A 31-year-old diamond miner with chronic and relapsing skin ulcers. What is the diagnosis?

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A previously healthy 31-year-old man presented in September 2007 to his doctor with a recurrence of painless ulcers on his left leg. He had traveled into Angola over 10 years for work involving diamond alluvial mining. He stayed mainly in the diamond province of Lunda Note, in an area called Capemba Camulembe. He was exposed to fresh water while working on the open mines on the banks of the rivers as well as while swimming, bathing or taking a shower in the tributaries of the Cuango River. During this time he frequently experienced cuts/abrasions of his skin. In 10 years he had been treated for malaria three times.

The first time he developed these lesions was in 2004 when he noticed a small ‘hole’ developing in the calf of his left leg. The lesion was painful at the time and grew in size over the subsequent months. He was treated with numerous combinations of antibiotics over those months with no response.

At the time of his first presentation, results of swabs and tissue biopsies were positive for numerous acid-fast bacilli (AFB) but mycobacterial cultures were negative. There was some bacterial growth (Staphylococcus aureus/Acinetobacter spp) but this was deemed to be superinfection. Histology of the tissue was also positive on staining for numerous AFB.

In 2004, he was started on a regimen of clarithromycin, doxycycline and co-trimoxazole but did not respond. He then underwent wide excision of the lesions followed by skin grafting, and did well, with no recurrence on the calf area to date. (See Figure 1)

In January 2007 he initially presented with a lump on his left thigh. The surgeon suspected a lipoma and resected this. Histology showed fat necrosis. Then about two months later, little red dots developed in the same area, these then progressed to ulcers (see Figure 2). He was then referred to a dermatologist with these multiple ulcerating lesions on the left thigh. There were some subcutaneous nodules present as well. Biopsies of these revealed numerous AFB. Mycobacterial culture was positive by a radiometric liquid culture method for a ‘mycobacterium other than Mycobacterium tuberculosis’ (MOTT/non-tuberculous mycobacterium [NTM]) but further identification tests were inconclusive. Culture with incubation at 25°C was negative.

Three punch biopsies were taken from the edges of these ulcers. One was sent to the Mycobacteriology Referral Laboratory in Johannesburg for microscopy and culture. Two specimens were sent to the Mycobacteriology Unit in the Institute of Tropical Medicine in Antwerp, Belgium.

What is the diagnosis?
PCR testing in the laboratory of the Institute of Laboratory Service in Johannesburg, the direct Ziehl-Neelsen smear of the biopsy was positive for presence of acid-fast bacilli (AFB)(i) (FD) ++++. Half the specimen was decontaminated as recommended with mild HCl/NaOH. The other biopsy was inoculated directly (no decontamination) onto media. Media used were Löwenstein-Jensen (LJ) slopes and MGIT liquid broth (BD Diagnositic Systems). The LJ slopes were incubated at 25, 32, 37, 40 and 45°C.

Results of the IS2404 PCR testing in the laboratory of the Institute of Tropical Medicine, Antwerp in Belgium were positive for Mycobacterium ulcerans DNA. Culture in both laboratories remained negative after incubation for 12 months at 32°C.

The patient was started on a regimen of ciprofloxacin and rifampicin and surgical excision was recommended. He continued on this regimen until he presented again, for the third time, to his GP, nine months later.

This time, he complained of subcutaneous nodules in the left thigh. Though they were painless, they were pruritic. On examination by the GP, the old ulcers were still present but seemed to be decreasing in size, but there were prominent, firm, subcutaneous nodules. The overlying skin was intact and not discoloured. An MRI scan of the leg showed six subcutaneous nodules that were 1-2 cm in diameter. There was no bone involvement. Antibiotic therapy was not started; instead the patient underwent excision of the nodules as delineated by the MRI scan. They were inoculated in transport media and sent to the laboratory in Belgium. Ziehl-Neelsen (ZN) stain was positive for AFB and Mycobacterium ulcerans DNA was again detected by PCR in the laboratory in Belgium. Molecular fingerprinting was performed directly on the specimens by variable number of tandem repeat (VNTR) analysis. This was successful and confirmed that the isolate was in fact part of the West African cluster of M. ulcerans (see discussion).

Histology was performed on one ulcer and on one nodule. Histology of the ulcer showed the presence of chronic inflammatory infiltrates, necrosis, was negative for AFB and histological assessment was that it was healing; it was negative on Ziehl-Neelsen stain for AFB and negative by PCR in the laboratory in Antwerp. A subcutaneous nodule that underwent histological examination was caseous, and showed friable acute and chronic inflammatory cells, a moderate number of AFB and some intracellular caseous-like necrosis. This nodule was positive on Ziehl-Neelsen for AFB as well as positive by PCR at the laboratory in Antwerp. Thus the histology correlated with the microbiological assessment. In total, two specimens were AFB smear-negative, PCR-positive, while four others were both AFB- and PCR-positive. Two of the positive Ziehl-Neelsen/PCR samples had VNTR analysis performed directly on them – this confirmed that the patient was infected by an M. ulcerans strain that has a West African genotype typical for strains isolated from countries along the Atlantic Ocean. This is therefore evidence that the patient was infected in Angola during his artisanal alluvial mining activities along the banks of the Cuango River.

Discussion

Buruli ulcer (BU) disease, caused by the bacterium M. ulcerans, is an indolent necrotising disease of the skin, subcutaneous tissue and bone. After tuberculosis and leprosy, it is the third most common mycobacterial disease. BU is a major problem in several West and Central African countries. It is endemic in Central America, subtropical climates of Southeast Asia and Australia, but countries in Africa in the past decade have recorded increased incidence rates in some communities exceeding that of tuberculosis.

BU has been reported from at least 30 countries, principally subtropical and tropical regions. Endemic foci of BU are most common near rural permanent wetlands in warm geographical regions, especially in areas prone to seasonal flooding. A rapid re-emergence of BU began in the 1980s and is thought to be attributable to environmental factors such as deforestation, dam and irrigation systems implantation, enlarging populations engaged in basic manual agriculture in wetlands, and possibly global climatic changes. Mode(s) of transmission, natural reservoir(s) and other key aspects of the epidemiology of BU are not fully understood. M. ulcerans is likely to be an environmental pathogen because of the association of BU-endemic foci with wetlands and overflowed riverbanks and the detection of M. ulcerans-specific sequences in mud, water, aquatic insects and plants. It is thus considered non-communicable. The most plausible mode of transmission is by skin trauma at sites contaminated by M. ulcerans.

BU afflicted mostly children up to 15 years of age in endemic countries. The mean incubation time is estimated to be two to three months.

This patient developed symptoms about a year after leaving the area. Delayed onset of disease has been described and probably represents reactivation of latent infection. It has been observed that individuals who originally resided in a BU-endemic country may develop BU at a body site where trauma occurred several years after leaving the endemic area. Our patient did not recall any trauma to his leg.

The genetic diversity of M. ulcerans is known, with four worldwide foci. The genetic diversity within Africa was first reported by Stragier
et al who described two main genotypes in Africa: the Atlantic African genotype and the East African genotype, using the VNTR typing method.

BU is recognised as a spectrum of clinical disease that includes nodules, plaques, oedema, characteristic skin ulcers, sometimes massive, and osteomyelitis. The differential diagnosis for painful nodular lesions includes insect bites, furuncles, anthrax, abscesses, infected sebaceous cysts, erythema nodosum (usually multiple), hydrenephrosis, furunculoid myiasis, and for painless or mildly painful nodules includes lipoma, onchocercal nodules and nodular forms of leishmaniasis. Isolated, non-infamed nodules may also be due to granulomas caused by foreign bodies, dermatofibromas or histiocytomas and gummatas (subacutae panniculitis) suggestive of sporotrichosis, actinomycosis, cold pyococcal infection, tuberculosis, tertiary syphilis or yaws. The differential diagnosis for ulcerative lesions includes phagedenic ulcers, ulcers of venous origin, ulcers of arterial origin, ulcerative cutaneous tuberculosis, African histoplasmosis, necrotising fasciitis, sporotrichosis, ulcerated carcinomas or melanomas, cutaneous leishmaniasis, sickle-cell ulcers.

BU bone lesions may include reactive osteitis or osteomyelitis beneath skin lesions or metastatic osteomyelitis from lymphoheamatogenous spread of *M. ulcerans*. Pathogenesis is related to a necrotising and immunosuppressive toxin produced by *M. ulcerans*, called mycolactone. In endemic countries, BU is considered a major public health and psychological problem because of potential disabling sequelae including major disabling scarring and destruction of bone, estimated to occur in at least 60% of patients. HIV infection may increase risk for BU, and renders BU highly aggressive. Improved understanding of the molecular biology of *M. ulcerans* will help elucidate these differences in clinical manifestations.

Clinical diagnosis is often used in endemic areas, especially when typical ulcerated lesions are present. Laboratory diagnosis consists of direct smear, culture, PCR and histopathology. In ulcerative forms, a Ziehl-Neelsen stain of exudate from the undermined edge will reveal clusters of strongly AFB. The same material may be cultured, after decontamination, onto Löwenstein-Jensen medium. Egg-based media seem to give better yields. Most methods of decontamination (used for cultivation of *Mycobacterium tuberculosis*) result in decreased yield of the organism and this may play a role in the difficulty of isolation in the laboratory, as it is susceptible to the usual NALC-NaOH method used for *M. tuberculosis* culture. The optimal incubation temperature is 30-32°C; the organism is strikingly sensitive to temperatures of 37°C and higher. If culture cannot be performed locally, transport media may be inoculated and maintained at 4°C while transported to a specialised laboratory. (The transport media are composed of Middlebrook 7H9 broth supplemented with antibiotics). 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*.

Molecular diagnosis is becoming increasingly more important in the diagnosis of BU disease. PCR for the identification of *M. ulcerans* is available, convenient and growing in popularity.

Tissue for histopathological analysis must be obtained from the edge of the ulcer or presumed centre of the lesions; it must have all levels of the integument, including the fascia. Fixation in 10% formalin is adequate.

Treatment options for BU include antibiotics and surgical intervention. The choice is usually based on the morphology and extent of the lesions, as well as the availability of antibiotics and surgical facilities. Physiotherapy is imperative for all BU patients. Historically surgery has been the standard of treatment for all cases of BU. Recently, to minimise the possibility of *M. ulcerans* spread and to increase cure rates, surgeons sometimes administer ciprofloxacin and rifampicin for one to two days before surgery, and continue this therapy for several weeks following surgery. However, adjunctive antibiotic therapy with surgery is of variable efficacy.

For papules and pre-ulcerative nodules, wide excision and primary closure are usually curative. Plaques and oedematous forms are excised widely down to fascia, or through fascia if this is necrotic. If muscle is damaged, the excision is extended into muscle. Blunt dissection helps determine the limit of induration and necrosis. Minor BUs may be excised and closed primarily. Major BUs must be excised widely. Split skin autographs are then applied after a bed of granulation tissue has formed. Recurrences after surgical treatment are not uncommon.

Successful antibiotic therapy, especially for early nodular or ulcerative lesions have been reported. In 2004, the WHO recommended a multiple antibiotics regimen consisting of at least eight weeks of oral rifampicin (10 mg/kg) and intramuscular streptomycin (15 mg/kg), both given daily under direct observation; a full oral antibiotic regimen is the goal.

Untreated BU may lead to deforming scars, contracture deformities, or amputations. Metastatic lesions and local recurrences occur frequently enough and warrant vigilant follow up. Reported disease recurrence rates vary from 6% to >20%. It is unknown whether this is due to exogenous reinfection or dissemination of the pathogen from previous lesions.

BCG vaccination provides short-term protection against *M. ulcerans* infection and prevents osteomyelitis.

Better disease control includes better diagnostics, improved knowledge of the reservoir and the epidemiology of the disease.

**References**