No human transmission of *Mycobacterium malmoense* in a perfect storm setting

To the Editors:

Nontuberculous mycobacteria (NTM) are opportunistic pathogens that are widely present in our environment, *i.e.* in the soil and in natural and processed water. This group of organisms, with varying biological properties and clinical relevance, has gained notoriety over the past two decades due to their capability to cause severe disease in patients with immunodeficiency and/or chronic lung disease [1]. Despite their rising isolation frequency and growing clinical importance, NTM infections receive little scientific or public health attention. This partly results from the dogma that these NTM infections are not transmitted from male-to-male [1].

During our previous research of *Mycobacterium malmoense* infection in the Netherlands [2], we studied two patients with *M. malmoense* pulmonary infection with a strong epidemiological link. Human transmission was suspected because an acid-fast bacilli (AFB) smear-positive patient was in close contact with an immunocompromised patient. Herein, we present details of the cases and the results of the molecular diagnostics that were performed. The patients gave informed consent; ethical approval was not required for this retrospective study.

A 38-yr-old male with a medical history of chronic obstructive pulmonary disease and spontaneous pneumothorax reported to the respiratory physician with progressive dyspnoea, productive cough, fever, night sweats, malaise and weight loss. A chest radiograph revealed infiltrates with cavities in the upper lobes. Laboratory diagnostics showed a raised erythrocyte sedimentation rate (90 $mm \cdot h^{-1}$) and leukocytosis (white blood cell count 22.4 $cells \cdot mL^{-1}$); serological tests for HIV were negative. Three sputum smears were negative for AFB on direct microscopy. Bronchoalveolar lavage was performed, which was positive for AFB on direct microscopy. A regimen of isoniazid, rifampicin, ethambutol and pyrazinamide was initiated, based on a presumptive diagnosis of pulmonary tuberculosis. Later, sputum appeared to be positive for AFB. All corresponding cultures yielded *M. malmoense*, identified by Inno-LiPA Mycobacteria v2 reverse line blot assay (Innogenetics, Ghent, Belgium). Since the patient met the American Thoracic Society diagnostic criteria for clinical disease [1] the regimen was changed to 24 months of rifampicin, ethambutol and clarithromycin and the patient slowly improved.

11 months after the diagnosis was made in patient one, a second patient was diagnosed with *M. malmoense* disease in the same hospital. This patient, a 59-yr-old male, had a history of kidney transplantation and subsequent immunosuppressive treatment with cyclosporine and prednisone. He presented with productive cough, malaise, fever and weight loss. Erythrocyte sedimentation rate was 110 $mm \cdot h^{-1}$ and the white blood cell count was 8.5 $cells \cdot mL^{-1}$. A chest computed tomography scan revealed an infiltrate with cavitation in the left upper lobe and mediastinal lymphadenopathy. A transbronchial biopsy showed granulomatous inflammation with central necrosis and visible AFB on histological examination. Sputum samples revealed AFB on direct microscopy and corresponding cultures, and two simultaneously obtained blood cultures yielded *M. malmoense*. A regimen of rifampicin, ethambutol and clarithromycin was initiated; owing to clarithromycin intolerance, this drug was changed to ciprofloxacin. After initial progression of disease, including sepsis, progressive pulmonary infiltrates and spontaneous rupture of a necrotising mediastinal lymph node to the oesophagus, the patient recovered slowly. Both patients completed 24 months of drug therapy and have remained culture negative during 2 yrs of follow-up. Patient two died after the 2-yr follow-up period due to a Grawitz tumour.

Epidemiological investigation revealed that the two patients were acquainted and had intensive contact because they met very regularly in the same bar. The first patient worked in a flower bulb processing factory. Isolates of both patients, as well as unrelated patients from the same region and bordering regions, were subjected to genotyping by repetitive element (rep)-PCR typing. The results were confirmed using amplified fragment length polymorphism (AFLP) typing as described previously [3, 4]. Rep-PCR typing revealed a 97.4% similarity between isolates of both patients, suggesting they were possibly the same single strain. However, this same (>97% similarity) pattern was also observed from the *M. malmoense* isolate of an elderly female with pulmonary *M. malmoense* disease living in a nearby city; this patient was not epidemiologically related. Other strains from nearby and more distant regions, as well as from the same region, although at a later period, revealed distinct patterns by rep-PCR typing (fig. 1a). However, AFLP typing showed that the isolates of both patients had only 90% similarity. AFLP included the strain of a patient with pulmonary *M. malmoense* disease from the neighbouring region (05–1477) in the cluster with isolates of both our patients (fig. 1b).We subsequently sequenced the 16S rDNA gene, 16S–23S internal transcribed spacer (ITS), as well as partial *hsp65* and *rpoB* genes as previously described [5]. The isolates of both patients had identical 16S, ITS and *rpoB* sequences; the 441bp Telenti-fragment of the *hsp65* gene showed two base-pairs difference between both isolates [6].

Although there is a strong epidemiological link between both patients with a perfect scenario for human transmission (an AFB positive and coughing source in close contact with an immunocompromised receiver), the results of AFLP and the *hsp65* gene sequences, showing two base-pairs difference between the strains of both patients, render human transmission in this case unlikely. Cases of NTM human transmission have been suggested previously, as reviewed by WOLINSKY [7]. One setting

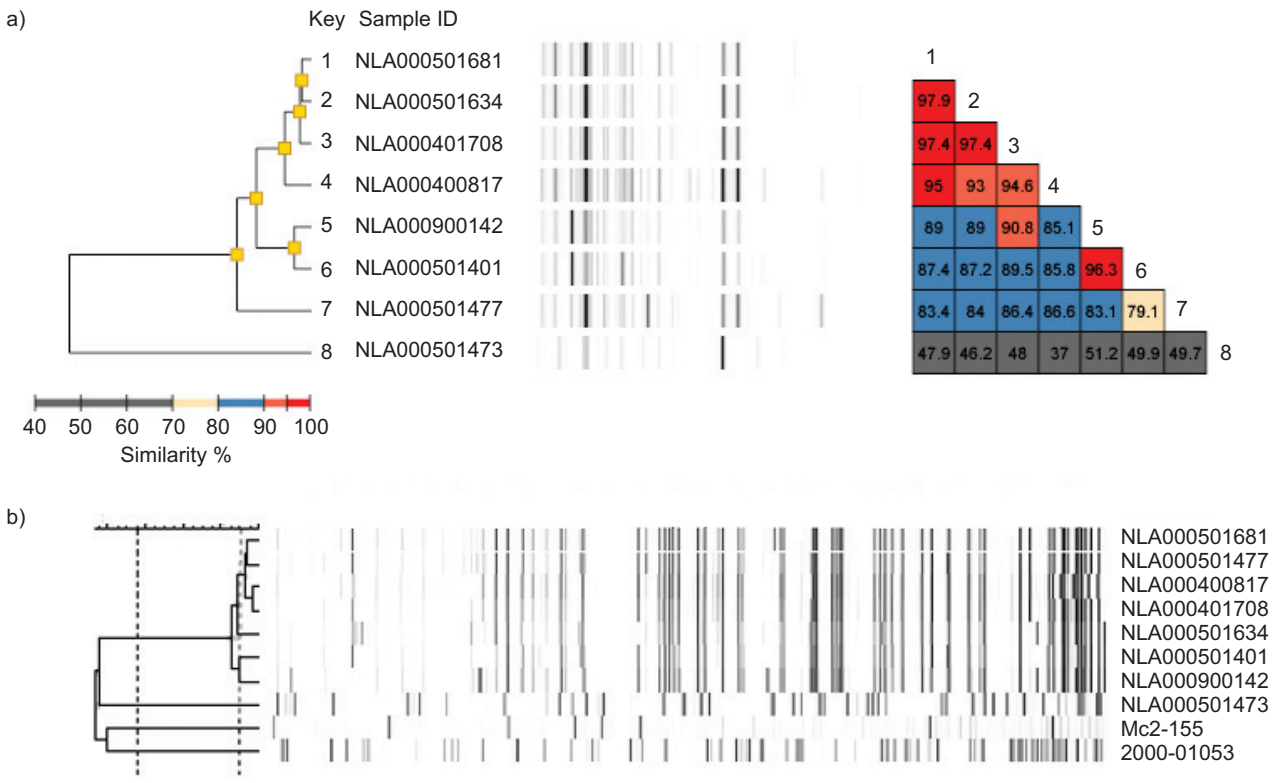

FIGURE 1. Gene sequencing results of the patient's isolates including related strains isolated from other patients. 04–1708 is the strain isolated from patient one and 05–1634 was isolated from patient two. a) Typing results obtained by repetitive element (rep)-PCR typing (DiversiLab; BioMerieux, Marcy l'Etoile, France). 97.4% similarity was calculated between the two patient isolates using rep-PCR. b) Amplified fragment length polymorphism (AFLP) typing results. 90% similarity was calculated between the two patient isolates using AFLP. Strain 05–1681 was isolated from an elderly female with pulmonary *Mycobacterium malmoense* disease in a nearby city; 04–817 was isolated from a child with lymphadenitis in the same region; and 05–1477 was isolated from a patient with pulmonary *M. malmoense* disease in a neighbouring district. More distantly related 09–142 and 05–1401 were isolated in the same region but 4 yrs later and in a region in another part of the country, respectively. Strain 05–1473 is a *Mycobacterium fortuitum* isolate used as an out group. *Mycobacterium smegmatis* mc2–155 and *Mycobacterium marinum* 2000–01053 are control strains.

showed some similarities to ours with two brothers who developed pulmonary *Mycobacterium kansasii* disease, with an interval of 1 yr [8]. Still, human transmission of NTM has, however, never been proven.

The interpretation of genotyping results in order to make definite conclusions about human NTM transmission is complicated: even if both strains had been fully identical by the methods described, this cannot rule out the possibility of infection from an identical environmental reservoir. The similarity with other strains from the same region (fig. 1) suggests the presence of a local ecotype. If local ecotypes exist, as our typing data suggests, the environmental source need not even have been identical, they might just have been infected in the same region. In conclusion, we couldn't establish human transmission in this case of an AFB positive and coughing source in close contact with an immunocompromised receiver.

Wouter Hoefsloot*, Martin J. Boeree*, Cees van Nieuwkoop[#,¶], Alexandra T. Bernards[+], Paul H.M. Savelkoul[§], Jakko van Ingen[f] and Dick van Soolingen[f,]**

*Dept of Respiratory Diseases, Radboud University Nijmegen Medical Center, [f]Dept of Medical Microbiology, Radboud University Nijmegen Medical Center, Nijmegen, [#]Dept of Infectious Diseases, Leiden University Medical Center, [+]Dept of Medical Microbiology, Leiden University Medical Center, Leiden, [¶]Dept of Internal Medicine, Haga Hospital, The Hague, [§]Dept of Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, and **National Mycobacteria Reference Laboratory, National Institute for Public Health and the Environment, Bilthoven, The Netherlands.

Correspondence: W. Hoefsloot, Dept of Pulmonary Diseases, Radboud University Nijmegen Medical Center, PO BOX 9101, 6500HB Nijmegen, the Netherlands. E-mail: w.hoefsloot@long.umcn.nl

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REFERENCES

1 Griffith DE, Aksamit T, Brown-Elliot BA, *et al.* An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007; 175: 367–416.

2 Hoefsloot W, van Ingen J, de Lange WC, *et al.* Clinical relevance of *Mycobacterium malmoense* isolation in the Netherlands. *Eur Respir J* 2009; 34: 926–931.

3 Bruijnesteijn van Coppenraet LE, Savelkoul PH, Buffing N, *et al.* Amplified fragment length polymorphism analysis of human clinical isolates of *Mycobacterium haemophilum* from different continents. *Clin Microbiol Infect* 2009; 15: 924–930.
4 Cangelosi GA, Freeman RJ, Lewis KN, *et al.* Evaluation of a high-throughput repetitive-sequence-based PCR system for DNA fingerprinting of *Mycobacterium tuberculosis* and *Mycobacterium avium* complex strains. *J Clin Microbiol* 2004; 42: 2685–2693.
5 van Ingen J, Boeree MJ, Kösters K, *et al.* Proposal to elevate *Mycobacterium avium* complex ITS sequevar MAC-Q to *Mycobacterium vulneris* sp nov. *Int J Syst Evol Microbiol* 2009; 59: 2277–2282.
6 Telenti A, Marchesi F, Balz M, *et al.* Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. *J Clin Microbiol* 1993; 31: 175–178.
7 Wolinsky E. Nontuberculous mycobacteria and associated diseases. *Am Rev Respir Dis* 1979; 119: 107–159.
8 Lichtenstein MR, Takimura Y, Thompson JR. Photochromogenic mycobacterial pulmonary infection in a group of hospitalized patients in Chicago. II. Demographic studies. *Am Rev Respir Dis* 1965; 91: 592–595.

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Successful treatment of progressive diffuse PEComatosis

To the Editors:

A 43-yr-old, nonsmoking female undergoing gynaecological surgery for menorrhagia was noted to have reduced arterial oxygen saturation and, on direct questioning, reported long-standing, mild, exercise-limiting dyspnoea. Treatment with inhaled bronchodilators had had no effect on her symptoms. Over the succeeding 2 yrs she developed progressive dyspnoea with corresponding deterioration in exercise tolerance. At that time, standard section computed tomography (CT) showed diffuse changes that were considered nonspecific, consisting of difficult-to-characterise opacities without zonal predominance; specifically, there were no conspicuous cysts. The features were suggestive of an infiltrative process and lung function assessment disclosed a forced expiratory volume in 1 s (FEV_1) of 2.12 L (80% predicted), forced vital capacity (FVC) of 2.66 L (88% pred) and transfer factor of the lung for carbon monoxide ($T_{L,CO}$) of 49% pred. A surgical lung biopsy was performed. Ultrasound of the kidneys disclosed no abnormality; specifically, there was no evidence of angiomyolipoma.

Lung histology showed an interstitial proliferation of cytologically bland, mainly rounded cells with predominantly clear cytoplasm (fig. 1a) and without obvious mitotic activity. In areas, these cells formed nodules in the alveolar parenchyma, while elsewhere, the proliferation was more diffuse. Focally, the cells showed a more spindled morphology, having blunt-ended nuclei and moderate volumes of eosinophilic cytoplasm (fig. 1b), a characteristic of lymphangioleiomyomatosis (LAM). All cells were positive for smooth muscle actin (SMA) and desmin, with focal positivity for HMB45. They were also positive for progesterone and oestrogen receptors. A periodic acid–Schiff stain for glycogen was focally positive. Following clinicopathological review, it was felt that the lack of imaging features characteristic of LAM, the lack of S-100 positivity characteristic of a clear-cell sugar tumour (CCST) and the overall perivascular epithelioid cell (PEC)omatous phenotype meant the case was best described as diffuse PEComatosis.

Because of the overlap between LAM and PEComatous disorders, treatment with hydroxyprogesterone was started. Genotyping for tuberous sclerosis was undertaken and proved negative. After 18 months of treatment, the patient's exercise tolerance had deteriorated further, and she also reported development of swelling of her left leg extending up to the gluteal region. Her lung function had deteriorated, with an FEV_1 of 1.58 L (63% pred), FVC of 2.03 L (70% pred) and $T_{L,CO}$ of 3.43 L (43% pred). CT showed interlobular septal thickening, a widespread micronodular pattern and a right-sided pleural effusion, subsequently confirmed to be chylous, but again no cysts (fig. 1c). CT of the abdomen and pelvis showed large retroperitoneal paraortic lesions of fluid density that, on biopsy, were shown to be lymphatic in nature. Lymphangiography showed decreased inguinal lymph drainage with dilatation of the pelvic lymphatic system. Following biopsy, significant chylous ascites developed. Because of the rapid clinical deterioration, combined with the lymphatic abnormalities, chylous pleural and abdominal effusions, a multimodal approach to therapy was adopted. The patient was commenced on: sirolimus (Rapamune, Wyeth, NJ, USA), at an initial dose of 0.25 $mg \cdot m^{-2}$ body surface area, increased over a period of 4 months to achieve serum sirolimus levels of 10–15 $ng \cdot mL^{-1}$; subcutaneous octreotide (100 μg three times daily); and a medium-chain triglyceride (MCT) diet (aiming for a dietary fat content of <14 $g \cdot day^{-1}$).

At review, 3 months after the initiation of this therapy, there had been a dramatic improvement in symptoms with improved exercise tolerance and virtual resolution of lower limb oedema, chylous ascites and pleural effusion (fig. 1d). Lung function tests disclose a marked improvement in both spirometry (FEV_1 2.26 L, 91% pred; FVC 2.97 L, 102% pred) and $T_{L,CO}$ (51% pred). The patient remained clinically and radiographically stable 24 months after stopping octreotide and having relaxed the MCT diet. She continues on sirolimus with target serum levels of 5–10 $ng \cdot mL^{-1}$.

The clinical presentation in this case is suggestive of LAM. However, in typical cases, spirometry shows an obstructive defect with widespread pulmonary cysts [1], while this patient presented with restrictive disease and no cysts. The presence of multiple nodules and an interstitial proliferation of cells with striking epithelioid/clear cell morphology are more character-