Antigen and antibody detection assays for the diagnosis of tuberculosis

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CHAPTER 2 a

First reported Case of *Mycobacterium ulcerans*

Infection in a Patient from China

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Abstract

Buruli ulcers have not been previously described in China, and only once at higher latitudes on the northern hemisphere. A patient who travelled in the Shan Dong Province in the People’s Republic of China developed an ulcer which was proven to be a Buruli ulcer. The clinical picture and histopathological findings from biopsy specimens are characteristic for a Buruli ulcer, and also the growth in culture (Coesloos medium) at a restricted temperature of 30°C. A multiplex polymerase chain reaction (PCR) based on the amplification of the gene encoding for 16S ribosomal RNA and a nested PCR based on the Mycobacterium ulcerans specific repeated sequence 2404 were performed. These PCR investigations identified the bacteria as *M. ulcerans*, subspecies *shinshuense*. The patient was initially treated with clarithromycin and rifampicin, which was changed to ciprofloxacin and rifabutin when rifampicin resistance of the first isolate was established. There were no signs of reactivation of the disease 6 months after the end of treatment. *M. ulcerans* infection occurs above 30° latitude on the northern hemisphere in China and is caused by *M. ulcerans*, subspecies *shinshuense*. This case appears to be cured by chemotherapy alone, in contrast to the general experience that surgical treatment is indicated. The granulomatous reaction with only fragments of acid-fast bacteria in the biopsy at the end of treatment may indicate the development of an adequate cell-mediated immune response leading to resistance to the infection.

Introduction

*Mycobacterium ulcerans* infections are most common in tropical regions but have also been observed in temperate climatic areas of Australia (5). Until now, only one patient from Japan infected above the latitude 30° N has been reported (14).

Recently, we observed a leg ulcer in a woman who had travelled in the Shan Dong province, Peoples Republic of China. In this report we describe the properties of the *Mycobacterium* isolate from this lesion. It is the first reported case of Buruli ulcer in the People’s Republic of China, and proves that *M. ulcerans* infection is also found in the temperate climatic zone of the Northern Hemisphere.
Case report

Clinical findings. A 40-year-old Chinese woman, who grew up in the People's Republic of China, had been living in Europe since 9 years. She travelled in July and August 1997 in the People's Republic of China where she walked barelegged in the grass between fruit trees outside Ri Zao city, 200 km south of Qingdao city, Shan Dong province. She noticed many so-called 'mosquito bites'. Three months later she noticed a swelling at the front of her left lower leg which later developed a pale slightly depressed center. There were no systemic symptoms. Four months later, when again visiting China, a surgeon excised the lesion; according to the patient there was no bleeding and the excised tissue was pale and firm. The lesion did not heal and showed ulceration. A Chinese doctor, specialised in infectious diseases, who was visiting her in The Netherlands, suggested that it might be tuberculosis of the skin, and a smear of the ulcer bed was made by her general practitioner. Ziehl-Neelsen staining revealed acid fast bacteria. She was referred to a dermatologist (J.T.) and subsequently to the Department of Dermatology, Section Tropical Dermatology of the Academic Medical Center, Amsterdam.

On examination there was a painless ulcer with a diameter of $3 \times 3.5$ cm with an undermined border and a necrotic ulcer bed. At the cranial side there was a small skin-coloured swelling (Fig. 1). There were no other skin lesions.

![FIG. 1. Ulcer with necrotic base and undermined border on the lower leg of the Chinese woman.](image1)

![FIG. 2. Histopathology of the Buruli ulcer, showing eosinophilic necrosis, mild mononuclear infiltrate, and a thrombosed and recanalized vessel (haematoxylin and eosin; original magnification ×80).](image2)

Diagnostic procedures. Histopathological examination of the biopsies taken from the ulcer border showed acanthotic broadening of the epidermis, extensive eosinophilic necrosis in the deeper dermis and the subcutaneous tissue, with a mild scattered mononuclear-cell infiltrate.
In the basis of the lesion there was a large thrombosed and recanalized vessel (Fig. 2). In the Ziehl-Neelsen staining, acid-fast bacteria were seen scattered and in clumps.

A biopsy from the ulcer border was cultured on Coletsos medium at 28, 30 and 37°C. After 40 days, pale-cream to yellow colonies grew only on the medium incubated at 30°C. The mycobacterial isolate was identified as described previously (9).

For the multiplex polymerase chain reaction (PCR) analysis the tissue biopsies were first treated with proteinase K and the DNA was isolated by the guanidine thiocyanate method as previously described (7). For the amplification of the gene encoding for the mycobacterial 16S ribosomal RNA, we used the 5′-biotinylated primers pMyc14bio and pMyc7bio. The primers Ptl88 and 5′-biotinylated INS2bio, amplifying the \textit{M. tuberculosis} complex specific insertion sequence IS6110, were included in the PCR mix. The PCR and the analysis of the PCR product by reverse cross blot hybridization were performed as previously described (7). The multiplex PCR assay showed hybridization with the probe specific for \textit{M. ulcerans} / \textit{M. marinum}. No reaction was found with the probes specific for \textit{M. tuberculosis}, \textit{M. leprae}, \textit{M. kansasii}, \textit{M. avium}, \textit{M. intracellulare}, \textit{M. fortuitum}, \textit{M. scrofulaceum}, \textit{M. xenopi}, \textit{M. chelonae}, \textit{M. genavense} (8). The presence of \textit{M. ulcerans} DNA in tissue biopsies and culture was confirmed by a nested PCR based on the \textit{M. ulcerans}-specific repeated sequence 2404 (12), as previously described (3).

\textbf{Treatment.} Treatment was started with rifampicin 600 mg plus clarithromycin 500 mg daily. After 2 months, treatment was changed because \textit{in vitro} the \textit{Mycobacterium} was found to be resistant to rifampicin. At that time the ulcer was nearly completely healed with only a small defect with purulent exudate in which acid-fast bacteria were found with the Ziehl-Neelsen staining; culture was negative. Treatment was changed to ciprofloxacin two times 750 mg and rifabutin two times 150 mg daily as the original isolate was sensitive \textit{in vitro} to these drugs. After four weeks, treatment had to be stopped because of leucopenia, and a rash with fever on reintroduction after one week. The ulcer was healed with conspicuous scarring and slight erythema of a small part of the border. A biopsy taken from this area showed a granulomatous reaction with only fragments of acid-fast bacteria; culture was negative. Three months later, on request of the patient, this erythematous part was completely excised. On histopathological examination a granulomatus reaction without mycobacteria was seen; culture was negative. Since then there has twice been some purulent discharge from a small erythematous induration. At the second time, 6 months after the end of antibiotic treatment, this induration was removed \textit{in toto} by biopsy; histopathological examination showed scar tissue, Ziehl-Neelsen staining was negative; also the culture was again negative.
Mycobacterium ulcerans infection in a patient from China

Discussion

The clinical picture and histopathological findings in this patient are characteristic for Buruli ulcer. In contrast to other mycobacterial infections, no granulomatous inflammatory reaction is seen except in the healing stage (4). Furthermore, the typical growth of the bacteria at a restricted temperature of 30°C is compatible with \textit{M. ulcerans}, and the diagnosis was confirmed by PCR investigations. PCR analyses of the biopsies and the culture performed at different periods in time produced identical results. Using classical identification schemes the isolate was identified as \textit{M. ulcerans}, subspecies \textit{shinshuense} (10).

The discovery of \textit{M. ulcerans}, subspecies \textit{shinshuense} in a patient infected in the Shan Dong province in China confirms that the disease is not only found in tropical regions and temperate climatic areas of Australia, but occurs also above latitude 30° N. Until now, only one case has been reported above this latitude. In October 1979 an ulcer was seen on the left arm of a 19-year-old girl who lived in Japan (14). Acid-fast bacilli were isolated on two occasions from the lesion. Taxonomic study of these isolates demonstrated that they clustered together with \textit{M. ulcerans} but belonged to a distinct subgroup. The authors named their isolates \textit{M. ulcerans}, \textit{subspecies shinshuense} (15).

We consider "\textit{M. shinshuense}" as representing a subgroup within the species \textit{M. ulcerans} as do the African, Australian and American strains (10). We propose that \textit{M. shinshuense} should be considered an Asiatic variant of \textit{M. ulcerans}.

Buruli ulcer occurs mainly in patients aged <15 years; this might be explained by an absence of antimycobacterial immunity at this age, combined with frequent contact with the microbial source (6, 13). Also elderly people (aged >60 years) are more commonly affected, which could be owing to a diminishing immune response or perhaps reactivation of a latent infection (1).

As mentioned before, medical treatment of Buruli ulcer is usually disappointing, leaving wide surgical excision followed by skin grafting as the only alternative (2). Only pre-ulcerative nodular lesions and early small ulcerated lesions can be effectively treated by rifampicin alone (11). The Japanese patient described in 1982 presented with an ulcer with a maximum diameter of about 5 cm. The lesion healed after only two weeks of oral rifampicin. Failure of antibiotic regimens for advanced \textit{M. ulcerans} infection has been attributed to poor penetration of drugs into necrotic tissue (14). The rapid success of rifampicin therapy in the Japanese patient may be due to the absence of significant necrosis, therefore permitting the penetration of the drug into the lesion. Our patient was initially treated with a combination of rifampicin and clarithromycin as both drugs have been shown to have strong activity against \textit{M. ulcerans in vitro} (11). The favourable response in our patient suggests that this subtype may be more sensitive to
antimycobacterial drugs in vivo.

Because of the potentially disfiguring character of the disease an early diagnosis is important. First, people in the endemic areas must become familiar with the clinical signs, to minimize patients’ delay. Secondly, medical doctors should be aware of the fact that Buruli ulcer is a mycobacterial disease that does not occur in tropical regions only. Moreover, as this case shows, Buruli ulcer can also be seen as an imported skin disease in Western countries. Since adventurous journeys have become more popular, imported ‘tropical’ skin diseases are seen more frequently and doctors in Western countries should be more aware of imported skin diseases.

The multiplex PCR offers a reliable and rapid method of diagnosis enabling treatment to be started at an early stage when minimal damage has occurred (8).

In conclusion, we believe this to be the first reported case of *M. ulcerans*, subspecies *shinshuense* infection in the People’s Republic of China as no other report of Buruli ulcer has been received by WHO (WHO, personal communication). Our findings show that *M. ulcerans* infection also occurs above 30° latitude on the Northern Hemisphere and must be considered in the differential diagnosis of skin ulcers in those returning from travel in China.

References

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