

Detection of *Mycobacterium leprae* by PCR Testing of Sputa from a Patient with Pulmonary *Cryptococcus* Coinfection in Northern Australia

Laura J. Edwards,^{a,*} Ric N. Price,^{b,c,d} Vicki L. Krause,^a Sarah E. Huffam,^c Maria Globan,^e Janet Fyfe,^e Krispin M. Hajkowitz^{c,*}

Centre for Disease Control, Department of Health, Darwin, Northern Territory, Australia^a; Global and Tropical Health Division, Menzies School of Health Research and Charles Darwin University, Darwin, Northern Territory, Australia^b; Division of Medicine, Royal Darwin Hospital, Darwin, Northern Territory, Australia^c; Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom^d; Victorian Infectious Diseases Reference Laboratory, Melbourne, Victoria, Australia^e

A case of fever, sepsis, and chest lesions evident on a computed tomography scan of an indigenous man in northern Australia following burns to the feet is described. Sputum PCR testing revealed *Mycobacterium leprae*, and a fine-needle aspirate of the chest lesions demonstrated *Cryptococcus* coinfection.

CASE REPORT

A 47-year-old indigenous man from East Arnhem, northern Australia, was referred with painful burns to the right foot, having recently stepped on a burning log from a campfire. He reported a cough productive of white sputum for 1 week and weight loss for several months. Medical history included chronic bilateral peripheral neuropathy, iron deficiency anemia, and chronic hepatitis B virus infection. He had a history of heavy alcohol and kava use but denied recent intake. There was no known history of contact with leprosy.

On examination he was febrile (40.8°C), tachycardic (heart rate, 130 beats/min), and hypotensive (blood pressure 85/45 mm Hg). His oxygen saturation was 100% on room air with a respiratory rate of 22. Respiratory examination revealed decreased breath sounds and crepitations bilaterally. He had full-thickness burns to the plantar aspect of the right great, second, and third toes and reduced sensation of the whole right foot in a stocking distribution. Laboratory investigations revealed a hemoglobin level of 93 g/liter (reference range [RR], 135 to 185) and a white blood cell count of 3.6×10^9 /liter (RR, 4.0 to 11.0), with 2.9×10^9 neutrophils/liter (RR, 2.0 to 7.5) and 0.6×10^9 lymphocytes/liter (RR, 1.5 to 4.0). He had a platelet count of 139×10^9 /liter (RR, 150 to 450) and a normal blood glucose level. Chest X-ray demonstrated opacification of the apical segment of the right lower lobe.

He was started on meropenem and azithromycin and placed in respiratory isolation. A computed tomography (CT) scan of the chest revealed two lesions of soft-tissue density within the right lower lobe (maximum diameters, 48 mm and 19 mm) but no lymphadenopathy (Fig. 1). A serum cryptococcal antigen test was positive (titer, 1:64; Remel, Lenexa, KS). Microscopy of a fine-needle aspiration biopsy specimen from one of the chest lesions showed numerous fungal organisms consistent with *Cryptococcus* species.

One of three sputum specimens demonstrated scanty acid-fast bacilli (AFB) by Ziehl-Neelsen staining. PCR for *Mycobacterium tuberculosis* complex was negative on this sample. A subsequent generic PCR for mycobacteria yielded a 220-bp product; restriction digestion of the amplified product using HaeIII and CfoI gave a differential identification of *Mycobacterium kansasii*, *Mycobacterium celatum*, or *Mycobacterium leprae* (1). Sequencing of the



FIG 1 CT chest scan.

amplified product resulted in a 100% match with the internal transcribed spacer (ITS) region of *M. leprae*.

On further clinical review, leonine facies and thinning eyebrows were noted (Fig. 2), but there was no evidence of dermatological hypopigmentation. The left greater auricular, right median, bilateral ulnar, and bilateral popliteal nerves were enlarged, and there was sensory loss on pinprick testing of the right foot (2 on a scale of 10) compared to the left. Slit-skin examination of the eyebrows, earlobes, and nose showed AFB with a bacillary index of 4 consistent with multibacillary leprosy.

The patient was initially started on standard treatment for tuberculosis following the finding of sputum AFB positivity. When

Received 28 April 2014 Returned for modification 14 May 2014

Accepted 3 July 2014

Published ahead of print 23 July 2014

Editor: B. A. Forbes

Address correspondence to Laura J. Edwards, laura.edwards@nt.gov.au.

* Present address: Laura J. Edwards, Top End Remote Health, Department of Health, Darwin, Australia; Krispin M. Hajkowitz, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia.

Copyright © 2014, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JCM.01060-14

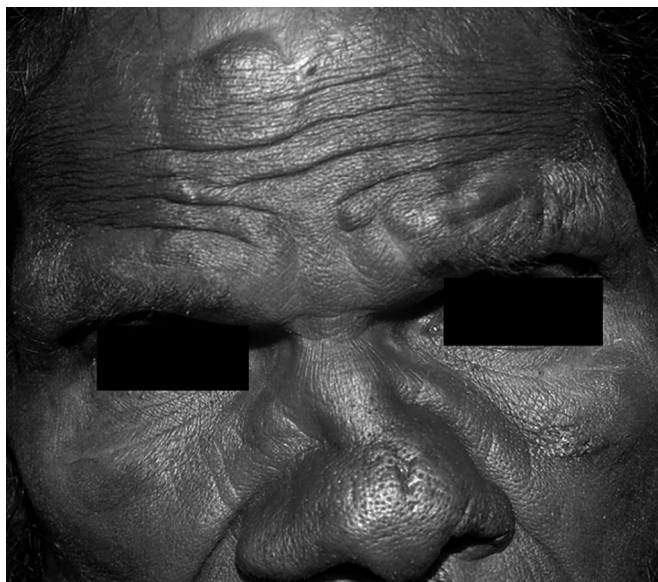


FIG 2 Leonine facies and thinning eyebrows.

PCR and sequencing results became available, the tuberculosis treatment was stopped and rifampin, clofazimine, and ofloxacin were commenced in accordance with the Guidelines for Leprosy Control in the Northern Territory (2). The cryptococcal infection was treated with 6 weeks of intravenous amphotericin and flucytosine and then oral fluconazole. Ofloxacin was substituted for dapsone to prevent the potential interaction between fluconazole and dapsone. The patient was treated for both conditions for a total of 24 months and made a full recovery.

The diagnosis of leprosy is usually made based on clinical features in patients with known risk exposure and confirmed by slit-skin examination or nerve biopsy. PCR on DNA extracted from various tissues (mainly skin) has been applied as an additional confirmatory test (3). Our patient was unusual in that the primary diagnosis was made following a positive Ziehl-Neelsen stain result using a generic mycobacterial PCR and confirmed on clinical review and slit-skin examination. The Ziehl-Neelsen stain is not sensitive for *M. leprae* but was positive in this case, which enabled the performance of further rapid testing for mycobacteria. To our knowledge, the use of PCR on sputum samples to differentiate between tuberculosis and leprosy has been previously reported only in Nepal (4). The most specific PCR to detect *M. leprae* targets the repetitive element (RLEP) gene (5, 6). This PCR was not available at the time of presentation but has subsequently been performed on the patient's sputum with a positive result.

The most prevalent mycobacterial disease within Australia is tuberculosis, which occurs in the indigenous population at rates up to 7 times those of nonindigenous Australians (7). Leprosy, caused by *Mycobacterium leprae*, was endemic in the indigenous population in northern Australia until the 1960s but over the last 10 years has been diagnosed infrequently across Australia, with 5 to 13 cases per year. A high proportion of infections continue to occur in the indigenous population (8). Cryptococcosis is also more common in the indigenous population of northern Australia

(9). While leprosy and tuberculosis are often endemic in similar geographic regions, we are aware of only three reports of concurrent leprosy and cryptococcal infection (10, 11).

Due to its rarity within Australia, the diagnosis of leprosy is often delayed, the median interval between onset of symptoms and diagnosis being 2 years (12). This case highlights the need for ongoing awareness of leprosy in low-prevalence countries, particularly in at-risk populations such as the indigenous population in northern Australia or in people born in higher-prevalence countries. It also provides evidence that leprosy can be diagnosed from sputum and that PCR can be effectively utilized to distinguish between different mycobacterial species in sputum samples positive for AFB. This was especially important in this case, where the organism was one that is unable to be routinely cultured and that without the PCR/sequencing result, the leprosy diagnosis may have been missed or delayed.

ACKNOWLEDGMENTS

K.M.H. has received speaker's fees from MSD pharmaceuticals, which he donated to charity (Médecins Sans Frontières).

L.J.E. drafted the manuscript with assistance from R.N.P., V.L.K., K.M.H., and S.E.H. M.G. and J.F. contributed technical laboratory content. All authors contributed important intellectual content. All authors approved the final version for publication.

REFERENCES

- Roth A, Reischl U, Streubel A, Naumann L, Kroppenstedt RM, Habicht M, Fischer M, Mauch H. 2000. Novel diagnostic algorithm for identification of mycobacteria using genus-specific amplification of the 16S-23S rRNA gene spacer and restriction endonucleases. *J. Clin. Microbiol.* 38: 1094–1104.
- Scott L, Zweck N. 2010. Guidelines for the control of leprosy in the Northern Territory, 3rd ed. Centre for Disease Control, Department of Health and Families, Northern Territory, Australia.
- Kampirapap K, Singham N, Klatser P, Wiriyawipart S. 1998. DNA amplification for detection of leprosy and assessment of efficacy of leprosy chemotherapy. *Int. J. Lepr. Other Mycobact. Dis.* 66:16–21.
- Sapkota BR, Ranjit C, Macdonald M. 2007. Rapid differentiation of *Mycobacterium tuberculosis* and *Mycobacterium leprae* from sputum by polymerase chain reaction. *Nepal Med. Coll. J.* 9:12–16.
- Wichitwechkarn J, Karnjan S, Shuntawuttisetee S, Sornprasit C, Kampirapap K, Peerapakorn S. 1995. Detection of *Mycobacterium leprae* infection by PCR. *J. Clin. Microbiol.* 33:45–49.
- Martinez AN, Lahiri R, Pittman TL, Scollard D, Truman R, Moraes MO, Williams DL. 2009. Molecular determination of *Mycobacterium leprae* viability by use of real-time PCR. *J. Clin. Microbiol.* 47:2124–2130. <http://dx.doi.org/10.1128/JCM.00512-09>.
- Barry C, Konstantinos A, National Tuberculosis Advisory Committee. 2009. Tuberculosis notifications in Australia, 2007. *Commun. Dis. Intell. Q. Rep.* 33:304–315.
- NNDSS Annual Report Writing Group. 2013. Australia's notifiable disease status, 2011: annual report of the National Notifiable Diseases Surveillance System. *Commun. Dis. Intell. Q. Rep.* 37:E313–93.
- Fisher D, Burrow J, Lo D, Currie B. 1993. *Cryptococcus neoformans* in tropical northern Australia: predominantly variant *gattii* with good outcomes. *Aust. N. Z. J. Med.* 23:678–682. <http://dx.doi.org/10.1111/j.1445-5994.1993.tb04726.x>.
- Furuta M, Obara A, Harada N, Sokai K, Hiroshi I. 1991. *Cryptococcus neoformans* can be misidentified as a microsporidian: studies of lung lesions in leprosy patients. *J. Protozool.* 38:955–965.
- Azulay RD, Mendonça IRM, Santos CM, Monteiro PCF, Lazera MS, Kac BK, Schechtman RC. 2001. Cutaneous cryptococcosis associated with lepromatous leprosy. *Int. J. Dermatol.* 40:412–414. <http://dx.doi.org/10.1046/j.1365-4362.2001.01246-5.x>.
- van der Oest C, Fairley M, Scott L, Krause V, Zweck N. 2005. Leprosy in the Northern Territory: a descriptive epidemiological study of all notified cases from 1991 to 2004. *NT Dis. Control Bull.* 12:19–21.