Introduction and outline of the Thesis
Buruli ulcer

Buruli ulcer is a neglected tropical skin disease, caused by infection with *Mycobacterium ulcerans* (1). The disease was first described in 1897 by Albert Ruskin Cook, a medical missionary in Uganda. He described a number of cases of large, slow-healing skin ulcers. An infectious etiology was suspected, but could not yet be demonstrated. In 1948 MacCallum described a series of slow healing ulcers in the Bairnsdale district of Victoria, Australia, where the margins were “teeming with acid-fast bacilli”, establishing “a new mycobacterial infection in man”, next to the then well-known and feared mycobacterial diseases of tuberculosis and leprosy, and named the species *Mycobacterium ulcerans* (2).

In the late 1950s and early 1960s, a large outbreak occurred in the Buruli district of Uganda (3), and the disease became known as Buruli ulcer. For reasons not fully understood, the disease prevalence in East Africa gradually declined, until it disappeared completely and the epicentre of the disease moved to West Africa, where the first large outbreaks were documented in Ghana and Benin in the 1980s (4, 5). Buruli ulcer has been reported in 32 countries, with the majority of cases occurring in West Africa, with an estimated annual incidence of between 6,000 and 10,000 cases (6). It is a predominantly rural disease, with a highly focal epidemiology. In most endemic areas, children are more affected than adults (7, 8). The mode of transmission is not fully understood. Transmission appears to be related to environmental exposure (9), and human to human transmission has only been described twice (10, 11). Proximity of outbreaks to slow-flowing or stagnant water has raised suspicion of an aquatic reservoir (12, 13), and mosquitoes and domestic animals have also been implicated (13, 14). In addition, the exposure of unprotected skin to the environment, possibly in combination with skin trauma, appears to play a role (15, 16).

After an estimated incubation period of several—usually 3–4 – months (17), the disease usually starts with a firm but painless nodule. This is followed by induration and ulceration of the surrounding skin. The tissue necrosis is caused by production of mycolactone, a polyketide toxin. A typical Buruli ulcer is characterised by undermined edges, indurated skin, and yellow slough. In the absence of osteomyelitis, systemic symptoms such as malaise or fever are usually absent. The disease is usually self-limiting, but can result in extensive lesions causing scars and contractures leading to severe functional limitations (18–20). In rare cases, amputations are necessary.

The combined features of an exsudative ulcer with undermined edges, surrounded by indurated skin, when found in a patient from an endemic area, allow for a relatively accurate clinical diagnosis. *M. ulcerans* is notoriously difficult to culture, and when successful requires 1 two 2 months for confirmation. This limits the usefulness of culture to research purposes. Laboratory confirmation of the diagnosis can be obtained through microscopy and Ziehl-Neelsen staining of biopsies, or through detection of *M. ulcerans* DNA the insertion sequence IS2404 using Polymerase Chain Reaction (PCR), which is gradually replacing microscopy (21).
An early trial of monotherapy with clofazimine proved unsuccessful (22), and surgical excision long remained the only treatment option. From the early 2000s onwards, evidence started to emerge on the efficacy of combination therapy with injected streptomycin and oral rifampicin (23, 24). Currently, the World Health Organisation (WHO) recommends eight weeks of streptomycin and rifampicin therapy, and this regimen has been proven to result in high rates of cure (25).

In early 1998 the WHO founded the Global Buruli Ulcer Initiative and later that year a conference in Yamoussoukro, Ivory Coast led to the Yamoussoukrou declaration; an affirmation of support to research and control efforts for Buruli ulcer. In 2004, the World Health Assembly adopted a resolution on Buruli ulcer, calling for increased efforts in surveillance, control and case management. These developments have led to an increased humanitarian and scientific interest in the disease, which among other things have yielded successful drug treatment, reliable diagnostics, and national control programmes in some of the most affected countries.

However, several challenges remain. Although drug treatment is successful, it is lengthy and painful. In addition, for patients to fully benefit from this drug treatment, they should report to the hospital early to prevent hospitalisation, and the disabling consequences of the disease. When hospitalised, they should receive a high standard of wound care, in order to prevent super-infections of the wound and reduce the time to healing. In this thesis, we studied these aspects of Buruli ulcer case management. In the following sections, we introduce studies on refining the drug treatment for Buruli ulcer, assessing and improving wound care, and evaluating public health aspects of the disease.

**Drug therapy for Buruli ulcer**

The current WHO-recommended drug treatment for Buruli ulcer is eight weeks of intramuscular streptomycin and oral rifampicin. Although this regimen is highly successful in clearing the infection, it suffers from several shortcomings. Streptomycin administration requires 56 days of consecutive intramuscular injections, which are not only painful, but also pose a risk of infection at the injection site. In addition, they require the presence of a health care facility, or at least of trained personnel, often forcing patients to travel several hours daily, mostly by foot, to the nearest health care facility. Third, streptomycin, when administered over long periods of time, can cause damage to the inner ear and kidneys (26–29). In chapter 2, we describe a study on the long-term effects of streptomycin on the hearing and kidney function of former BU patients.

The currently recommended duration of drug treatment for Buruli ulcer is 8 weeks. This duration is based on a study where Buruli ulcer lesions were excised after increasing periods of antimicrobial therapy. In this study, no *M. ulcerans* could be cultured af-
ter at least 4 weeks of antimicrobial therapy (24). Based on expert opinion, the period of 8 weeks was then chosen to establish a safety margin. Reducing the number of days of treatment, however, would reduce costs, adverse medication effects, and discomfort and time spent walking and waiting for streptomycin injections. If treatment duration could be tailored to the need of each individual patient, perhaps treatment compliance could be improved as well. A recent study in Buruli ulcer patients from Australia suggests that selected patients will heal when treated for shorter periods of time (30). In chapter 3, we describe a follow-up of a group of Buruli ulcer patients from Ghana that, because they defaulted from antimicrobial therapy, has not received the full 8 weeks of antibiotics.

Whether drug therapy can be shortened or not, an oral regimen for Buruli ulcer treatment is urgently needed. In the mouse footpad model for Buruli ulcer, combination therapy of rifampicin and clarithromycin has been demonstrated to be effective (31). Its efficacy was further demonstrated in a small pilot study (32), and in a larger drug trial, where patients were randomised to receive either 8 weeks of streptomycin and rifampicin or 4 weeks of streptomycin and rifampicin followed by 4 weeks of clarithromycin and rifampicin (25). Currently, a randomised controlled trial comparing 8 weeks of streptomycin and rifampicin to 8 weeks of clarithromycin and rifampicin is ongoing (33).

Although the clinical response to clarithromycin and rifampicin combination therapy is promising, a pharmacokinetic side study to the previous drug trial found significant interactions between these drugs. Rifampicin induces hepatic enzymes that quickly metabolise clarithromycin, yielding sub-optimal serum concentrations (34). Therefore, in the current drug trial, a double dosage of clarithromycin was given in an extended release formulation next to rifampicin. Chapter 4 describes the pharmacokinetics of this combination.

Other oral antibiotics have also been proven effective against Buruli ulcer in vivo and in vitro (35). Among these, the fluoroquinolones have been used extensively in Australia (30, 36, 37). It has been argued that compared to clarithromycin, these antibiotics are to be preferred, especially for their presumed usefulness in the event of Buruli ulcer-tuberculosis co-infection, a disease combination which has not yet been described in the literature to date (38). Although these drugs do not suffer from the pharmacokinetic interaction mentioned above, we believe there are several disadvantages to their use, which we discuss in chapter 5.

Sometimes during drug therapy for Buruli ulcer, a transient increase of the size of the lesions occurs. These paradoxical reactions are thought to represent an immune response that occurs after the production of the immunosuppressant toxin mycolactone has been inhibited by antimicrobial therapy (39–41). However, these reactions are poorly understood and it is not clear if a change of management consisting of additional antibiotics, corticosteroids or surgery is warranted (42). In chapter 6 we study the risk factors and outcomes of paradoxical reactions in a cohort of Buruli ulcer patients.
Wound care

Despite effective antimicrobial therapy, most Buruli ulcer patients are still faced with large, slow healing ulcers (41). In order to optimize time to healing and prevent wound infections, a high standard of wound care is essential. The WHO has published guidelines for wound care in Buruli ulcer, stressing the importance of protecting the wound from the environment, maintaining a clean wound bed, and maintaining a moist wound environment (43, 44). However, little is known on how these guidelines can be implemented in endemic countries. Therefore, in chapter 7, we report on the current standard of wound care in a sample of several Buruli ulcer treatment centers and associated health centers in Ghana and Benin.

To date, only gauze dressings have been used for Buruli ulcer wound care, as these are cheap and easily available. Gauze dressings have several disadvantages, as a moist wound environment cannot be maintained, and the removal of dried-out gauze from a wound causes pain and bleeding, and disrupts fibroblasts and epithelial cells and therefore, tissue repair (45–47). Experience from the treatment of chronic ulcers in Western countries has shown that modern, more absorbent foam-containing dressings can maintain a moist environment and do not disturb tissue repair (48–50). In addition, these materials can remain on exudative wounds for a longer period of time, conceptually reducing staff time, travel time and patient discomfort. In chapter 8, we report on a pilot with a hydrofoam dressing in Buruli ulcer patients in an endemic area in Ghana.

A high standard of wound care, particularly hygiene, is important in order for the wounds not to become infected. Secondary infection of BU Buruli ulcer lesions by other pathogens has been assumed to be a rare event. However the prevalence of this super-infection is unknown. In addition, colonization of wounds with other bacteria without clear signs of infection might also increase the time to healing. On the other hand, Buruli ulcer patients already receive an intensive course of antibiotics, and further administration of antibiotics should be reserved for those cases where the clinical suspicion of a secondary, complicating infection is high. A rationale for the diagnosis and treatment of super-infections is desirable. Therefore, in chapter 9 we present findings on Buruli ulcer super-infections, and a culture-based guideline for antimicrobial treatment.

Public health aspects

In spite of all recent scientific advances, the burden of Buruli ulcer on patients and endemic districts remains high (51–53). Loss of productivity caused by lengthy hospital stays is a concern, as are the disabling contractures that frequently occur. As with many diseases in underprivileged areas of the world, a key limitation to effective treatment and prevention of these disabilities is late presentation. Although Buruli ulcer treatment is provided
free of charge in Ghana and Benin, fear of costs for transportation and hospitalisation are a barrier to early presentation. Patients often resort to methods of traditional healing before visiting the hospital. Late presentation might also result from the stigma surrounding the disease, and fears of the consequences of treatment, such as amputation (54–56).

Community based liaisons are an important tool both for combating this stigma and misinformation, and for early detection of BU cases (57, 58). Previous experience with these community based surveillance volunteers in other infectious diseases, such as Guinea worm, has shown to be highly effective (59). In contrast to health care personnel, they are trusted members of the target community. Conceptually, these volunteers may help detect Buruli ulcer in an early stage. They are present in their community on a daily basis, whereas outreach teams might visit a certain community only once every few weeks. In Chapter 10 we describe the nature and impact of a network of community based surveillance volunteers in a highly endemic district in Ghana.

Once the patient has reported to the health care system and drug therapy has been initiated, it should be completed as prescribed. Experience from tuberculosis indicates that in spite of directly observed therapy, there is a significant risk for non-compliance (60). The administration of streptomycin through injections forces patients to visit the health care center, and in this way provides a form of monitoring by health care personnel. These injections will likely be replaced by an oral regimen in the near future, raising concerns about monitoring adherence to therapy. Chapter 11 presents the first data on therapy compliance and risk factors for non-compliance in Buruli ulcer.

It has been well established that Buruli ulcer can cause severe functional limitations due to scarring and contractures that persist long after treatment has been completed. These Buruli ulcer sequelae can cause problems in different areas of life such as work, schooling, and social and romantic relationships (18, 19). However, the severity of these sequelae might be reduced by early treatment of small lesions, resulting in minimal scarring and functional limitations. In Chapter 12 we present the first data on the long-term quality of life of former Buruli ulcer patients with small lesions that took part in a drug trial in Ghana.
References


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Part I

Drug treatment for Buruli ulcer