Diagnosis of *Mycobacterium ulcerans* disease: be alert to the possibility of negative initial PCR results

Daniel P O'Brien[1], Maria Globan[2], Janet M Fyfe[2], Caroline J Lavender[2], Adrian Murrie[3], Damian Flanagan[4], Peter Meggyesy[4], Jonathan T Priestley[4], David Leslie[2]

Mycobacterium ulcerans causes necrotising infections of the skin and soft tissue (Buruli ulcer), a disease that is endemic in the coastal regions of Victoria and northern Queensland. Most lesions (> 85%) are painless ulcers, but some are non-ulcerative.[1] As the incidence of Buruli ulcer rises in Victoria,[2] Australian health practitioners are increasingly required to recognise this disease in people who reside in or have travelled to endemic areas, with early diagnosis vital for good outcomes.[3]

The most important diagnostic method for Buruli ulcer in terms of accuracy, speed, and ease of performance is the polymerase chain reaction (PCR) assay of lesion tissue for the DNA insertion element IS*2404*.[4] In Australia, the sensitivity and specificity of the assay are each reported to be 100%.[4] However, some often unrecognised pitfalls can lead to missed diagnoses and serious adverse outcomes.

We report our experience in Barwon Health, Victoria. Of 551 patients with prospectively confirmed *M. ulcerans* disease diagnosed between 25 March 1998 and 13 February 2018, the PCR result for the initial swab specimen was negative in 34 cases (6.2%), but PCR results for repeat samples were positive (biopsy samples, 15; swab specimens, 19). The initial negative test led to a diagnostic delay of as long as 74 days (median, 17 days; interquartile range [IQR], 9–33 days).

We analysed risk factors associated with a negative initial PCR result for patients with subsequently confirmed *M. ulcerans* disease. At the time of the initial PCR test, the median duration of symptoms was significantly shorter for patients with negative results (22 days; IQR, 14–41 days) than for those with positive results (42 days; IQR, 28–84 days; $P < 0.001$). Further, a significantly larger proportion of children (15 years old or younger) had negative initial results (11/49, 22%) than of adults (16–50 years, 11/160, 6.4%; ≥ 51 years, 12/331, 3.6%; χ^2 test, $P < 0.001$).

Our findings indicate that health practitioners should be cautious when interpreting negative PCR results from people with lesions suggestive of *M. ulcerans* disease, especially when testing early lesions or children. The lesion may not yet have ulcerated when the swab sample is collected (nine of the 34 lesions with negative initial results were non-ulcerative at the time of diagnosis). PCR results for swab samples from non-ulcerative lesions can be negative because the lesion surface is free of bacteria. There may also be fewer organisms in these lesions; all mycobacterial smears and cultures performed at the time of negative initial PCR tests were also negative.

Incorrect swabbing technique probably explains negative initial PCR results, with insufficient clinical material collected for detecting bacteria. It is imperative that swab samples are taken by circling the entire undermined edge of a lesion, and checking that clinical material from the lesion is visible on the swab surface.[5] Errors can also be introduced if single swabs are processed in the laboratory for smear and culture before referral for PCR testing. A separate swab sample should be collected for PCR testing (Box).[5]

Key points for interpreting polymerase chain reaction (PCR) test results in cases of *Mycobacterium ulcerans* disease

- IS*2404* PCR testing of lesion material is the most accurate method for diagnosing *M. ulcerans* disease (Buruli ulcer).
- The PCR result for an initial swab specimen from a patient with *M. ulcerans* disease is not infrequently negative, particularly for samples from early lesions or children.
- A negative PCR test result for a suspicious lesion does not exclude *M. ulcerans* disease; the test should be repeated, preferably with a punch biopsy specimen.
- The PCR result for a swab sample from the surface of a non-ulcerative lesion is usually negative. PCR testing of non-ulcerative lesions requires biopsy (for fresh tissue) or fine needle aspiration (for tissue fluid).

Competing interests: No relevant disclosures.

Received 14 September 2018, accepted 5 December 2018 ■

1 Boyd SC, Athan E, Friedman ND, et al. Epidemiology, clinical features and diagnosis of *Mycobacterium ulcerans* in an Australian population. *Med J Aust* 2012; 196: 341–344. https://www.mja.com.au/journal/2012/196/5/epidemiology-clinical-features-and-diagnosis-mycobacterium-ulcerans-australian

2 O'Brien DP, Athan E, Blasdell K, De Barro P. Tackling the worsening epidemic of Buruli ulcer in Australia in an information void: time for an urgent scientific response. *Med J Aust* 2018; 208: 287–289. https://www.mja.com.au/journal/2018/208/7/tackling-worsening-epidemic-buruli-ulcer-australia-information-void-time-urgent

3 O'Brien DP, Friedman ND, McDonald A, et al. Wound healing: natural history and risk factors for delay in Australian patients treated with antibiotics for *Mycobacterium ulcerans* disease. *PLoS Negl Trop Dis* 2018; 12: e0006357.

4 Fyfe JA, Lavender CJ, Johnson PD, et al. Development and application of two multiplex real-time PCR assays for the detection of *Mycobacterium ulcerans* in clinical and environmental samples. *Appl Environ Microbiol* 2007; 73: 4733–4740.

5 Department of Human Services (Victoria). *Mycobacterium ulcerans* infection. Health.vic; 2018. https://www2.health.vic.gov.au/public-health/infectious-diseases/disease-information-advice/mycobacterium-ulcerans (viewed May 2018). ■

[1] University Hospital Geelong, Geelong, VIC. [2] Mycobacterium Reference Laboratory, Victorian Infectious Diseases Reference Laboratory, Melbourne, VIC. [3] Sorrento Medical Centre, Sorrento, VIC. [4] South Coast Medical, Blairgowrie, VIC. ✉ DANIELO@BarwonHealth.org.au ▪ doi: 10.5694/mja2.50046

Podcast with Daniel O'Brien available at https://www.mja.com.au/podcasts