

# The tough process of unmasking the slow-growing mycobacterium: case report of *Mycobacterium microti* infection

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## Abstract

*Mycobacterium microti* belongs to the *Mycobacterium tuberculosis* complex (MTBC). It can cause pulmonary and extrapulmonary tuberculosis in humans. Compared to *M. tuberculosis*, which is the most prevalent subspecies of the MTBC, *M. microti* infection has a different etiology. Moreover, establishing the diagnosis with conventional bacteriology can be difficult. We will illustrate this with a case of an extrapulmonary tuberculosis of the hip caused by *M. microti* in an immunocompetent patient in The Netherlands.

## CASE REPORT

A 45-year-old man presented at the emergency department of our tertiary teaching hospital with a septic arthritis of the left hip. Four weeks before presentation the hip became painful. Since then the pain worsened and the patient became febrile. He did not suffer from night sweats.

The patient had no significant medical history. He originated from former Yugoslavia, where he spent the holidays once or twice a year. His occupation was supervisor in a metal recycling company in the harbour of Rotterdam.

On physical examination the hip was mildly swollen and painful at palpation. Laboratory investigation showed an increased C reactive protein (CRP) of 234 mg l<sup>-1</sup> (normal <10) with a normal leukocyte count. Magnetic resonance imaging (MRI) of the hip was performed, which showed a high-intensity signal and a lot of intra-articular fluid at the swollen side of the hip. With an ultrasound guided biopsy synovial fluid was aspirated and sent to the microbiology department.

The working hypothesis was an ordinary septic arthritis. However, a gram stain of the synovial fluid did not show any micro-organisms. Antibiotic treatment was started immediately with intravenous cefuroxime and one dose of

gentamicin, according to local antibiotic guidelines. After starting antibiotic treatment the fever and increased CRP persisted. Therefore, a chest radiograph was taken, which showed bilateral, perihilar infiltrates consisting of several small nodules. A chest computed tomography (CT) showed perihilar diffuse nodular infiltrates (Fig. 1).

With a differential diagnosis of pulmonary and extrapulmonary tuberculosis, the hip synovial fluid was stained with auramine-rhodamine and then acid-fast bacilli could be visualized. Next, GeneXpert MTB/Rif (Cepheid, Sunnyvale, CA, USA), a molecular test based on nucleic acid amplification, confirmed *M. tuberculosis* complex in the sample from the synovial fluid. The normal aerobic and anaerobic cultures were negative. The mycobacterial culture, using our BD BACTEC MGIT 960 mycobacteria culture system (BD Diagnostics, Sparks, MD, USA) was negative after 6 weeks of culture. Given the positive GeneXpert and no history of pre-treatment, it was decided to extend the culture period for another 6 weeks. After a total of 75 days of culture, the mycobacterial culture became positive (Fig. 2). In order to differentiate within the *M. tuberculosis* complex we used the GenoType MTBC molecular genetic assay (HAIN Lifescience, Nehren, Germany) and identified the strain as an *M. microti*. Additionally, the GenoType MTBDR<sub>plus</sub> (HAIN Lifescience)

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**Abbreviations:** CRP, C reactive protein; CT, Computed Tomography; INH, Isoniazid; MRI, Magnetic Resonance Imaging; MTBC, Mycobacterium Tuberculosis Complex; RMP, Rifampicin.

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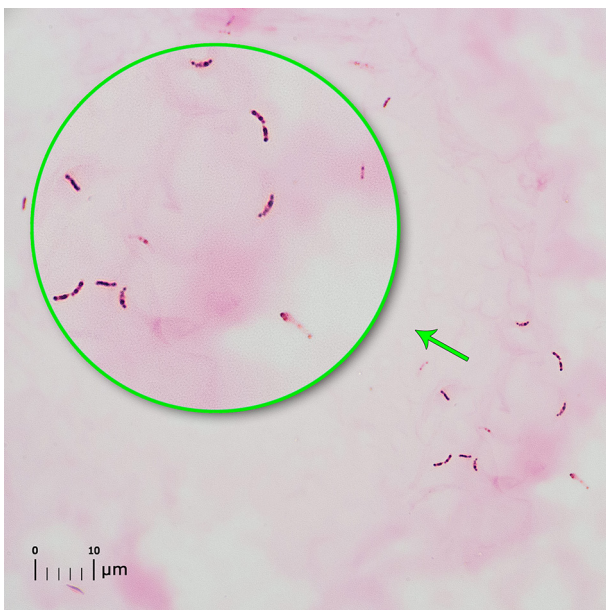


**Fig. 1.** Chest computed tomography, showing perihilar consolidations at both sides with underlying bronchiectasis. In the periphery of the lungs smaller nodular infiltrates are visible. These are indicated by the arrows.

was performed in order to identify molecular genetic resistance towards isoniazid (INH) and rifampicin (RMP). This hybridization assay showed no mutations in the *rpoB* gene (associated with RMP susceptibility) and no mutations in the *katG* or *inhA* genes (associated with INH susceptibility).

The sputum of the patient was also tested for *Mycobacterium*, but turned out to be negative by auramine-rhodamine staining, GeneXpert and (mycobacterial) culture. Eventually, bronchoalveolar lavage was performed, but with the previous three tests *M. microti* could not be detected.

The *M. microti* infection was treated with INH, RMP, pyrazinamide and ethambutol according to the World Health Organization recommended dosage [1]. Initially the patient



**Fig. 2.** Microscopy after kinyoun staining of *M. microti* in culture. This culture became positive after 75 days of incubation. The microscope used was a Zeiss Axioskop, the magnification  $\times 100$ .



**Fig. 3.** Computed tomography of the left hip, showing bone destruction of the anterior side of the collum. Next to this site, multiple fluid collections are visible, which are highly suspicious for abscesses. These collections infiltrate in the adjacent muscles. The largest collection in this view measures 64.5 by 37.1 mm and has a very thick wall.

recovered and the fever and pain in the hip disappeared. Therefore, the pyrazinamide and ethambutol were discontinued after 2 months. However, shortly thereafter the former complaints reoccurred and the pyrazinamide and ethambutol were restarted. A CT scan of the hip revealed bone destruction at the anterior side and multiple abscesses in the adjacent muscles (Fig. 3). A radiological drainage of these abscesses was performed. Therapeutic drug monitoring of RMP and INH showed that drug levels of both drugs were subtherapeutic. Hereafter, the dose of INH was increased to 900 mg per day and RMP to 1800 mg per day. Shortly after this treatment modification the patient got complaints of polyneuropathy. Therefore, the INH was discontinued. The other drugs were continued and eventually, the patient improved clinically.

## DISCUSSION

This case report presents a patient with an unexpected diagnosis of *M. microti* tuberculosis.

*M. microti* is part of the MTBC complex, which among others include *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canettii*, *M. pinnipedii* and *M. caprae*.

Studies conducted in France and Great Britain showed that *M. microti* is endemic in small rodents, such as mice and voles, who serve as the reservoirs [2, 3]. Infections of other animals, such as pigs, ferrets, cats and dogs, have also been described. Until 1998 it was believed that *M. microti* exclusively caused disease in animals, but then the first four cases were described in humans [4]. Since then, *M. microti* is considered a zoonotic pathogen, which can infect humans through spillover hosts, such as dogs or cats or through faecal contamination of the environment. One case series suggests that human-to-human transmission may have occurred [4]. For our patient the source of infection was not clear.

In 2010, only 27 human cases of *M. microti* had been described in the literature [5]. It is believed that the prevalence of infections with *M. microti* is underestimated. One reason is that it is easily missed in routine bacteriology. *M. microti* is extremely slow growing and to culture it, it often takes more than 6–8 weeks. *M. microti* can be distinguished from *M. tuberculosis* by the typical curved morphology of the bacteria at Ziehl–Neelsen staining. However, given the subtle differences, this might be missed and regarded *M. tuberculosis* if no additional genotypically testing is performed. Therefore, nowadays, molecular assays, such as the GenoType MTBC, have become part of routine laboratory diagnostics in high- and middle-income countries. These assays can distinguish between the members of the MTBC. Moreover, they can be used on direct patient material instead of cultured strains if the mycobacterial load is high enough, which speeds up the time to diagnosis [2, 6].

It is expected that with increased use of molecular techniques in diagnostic procedures, more infections with *M. microti* will be discovered. In a recent study, conducted in South Africa, genotyping of 215 *M. tuberculosis* isolates showed that 1.9% were *M. microti* [7].

*M. microti* can cause disease independently of the immune status of the patient. In a report from Panteix *et al.* from the 27 cases, ten were immunocompetent [5]. From the other 17 cases, the immunological status was compromised by HIV infection, kidney transplantation or diabetes. Our patient proved HIV negative and had no other signs or test results indicative of a compromised immunological status.

In the majority of cases *M. microti* causes pulmonary tuberculosis, but extrapulmonary tuberculosis has also been described. Of the cases with extrapulmonary tuberculosis two patients had an abdominal infection and one an osteomyelitis of the hip [8]. Our case would be the fourth case of extrapulmonary *M. microti* infection ever published.

The empirical treatment chosen in this patient, is based on the fact that *M. microti* usually responds well to conventional treatment. However, in the case series from Panteix *et al.* resistance to pyrazinamide was reported in one of the six cases described [5].

In conclusion, we summarized a patient who was diagnosed after a significant time delay with *M. microti* tuberculosis. Establishing the diagnosis with conventional bacteriology can be difficult because this mycobacterium takes longer to grow in culture than *M. tuberculosis*. Molecular assays can identify *M. microti* subspecies and decrease diagnostic delay.

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#### Conflicts of interest

The authors declare that there are no conflicts of interest.

#### Ethical statement

The patient, described in this case report, has read the manuscript and signed for consent to publish. The corresponding author has obtained written informed consent from the patient before submission.

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