Delayed Diagnosis of Disseminated *Mycobacterium genavense* Infection in a Human Immunodeficiency Virus–Negative Young Woman

Enrico Tortoli, ScD, Roberta Mannino, ScD,* Anna Cristina Carvalho, MD,† and Alberto Matteelli, MD†*

Abstract: We report here a case of *Mycobacterium genavense*–disseminated infection in a human immunodeficiency virus–negative young patient under immunosuppressive treatment for autoimmune chronic hepatitis type I. The diagnosis was achieved thanks to the detection of *Mycobacterium genavense*–specific DNA in a hepatic biopsy embedded in paraffin for histologic examination 2 years before.

Key Words: *Mycobacterium genavense*, immunosuppression, case report, autoimmune disease

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The widespread awareness of the possibility of *Mycobacterium genavense* infections led, in the 1990s, to its frequent diagnosis in patients with acquired immunodeficiency syndrome.[1,2] With the introduction of highly active antiretroviral treatments, which allow an efficient prevention of opportunistic infections, finding *M. genavense* has become nowadays extremely rare with the consequent loss of the aforesaid awareness. We report here a case of *M. genavense* infection in which the diagnosis was achieved with a 2-year delay.

CASE REPORT

The history of the patient is closely interlaced with immunosuppressive treatments since childhood. She first took steroids for several months at the age of eight when cryoglobulinemia type III was diagnosed after an episode of purpura with manifestations of cutaneous vasculitis of the legs.

Fifteen years later, the girl was hospitalized because of severe jaundice attacks. On that occasion, hepatomegaly was detected, and the hepatic biopsy revealed hepatitis with signs of necrosis.

A diagnosis of autoimmune chronic hepatitis type I was made, and the treatment with steroids was resumed with the adjunct of azathioprine. Genetic investigation revealed the patient was a carrier of α-1-antitrypsin deficiency. In subsequent years, the patient was monitored with frequent hepatic biopsies, which invariably revealed fibrosis, inflammatory infiltrations of mononucleate cells, and degenerative lesions of the biliary ducts. Immunosuppressive treatment was continuously assumed at high doses.

In 2007, when she was 32, the woman was hospitalized in the Infectious Diseases Unit because of high fever and severe weight loss. The fever was irresponsive to antimicrobials and blood cultures remained negative. Echographic investigations showed hepatomegaly, splenomegaly, and the presence of swelling mesenteric and lumbar aortic lymph nodes. Biopsies of the bone marrow, liver, and lymph nodes revealed presence of histiocytes containing acid-fast bacilli; in the liver, granulomatous lesions also were seen. No growth of mycobacteria in culture was however obtained. The immunosuppressive drugs taken continuously for 7 years were discontinued and an antimycobacterial treatment was undertaken and carried out for 14 months. It included, at first, levofloxacin, isoniazid, rifabutin, and clarithromycin but was modified 2 months later, with the elimination of clarithromycin and by adding pyrazinamide for 5 months. The patient clearly improved and regained partially the weight lost. Upon follow-up, hepatic biopsies confirmed however the presence of granulomatous lesions with acid-fast bacilli and a tomographic scan drew attention to enlarged abdominal lymph nodes. In October 2009, because of the clear deterioration of the patient's condition, she complained of persistent abdominal pain, the patient was again hospitalized in an infectious diseases ward where a new course of antimycobacterial therapy was started. The initial regimen with isoniazid, ethambutol, and pyrazinamide was changed after 1 month, in the hypothesis that the infection was due to nontuberculous mycobacteria, by replacing pyrazinamide with clarithromycin plus rifampicin.

A bone marrow biopsy was performed. This showed heavy infiltration of granulomatous lesions and acid-fast bacilli, but no mycobacteria were grown in culture.

While the aforesaid treatment was in its fifth month, the Mycobacteria Reference Center of Florence was involved in the diagnostic process and was requested to attempt the identification of the acid-fast bacilli visible in the paraffin-embedded biopsy specimens.

The tissue was freed from paraffin by xylene immersion and subsequently rehydrated with serial ethanol baths of decreasing concentration. The DNA extracted from the biopsy specimen using UltraClean Microbial DNA Isolation kit (Mo Bio, Carlsbad, Calif), following the modified protocol for mycobacterial DNA, was amplified using the primers provided in the commercial kit GenoType Mycobacterium (Hein, Nuhren, Germany). Such primers define a region of 23S rRNA gene, which, thanks to its variability, can be used for the identification of a number of mycobacterial species. The amplification product incubated with the strips provided by the aforesaid kit, on which are immobilized several DNA-probes specific for various mycobacteria, produced a hybridization pattern compatible with the species *M. genavense*.

With the aim of isolating the mycobacterium in culture, a new hepatic biopsy was performed; the fresh tissue revealed the presence of acid-fast coccobacillar forms, which were identified, by molecular investigations, as *M. genavense*. However, the culture in liquid medium (MGIT; Becton Dickinson, Towson, MD) supplemented with 0.2% mycobactin J[3] remained negative

From the *Regional Reference Center for Mycobacteria, Careggi University Hospital, Florence and †Institute of Infectious and Tropical Diseases, University of Brescia, Brescia, Italy.

Correspondence to: Enrico Tortoli, Centro Regionale di Riferimento per la Diagnostica dei Micobatteri, Piastra dei Servizi, A.O.U. Careggi, largo Brambilla 3, 50134 Firenze, Italy. E-mail: e.tortoli@libero.it.

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despite the fact that the incubation was prolonged until the 12th week. Nevertheless, the genetic amplification was able to detect *M. genavense* DNA in the broth.

The antimycobacterial treatment was modified, and the patient was started on a regimen containing clarithromycin, amikacin, moxifloxacin, and rifabutin. At that time, the bone marrow biopsy showed heavy infiltration of granulomatous lesions and acid-fast bacilli. Immunological investigations revealed $CD4^+$ lymphocytes deficiency but confirmed the woman was human immunodeficiency virus (HIV) negative.

At the fourth month follow-up, the general conditions remain unchanged, and acid-fast bacilli are still visible in the bone marrow.

Many cases of *M. genavense* infection have been reported in patients with acquired immunodeficiency syndrome[1,2]; on the contrary, the literature concerning HIV-negative subjects is scanty.

A part from 2 patients with cervical lymphadenitis,[4,5] for whom no immunodeficiency could be demonstrated, all the reported cases of infection due to *M. genavense*, in HIV-negative patients, concern individuals under immunosuppressive treatment. The motivations were prevention of rejection after organ transplantation[6–8] and treatment of autoimmune diseases[9–11] and of chronic lymphocytic leukemia.[12] In one case only, disseminated infection has been reported[11]; in the others, abdominal lymphadenopathy, with occasional diffusion to axillary, inguinal and cervical lymph nodes, was the prevalent manifestation. One localized infection[9] was probably due to incidental inoculation because it developed in the site of a previous bone marrow biopsy.

In most of the cases, acid-fast bacilli were seen in clinical specimens (subcutaneous tissue, lymphonodal biopsies, blood or bone marrow). Growth of *M. genavense* in culture was obtained in about half of the cases; in the others, it was only possible to demonstrate by polymerase chain reaction the presence of *M. genavense*–specific DNA.

The lack of standardized methods and the extremely slow growth of *M. genavense* render in vitro susceptibility testing unreliable. A common denominator of reports concerning successfully treated infections is having resorted to drugs associations, which invariably include clarithromycin; evidence also exists about the efficacy of rifabutin. The role of ethambutol is still debated but, likewise in *M. avium* complex infections, its synergistic activity in drug combinations seems likely. The addition of other drugs (quinolones, amikacin, or clofazimine) has been only occasionally reported. The duration of the treatment ranged from 6 to 18 months. Almost all the patients responded to the treatment, and only in 1 case the infection relapsed, 4 years later. The case of disseminated infection had a fatal outcome, whereas the death of another patient was not related to the infection by *M. genavense*.

The present case confirms data reported in literature. *M. genavense* is an opportunistic organism occasionally responsible for disseminated or localized infections in patients with impairment of the immune system. The distinction of such infections from the ones due to *Mycobacterium avium* complex is possible only at microbiological level. The well-known difficulty of growing *M. genavense* in culture, which could be overcome in the past with the use of radiometric liquid medium (Bactec 12B; Becton Dickinson), appears increased today because the new commercial liquid media do not seem as able to support the growth of such fastidious mycobacterium, and the molecular approach remains the most reliable when a *M. genavense* infection is suspected.

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