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Iwata, Kentaro
Takai, Yoshie
Kitada, Nozomi
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【 CASE REPORT 】

Mandibular Nodule Caused by *Mycobacterium marinum* with False Positive Interferon-γ Release Assay

Kentaro Iwata[1], Yoshie Takai[2], Nozomi Kitada[3], Naomi Morishita[4] and Hiroaki Kiyona[3]

Abstract:
Mycobacterium marinum is a ubiquitous organism inhabiting both fresh and salt water. It can cause human diseases such as skin and soft tissue infection. The organism is also known to cause a false positive reaction to interferon-γ release assay, the test to diagnose latent tuberculosis infection. Here, we present a case of submandibular nodule caused by *M. marinum* with positive T-SPOT®.TB test, which was likely to be false positive.

Key words: *Mycobacterium marinum*, interferon-γ release assay, false-positive

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Introduction

Mycobacterium marinum is a slow-growing nontuberculous photochromogenic mycobacteria. It is a ubiquitous waterborne organism, inhabiting fresh and saltwater and causes disease in many fish species and occasionally in humans (1, 2). It is also known to cause a false positive reaction to interferon-γ (IFN-γ) release assay (IGRA) for the diagnosis of latent tuberculosis infection (LTBI) (3, 4). Here, we report a case of submandibular nodule caused by *M. marinum*. IGRA during the work up was positive but it was considered to be a false positive reaction to *M. marinum* infection.

Case Report

A 63-year-old woman presented to a plastic surgery clinic for a submandibular mass lesion lasting for about a month. There was no fever, weight loss, or other generalized symptoms. There was no tenderness on palpation. Atheroma formation was suspected and the mass was surgically excised. However, the skin lesion recurred shortly, and granuloma formation was suspected. The excised lesion at the plastic surgery clinic was sent to the pathology department, and it found granulomatous formation (Fig. 1A) High power field revealed numerous epithelioid cells (Fig. 1B). The Ziehl-Neelsen staining and the Grocott staining failed to identify any microorganisms. The patient was referred to the dermatology clinic of our hospital. On palpation of the lesion, yellowish lucent fluid came out and the sample was sent to the microbiology laboratory. The auramine-rhodamine stain of the fluid with fluorescent microscopy did not reveal mycobacteria. Polymerase chain reaction tests for *Mycobacterium tuberculosis* and *Mycobacterium avium-intracellulare*-complex DNA was negative. An IGRA, T-SPOT®.TB test (Oxford Immunotec), was positive (ESAT-6: 10, CFP-10: 5), but the tuberculin skin test (TST) was negative. The patient was referred to the infectious diseases clinic of the hospital.

On physical examination, she was afebrile and other vital signs were also normal. There was submandibular swelling with skin discoloration (Fig. 2A). The lesion was soft to but non-tender. Blood tests, including complete blood count, electrolytes panel, liver function tests, creatinine level, and C-reactive protein were all normal. The computed tomography scan of the chest did not reveal any lung lesions or lymphadenopathy. The patient was not able to expectorate sputum.

The culture of discharged fluid from the skin lesion became positive at the liquid medium 3 weeks later, while

[1]Division of Infectious Diseases, Kobe University Hospital, Japan, [2]Department of Dermatology, Hyogo Prefectural Kakogawa Medical Center, Japan, [3]Department of Laboratory Medicine, Hyogo Prefectural Kakogawa Medical Center, Japan and [4]Department of Nursing, Hyogo Prefectural Kakogawa Medical Center, Japan
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Correspondence to Dr. Kentaro Iwata, kentaroiwata1969@gmail.com

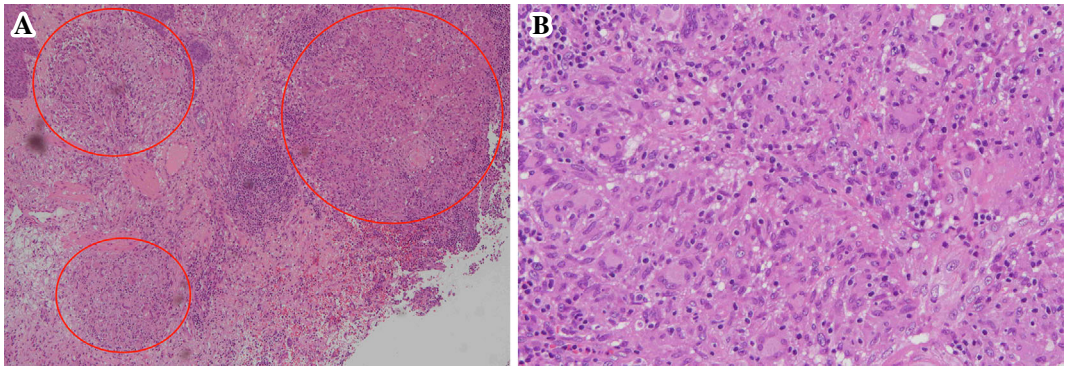

Figure 1. **Low power field view of Hematoxylin and Eosin staining of the submandibular lesion reveals multiple granulomas (red circles) (A). A high power field view of the same lesion reveals many epithelioid cells (B).**

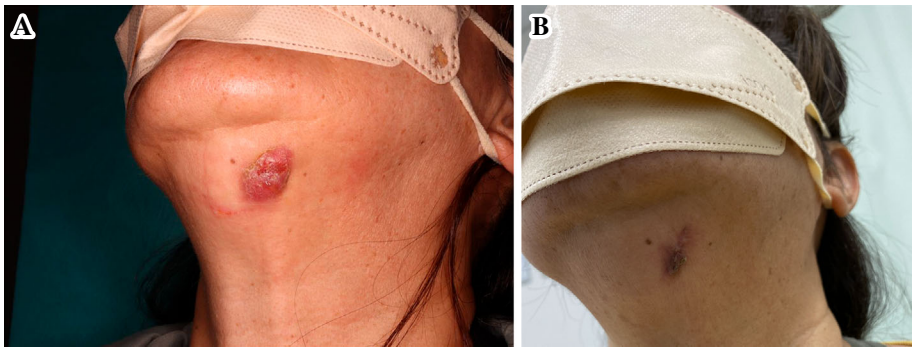

Figure 2. **Submandibular lesion of the patient at the presentation (A) and after the healing (B).**

Ogawa solid medium did not grow organisms. The organism was later identified as *Mycobacterium marinum*. The identification of the organism was provided by using Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS), with the score value of 2.34. The minimum inhibitory concentration (MIC) of antimicrobials is derived using BrothMIC NTM® (Kyokuto Pharmaceutical Industrial), shown in the Table. The patient was treated with clarithromycin 400 mg twice and ethambutol 750 mg once daily for 4 weeks with clearance of the lesion (Fig. 2B).

The patient later denied any access to the sea area or any other waterfront, before the onset of the symptoms, and the source of the inoculation of the organism remained unknown.

Discussion

The patient developed a submandibular nodule caused by *M. marinum*. The lesion persisted after the excision of the lesion at the plastic surgery clinic.

IGRAs, including T-SPOT®.TB and QFT-G, display a higher sensitivity compared to the TST for specific detection of latent tuberculosis, pulmonary TB, or extrapulmonary TB, based on the T-cell mediated IFN-γ release induced by specific *M. tuberculosis* antigens, including the 6-kDa early secretory antigenic target (ESAT-6) and 10-kDa culture filtrate protein (CFP-10). False positive IGRA test caused by *M. marinum* is well-documented (5), but actual clinical cases reporting the phenomenon are scarce. Most report false positive cases caused by *M. kansasii* (3, 5). Lai et al. reported the value of IGRA among cutaneous mycobacterial infections. Among 23 cases of cutaneous mycobacterial infections confirmed microbiologically, 13 had positive IGRA; 9 with *M. tuberculosis*, 3 with *M. marinum*, and 1 with *M. kansasii* (6). Therefore, it might be useful to consider *M. marinum* or *M. kansasii* infection when one encounters a cutaneous lesion with positive IGRA. The genes encoding ESAT-6 and CFP-10 are situated within "region of difference 1" (RD1) of *M. tuberculosis* but are also present in some nontuberculous mycobacteria (NTM). NTMs such as *M. kansasii*, *M. gastri*, *M. marinum*, *M. szulgai*, and *M. riyadhens*e share similar RD1 areas. Therefore, although IGRAs present high sensitivity for discriminating most NTM and TB, some NTMs could still show false positive results (3, 4).

The present case is a likely example of a false positive result of IGRA due to *M. marinum* infection. The patient did not have significant risk factors for developing tuberculosis, and there was neither a primary lesion nor reactivation of tuberculosis in the lungs. The TST, which could cause false positive results with other mycobacterial infections, such as *M. avium-intracellulare* complex (MAC), was also negative. Putting it all together, the patient is likely to be infected

Table. **MIC of *Mycobacterium marinum* of the Case, Using BrothMIC NTM®.**

Antimicrobials	Minimum inhibitory concentration (MIC) μg/mL	Breakpoint for being susceptible (S)
Amikacin	≤0.5	≤2
Clarithromycin	1	≤8
Levofloxacin	1	≤1
Sulfamethoxazole-trimethoprim	1	
Rifampin	0.25	≤0.5
Ethambutol	1	≤2
Kanamycin	1	≤2
Ethionamide	4	≤1
Rifabutin	0.12	

Note That the Product was Aimed at Deriving MIC with the Presumptive Breakpoint for *Mycobacterium Avium-intracellulare* complex. the Breakpoints are as a Reference, Lacking Some for Certain Antimicrobials, and Is Not Validated Specifically for *M. Marinum*. MIC, Minimal Inhibitory Concentration.

with *M. marinum*, but not with *M. tuberculosis*. False positive results of IGRA due to the presence of *M. marinum* were well known from a theoretical basis, but few reported the actual cases of *M. marinum* infection with false positive IGRA. When IGRA was positive and there was no evidence or high risk of tuberculosis, one should consider the possibility of false positive test results and mycobacterial infection caused by certain NTMs.

We were able to treat the disease using the regimen recommended by the Sanford guide (7).

The Clinical and Laboratory Standards Institute (CLSI) recommends microdilution for *M. marinum* drug susceptibility testing (DST). Susceptibility data on *M. marinum* are scarce and rely upon the small numbers of strains and antibiotics tested. As a consequence, intrinsic antibiotic susceptibilities of *M. marinum* are not well defined, and methods for their routine determination have not been evaluated (8). As the wild-type susceptibility pattern of *M. marinum* is well known and acquired resistance has not been described so far, antimicrobial susceptibility testing is not recommended except in the case of treatment failure and relapse.

There is little evidence to support the choice of antibiotics and the duration of the treatment. The exact role of DST in the management of *M. marinum* is also not established, in part because most isolates show low MICs to the most frequently used antimycobacterial drugs (9). A combination of antimicrobials including clarithromycin, ethambutol, rifampin, and minocycline is often used. It is resistant to antimicrobials such as azithromycin, isoniazid, and pyrazinamide (7, 10).

In conclusion, our case showed a well-known, but rarely reported example of *M. marinum* cutaneous infection with a false-positive IGRA test. One needs to be mindful of the phenomena when encountering a skin lesion likely to be caused by mycobacteria.

The authors state that they have no Conflict of Interest (COI).

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