Disseminated Infection with *Mycobacterium tilburgii* in a Male Immunocompromised Patient

Sarah Temmerman, Linos Vandekerckhove, Erica Sermijn, Dirk Vogelaers, Geert Cleeyts, Mario Vaneechoutte, Piet Cools, Steven Callens

Departments of General Internal Medicine and Infectious Diseases* and Microbiology, Ghent University Hospital, Ghent, Belgium

*Mycobacterium tilburgii* is a nonculturable nontuberculous mycobacterium identifiable only by molecular methods. We report a case of disseminated *M. tilburgii* infection illustrating the importance of 16S rRNA gene sequencing to determine the responsible mycobacterial pathogen and the difficulties in tailoring antitymocobacterial treatment in the absence of a culturable organism.

CASE REPORT

A 41-year-old male of Belgian origin was admitted in December 2008 with pain in the left hypochondrium, weight loss of approximately 5 kg, dyspnea, and splenomegaly.

In 2004, he suffered an episode of anorexia, weight loss, and fever.

Computed tomography revealed pulmonary nodular infiltrates. Biopsy of the infiltrates showed inflammatory changes, which subsequently regressed spontaneously. A diagnosis of pulmonary sarcoidosis was assumed.

In 2007, he developed exercise-induced dyspnoea and a dry cough. Bilateral lung infiltrates recurred, together with hilar lymphadenopathy. A bronchoscopy showed no intraluminal lesions, and a lung biopsy specimen yielded important squamous metaplasia in the absence of granulomas or morphological evidence for malignancy. A tuberculin skin test (TST) was negative. Stage III sarcoidosis was presumed, and corticosteroids were initiated.

At the end of 2008, while still receiving steroid treatment, the patient was hospitalized because of pain in the left hypochondrium with referred pain to the left shoulder. Clinical examination revealed splenomegaly with no signs of hemorrhage. The corticosteroid dosage was increased to 16 mg methylprednisolone daily with an initial favorable effect on the pain.

Two weeks later, the pain recurred and the patient was referred to our hospital.

On admission, the body temperature was 37.1°C, with a regular pulse of 72 beats per min and blood pressure of 90/56 mm Hg. No peripheral lymphadenopathy was noted. Cardiopulmonary auscultation was normal. Abdominal palpation was painless, with significant splenomegaly (cranio-caudal diameter of 18 cm).

The patient did not travel outside Europe and had recently stopped smoking, after a cumulative cigarette consumption of 24 pack-years.

Blood analysis showed a mildly accelerated erythrocyte sedimentation rate of 18 mm in the first hour and a C-reactive protein level of 27 mg/liter. There was a microcytic anemia with a hemoglobin of 10.8 g/dl and a mean corpuscular volume of 78 fl. The white blood cell count was 8,620/μl. A mild thrombocytopenia of 102,000/μl was observed. The CD4⁺ cell count was 1,000/μl (64%) with a CD4/CD8 ratio of 2.91. Liver enzymes were mildly elevated at 48 U/liter for aspartate transaminase (AST), 111 U/liter for alanine transaminase (ALT), and 72 U/liter for gamma glutamyl transpeptidase (γ-GT). Lactate dehydrogenase (LDH) and autoimmune serology (rheumatoid factor and antinuclear factor) results were normal. A TST (as in 2007) and HIV testing proved negative.

A positron emission tomography-computed tomography (PET-CT) scan showed diffuse supra- and subdiaphragmatic lymphadenopathy with some pulmonary infiltrates, as well as spleen and bone marrow involvement, suggestive of the diagnosis of lymphoma.

Bronchoalveolar fluid and transbronchial biopsy specimens were normal, but many acid-fast bacilli were observed by direct microscopy.

Histological examination of an excised cervical lymph node showed granulomas consisting of foamy histiocytes without necrosis or giant cells or arguments for malignancy. The acid-fast staining revealed multiple acid-fast rods. Antitymocobacterial treatment with isoniazid, rifampin, and pyrazinamide was initiated.

Cultures were performed by incubation on Lowenstein agar and in liquid media processed with BacT/Alert MP bottles (bioMérieux, Marcy l’Etoile, France) after decontamination and, except for the biopsy specimen, liquefaction with the acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method.

Since cultures remained negative, despite the strongly positive microscopy, and histological examination of the cervical lymph node was not suggestive for *M. tuberculosis* infection, the possibility of atypical mycobacterial infection was considered and, in early January 2009, treatment was switched to rifampin at 300 mg twice a day (b.i.d.), clarithromycin at 500 mg b.i.d., ethambutol at 400 mg three times a day (t.i.d.), levofloxacin at 500 mg b.i.d., and amikacin at 2 g every 36 h. Revaluation of the lung biopsy specimens taken in the referring hospital confirmed their initial results.

While the corticosteroid treatment was tapered to 4 mg methylprednisolone daily, the pain in the left hypochondrium worsened and an acute drop in hematocrit associated with thrombo...
cytopenia, dysfunctional coagulation, and an increase in LDH, AST/ALT, and ferritin levels were seen. Bone marrow showed a high load of acid-fast bacilli.

A repeat CT scan disclosed an increase in the splenomegaly with multiple hematomas. Ejective splenectomy was performed. Histological examination revealed massive infiltration of the spleen as well as of the liver (cylinder biopsy specimen) by granulomas positive for acid-fast mycobacteria.

Direct PCR with the 16S rRNA gene primers MBUZ1 and MBUZ2, specific for the order Actinomycetales (1), on different specimens (sputum, cervical lymph node, bone marrow, and spleen) followed by sequencing, revealed a sequence of 803 bases (GenBank accession no. KF724951) for the latter three specimens that was 99% identical to the 16S rRNA gene sequence of Mycobacterium tilburgii (accession number AJ580826.1) (Table 1).

Mycobacterial culture remained negative after up to 3 months of incubation on Lowenstein agar and in BacT/Alert MP bottles. Clinical and biochemical evolution was favorable under conditions of treatment with rifampin at 300 mg b.i.d., clarithromycin at 500 mg b.i.d., ethambutol at 400 mg t.i.d., levofloxacin at 500 mg b.i.d., and amikacin at 2 g every 36 h, and a repeat bone marrow analysis revealed a decrease in the M. tilburgii load as determined by microscopy.

Amikacin therapy was stopped after 3 months of treatment due to significant bilateral hearing loss.

Intermittent fever and night sweats recurred in May 2009. A bone marrow examination showed an increase in the amount of acid-fast bacilli. Linezolid at 600 mg b.i.d. was then added to the ongoing treatment consisting of rifampin, clarithromycin, ethambutol, and levofloxacin followed by a favorable clinical course.

An extended screening for underlying primary immunodeficiency (ID) did not indicate the presence of cellular or humoral ID. In particular, there was no evidence for a defect in the interleukin-12/gamma interferon (IL-12/IFN-γ) axis.

In August 2009, the patient developed severe peripheral neuropathy and linezolid therapy was stopped. A bone marrow examination revealed the continued presence of acid-fast bacilli. γ-IFN administered at 50 μg/m² three times weekly was added to the treatment.

In February 2010 (13 months after initiation of treatment), an extensive reevaluation showed no inflammatory lung pathology and a decrease of the intra-abdominal lymphadenopathy. Histology of liver and bone marrow specimens still demonstrated the presence of acid-fast bacilli, but their density was significantly diminished.

After 2 years of continuous treatment with rifampin, clarithromycin, ethambutol, levofloxacin/moxifloxacin, and subcutaneous γ-IFN, treatment was switched to a lifelong suppressive treatment with moxifloxacin at 400 mg once a day (q.d.) and clarithromycin at 500 mg b.i.d.

The patient was in stable clinical condition at his latest checkup in October 2013.

Nontuberculous mycobacteria are being increasingly identified as causative pathogens in a broad spectrum of diseases. This is certainly partly due to factors affecting the human host’s immune system (AIDS epidemic, increased numbers of organ and bone marrow transplantations, use of chronic corticosteroid therapy) but is also due to improved diagnostic techniques (2–5).

M. tilburgii has so far been reported in 8 patients, 6 of whom were adults.

It was described for the first time as the probable pathogen responsible for a disseminated infection of the bladder and intestine in a previously healthy woman (with known cutaneous energy) (6, 7). This was followed by 2 case reports of disseminated disease and 1 case report of localized pulmonary disease, all in HIV-infected patients with low CD4+ cell counts (7–9). A fifth published case described disseminated infection in a man with history of sarcoidosis and atherosclerotic cardiovascular disease. This adult patient had a history of prolonged corticosteroid treatment preceding the onset of symptoms by more than 10 years (10).

The next two case reports described disseminated infections with generalized lymphadenopathy and hepatosplenomegaly in 2 children with an unremarkable medical history. Further investigations revealed that both children had a deficiency in the IFN-γ/IL-12 pathway, known to increase susceptibility to mycobacterial infection (11).

Recently, an eighth case was published describing a disseminated M. tilburgii infection in a 33-year-old woman who was found to carry biallelic mutations of the gene encoding the β1 chain of the IL-12 receptor (12).

As in the fifth published case report, our patient did not have a known immunodeficiency and diagnosis of sarcoidosis was presumed a few years prior to the diagnosis of M. tilburgii infection. Our patient was still under steroid therapy when disseminated M. tilburgii infection was diagnosed, while it had been more than 10 years since the other patient had stopped this treatment.

The possibility that our patient had first been infected with M. tilburgii in 2004 (when diagnosis of sarcoidosis was assumed) and had experienced a flareup of the infection while under steroid therapy in 2008 needs to be considered.

We could not identify any environmental source of infection. In fact, the M. tilburgii species has been isolated only from clinical human specimens thus far, and no environmental source could be identified in any of the described cases.

Microscopy of several samples (sputum, bone marrow, lymph node, spleen, and liver) from the patient showed massive amounts of acid-fast bacilli. The cultures remained negative, which was unexpected considering the abundance of acid-fast bacilli. Molec-
ular methods such as amplification with specific primers or amplification using universal bacterial primers followed by 16S rRNA gene sequencing represent an interesting opportunity for rapid identification and the only alternative for mycobacterial species that cannot be cultured (1, 13–18).

Amplification by PCR using different specimens from the patient, followed by sequencing of the 16S rDNA gene (GenBank accession no. KF724951), showed 99% homology with the 16S rRNA sequences of M. tilburgii (accession number AJ580826.1) (Table 1).

We considered M. tilburgii to be the causative pathogen in our patient because of the following reasons: clinical symptoms compatible with mycobacterial infection; numerous specimens which revealed massive amounts of acid-fast bacilli; the involvement of normally sterile locations; the repeated histopathological finding of granulomas consisting of foamy histiocytes containing large numbers of acid-fast bacilli; and the progressive improvement under antimycobacterial treatment.

In the case of non culturable Mycobacterium species, no susceptibility test results are available. The exact identification of the causative species is therefore useful in tailoring the antimycobacterial treatment (2).

Many difficulties in efforts to define an efficient, safe, and well-tolerated treatment for our patient were faced. We based his treatment on previous experience with atypical mycobacterial infections and published case reports (7–11). The composition and duration of treatment were adapted based on clinical and histological features and the appearance of side effects.

A lifelong suppressive treatment with clarithromycin and moxifloxacin is proposed for our patient since acid-fast bacilli remain present in the patient’s samples (though they are significantly reduced in density) and a future lung transplantation in the context of chronic obstructive pulmonary disease (COPD), GOLD grade IV, is probable.

In conclusion, we report a hitherto rarely reported case of disseminated infection with nonculturable M. tilburgii, proven by PCR, and treated with a fully empirical combination of antimycobacterial drugs, in combination with gamma interferon.

**Nucleotide sequence accession number.** The nucleotide sequence determined in this work was submitted to GenBank under accession no. KF724951.

**REFERENCES**