Clinicopathologic Classification of *Mycobacterium ulcerans* disease (Buruli ulcer)

IAP Montréal 2006
Collaborators

**Armed Forces Institute of Pathology, Washington D.C.**
Wayne M. Meyers
Melanie Malecombe-Usher
Fides Abalah-Norrie

**Centre Sanitaire et Nutritionnel Gbempoten, Zagnano, Bénin**
Julia Aguilar

**Programme National de Lutte contre L’ulcère de Buruli, Cotonou, Bénin**
P. Christian Johnson
Augustin Guédénon

**Institute of Tropical Medicine, Antwerp, Belgium**
Martine Debacker
Françoise Portaels

**Walter Reed Army Institute of Research (WRAIR), Silver Spring, Md., USA**
Douglas Walsh
Clancey et al., in 1961, named *M. ulcerans* disease “Buruli ulcer” after the geographic area of the first large epidemic area investigated. This area was in Buruli county (now Nakasongola District) in Uganda. The disease was also first described in Uganda by Cook in 1897. However, the recent trend is to call the disease “Mycobacterium ulcerans infection”, or “Mycobacterium ulcerans disease.”

Other names include “Bairnsdale ulcer”, Searle ulcer”, “Kumusi ulcer”, “Kakerifu ulcer” and “Toro ulcer”.
Most known *M. ulcerans* infections are in West and Central Africa. In Australia the disease has been noted only in the eastern one-third of the country. Côte d'Ivoire has identified the most cases of *M. ulcerans* (>22,000). Eight patients have been reported in Mexico. The disease has been observed in approximately 30 countries.
Depicted here is a backwater shallow pool along the Ouémé River near Sagon in Benin, a highly endemic area of Buruli ulcer. Note the various activities, swimming, bathing, laundering and mud brick making.

The reemergence of Buruli ulcer may, in part, be attributable to: deforestation, causing increased flooding, man made topographical alterations (dams, irrigation developments), climatic changes, increased population at risk and persistent use of antiquated agricultural techniques.
One scenario for transmission of *M. ulcerans*: aquatic insects of the order Hemiptera (Naucoridae and Belostomatidae) can become infected and harbor *M. ulcerans* in their salivary glands. Experimental transmission by the bite of infected Hemiptera has been reported. Transmission by mosquito has been postulated. Koalas and possums acquire the disease in nature in Australia. The ultimate natural host for the etiologic agent has not been established. Freshwater branchiopod crustaceans such as Daphnia seem to tolerate ingested *M. ulcerans* in the laboratory (personal observation). Free-living amoebae as natural hosts are under study.
Mean incubation times are 2 to 3 months in patients who had antecedent trauma at the lesion site. Incubation times of 2 weeks to 3 years, possibly longer, have been reported.

Risk factors are not well understood. HIV infection does not seem to be a risk factor for infection, but the disease can be very aggressive in AIDS patients. Infection is most frequent in children <15 years old, and the elderly.

Acid-fast bacilli (AFB) are extracellular in areas of necrosis; however, recent observations reveal that AFB at reactive sites can be intracellular (not shown here).
Sensitivity for the above tests is estimated as follows;

Smear -- 40-80%
Culture -- 20-60%
Histopathology -- >90%
PCR -- >90%

Ziehl-Neelsen is most applicable for field use.
PCR is now being studied for field application using dry regents.
The WHO standard for confirmed diagnosis is under evaluation – one positive laboratory test may be sufficient for an experienced field team.
This is a proposed classification and natural history of the development of the various forms of *M. ulcerans* disease, and of course, is subject to modification. Type of disease depends on many factors, known and probably unknown. Some suggested factors are: size of inoculum of *M. ulcerans*; depth of inoculation (superficial vs. deep, in skin); virulence of strain of *M. ulcerans*; and immune response of host (Th1 response protective).
Clinically, nodules are movable, painless but often pruritic, and usually 2-4 cm in diameter. There may be incipient ulceration. Histopathologically, there is coagulation necrosis of the lower dermis and subcutaneous tissue and acid-fast bacilli (AFB) in the center of the necrosis. Note that necrosis extends far beyond the concentration of AFB in ZN stained sections *(lower right).*
Two clinical minor ulcerative lesions are shown on the left. The gross pathologic and histopathologic features are presented in the remaining frames. Clinically, the ulcers are small, sharply limited and undermined. These lesions are sometimes seen in surveys conducted in villages. The lesions may have started as nodules, and our impression is that they usually self-heal. Histologically, the AFB are in the central necrotic slough, with scarring in the surrounding dermis. Untreated, we believe the AFB are discharged in the extruded necrotic slough, allowing the ulcer to heal.

This form requires more prospective study to establish our speculations.
The major ulcerative lesion is the classic form of *M. ulcerans* disease. Two examples are shown on the left: a lesion on the deltoid area (*upper photo*) that followed the inoculation of an anti-cholera vaccine, and the lesion on the abdomen (*lower photo*) followed local penetrating trauma. Lesions of this form are large, undermined, well demarcated ulcers with delayed healing and occasional dissemination. Histologically, there are acid-fast bacilli (AFB) in the necrotic base and adjacent areas, and wide undermining. The distribution of AFB is shown with ink dots in a low power photomicrograph (*right lower corner*). The undermining and necrosis, with vasculitis (*insert, upper right*), and the necrosis of fat (*lower middle*) are striking features. This form often produces disabling contractures if untreated, or inappropriately managed.
Clinically, the contiguous, nonulcerative form presents as small or large edematous indurated plaques. Such lesions are often referred to as the edematous form, especially if they cover large areas. Top left represents a massive edematous lesion in a 9-year-old boy. Lesion covers the left side of the trunk from the axilla to the iliac crest. Successively, the photos to the right show the extent of the lesion, the excised skin, and an 18 cm portion that was evaluated histopathologically to determine the distribution of the AFB. Histopathologic study revealed no evidence of nodular changes, but massive necrosis and large numbers of AFB (see the 4 photomicrographs). Spread of AFB tended to follow the fascial planes (lower right). The remaining clinical postoperative photographs of two other patients point out the extent of the involvement. The two patients on the upper left died at 2 days, and 30 days post-surgery, respectively.
Above are multiple examples of ulceration in untreated contiguous disseminated lesions. The two lower right photographs show a patient with this form after the original excision in 1994, and 5 years after autologous mesh skin grafting.
Although clinical lymphadenopathy is not a consistent feature of Buruli ulcer, histopathologic evaluation of local and regional lymph nodes may show extensive necrosis and invasion by AFB (top 3 photomicrographs). Presumably metastatic lesions arise from lymphatic-hematogenous spread.

The lower photograph shows severe osteomyelitis of the ankle that developed following a major ulcerative lesion on the buttock. The history of this patient is given on Slide 21.
Histopathologically, the healing stages are shown in the top 3 photomicrographs: organizing stage, granulomatous stage, and ultimate scarring.

Scars of Buruli ulcer typically are depressed (*bottom left*). Without appropriate skin grafting and physiotherapy, contracture deformities develop if the lesion traverses an articulation (*bottom middle*). Without skin grafting, the healed lesion often does not repigment and actinic rays may provoke cancers (see Slide 18).
Disabilities develop in many patients: most are preventable by appropriate therapy. *Top left:* Contracture of knee with early epithelioma. *Top middle:* Contracture of knee with advancing cancerous changes. *Top right:* Contracture of foot and ankle with far advanced squamous cell carcinoma. Patient died of metastatic squamous carcinoma. *Lower left:* Lesion in groin required excision of skin of perineum, scrotum and penis. *Lower middle and right:* Lesion of face required enucleation. The eyeball was not involved, but adequate expertise for reconstructive surgery of the eyelids was not available. The left eye was preserved.
Approximately 10% to 15% of Buruli ulcer patients develop osteomyelitis. This includes both contiguous bone involvement from an overlying lesion in the skin, and metastatic *M. ulcerans* osteomyelitis.
CONTIGUOUS OSTEOMYELITIS

Radiograph shows a sclerosed sequestrum in the distal end of the radius immediately under the lesion of the forearm. “Outside in”

Six year old Congolese girl with ulcerating lesion of *M. ulcerans* of forearm.

The sequestrum contained acid-fast bacilli that had initially invaded the periosteum.
Four year old Angolan boy admitted with nodule on cheek and ulceration of skin of right hip and left knee with contiguous osteomyelitis, and osteomyelitis of the right tibia and right ankle. Had scars on shoulder and hip typical of healed *M. ulcerans*. Developed additional bone lesions while in hospital in Germany. Patient had malaria and sickle cell trait.
METASTATIC OSTEOMYELITIS

Ulcerated plaque on buttock of 12 year old schoolboy in Benin. Treated successfully by excision and grafting at medical center.

Spread to ankle 6 months after discharge from hospital. Lesion began with painful swelling followed by sinus formation.

This 13 year old Benin girl had successive amputations of lower extremities following cutaneous *N. ulcerans*. Months later bilateral lesions of the metacarpal bones developed and are now breaking through the skin.
METASTATIC OSTEOMYELITIS
Microscopic Features

Necrosis of a cancellous area of cortical bone
(H & E Stain)

Necrosis of bone marrow
(H & E Stain)
METASTATIC OSTEOMYELITIS
Microscopic Features (cont’d)

AFB (M. ulcerans) in necrotic bone with erosion of trabecula.
(Ziehl-Neelsen stain)

AFB (M. ulcerans) in necrotic marrow
(Ziehl-Neelsen stain)
While surgical excision of lesions is the usual therapy for Buruli ulcer, today (2006) there are multiple controlled studies on antimicrobial therapy. However, the efficacy of systemic therapy with rifampin for small lesions and nodules has been known since the early 1970s. Above are two examples of patients with small lesions of Buruli ulcer who were treated by monotherapy with rifampin, 600 mg daily. Neither lesion recurred during several years follow-up.
ACKNOWLEDGMENTS

We thank the following for their interest and help over the last 40 years:

• The patients and their families who allowed us the privilege of working with them.

• The numerous fellow-workers who helped make these studies go forward:
  – Chauffeurs, mechanics, office workers, lab. techs., nurses, other paramedical workers and physicians.

• For financial support:
  – Damien Foundation
  – The American Registry of Pathology
  – American Leprosy Missions
  – Nippon Foundation
  – WHO

www.afp.org/hot-topics.html