What does detection of *Mycobacterium ulcerans* DNA in the margin of an excised Buruli ulcer lesion tell us?

Simona Rondini¹*, Ernestina Mensah-Quainoo², Thomas Junghanss³, and Gerd Pluschke¹

Molecular Immunology, Swiss Tropical Institute, Basel, Switzerland¹, Ghana Health Service, Ga West District, Ghana², and Section Clinical Tropical Medicine, University Hospital Heidelberg, Heidelberg, Germany³

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**Corresponding author:**

Simona Rondini, Department of Medicine, Division of Infectious Diseases and International Health, Weill Medical College of Cornell University 1300 York Avenue, New York, NY 10021.

Phone: +1-212-746-6324

Fax: +1-212-746-8675

e-mail: sir2004med.cornell.edu
Abstract

We determined the distribution of *Mycobacterium ulcerans* DNA by real-time PCR in the excised lesions of Buruli ulcer patients. In one of the patients a new lesion developed adjacent to the site of excision. The excised margin around the primary lesion contained a small amount of mycobacterial DNA in the area where the secondary lesion developed. These results suggest that a relatively small number of infiltrating mycobacteria can lead to the development of a recurrence.
CASE REPORT

A 23-year-old man from the Ga district of Ghana presented at the Amasaman Health Centre in November 2002 with an ulcerated plaque (23 x 15 cm) on the right forearm (Fig 1a). Within the plaque an ulcer (3 x 2 cm) was observed. Laboratory analyses (PCR, culture and histopathology) confirmed the clinical diagnosis of BU. After clinical diagnosis of BU, the plaque lesion was surgically removed *en block* on 18\(^{th}\) November 2002 with a margin of 2-3 cm of healthy-looking tissue (Fig. 1b). For the analysis of the spread of *M. ulcerans* in the lesion, samples were immediately collected along the long axis of the excised tissue (Fig. 1b) and individual specimens of about 100mg each were stored in liquid nitrogen until analyzed. The risk of cross contamination was minimized by taking the samples starting with the macroscopically healthy looking margins and working towards the centre of the lesion, using a separate disposable scalpel to cut each piece. Twenty-two specimens adjacent to each other were obtained from this strip of tissue. Two extra samples (23 and 24 in Fig. 1b) were taken from the margin in a region where, based on the macroscopic appearance of the tissue, the surgeon had decided to extend the excision beyond the originally decided margins (Fig. 1b). Fifty nanograms of DNA/sample were used for *M. ulcerans* real-time PCR quantification as described (11). This analysis revealed a focal distribution of *M. ulcerans*, with the highest mycobacterial burden near the centre of the ulcer (sample 14 in Fig. 2). While the *M. ulcerans* content was >100 fold higher in this sample than in any other sample, smaller peaks were also observed at the edges of the ulcer (samples 8 and 11). No significant amounts (< 50 genome copies/ml) of *M. ulcerans* DNA were found in some of the samples from the
healthy appearing margins of the excised tissue (samples 1, 2 and 24) while mycobacterial spreading had extended to others (samples 22 and 23).

The patient underwent skin grafting of the post-operative wound 50 days later on 6th Jan 2003. The wound healed completely with a sound scar and he was discharged in good condition on 17th Feb 03. On 15th March 2003, one month after discharge – nearly 4 months after the first excision –, he presented to the Health Centre with a new BU lesion located adjacent to the site where the excised margin contained a significant amount of M. ulcerans DNA (sample 23). Administrative delays prevented excision of the new lesion until on 8th April 2003, 3 weeks later. After the repeated excision, successful skin grafting on 20th May 2003 (Fig. 1c), and final discharge on 21st July 2003, no further lesion has developed till the last follow-up observation in March 2006.

M. ulcerans disease, commonly called BU, is a progressive necrotizing infection of the skin and the subcutaneous tissue (2). The mode of transmission of BU is not entirely clear, but once M. ulcerans is introduced into the dermis or subcutaneous tissue, it proliferates and produces a toxin, known as mycolactone (6). This polyketide toxin has cytopathic activity (7) and causes necrosis of the dermis, panniculus, and fascia, usually leading to relatively painless manifestations like subcutaneous nodules, ulcers, oedema, plaques and ulcers. Focal distribution of mycobacteria with tissue destruction extending into areas with low mycobacterial burden is a common feature of BU lesions. Additional peaks of mycobacterial DNA mark sites where satellite lesions in the vicinity of the primary focus are developing (10). M. ulcerans may also spread, presumably by lymphatic and haematogenous pathways, to distant locations, where metastatic skin and
occasionally bone lesions arise (9). Until recently, the only definitive treatment of BU was the surgical removal of the infected tissue (4), although it does not always ensure complete removal of bacilli (3). Frequent delays between the first appearance of lesions and admission to a health facility, often result in spreading of the disease, necessitating extended surgical interventions and long periods of hospitalization. Recurrence rates in hospital-treated BU patients between 6.1% (5) and 47% (13) have been reported. Amofah et al found a local recurrence rate of 16% at the same site within a year of follow-up (1).

In the primary BU lesion described in this report, the highest mycobacterial load was detected at one side of the ulcer, while most of the mycobacteria were obviously washed out from its centre, which represented the primary focus. Small amounts of bacterial DNA were also detected in an area of excised healthy appearing tissue that during surgery showed slight changes in texture (sample 23). Within four months after the primary excision, a new lesion developed in a distance of about 3 cm from the dorsal margin of the primary lesion (see fig 1b), and was excised (see fig 1c). The location of the new lesion strongly suggests that small numbers of remnant mycobacteria were enough to provoke a recurrence. We have recently demonstrated for the first time genetic diversity of *M. ulcerans* in an African country (8). However, the discriminative power of the newly developed VNTR fingerprinting method used was not sufficient to differentiate between re-infection and recurrence in the patient described here, since all 47 analysed recent isolates from the Ga District of Ghana had the same allele combination (8).

However, re-infection appears very unlikely in the described case, considering both the location of the new lesion, which was adjacent to an area where mycobacterial DNA was detected, and first signs of texture changes of the excised tissue margin noticed at the
time of surgery at this site. Additionally, the same site recurrences reported in the literature (5) well mirror the case described here. Although there is good evidence that wide excision reduces the risk of recurrence (13), the mycobacterial threshold levels for a new lesion to develop are far from clear. The genetic background (12) and the immune status of a patient are additional factors which may determine the mycobacterial load that can still be successfully contained. Previous work has shown that mycobacteria can contiguously disseminate and give rise to satellite lesions, even when granulomas provide evidence for the development of cell-mediated immunity (10). It appears that the mycobacteria can spread to some extent locally and diffuse across tissue affected by the disease (10). Satellite lesions therefore do not seem to appear exclusively downstream the lymphatic flow. As soon as a microcolony containing a critical number of *M. ulcerans* cells has developed by focal bacterial multiplication, a cloud of mycolactone may impair the cellular immune system locally, permitting the development of a new lesion.

This is the first report correlating the development of a new lesion in a BU patient who underwent surgical treatment with the spatial distribution of the mycobacteria within the removed tissue. Although the primary lesion had a focal *M. ulcerans* distribution, this case suggests that small numbers of spreading mycobacteria are sufficient to establish a new infection focus at the edge of the previously excised lesion.

Careful clinical and laboratory examination of excised tissue margins around BU lesions may help to assess the risk for local recurrences.
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None of the authors have conflict of interests.
References


Figure legends

Fig. 1
(a) BU patient presenting an ulcerated plaque on the right forearm before surgery (November 2002). The dashed line represents the incision margins. Samples were collected along the longitudinal axis of the excised tissue.
(b) Surgically excised tissue and location of collected samples.
(c) Extension of the skin-graft (R) following excision of recurrent lesion (July 2003).

Fig. 2
Real-time PCR quantification of mycobacterial genome copies corresponding to 50ng of extracted DNA. The threshold level was set to 50 genome copies/ml.
Fig. 2

The bar chart shows the distribution of genome copies across 24 samples. The y-axis represents the number of genome copies ranging from 0 to 100,000,000, while the x-axis lists the sample numbers from 1 to 24.

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