – urine	Conventional culture Tbc	Negative
6.5.2010	Patient – urine	Conventional culture Tbc	Negative
11.5.2010	Patient – urine	Conventional culture Tbc	Negative
12.5.2010	Patient – urine	Conventional culture Tbc	Negative
24.52010	Patient – skin swab	Culture – aerobic bacteria	Negative
21.5.2010	Patient – abscess swab	Bacterioscopy	No acid-resistant Mycobacteria found
2010–05–07	Patient – sputum	Bacterioscopy	No acid-resistant Mycobacteria found
6.5.2010	Patient – sputum	Bacterioscopy	No acid-resistant Mycobacteria found

[1] Institute for Tuberculosis and Pulmonary Disease in Warsaw.

induced in the Rt23 Mantoux screening test was 9 mm. Microbiologic cultures for atypical bacteria were negative.

Microbiologic examination of the water from the aquarium yielded nontuberculous bacilli (MOTT). Staining and microscopic inspection revealed single acid-fast bacilli. Additionally, the Microbiology Department of the National Veterinarian Institute in Puławy isolated an atypical strain of Mycobacterium from the internal organs of the fish, which was then precisely identified by the ITaPD. The genetic analysis was performed with the GenoType Mycobacterium MTC protocol and DNA of the nontuberculous bacilli *Mycobacterium peregrinum* belonging to the *Mycobacterium fortuitum* complex was identified.

DISCUSSION

In summary, the results of the performed examinations indicate that the patient was infected with *Mycobacterium tuberculosis* (positive Mantoux and QuantiFeron test) and probably had cutaneous tuberculosis (genetic tests). Granulomas, which were identified histopathologically, can be associated with tuberculosis or mycobacteriosis of the skin. Genetic analysis of the fish tissues suggests MOTT mycobacteriosis. In the water from the aquarium, acid-fast bacilli (MOTT?, *M. tuberculosis*?) were found microscopically and atypical MOTT bacilli were grown in the Bactec culture. A scheme is shown below of the epidemiologic sequence in the described case:

fish (MOTT) – > water (MOTT), (M.tbc?) – > human (M.tbc)

An interpretation of the results is difficult, finding a

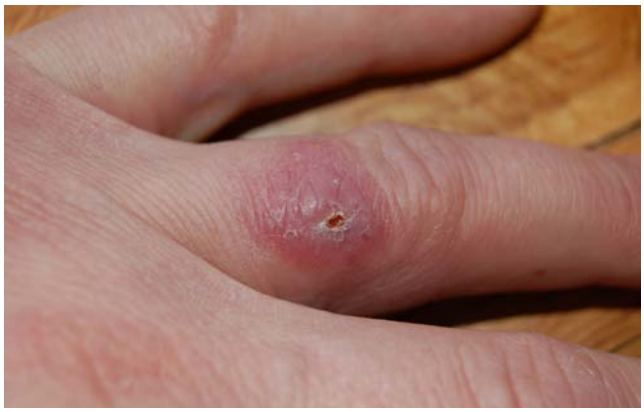

Figure 1. Primary skin lesion – finger, 25.02.2010

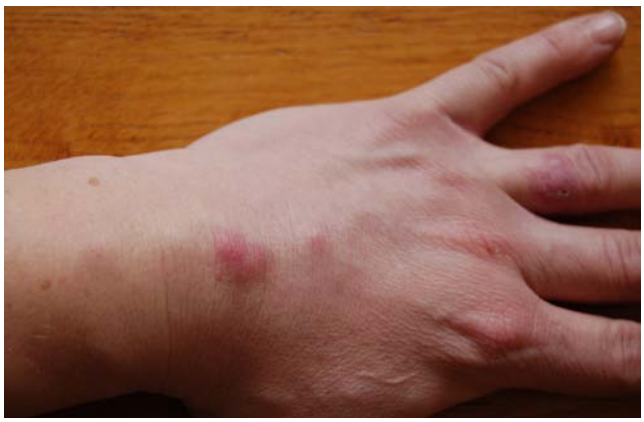

Figure 2. Secondary lesions – wrist, 25.02.2010

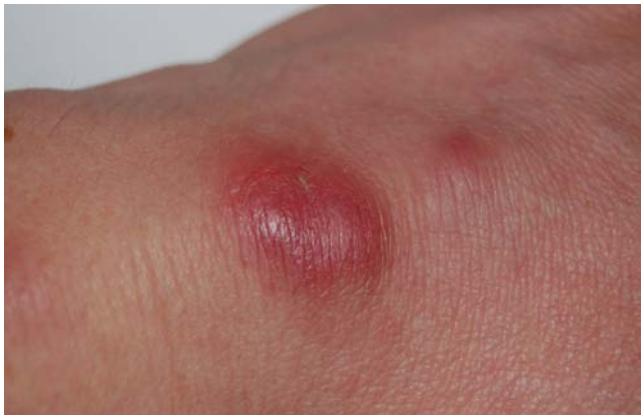

Figure 3. Secondary lesion I – evolution, 10.03.2010

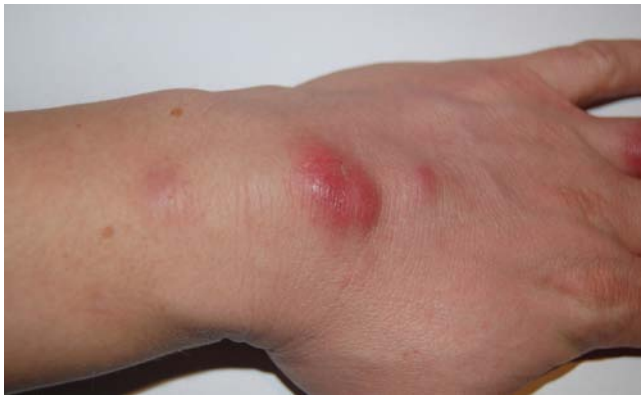

Figure 4. Secondary lesion I – evolution and new, smaller, satellite lesions, 10.03.2010

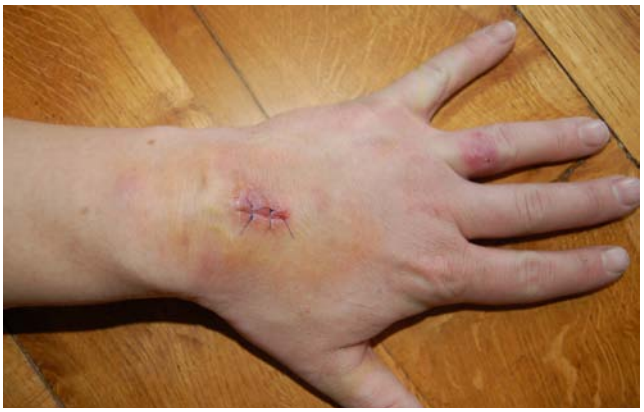

Figure 5. Secondary lesion I – after debridement, 13.03.2010

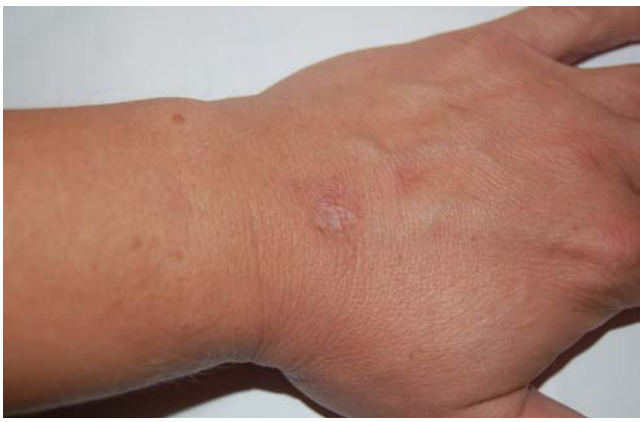

Figure 6. Wound healing (scarring) 10.09.2012

Figure 7. Iridescent shark

causative link – detection of the etiologic agent, path of transmission and the pathogen in the infected organism, the essence of the infective disease – even more so. Diagnosis of tuberculosis is neither easy nor quick. In tuberculosis of the integumentary system, staining of the samples for bacilli produces positive result in only 5.8–54.1 %. Incubation of the material harvested from the skin lesions (so-called bacilli scarce material) confirms the diagnosis only in 6.0–47.5% of the cases, and the incubation takes several weeks [15]. With the insufficient efficacy of the classic microbiologic methods (culture and staining), the importance of the molecular techniques increases. To date, the reference method applied in the identification was the analysis of the mycolic acids with the High-Performance Liquid Chromatography (HPLC) technique. At present, clinically

certified molecular tests are introduced into the diagnosis. One of the recommended methods are complementary tests certified in the EU: GenoType MycobacteriumCM and GenoType Mycobacterium AS. In the presented study, the available methods of identification of Mycobacterium, including culture, staining and genetic tests were used.

Clinically, both *Mycobacterium tuberculosis* and nontuberculous bacilli penetrating the damaged skin can induce a formation of similar primary focal lesion, which becomes visible after approximately one month. In both cases, if untreated, the lesion lingers for around a year. If the disease does not spread, the lesion transforms into a scar. With proper treatment, earlier improvement can be achieved in both types of infection (on average after 3 -6 weeks). In complication-free cutaneous tuberculosis, the recommended treatment is comprised of 3 tuberculostatic drugs: Isoniazid, Rifampicin and Pyrazinamide for a period of 6 months (most cases of skin tuberculosis belong to the III category in the WHO recommendations for treatment regimen) [4]. In the case of a *M. peregrinum* infection, similarly to other fast-growing nontuberculous bacilli, the recommended therapy includes: Macrolides and one of the following: Quinolones, Doxycycline or Cotrimoxazole, usually for 3–4 months.

In the presented case study, 2.5 months after the skin laceration in the aquarium, the patient received Cotrimoxazole for 5 months, as well as Doxycycline and Macrolide antibiotic for 2 weeks (the empirical choice of the drugs was in accordance with recommendations, although the time of administration was shorter). However, the tuberculostatic medication was not introduced until 4 months after the patient had been stung by the fish, followed by the therapy being continued for 6 months, with noticeable improvement after 4–6 weeks.

Taking into consideration the tendency to spontaneous resolution in both tuberculosis and MOTT infections – it is difficult to definitely assess the efficacy of the applied therapy and distinguish the etiology of the disease.

Therefore, in the presented situation, neither the clinical history nor the macroscopic / microscopic picture of the nodules, or the positive effect of the therapy (the used drugs are efficient against MOTT mycobacteriosis as well as tuberculosis; additionally, spontaneous recovery is possible in both diseases) helped to ascertain a clear diagnosis. In practice, in the absence of microbiologic evidence, the clinical picture of skin disease and the results of the additional tests must be sufficient to start a tentative tuberculostatic treatment. In the discussed case, it had to be assumed that atypical bacilli were present in the internal organs of the fish, *Mycobacterium tuberculosis* was present in the organism of the patient, and atypical bacilli and possibly M. tuberculosis in the water of the aquarium. However, no answer was found to the question whether the patient's skin lesions, which developed directly after the fish injured him (the first nodule formed in the exact same spot on the skin of the hand) were cutaneous tuberculosis or mycobacteriosis. Perhaps, as the skin was pierced, the Mycobacteria tuberculosis present in small numbers in the tank water, penetrated into the tissue and caused skin tuberculosis. Another possibility (purely hypothetical): an external stimulus – the skin laceration and superinfection with atypical bacilli induced the latent *M. tuberculosis* and led to the development of the cutaneous tuberculosis. Thirdly, a possible though unlikely contamination of the sample sent to the laboratory or PCR environment (presence of contamination: DNA of *Mycobacterium peregrinum* or *Mycobacterium tuberculosis* complex). Finally, the fourth scenario: both the fish and the patient had a symptomatic skin MOTT infection, *M. tuberculosis* detected with the genetic methods in the samples from the patient's skin being coincidental and clinically insignificant. Microbiologically, *M. tuberculosis* was not detected in any of the samples (sputum, urine, skin biopsies and swab, swab biopsy from the abscess) except for one examination, i.e. skin biopsy, in which the genetic material of *M. tuberculosis* was identified using the ProbeTec (B-D) method. This examination confirmed the suspicion of cutaneous tuberculosis. According to WHO criteria, the clinical picture, positive microscopic and genetic examination constitute reliable proof for a diagnosis of tuberculosis [1]. The incubated tissues obtained from the fish yielded *M. peregrinum*, the aquarium water – *M. fortuitum* complex, since *M. peregrinum* belongs to the *M. fortuitum* complex, it could be assumed that the same species of atypical bacilli was present in both cultures. Positive Mantoux test and Interferon Gamma Release Assay, microbiologic and genetic tests, as well as recovery after the standard tuberculostatic protocol, indicate that the patient suffered from tuberculosis. On the other hand, the patient was treated for a short period of time with Cotrimoxazole and Macrolide, drugs recommended for cutaneous mycobacteriosis produced by the bacilli of the *M. fortuitum* complex. Following the guidelines issued by the American Thoracic Society [16], severe skin, bone and soft tissue infections caused by *M. fortuitum*, should be treated with at least 2 drugs, the efficacy of which has been proved *in vitro.* In order to improve the results, the therapy should be continued for 4 months.

M. fortuitum belongs to the rapidly-growing bacilli (RB) which are less pathogenic. Historically, this group consisted of 3 species *M. fortuitum*, *M. peregrinum* and a third, unknown strain. Recently, the species *M. houstonense*, *M. boenickei* and others have been included into that group. The differentiation of these species is only possible with the techniques of molecular biology [3]. There are descriptions of pseudo-epidemics caused by the bacilli of *M. fortuitum* complex in healthcare environments, beauty and manicure salons. After epidemiologic molecular enquiries, it turned out that the bacteria came from the tap water [2, 17]. If the aquarium was filled with tap water, *M. fortuitum* complex bacilli might have been introduced into the tank in the germ-infested water. Could the bacilli have caused soft-tissue infection in the aquarist? Because the tests for tuberculosis were positive, the disease might have manifested itself coincidentally as the patient was cleaning the tank.

At the same time, from the immunologic and genetic perspective, a possible etiology of the cutaneous condition in the patient could be an infection of *Mycobacterium peregrinum*, nontuberculous bacilli from the infested fish. To corroborate this hypothesis, it is necessary to explain the positive results of the 2 tests. The positive QuantiFeron TB test suggests an infection of *Mycobacterium tuberculosis*, *kansasi*, *shulgai* or *marinum;* however, there is no evidence that the composition of amino acids in the epitopes of *Mycobacterium peregrinum* antigens is not similar to the antigens of the above-mentioned group. Consequently, memory T lymphocytes immunized with antigens of *Mycobacterium peregrinum* could acquire the capability to produce INF gamma when exposed to *Mycobacterium tuberculosisin vitro*.

Moreover, QuantiFeron TB test can be positive a long time after a previous asymptomatic Mycobacterium tuberculosis infection, which could be the case in the discussed patient. The genetic examination of the material from the patient was carried out with PCR using IS6110 fragments of bacilli as starters. Such starters could lead to an amplification of similar nucleotide sequence of the DNA *Mycobacterium peregrinum* DNA. Thacker et al. [18] provide evidence for such a sequence of events: 'The IS41/43 primer pair also detected *M. fortuitum,* whereas the IS6110 primer pair cross-reacted with *M. peregrinum* and *M. chelonae*.' The GenoType examination of the tissue of the fish should be considered the most reliable of the tests performed in the discussed case, because the molecular probes used in this assay are most specific for the DNA sequence of Mycobacterium peregrinum. Considering all this information, it can be assumed that the patient had a cutaneous mycobacteriosis caused by *Mycobacterium peregrinum*. According to the ichthyologic investigation, none of the fish in the aquarium (*Poecilia sphenops*, *Poecilia reticulata*, *Xiphophorus helleri*, Danio rerio and *Pangasius hypophthalmus*, whose fin caused the wound) showed symptoms of the disease. The internal organs: liver, kidney, spleen and heart of the fish were free of the nodules typical for tuberculosis in those animals. The specimen prepared from the internal organs of the fish using Ziehl-Neelsen stain, revealed numerous clusters of acid-fast bacilli. The samples were also incubated on Petragnani and Stonenbrinck media using the recommended protocol. The grown colonies were then stained with the Ziehl-Neelsen method and identified with Geno Type Mycobacterium CM (Hain Lifescience GmbH, Nehren, Germany). This method relies on PCR of species-specific regions in the gene for 23S rRNA and hybridization of the amplified products to the probes on a nitrocellulose slides.

The presented case should encourage further research on the presence of various species of mycobacteria in water environments, with particular emphasis on fish and aquatic organisms available to private customers. It is especially important in a country which imports fish from all around the world, some of them coming from wild fisheries. Moreover, it is necessary to develop proper algorithms for diagnosis and treatment of mycobacteriosis common in fish and humans.

The diagnosis of a mycobacteriosis is a difficult process and often requires cooperation of several specialists. In the presented case, pulmonologists, microbiologists, ichthyologists and other specialists launched a complex diagnostics aimed at both tuberculosis and mycobacteriosis. In the opinion of the authors, the presented interdisciplinary discussion will allow further exploration of the problem of differential diagnosis of cutaneous tuberculosis and nontuberculous cutaneous mycobacteriosis and its challenges. The model of the presented discussion shows how difficult it is to draw a common conclusion and proves the necessity of collaboration of specialists from many fields; however, in the end, it is always the clinician taking care of the patient who makes the final therapeutic decision.

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