9 Buruli ulcer

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9.1 INTRODUCTION

Buruli ulcer (BU), caused by *Mycobacterium ulcerans*, is an indolent necrotizing disease of the skin, subcutaneous tissue and bone. BU is the third most common mycobacterial disease of humans, after tuberculosis and leprosy, and the least understood of the three diseases.

In 1998 the World Health Organization recognized BU as an emerging health problem, primarily because of its frequent disabling and stigmatizing complications. The disease was named after the geographic area of the first large epidemic investigated in Uganda (in 1961), in a county named 'Buruli' (now called 'Kasongola'), near lake Kyoga.

BU is endemic in Africa, particularly in West African countries. The disease is also endemic outside Africa, but remains uncommon in non-African countries.

In sub-Saharan Africa more than 70% of all patients are children. Individuals 60 years or older are also at increased risk of BU.

The infectious agent, *M. ulcerans*, is an environmental mycobacterium that grows optimally at 30 to 32 °C on mycobacteriologic media such as Löwenstein-Jensen. *M. ulcerans* contains a large plasmid that encodes enzymes which produce a necrotizing toxin, mycolactone, in tissues (1). This toxin diffuses and destroys tissue well beyond the location of the colonies of mycobacteria.

There are genetic and virulence variations in isolates from different geographic origins, with African isolates being much more virulent than those from elsewhere (2).

9.2 EPIDEMIOLOGY

9.2.1 Geographic distribution, incidence and prevalence

BU is focally endemic in rural wetlands of tropical countries of Africa, America, Asia and Australia. A few cases have been reported in non-tropical areas of Australia, Japan and China. Seasonal variations have been reported in Uganda, Papua New Guinea, Cameroon and Australia (3).

Incidence rates vary greatly by continent, country, and in areas within a country. Known incidence rates currently are highest in West Africa, particularly in Benin, Côte d'Ivoire and Ghana. More than 20 000 cases were reported in West Africa during the last decade (4).

In Benin, comparison of detection rates of BU in the Zou region with those of leprosy and tuberculosis in 1999 showed a higher rate of BU (21.5/100 000) than leprosy (13.4/100 000) and tuberculosis (20.0/100 000) (5).

Little is known about the focal epidemiology of BU. Incidence, prevalence, and other data are usually reported at the national or district level. These data show the importance of the disease but do not reveal the wide variations that often exist at the village level within a given district. Prevalence and incidence of BU must therefore be determined at geopolitical divisions below district or national levels (6).

In several countries, over many decades, only a few cases of BU have been reported. In the Americas, the disease seems more common in French Guiana (however, still less than 200 cases over 35 years), than in Surinam, Mexico or Peru, where very few cases have been confirmed. This may be related to gross underreporting, spontaneous healing, environmental and socioeconomic factors, or widespread use of traditional medicine. In Peru some patients showed favorable responses to antituberculous therapy. Although their lesions were extensive, the ulcers were less severe than those often seen in African patients.

The small numbers of BU cases in these countries may be related to a low virulence of the strains of *M. ulcerans* and a more effective immune response of patients. Infrequent contact with contaminated water is clearly not the reason. As in Africa, the populations living in BU endemic areas (e.g. the Amazon basin) have frequent contact with water for domestic use. Similarly, the low incidence of BU in Peru does not seem to be related to the absence of *M. ulcerans* in the environment; IS*2404* PCR positivity of the environmental specimens collected in BU endemic foci in Peru (13.8% positivity) and in Benin (10 to 20% positivity) are comparable.

Prevalence of BU is low in Asia and Oceania. Since 1971, more than 400 cases have been detected in Papua New Guinea while in other Asian countries such as Malaysia, China and Japan, only a few cases have been confirmed. In Australia, the disease remains uncommon (4).

9.2.2 Reservoir(s)

The epidemiology of BU is strongly associated with wetlands, especially those with slow-flowing or stagnant water (ponds, backwaters and swamps).

In Benin, we found an inverse relationship between the prevalence of BU and distance from a river. Prevalence in one study increased gradually from 0.6 to 32.6/1000 as the distance from the river decreased from 10 to 1 km. Recently, aquatic

insects have been considered potential 'vectors' of *M. ulcerans* (6). These insects can fly many kilometers from their source. This may help explain how patients living some distance from their source of water become infected but not as often as those who live nearer. Considering domestic water sources, only villages near the river used water directly from this river, while other villages employed protected water sources for domestic purposes (boreholes, cisterns, or piped water from artesian wells). These results are similar in Uganda: families who used unprotected sources of water for domestic purposes, had higher prevalence rates of BU than those who used boreholes. Consequently, in addition to the probable influence of distance from the river on disease prevalence through potential carriers such as insects, the use of river water for domestic purposes may play a role in the elevated prevalence rates of the disease in some villages (7).

DNA of *M. ulcerans* has been identified by direct PCR in water and related detritus in Australia, and more recently in aquatic insects, aquatic snails and small fish from endemic areas in Africa (3). Primers targeting the repetitive sequence IS*2404* were used to detect *M. ulcerans* DNA in the environment. This target was considered specific for *M. ulcerans* until recently when several studies reported that mycobacteria other than *M. ulcerans* could harbor the IS*2404* sequence. However, search for environmental *M. ulcerans* DNA in Benin detected variations in positivity rates of aquatic insects (*Hemiptera*) over time, and these changes were reflected in corresponding alterations of frequency of BU patients in the same foci (5). This spatiotemporal relationship between the IS*2404* positivity rates of environmental specimens and the coincident rates of the disease in humans, supports the hypothesis that the IS*2404* positive environmental specimens were positive for *M. ulcerans*.

There is now sufficient evidence from microbiological and epidemiological data (including studies of risk factors) to consider BU as a water-related disease. Aquatic insects, other aquatic invertebrates and fish could be reservoirs or intermediate hosts of *M. ulcerans*.

Despite numerous attempts, *M. ulcerans* had never been cultivated from environmental sources (8) until recently when two pure cultures were obtained form aquatic insects from Benin and from Côte d'Ivoire (6).

9.2.3 Mode(s) of transmission

The exact mode(s) of transmission from the environment and the ultimate natural source(s) of infection remain obscure, and must remain active areas of inquiry. Since the initial discovery of *M. ulcerans* DNA in water bugs in Benin, insects have been suspected as reservoirs for transmission (3,6).

Passage of *M. ulcerans* to mice by the bite of infected water bugs has been reported, suggesting that these insects may play a role in natural transmission (6).

Hemiptera should, however, be considered as 'passive reservoirs' or possible 'vectors'.

Studies on more than 1000 bacteriologically confirmed cases of BU in Benin indicate that trauma is the most frequent means by which *M. ulcerans* is introduced into the human body from the contaminated surface of the skin. Contamination of the skin may result from direct exposure to stagnant water, insect bite, aerosols, or fomites.

Research studies on reservoir(s) and mode(s) of transmission of *M. ulcerans* are urgently needed, especially to develop measures to protect individuals at risk of *M. ulcerans* infection.

9.3 CLINICAL PICTURE

9.3.1 Infection vs disease

Contact with environmental mycobacteria may result in colonization or primary infection of humans or animals. Whether or not this causes clinical disease depends largely on host defenses. It is likely that most individuals exposed to *M. ulcerans* clear the infection and never develop BU disease. Pathogenic mycobacteria including strict pathogens such as *M. tuberculosis* and *M. leprae* or opportunistic pathogens such as environmental mycobacteria, are all capable of establishing persistent infections.

As in tuberculosis, exposure of cutaneous tissues to *M. ulcerans* may lead to early clearance of the infection, development of disease soon after infection (primary BU), or of a subclinical or asymptomatic infection (latent BU) that may subsequently reactivate and produce BU disease.

Mean incubation periods of primary BU are estimated to be 2-3 months. Delayed onset of disease after leaving an endemic area has been seen and probably represents reactivation of latent infection. For example, we have observed individuals who originally resided in a BU endemic area and developed BU at a body site where trauma occurred several years after leaving the endemic area.

Occasionally, the incubation period may be very short (< 15 days). Some patients with such short incubation periods developed the disease after cutaneous trauma without superficial damage to the skin (e.g. bruise or sprain). These cases also suggest reactivation of latent *M. ulcerans* infection by local trauma.

M. ulcerans disease presents in a spectrum of forms related partly to patient delay in admission to hospital. After infection, the disease may become localized by developing a nodule or papule which eventually ulcerates, or may disseminate directly, bypassing the nodular stage. The non-ulcerated form is thus the first stage of the disease in the nodular oedematous, or plaque form. Early stages of the disease are often ignored by the patients, and some may heal spontaneously. After variable periods

(a few weeks to several months), these forms ulcerate. Thus, unlike the term 'Buruli ulcer' suggests, the disease does not always present as an ulcer.

9.3.2 The disease

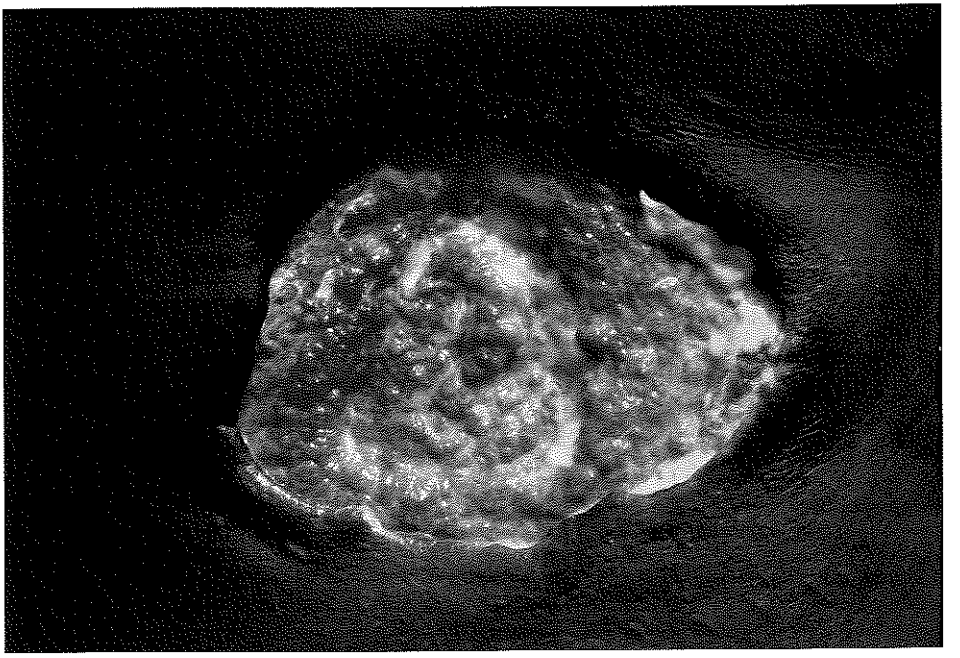
Clinically, BU disease may be divided into stages: stages 1 and 2 are active forms, and stage 3 is the healed or scarred lesion. In addition there are mixed forms that appear as combinations of any of the 3 stages at diagnosis.

Non-ulcerated lesions (stage 1)

There are 4 forms characterized as follows:

1. *Nodule*: subcutaneous, firm, palpable, painless or only slightly painful, attached to the skin but not to the deep tissue, up to 3 cm in diameter. The lesion gradually increases in size and is sometimes surrounded by an edematous indurated zone.
2. *Edematous lesion*: a more diffuse, firm, non-pitting swelling, with ill-defined edges, painless or mildly painful and not perceptibly inflamed.
3. *Plaque*: indurated, raised, more or less clearly demarcated, dry, painless and covered with discoloured papery skin.

Figure 9.1 Buruli ulcer puncture wound on abdomen

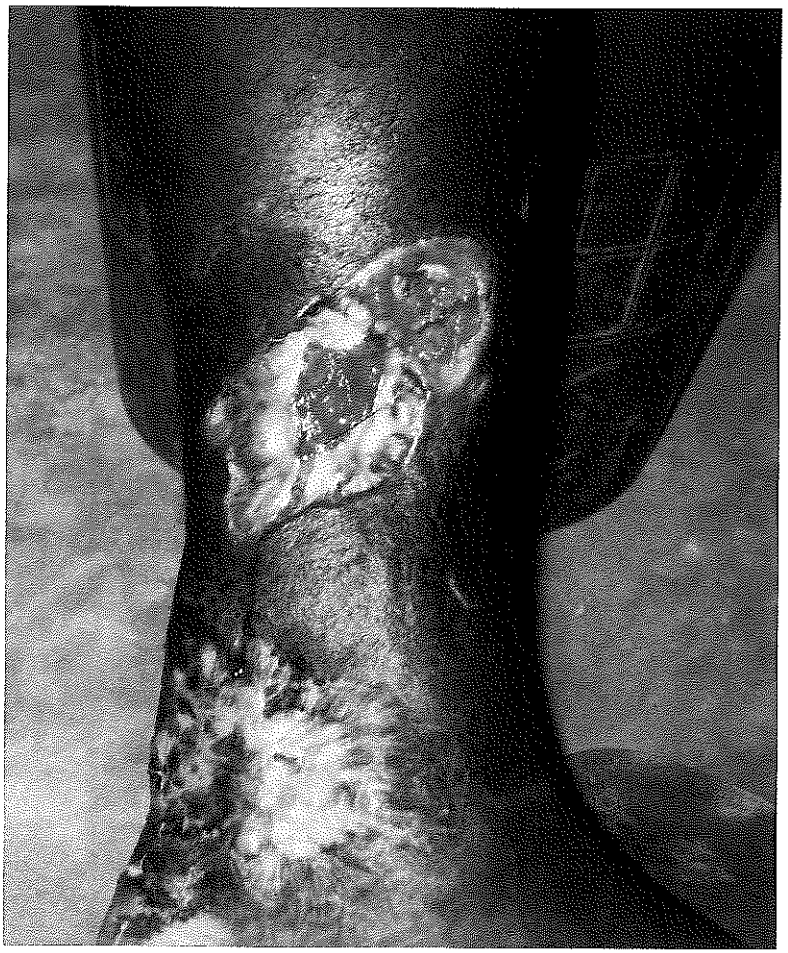


4 *Papule* (most often observed in Australia): raised skin lesion, less than 1 cm in diameter with erythematous surrounding skin.

Ulcerated lesions (stage 2)

More or less extensive single or multiple ulceration, painless or only slightly painful, undermined, with a centre lined with yellowish-white necrotic slough and devitalized edges. The edges are sometimes hyperpigmented. No satellite adenopathies. These lesions are chronic and rarely heal spontaneously. Figure 9.1 shows a typical ulcer with undermined edges.

Figure 9.2 Scarring lesion alongside an ulcerative lesion at the same site (left leg)



Scar lesions (stage 3)

Atrophic scar, generally following stages 1 or 2. When this develops over a joint, it may lead to severely disabling sequelae. As a result of the adhesion and shrinking of periarticular scars, the range of joint movement is reduced and joints may ankylose and become totally immobile.

Mixed forms

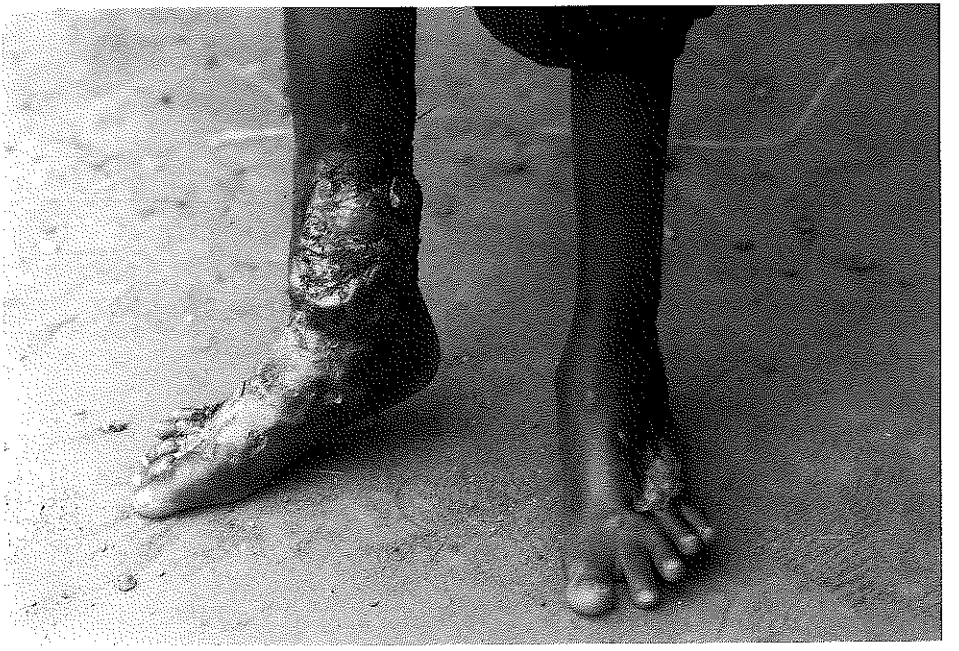
Some patients present mixed forms: the simultaneous presence of different forms in the same patient, at the same or different sites. When the lesions are on different parts of the body they usually represent disseminated forms.

Figure 9.2 shows a mixed form with scarring lesion (inactive) alongside an ulcerative lesion (active) at the same site (left leg).

Disseminated forms

Disseminated forms are characterized by the presence of clinical forms which may or may not be similarly situated at different places on the body. The concept of dissemination denotes spreading of the disease to other parts of the body by contiguous spread or septic metastasis. It is therefore important to examine the patient all over, looking for new or old lesions since the patient may not always be aware of scars of

Figure 9.3 Disseminate form: both legs are affected



healed infections, for example. Figure 9.3 shows a disseminated lesion: there is an ulcer on the right leg which followed the scarring lesion on the left foot.

Osseous forms

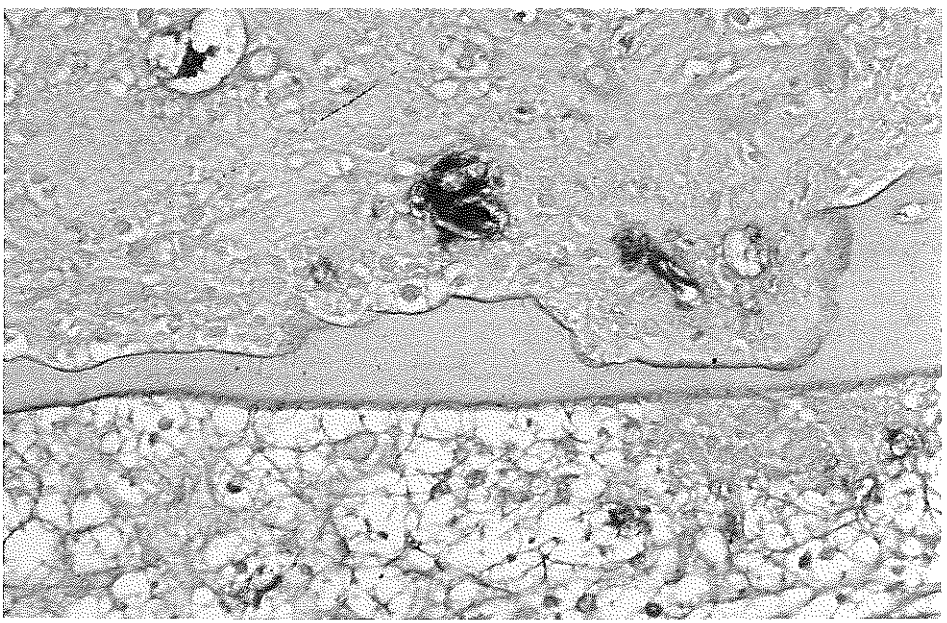
The bacterium infects the bone by 2 different routes:

1 *by contiguity*: *M. ulcerans* reaches the periosteal tissue and bone directly from a BU lesion in the overlying skin and subcutaneous tissue;
2 *by septicemic metastasis*: in this case, infection of soft tissues surrounding the bone is secondary to infection arising within the bone; this occurs at a distance from the initial skin lesion which may already have formed a scar, and of which the patient may be unaware.

Figure 9.4 shows an osteomyelitis of a tibia with eroded bone trabecula and AFB, present in varying numbers in the necrotic bone marrow.

A diagnosis of osteomyelitis should be suspected in the following conditions: presence of edema, slight discomfort early but subsequently painful, with functional disability. Diagnosis is best made by radiological examination.

Figure 9.4 Osteomyelitis of tibia showing eroded bone trabecula and varying numbers of AFB in the necrotic bone marrow (ZN stain x 100)



If possible these patients should be referred without delay to the nearest recognized treatment centre for appropriate management. Delay in referral increases the risk for serious consequences such as amputation of limbs.

Frequency of the different clinical forms

The frequency of the different forms varies according to geographic areas, early detection rates and the delay between onset of disease and admission to a BU treatment center. A recent study in a rural hospital of southern Benin showed that the percentages of ulcerated and non-ulcerated forms were each approximately 50% (5). Delayed admission to the hospital results in an increased frequency of ulcerated forms compared to non-ulcerated forms.

9.4 DIAGNOSTIC PROCEDURES

The experienced health worker in endemic areas usually can make an accurate clinical diagnosis.

Clinical criteria for suspecting BU include:

1 presence of a chronically developing lesion (several weeks or months), i.e. a 'wound that will not heal';
2 no fever or regional lymphadenopathy;
3 typical nodular, indurated plaque or edematous lesion;
4 one or more painless chronic ulcers with undermined edges or a depressed scar (there may be pain, fever and even swollen lymph nodes when there is superinfection of the lesion by other bacteria);
5 swelling over a painful joint, suggesting bone involvement;
6 patient age < 15 years;
7 patient living or traveling in an endemic zone.

Microbiologic confirmation of BU is essential for several reasons. Among these reasons are:

1 determination of the true prevalence and incidence of BU in a given area;
2 confirmation of new foci;
3 appropriate management of the disease (by surgery and/or by drug therapy). This is becoming particularly important now that an increasing number of health professionals are using or planning to use antimycobacterial drugs to treat BU.

The clinical diagnosis of BU is usually easy when a child from a known endemic area presents with a typical painless ulcer characterized by undermined edges. Diagnosis should even be easier when health professionals dealing with BU are highly experienced and skilled.

It has now been demonstrated that in regions where health professionals are not highly experienced, few clinically diagnosed or suspected cases are confirmed by laboratory tests. In a study performed in Ghana, where health professionals were trained to recognize and treat early BU lesions, the accuracy of the clinical diagnosis of nodules in these 'experienced' hands, was 48% to 52%.

In another study performed in Côte d'Ivoire, also by 'experienced' health professionals, the accuracy of clinical diagnosis of early lesions was 30%. Among the 70% of misdiagnosed BU, histopathologic analyses performed at the Armed Forces Institute of Pathology, Washington DC, confirmed various types of cutaneous cysts (e.g. epidermoid cysts), Kaposi sarcoma, onchocerciasis, nonspecific chronic inflammation, calcinosis cutis, staphylococcal abscess, phaeohyphomycosis, dermatophytosis, leiomyoma (benign smooth muscle tumor) and even tuberculous lymphadenitis. This list is presented only to indicate that the clinical differential diagnosis of nodular forms of BU can be difficult, even for 'experienced' health professionals. Consequently, the number of declared BU cases may be overestimated, and management of patients with diseases other than BU can be totally inadequate.

Four laboratory tests are currently available to confirm the clinical diagnosis of BU:

1 direct smear examination for acid-fast bacilli (AFB) i.e. Ziehl-Neelsen (ZN) and auramine stain;
2 in vitro culture;
3 IS*2404* PCR;
4 histopathological examination.

These tests are described in detail in a freely available WHO manual (9). In endemic areas, the most often available diagnostic technique is the direct smear examination. Culture and histopathology are not widely available in regions were BU is endemic, and PCR can only be performed in well-equipped reference laboratories highly experienced in molecular techniques.

WHO recommends that at least two different laboratory tests be positive to consider a patient as a confirmed BU case.

Two positive tests are preferable to avoid misdiagnosis due to false positive or false negative results and should be required for confirmation of new BU foci, and when health professionals are not highly experienced in the clinical diagnosis of BU. When health professionals are highly experienced, however, one positive test may be sufficient to confirm the diagnosis of BU. Given the heterogeneous distribution of the mycobacteria in the lesions, we also advise that two tissue fragments from excised tissues or two punch biopsy specimens be studied to confirm the clinical diagnosis of BU. As with all laboratory tests, the positivity of the tests depends on the quality of the samples and their prompt delivery to the laboratory.

Laboratory analysis of > 1000 BU proven cases using a strict definition of having at least two different laboratory tests positive, gave a sensitivity of 60 to 80% for ZN staining for AFB, 20 to 80% for culture and > 90% for PCR and histopathology. Indeed, the positivity of the laboratory tests may vary according to the clinical forms. Direct smear examination and culture are less frequently positive for nodules (60% positivity) than for edematous forms (80% positivity). *M. ulcerans* is particularly difficult to cultivate from bone (only 20% positivity).

There is no difference in the positivity of PCR and histopathology results between the different clinical forms (> 90% positivity).

Mycobacteria other than *M. ulcerans* could harbor the IS*2404* sequence, but it does not interfere with the diagnosis of BU by PCR as there is no evidence that these *M. ulcerans*-like mycobacteria cause BU in humans.

9.5 TREATMENT

Currently, excisional surgery with or without skin grafting remains the recommended therapy of BU. In 2001 WHO published a manual for the surgical management of the different clinical forms of BU (10).

Drug treatment has usually been considered ineffective, although there have been anecdotal accounts of successful antibiotic therapy of early lesions. A number of prospective therapeutic drug trials are presently underway and some are promising.

Provisional guidelines proposed by WHO recommend the use of rifampicin plus streptomycin combined or not with surgery for the treatment of BU (11).

Several centers in Africa have started to treat patients with antibiotics according to WHO guidelines (11) and some studies seem to indicate that following drug therapy for 8 weeks ulcers may heal without surgery. Recurrence rates within the year following the end of treatment were less than 3%. These encouraging results, however, need to be confirmed on a larger number of patients with different clinical forms of BU, and recurrence rates should be evaluated after a follow-up of more than one year.

In a rural hospital of Benin, antimycobacterial therapy was administered as an adjunct to surgery for one week before surgery and continued after surgery for a total of 8 weeks. This study did not show any benefit compared to surgery alone. Antimycobacterial treatment of patients with osteomyelitis did not prevent dissemination to other bones after surgery. Despite some reported encouraging results, additional studies are required to determine the role of antimycobacterial drugs and their optimal use in the management of all clinical forms of BU.

The following aspects of treatment should be further investigated:

1 the duration of antibiotic treatment before and/or after surgery;

2 the possible use of an all-oral regimen;
3 the effect of antimycobacterial therapy without surgery on the regression and healing of lesions;
4 the effect of antimycobacterial therapy before surgery on the extent of surgical excision (when surgery is required);
5 the effect of antimycobacterial therapy on recurrence rates.

9.6 PREVENTION

In tropical rural settings where BU is endemic and scantily dressed people play and work, avoiding contact with the *M. ulcerans* contaminated environment is virtually impossible. Wearing protective clothing when farming and immediate cleansing of any skin injury may reduce rates of infection, but achieving these measures are seldom feasible.

The use of protected sources of water for domestic purposes reduces exposure to *M. ulcerans* contaminated sources and consequently may reduce prevalence rates of BU (7). Strategies that include drilling wells and supplying pumps, should be developed to provide protected water sources to villages at risk of BU. The problem of reducing risk factors for basic agricultural workers, fishermen and others who must put themselves at risk, remains, however, a serious concern. Appropriate educational programs for behavioral changes, although difficult to implement, should be included in all BU control strategies.

Vaccination programs remain the only viable prevention alternative. Short-term protective effect of BCG has been demonstrated in Uganda, and neonatal vaccination with BCG seemed to reduce the rate of osteomyelitis in BU patients. However, in two recent case-controlled studies performed in Benin, there was no evidence of a protective effect of BCG vaccination against BU (DeBacker et al. and Nackers et al., to be published).

Serious consideration of vaccination rationales should remain a topic of utmost concern in all BU control efforts.

9.7 CONCLUSION

Since BU was declared an emerging disease in 1998, much effort has been invested in research. Some aspects, however, remain unclear and thus require much more investigation, including reservoir(s) and mode(s) of transmission, risk factors, optimal management and preventive tools. BU remains inadequately recognized or understood by health professionals, both within and outside of endemic countries. Thus, research interests and funding are negatively influenced. Moreover, BU is not included in the health statistics of the majority of affected countries. Better strategies for early diagnosis and effective therapy compatible with the socioeconomic structures of BU endemic areas, should be developed. A multidisciplinary approach and

productive cooperation between scientists and health professionals remain indispensable for the improvement of BU control worldwide.

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