Rapid Identification of *Mycobacterium genavense* with a New commercially Available Molecular Test, INNO-LiPA MYCOBACTERIA v2

François Trueba,1* Michel Fabre,1 and Patrick Saint-Blancard2

Department of Clinical Microbiology1 and Department of Pathology,2 Percy Hospital, Clamart, France

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We report a rare mesenteric localized *Mycobacterium genavense* infection in a severely immunosuppressed human immunodeficiency virus-infected patient. An INNO-LiPA MYCOBACTERIA v2 test was performed directly on biopsy samples. This new molecular tool could be used for simultaneous identification of mycobacterium species from human specimens, but other studies are needed to validate our first results.

CASE REPORT

A 39-year-old human immunodeficiency virus type 1 (HIV-1)-infected homosexual man was referred to our hospital because of a 2-week history of intermittent left lower quadrant abdominal pain and constipation. The patient also noticed systemic symptoms, such as weakness, anorexia, and massive weight loss (12 kg in the last 2 months), without fever. There was no palpable mass on abdominal examination. The complete blood count was within the normal range. The erythrocyte sedimentation rate was 126 mm in the first hour, and the C-reactive protein level was 85 mg/liter. Laboratory studies were consistent with advanced immunodepression. The patient had a significantly decreased number of circulating CD4 lymphocytes (27 cells/mm3) and high HIV RNA levels in plasma (235,000 copies/ml). An enhanced computed tomography scan of the abdomen revealed a mesenteric mass with a hypodense center and abdominal lymphadenopathy. The tumor, suspected to be a lymphoma, was resected. The sample was processed by using the *N*-acetyl-l-cysteine–sodium hydroxide decontamination procedure, prior to testing by the Amplified Mycobacterium Tuberculosis Direct test (Gen-Probe, San Diego, Calif.), and was analyzed for the presence of inhibitors or interfering substances according to the manufacturer’s instructions (4). The sample was stained using the Ziehl-Neelsen method (9). It was inoculated onto a solid medium (Lowenstein-Jensen medium) and into a mycobacterial growth indicator tube (2). The inoculated culture media were incubated for a maximum of 8 weeks. Histology of the clinical specimen showed a macrophagic infiltration, and the Ziehl-Neelsen stain revealed numerous acid-fast bacilli. No *Mycobacterium tuberculosis* complex was identified by Amplified Mycobacterium Tuberculosis Direct test of the tumor sample. An INNO-LiPA MYCOBACTERIA v2 test (Innogenetics, Ghent, Belgium) was performed, and *Mycobacterium genavense* was identified from sample and from liquid culture. No stool specimens were tested. A positive growth in the mycobacterial growth indicator tube 960 system was detected 2 months later. The outcome was fatal in spite of anti-HIV tritherapy and treatment with clarithromycin, ethambutol, and rifampin.

*M. genavense* is a nontuberculous mycobacterium and was first described in 1992 by Böttger et al. (1). It has been identified as a cause of disseminated disease in AIDS patients (6). *M. genavense* infection should be considered in the differential diagnosis of AIDS patients with CD4 cell counts below 100 cells/mm3 presenting with multiple large retroperitoneal and mesenteric lymph nodes or circumferential wall thickening of the proximal small bowel (3). Realini et al. found that the addition of a polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA) antibiotic mixture to primary cultures impedes the in vitro growth of *M. genavense* (5). These bacteria grow poorly in vitro and were detected from liquid medium without the addition of PANTA 8 weeks after inoculation. Diagnosis was established using the new reverse hybridization multiple DNA probe assay INNO-LiPA MYCOBACTERIA v2. Biotinylated DNA material, obtained by means of a PCR amplification of the 16S to 23S rRNA polymorphic spacer region, is hybridized with 23 specific oligonucleotide probes immobilized as parallel lines on membrane strips. The addition of streptavidin labeled with alkaline phosphates and of a chromogenic substrate results in a purple-brown precipitate on hybridized lines (7). The mesenteric tumor biopsy sample had been decontaminated and directly tested without DNA extraction. The number of acid-fast bacilli in the sample and the substantial enlargement of the spectrum of species identifiable by INNO-LiPA MYCOBACTERIA v2 testing allowed us to detect *M. genavense*. The amplification product from the isolate detected by the test was sequenced. The DNA sequence was identical to the 16S to 23S rRNA spacer for *M. genavense* from GenBank (accession number Y14183), confirming the INNO-LiPA MYCOBACTERIA v2 result. Liquid medium culture was also tested 2 months after inoculation, and *M. genavense* was confirmed as the sole etiological agent. A recent evaluation of INNO-LiPA MYCOBACTERIA v2 revealed 100% sensitivity and specificity for the genus-specific probe. For the species-specific probes, the total specificity was 94.4% and the sensitivity was 100% (8).
Despite the fact that mycobacterial identification from biopsy samples using INNO-LiPA MYCOBACTERIA v2 is not recommended by the manufacturer, our example demonstrates the role of this new molecular tool, performed on biopsy specimens, for the rapid and simultaneous identification of mycobacterium species. More relevant studies are needed to validate our findings.

REFERENCES