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REVIEW

Buruli ulcer (*Mycobacterium ulcerans* infection)

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Review

Summary *Mycobacterium ulcerans* is an emerging infection that causes indolent, necrotizing skin lesions known as Buruli ulcer (BU). Bone lesions may include reactive osteitis or osteomyelitis beneath skin lesions, or metastatic osteomyelitis from lymphohematogenous spread of *M. ulcerans*. Pathogenesis is related to a necrotizing and immunosuppressive toxin produced by *M. ulcerans*, called mycolactone. The incidence of BU is highest in children up to 15 years old, and is a major public health problem in endemic countries due to disabling scarring and destruction of bone. Most patients live in West Africa, but the disease has been confirmed in at least 30 countries. Treatment options for BU are antibiotics and surgery. BCG vaccination provides short-term protection against *M. ulcerans* infection and prevents osteomyelitis. HIV infection may increase risk for BU, and renders BU highly aggressive. Unlike leprosy and tuberculosis, BU is related to environmental factors and is thus considered non-communicable. The most plausible mode of transmission is by skin trauma at sites contaminated by *M. ulcerans*. The reemergence of BU around 1980 may be attributable to environmental factors such as deforestation, artificial topographic alterations and increased manual agriculture of wetlands. The first cultivation of *M. ulcerans* from nature was reported in 2008.
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1. Introduction

Mycobacterium ulcerans causes an indolent, necrotizing cutaneous lesion known as Buruli ulcer (BU). BU was a name first used by Dodge and Lunn, referencing the geographic location of their observations in Buruli County, Uganda (now called Nakasongola District) (Dodge and Lunn, 1962). Dodge then reported the first large epidemic of BU (Dodge, 1964). Today, BU is recognized as a spectrum of clinical disease that includes nodules, plaques, edemas, characteristic skin ulcers (Figure 1), sometimes massive, and osteomyelitis. BU, after tuberculosis and leprosy, is the third most common and perhaps least understood major mycobacterial infection. In contrast to tuberculosis and leprosy, BU is related to environmental factors, and thus considered non-communicable (Wansbrough-Jones and Phillips, 2006).

In 1998, WHO recognized BU as a re-emerging infectious disease in West and Central Africa with an important public health impact (WHO, 2008). Today, BU is one of 13 'neglected' tropical diseases. In endemic countries, BU is considered a major public health and psychosocial

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Figure 1 Major Buruli ulcer in the deltoid area of a 12-year-old Angolan boy. This pristine lesion developed 3 months after an anti-cholera immunization at this site. Note undermining of symmetrical borders, necrotic base and induration with scaling of adjacent skin.

problem because of potential disabling sequelae, estimated to occur in at least 60% of patients (Aujoulat et al., 2003). The stigma of deformities and socio-economic burdens of the disease are marked (Asiedu and Etuaful, 1998). This is largely because BU in highly endemic countries most often afflicts children up to 15 years old. In some West African countries, the number of BU patients exceeds those with leprosy or tuberculosis (Debacker et al., 2004a).

2. Etiologic agent

In 1948, MacCallum in Australia was the first to isolate the etiologic agent of BU in culture from patients (MacCallum et al., 1948). *Mycobacterium ulcerans* has an optimal growth temperature of 30—32 °C on routine mycobacteriologic media, such as Löwenstein—Jensen, and is strikingly sensitive to temperatures of 37 °C or higher (Meyers et al., 1974a). The organism is a slow grower, often requiring several months of incubation to achieve isolation in primary culture. Microaerophilic conditions promote the growth of *M. ulcerans*. By the Ziehl—Neelsen stain, *M. ulcerans* is strongly acid-fast, similar to *M. tuberculosis*.

With the development of molecular biologic techniques for the identification of *M. ulcerans*, the organism was first detected in the environment in Australia and West Africa using PCR (Duker et al., 2006; Portaels et al., 1999; Ross et al., 1997; Stinear et al., 2000). After many attempts to cultivate the organism from the environment by many investigators over half a century, the first isolation of *M. ulcerans* from nature was reported in 2008 (Portaels et al., 2008). The isolate was from an aquatic insect called a water strider, collected from a BU-endemic area of West Africa.

In contrast to *M. leprae* and *M. tuberculosis*, *M. ulcerans* produces a necrotizing and immunosuppressive polyketide toxin called mycolactone (George et al., 1999; Pimsler et al., 1988; Read et al., 1974). Genomic sequences of the plasmid-encoded synthases that produce mycolactone have been described (Stinear et al., 2004).

The phenolic mycosides of *M. ulcerans* and *M. marinum* are identical, and sequences for the 16S rRNA gene differ by only one base pair (Ablordey et al., 2005; Daffe et al., 1992). Specific insertion sequences for *M. ulcerans* have been characterized and serve to identify the organism by PCR (Ablordey et al., 2005; Siegmund et al., 2005; Stinear et al., 1999). Variations in the 3′ end of the 16S rRNA gene are related to geographic origin, dividing the organism into African, American, Asian and Australian strains (Portaels et al., 1996).

3. Epidemiology

BU has been reported from at least 30 countries, principally tropical and subtropical regions (WHO, 2008). Endemic foci of BU are most common near rural permanent wetlands in warm geographic regions, especially in areas prone to seasonal flooding. Seasonal changes in climate may affect incidence in some foci (Revill and Barker, 1972). Focal prevalence within countries varies greatly, and must be assessed at the community level of geopolitical subdivisions. A rapid reemergence of BU beginning in the early 1980s is thought to be attributable to environmental factors such as deforestation, artificial topographic alterations (dams and irrigation systems), enlarging populations engaged in basic manual agriculture in wetlands, and possibly global climatic changes (Meyers et al., 1996).

It is estimated that more than 7000 people develop BU annually, with the highest incidence rates in the West African countries of Benin, Côte d'Ivoire and Ghana (WHO, 2008). Other African countries in which the disease has been reported include Angola, Burkina Faso, Cameroon, Congo, Democratic Republic of Congo, Equatorial Guinea, Gabon, Guinea, Liberia, Nigeria, Sudan, Togo and Uganda (Meyers et al., 1996; Phanzu et al., 2006).

In Asia, BU occurs most often in Papua New Guinea, with sporadic cases in China, Indonesia, Japan and Malaysia (Faber et al., 2000; Nakanaga et al., 2007). In Australia, the southern regions are endemic (Veitch et al., 1997). In the Americas, French Guyana is a known focus of BU, with sporadic cases in Peru, Suriname, Mexico and, most recently, Brazil (Coloma et al., 2005; Guerra et al., 2008; WHO, 2008). Travelers to endemic areas who develop BU occasionally present to European, American and Canadian clinics (Evans et al., 2003).

Individuals of all ages are affected, but children 15 years of age or younger constitute about 75% of all cases (Debacker et al., 2004b; Marston et al., 1995; WHO, 2008). Approximately 80% of lesions are located on the limbs, most commonly on the lower extremities. The genders are affected equally, and no ethnic predilection has been reported (Debacker et al., 2004b). Anecdotal observations of BU in children in families of multiple parentage have suggested a possible genetic predisposition, supported by molecular studies (Stienstra et al., 2006).

Some authorities believe that *M. ulcerans* is a saprophytic or commensal organism that thrives in the mud, flora or fauna of the cool microaerophilic environment of the bottom of stagnant water, well protected from the lethal ultravio-

let radiation that prevails in the tropics. Some speculate that *M. ulcerans* may colonize biofilms in certain ecological niches (Marsollier et al., 2007). Although the ultimate source of *M. ulcerans* remains obscure, the organism has been found in aquatic insects such as water bugs, firefly larvae and beetles obtained from stagnant water in endemic areas of West Africa (Marsollier et al., 2002; Portaels et al., 1999, 2001). In some cases, the organisms were found only in the salivary glands of aquatic insects, supporting the notion that insect bites may play a role in transmitting *M. ulcerans* (Marsollier et al., 2005).

In Australia, *M. ulcerans* DNA was found in mosquitoes captured during an outbreak of BU (Johnson et al., 2007). Koalas and possums in Australia acquire BU in the wild (Portaels et al., 2001), and investigations into BU as a zoonosis transmitted to humans from possums, via mosquitoes, is underway.

As *M. ulcerans* is an environmental organism, BU is rarely, if ever, contagious. The distribution of patients, even in highly endemic foci, is random, suggesting exposure to an environmental source. Studies in African populations suggest that some risk factors include exposure to swamp water, swimming or wading in rivers, use of unprotected water sources for domestic purposes, low levels of schooling and HIV infection (Johnson et al., 2008; Nackers et al., 2007; Pouillot et al., 2007).

Modes of transmission to humans have not been delineated completely. The most plausible route is by trauma at sites of skin recently contaminated by *M. ulcerans* (Duker et al., 2006; Meyers et al., 1974b). Many patients give a history of antecedent penetrating trauma at the site of the initial lesion, which may include wounds from a gunshot or land mine, thrown stones, human bite or hypodermic injection (as in Figure 1) (Debacker et al., 2003; Meyers et al., 1974b). The organism may be spread by aerosol from the surface of ponds or be carried by fomites or insects to skin surfaces. Insects may introduce *M. ulcerans* into the skin, but this means of transmission has not been confirmed in humans (Pouillot et al., 2007).

4. Pathogenesis and immunity

Initial infection is primarily related to two properties of *M. ulcerans*: optimal growth at 30—33 °C; and elaboration of the toxin, mycolactone. The temperature requirement of *M. ulcerans* favors development of lesions in the skin and subcutaneous tissue, and mycolactone destroys tissue and suppresses host immune responses. The spectrum of clinical signs and histopathological features suggests that some patients have strong innate or acquired immunity, others acquire immunity at various rates, and some never develop an effective resistance (Meyers, 1995).

The immune response to *M. ulcerans* infection is an important area of study that may contribute to the development of a vaccine. Skin tests with 'burulin', a sonicate of *M. ulcerans*, show that non-infected individuals or those with early infections do not have delayed-type hypersensitivity (DTH) to *M. ulcerans*, but develop a positive DTH response as healing begins. Mycolactone profoundly suppresses monocytes, B cells, and T cells, partly by inhibiting production of IL-2, TNF-α and other cytokines (Adusumilli et al., 2005; Coutanceau et al., 2005; Pahlevan et al., 1999). Similar to leprosy, nodular (granulomatous, often non-progressive) lesions contain abundant IFN-γ, whereas ulcerated (progressive) lesions contain abundant IL-10 and have higher *M. ulcerans* loads (Kiszewski et al., 2006). As minor BU (small nodules or ulcers) may self-heal early, study is warranted because these lesions may demonstrate fundamental elements of high host resistance.

Treatment with antibiotics (rifampin and streptomycin) kills *M. ulcerans*, reversing immunosuppression in tissues. This is associated with the development of organized lymphoid aggregates and initiation of healing (Schutte et al., 2007). In active lesions containing viable *M. ulcerans*, significant apoptosis occurs, probably an important element in tissue destruction (Walsh et al., 2005).

Progress has been made in understanding the natural history and pathogenesis of *M. ulcerans* infection. After inoculation of *M. ulcerans* deep into the skin or subcutaneous tissue, there is a latent phase during which the mycobacterium proliferates slowly, possibly intracellularly at first, and begins to elaborate small amounts of mycolactone causing necrosis, especially of fatty tissue. The necrosis provides a micro-aerophilic environment and perhaps nutrients favorable for the accelerated growth of *M. ulcerans* and elaboration of increased amounts of mycolactone. During this necrotic phase, immunosuppression inhibits a significant cellular response. Then, in some patients, a subcutaneous nodule begins to develop with clusters of *M. ulcerans* in the center, surrounded by a zone of necrosis (Figure 2). In highly resistant patients, this lesion may self-heal, perhaps without ulceration, or it may form a small, sharply defined ulcer. In others, the skin is undermined by the necrosis and eventually breaks down into larger ulcers with widely undermined skin. In the least resistant individuals, a nodule never develops and the necrosis spreads rapidly and widely to cover large body surface areas, but ulceration, if it takes place at all, is a late event. Eventually, the necrotic stage ceases in most patients, from reduced immunosuppression and reduction of viable *M. ulcerans*. At this time, a granulomatous stage begins to develop, followed by healing and scarring. Some of the variability in clinical presentation and

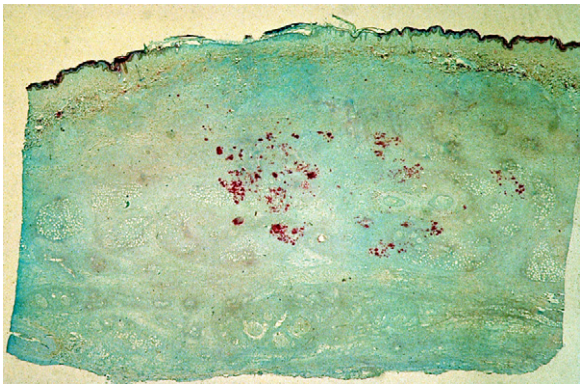

Figure 2 Microscopic section of early nodule of Buruli ulcer, showing clumps of extracellular acid-fast bacilli (AFB; red color) in the center of widespread necrosis. Necrosis extends far beyond the AFB (Ziehl—Neelsen stain, ×1000). These features prompted the idea that *Mycobacterium ulcerans* elaborates a diffusible necrotizing toxin.

progression of the disease may also be related to heterogeneity in the *M. ulcerans* genome, as well as in the plasmid that encodes the production of mycolactone (Ablordey et al., 2005; Mve-Obiang et al., 2003; Stragier et al., 2006).

Microscopically, an active ulcer shows extensive coagulation necrosis of the subcutaneous tissue down to, and often including, the fascia (Figure 3) (Guarner et al., 2003; Meyers, 1995). The deep layers of necrosis often contain masses of extracellular acid-fast bacilli (AFB) (Figure 2), frequently with mineralization and extensive vasculitis. Some believe the ischemia engendered by the vasculitis is partially responsible for the necrosis. Marked edema is present, and fat cells are enlarged and dead, leaving only their cellular ghost outlines. The dermis and surrounding tissue seldom contain AFB; however, a few intracellular AFB are sometimes seen in inflammatory exudates at the periphery of necrotic areas (Torrado et al., 2007).

BU skin lesions sometimes provoke a reactive (contiguous) osteitis that leads to necrosis of cortical bone and osteomyelitis. Metastatic lesions may develop in skin and bone from vascular or lymphatic spread of *M. ulcerans*, producing isolated or multifocal osteomyelitis distant to the original skin lesion. In mice, regional lymph nodes, especially in the capsule, may show massive necrosis and contain large numbers of AFB (Addo et al., 2005). Visceral organs are not known to be involved, but necropsy findings of patients who died with disseminated *M. ulcerans* disease have not been reported.

Experimental animal models that replicate the spectrum of features of BU found in human disease have been difficult to identify. Laboratory rats and mice develop skin lesions after intradermal inoculation with *M. ulcerans*, but lack extensive ulceration. In the mouse footpad, *M. ulcerans* multiplies but necrosis without ulceration destroys the limb and causes death (Read et al., 1974). Guinea pigs develop inflammatory lesions at the inoculation sites that may resolve without ulceration (Krieg et al., 1974; Read et al., 1974). Nine-banded armadillos develop cutaneous lesions after intradermal inoculation with *M. ulcerans* approximating those of human disease, but are phylogenetically distant from humans (Walsh et al., 1999). The cynomolgus monkey, a useful model for tuberculosis (Walsh et al., 1996), develops some of the clinical and histological features of human BU after intradermal inoculation of *M. ulcerans* (Walsh et al., 2007).

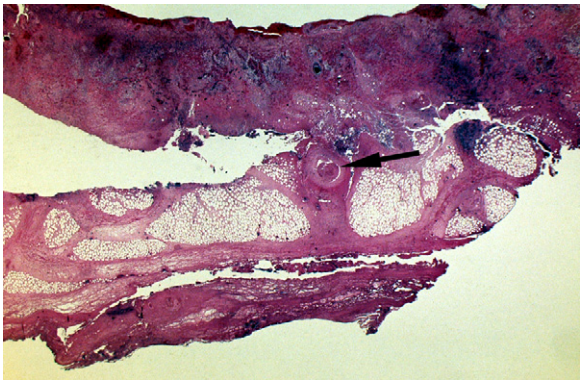

Figure 3 Microscopic section of the undermined edge of a major Buruli ulcer. Note contiguous coagulation necrosis of the panniculus and fascia, and vasculitis with thrombosis of a medium-sized vessel (arrow) (hematoxylin—eosin stain, ×40).

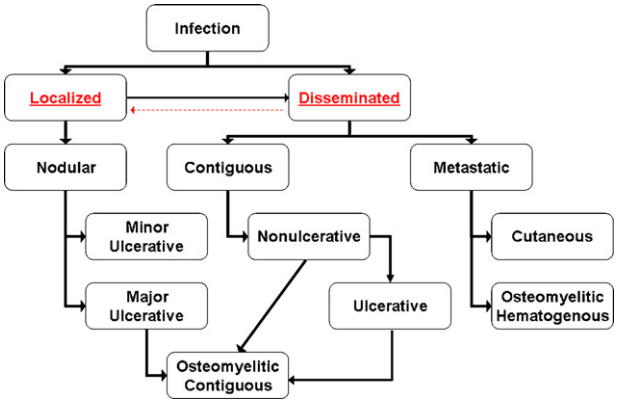

Figure 4 A proposed classification of clinical forms of active *Mycobacterium ulcerans* disease, and possible pathways of development if the disease is not treated.

5. Clinical features

Table 1 lists the spectrum of skin lesion morphologies of BU, according to WHO designations (WHO, 2004). Figure 4 presents a proposed classification of most of the clinical forms of BU and, without treatment, possible pathways of development as the disease progresses. In a study of trauma-related lesions, the incubation period ranged from 2 weeks to 3 years following trauma, with a mean of 3 months (Meyers et al., 1974b). Notably, most lesions are painless, which may be related to mycolactone (En et al., 2008).

Clinical differential diagnoses of ulcerative BU can include stasis, diabetic or tropical phagedenic ulcers, leishmaniasis, noma, deep fungal or atypical mycobacteria infections, other bacterial infections (ecthyma, yaws, anthrax, tularemia, cutaneous diphtheria), arthropod bites, pyoderma gangrenosum and autoimmune disease.

Beneath BU lesions that have destroyed overlying skin and soft tissue, reactive osteitis or contiguous osteomyelitis occasionally develops. Bone subjacent to a BU lesion may become devitalized and necrotic, with the development of sequestra (Figure 5).

Mycobacterium ulcerans-specific metastatic osteomyelitis develops in approximately 10% of all patients, most likely from lymphohematogenous spread of *M. ulcerans* from a skin lesion (Wansbrough-Jones and Phillips, 2006). Thus, BU should always be considered a potentially systemic disease. The skin overlying metastatic osteomyelitis is usually intact, but develops swelling and inflammation. Bone scans, if available, are helpful in diagnosis (Pszolla et al., 2003). If untreated, a draining fistula may develop. Contiguous and metastatic osteomyelitis often results in deformity and amputation.

6. Diagnosis

To an experienced observer in an endemic area, an accurate clinical diagnosis of BU often can be made, especially for ulcerative forms. For laboratory diagnosis, four methods currently in use include direct smear, culture, PCR and

Table 1 The spectrum of cutaneous lesions of *Mycobacterium ulcerans* infection, according to WHO designations

Lesion morphology	Major characteristics
Non-ulcerative	
Papule	- an initial lesion, most common in Australia - elevated, up to 1 cm, ulcerates early (sometimes called Bairnsdale ulcer) - painless
Nodule	- initial stage in most African patients - subcutaneous, firm, 1—2 cm, overlying skin may be discolored - painless, often pruritic
Plaque	- may or may not arise from a nodule - firm, elevated, well-defined, >2 cm, irregular borders, overlying skin discolored (Figure 5) - may ulcerate late, producing stellate pattern (Figure 6) - painless
Edematous form	- often no nodule, spread directly from initial nidus of infection - rapid spread, may cover entire limb or large portion of face or trunk (Figure 7) - diffuse, non-pitting swelling, vague margins, firm, color changes, scaling - sometimes fever - painless
Ulcerative[a]	- 'pristine' ulcer, symmetric, undermined edges, induration (Figure 1) - ulcer base shows whitish necrotic slough, sometimes eschars develop, oily exudate frequent - old ulcers begin healing superficially, activity continues in dependent portion - painless, unless secondary bacterial infection
Minor	- ≤2 cm, sharply delineated - self-heals early
Major	- >2 cm - chronic, self-heals late

[a] Both minor and major ulcerative forms generally begin as subcutaneous nodules and tend to self-heal.

histopathology. In the ulcerative forms, a Ziehl—Neelsen stain of exudate from the undermined edge obtained with a cotton swab will reveal clusters of extracellular AFB. The same material, obtained by swab after decontamination, may be used for culture on Löwenstein—Jensen or other suitable mycobacterial media. The incubation temperature must be 30—32 °C. If culture cannot be performed locally, transport media may be inoculated with material from the cotton swab and maintained at 4 °C while in transport to a specialized laboratory. The transport medium is composed of Middlebrook 7H9 broth supplemented with polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin and 0.5% agar (Eddyani et al., 2008). PCR for the identification of *M. ulcerans* is available, convenient and growing in popularity (Ablordey et al., 2005; Stinear et al., 1999). Tissue for histopathological analysis should be obtained from the edge of the ulcer or presumed center of edematous or plaque lesions, and must include all levels of the integument, including the fascia. Fixation in 10% buffered formalin is adequate. Rapid diagnostic tests to detect mycolactone or *M. ulcerans*-specific proteins, considered a high priority, are in development.

7. Treatment

Treatment options for BU include antibiotics and surgical intervention. As shown in Table 2, the choice is usually based on the morphology and extent of the lesions, as well as availability of antibiotics and surgical facilities (WHO, 2004). Physiotherapy is imperative for all BU patients (WHO, 2006).

Historically, surgical intervention has been the standard treatment for all forms of BU (Wansbrough-Jones and Phillips, 2006). Recently, to minimize the possibility of *M. ulcerans* spread and to increase cure rates, surgeons sometimes administer ciprofloxacin and rifampin for 1—2 days before surgery, and continue this therapy for several weeks after surgery. However, adjunct antibiotic therapy with surgery is of variable efficacy (O'Brien et al., 2007).

Papules and pre-ulcerative nodules are seldom diagnosed, even in endemic areas. However, when they are present, wide excision and primary closure are usually curative (Uganda Buruli Group, 1970). Plaques and edematous forms are excised widely down to fascia, or through the fascia if it is necrotic. Muscle is usually not damaged, but if so, the excision is extended into muscle. The lateral extent of excision is often difficult to determine. By careful palpation, the physician can establish an approximate limit of the disease. Exploratory incisions and blunt dissection may help determine the limit of induration and necrosis. Use of real-time PCR for determining extent of disease is under evaluation (Rondini et al., 2003). Minor BU (small ulcers) can be excised and closed primarily. Major BU (large ulcers) is excised widely, with borders determined by exploratory lateral excision and blunt dissection. Split-skin autografts are then normally applied after a bed of granulation

Table 2 Three categories of Buruli ulcer and suggested treatment

Category	Form of disease	Treatment[a]	1° aim	2° aim	Level of health care system	Diagnosis
I	Small, early lesions (papules, plaques, nodules, ulcers ≤5 cm and >5 cm diameter)	For papules and nodules, if excision available, start antibiotics 24 h before surgery and continue for 4 weeks Otherwise, treat lesions with antibiotics for 8 weeks	Cure without surgery, except for removal of necrotic tissue	Reduce or prevent recurrence	Community health centers and district hospitals	Clinical and laboratory
II	Non-ulcerated and ulcerated plaques, edematous lesions Large ulcers, >5 cm diameter Lesions on head or neck, especially face	Treat with antibiotics for at least 4 weeks, then surgery (if necessary), followed by 4 more weeks of antibiotics	Reduce extent of surgical excision	Reduce or prevent recurrence	District and tertiary care hospitals	Clinical and laboratory
III	Disseminated or mixed forms, such as osteitis, osteomyelitis, joint involvement	Treat for at least 1 week with antibiotics before surgery, then continue for at least 8 weeks	Reduce *M. ulcerans* infection and dissemination, before and after surgery	Reduce or prevent recurrence; reduce extent of surgical excision	District and tertiary hospitals	Clinical and laboratory

Source: Adapted from WHO, 2004.
[a] Antibiotic regimens are described in the text.

Figure 5 (A) Buruli ulcer plaque on the forearm of a Congolese boy invaded the deep tissues and bone, causing contiguous osteomyelitis. (B) Radiograph showing contiguous reactive osteitis and necrosis of the cortex of the radius, with formation of a large sequestrum (arrow).

tissue has formed. Recurrences after surgical treatment are not uncommon, but rates vary widely for reasons not well understood (Debacker et al., 2005).

Anecdotal reports of successful antibiotic therapy date back to the 1960s, especially for early nodular and ulcerative

Figure 6 Ghanaian boy with a well-developed plaque of Buruli ulcer on the right flank. The ulceration is remarkably stellate, a feature of plaque lesions.

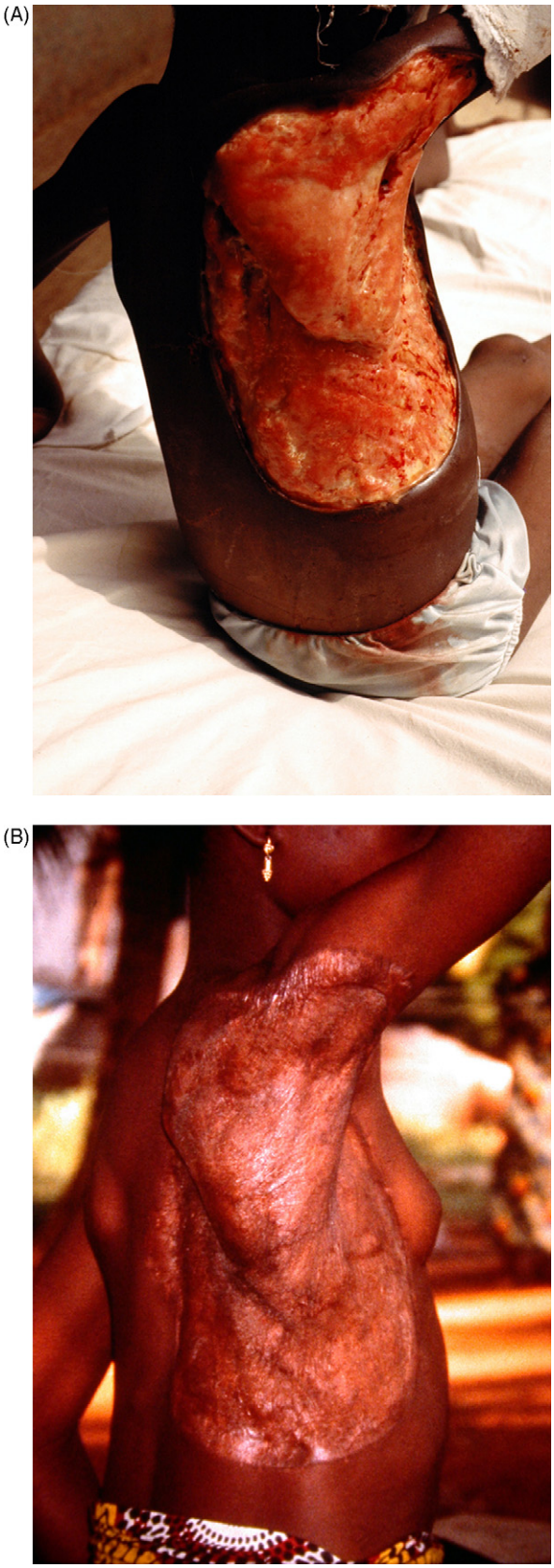

Figure 7 (A) An edematous Buruli ulcer lesion was excised in a 9-year-old Togolese girl. (B) Pockets of active disease at the borders were later excised and the lesion was grafted with autologous split-skin. (Photograph by G.B. Priuli, the treating surgeon.)

lesions. In 2004, although somewhat empiric, WHO formally recommended a multiple antibiotic regimen consisting of at least 8 weeks of oral rifampin (10 mg/kg) and intramuscular streptomycin (15 mg/kg), both given daily under direct observation (WHO, 2004). A fully oral antibiotic regimen is the goal. Published reports on antibiotic treatment results in Africa and Australia appear encouraging. Recently, short-term follow-up of 208 African patients treated with rifampin—streptomycin showed only a 1.4% recurrence rate (Chauty et al., 2007). Nonetheless, in the authors' opinion, antibiotic therapy for advanced lesions remains a subject for inquiry, and the rifampin—streptomycin regimen, although promising, does not constitute established recommendations.

Rifampin is supplied in tablet and syrup forms, the latter helpful for small children. Streptomycin and, to a lesser extent, rifampin, are associated with rare but important side effects, some of which require stopping treatment. For streptomycin, treatment should be stopped if hearing impairment or vertigo with nystagmus develops. For rifampin, treatment should be stopped if hepatitis, jaundice or renal failure develops; these side effects are generally associated with intermittent doses over 10 mg/kg. Streptomycin is contraindicated during pregnancy.

Heat therapy without surgical excision has been successful for appropriate lesions, but must be applied assiduously (Meyers et al., 1974a). Bone lesions are difficult to manage and should be referred to specialists (WHO, 2001).

Untreated BU may lead to deforming depressed scars, contracture deformities, or amputations. Several deaths attributed to severe edematous BU have been observed. With early appropriate treatment, including excision and grafting, the prognosis is excellent. However, metastatic lesions and local recurrences occur frequently enough to warrant vigilant follow-up (Aguiar and Steunou, 1997).

8. Complications

Infection may traverse the deep fascia and damage tendons, nerves, joints and genitalia. When eyelids and periorbital tissues are destroyed, enucleation of the eye may be required if expert reconstructive surgery is not available. Healing leads to fibrosis and scarring, and can severely limit movement with lifestyle alterations. The scar may form keloids and often causes major contraction deformities, especially in lesions that cross joints. Squamous cell carcinoma may develop in healed lesions, especially those that are non-pigmented. Skin grafting and physiotherapy will prevent most of these complications.

Most disease-related deaths result from septicemia, gas gangrene or tetanus. The authors postulate, however, that in massive edematous disease, large amounts of *M. ulcerans* mycolactone may cause fatal damage to vital organs.

9. Prevention

In a BU-endemic, tropical rural setting, where children are often scantily attired, prevention of contamination of the skin from environmental sources is virtually impossible. Protected water supplies in villages would reduce exposure somewhat; however, such protective measures are usually

futile in rural areas of developing countries. For adults, wearing trousers, especially when farming, may reduce infection (Pouillot et al., 2007). Other preventive measures include use of soap for washing, treating injuries with soap or antibiotic powder, and use of bed nets and insect repellent (Nackers et al., 2007; Pouillot et al., 2007; Quek et al., 2007).

BCG vaccination may provide moderate protection against *M. ulcerans* infections for 6—12 months (Uganda Buruli Group, 1969) and has been shown to prevent BU-related osteomyelitis in children (Portaels et al., 2004; Uganda Buruli Group, 1969). Studies are proposed to determine whether repeated BCG vaccination may render populations more immune to *M. ulcerans* infection, as found for leprosy (Karonga Prevention Trial Group, 1996). Other vaccines based on DNA engineering and virulence factors of *M. ulcerans* are also being studied.

10. HIV and Buruli ulcer

As BU is primarily a disease of children in rural areas, opportunities to study co-infection with HIV are limited. In Benin, a case—control study comparing HIV-1/HIV-2 seroprevalence in BU patients suggests that HIV seropositivity increases the risk for BU (Johnson et al., 2008). HIV infection may also render BU highly aggressive, with rapidly spreading osteomyelitis (Johnson et al., 2002; Toll et al., 2005).

Authors' Note: Informed verbal consent for inclusion of the clinical photographs was given by the child's parent or guardian.

Addendum

Patient in 7 (A) and (B) was treated by G.B. Priuli who contributed the photograph for 7(B). Interval between photographs 7(A) and (B) was 5 years.

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Conflicts of interest: None declared.

Ethical approval: Not required.

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