Summary

A review of the clinical features of Buruli ulcer is given in chapter 1. Briefly, Buruli ulcer is a skin disease caused by infection with Mycobacterium ulcerans. Although cases have been reported from over 30 tropical and sub-tropical countries, it is currently most prevalent in West-Africa. Although environmental aspects, insects and small mammals have been implicated in its occurrence, the mode of transmission is unknown. Typically, the disease starts with a small, painless nodule, which over the course of several weeks progresses to a slow-healing ulcer with yellow slough and undermined edges. The clinical picture is rather specific, and laboratory confirmation can be obtained through Ziehl-Neelsen staining or polymerase chain reaction. Drug treatment currently consists of eight weeks of streptomycin and rifampicin, and is successful in clearing the infection. However, a high standard of wound care and physical therapy are essential for the ulcer to heal, and to prevent future disabilities. Early presentation of the patient to the hospital limits the need for hospitalization, additional surgery, and advanced rehabilitation. The studies in this thesis explore and assess current Buruli ulcer patient management, and focus on drug treatment, wound care, and public health aspects of the disease.

Drug Treatment

The current WHO-recommended regimen of 8 weeks of oral rifampicin and intramuscular streptomycin has been widely applied by Buruli ulcer control programmes in West-Africa. However, like other aminoglycosides, streptomycin is known to be ototoxic, and, albeit transiently, nephrotoxic. In chapter 2, we assessed its long-term toxicity by following-up former Buruli ulcer patients that took part in a randomized controlled trial several years earlier. In that trial, patients were randomized to receive either 8 weeks of rifampicin and streptomycin, or 4 weeks of rifampicin and streptomycin, followed by 4 weeks of rifampicin and clarithromycin. This design allowed us to compare two groups of Buruli ulcer patients that were randomized to receive either 4 or 8 weeks of streptomycin. Overall, ototoxicity was present in 29% of adults and 25% of children. Adults who received 8 weeks of streptomycin had significantly worse hearing at long term follow-up, and this was most prominent in the high frequencies. In children, no differences between the two groups were found. Nephrotoxicity that had been detected in 14% of adults and in 13% of children during treatment, was present in only 2.4% of patients at long term follow-up. The findings indicate that caution should be exercised when prescribing streptomycin to adults for prolonged periods of time. Treatment regimens for Buruli ulcer that do not contain streptomycin are desirable and should be investigated.

The 8 week duration of drug treatment is based on limited evidence, that suggests that 4 weeks of therapy might suffice. In chapter 3, we studied the effects of shorter drug
therapy on a group of Buruli ulcer patients that defaulted from treatment. In a BU treatment center in Ghana, 54% of Buruli ulcer patients had defaulted from therapy because of side effects, but also for other reasons. This allowed us to study the effects of a shorter course of antimicrobial therapy. We followed-up on a cohort of patients that had defaulted from therapy, and thus had received less than the WHO-recommended 56 doses of streptomycin and rifampicin. Out of the 47 patients that were retrieved, 9 still had a non-healed Buruli ulcer. There was a significant effect of lesion size at presentation on rates of healing. In those that presented with small, WHO category I lesions, that received 32 days or less of antibiotics, the rate of healing was 94%, which is similar to known rates of healing in recent large cohort studies where patients received 8 weeks of antimicrobial therapy. However, our cohort was small, and due to the retrospective nature of the study a significant opportunity for bias exists. The findings indicate that shorter therapy is feasible for Buruli ulcer patients with small lesions, but this should be explored further in a well-designed randomized controlled trial.

Besides its toxicity, there are other concerns with streptomycin, related to the logistical challenges of its parenteral mode of administration in rural Africa, and to the fact that mass production is expected to stop in the near future as it is no longer considered a first-line drug against tuberculosis. A promising alternative to streptomycin is clarithromycin. We studied its pharmacokinetics in chapter 4. A previous pharmacokinetic study in Buruli ulcer patients showed that serum clarithromycin levels were reduced considerably by rifampicin induced CYP3A4 metabolism. In addition, there was a modest increase of rifampicin. Therefore, we doubled the dosage of clarithromycin from 7.5mg/kg to 15mg/kg, and used an extended release formulation to be able to maintain a once daily dosing scheme. We obtained pharmacokinetic curves from 20 patients receiving clarithromycin and rifampicin, and from 10 patients receiving streptomycin and rifampicin, who served as controls. Samples were stored as dried blood spots – a new technique that offers logistical advantages for rural African settings, and were analyzed using a previously validated LC-MS/MS assay. All 30 patients healed without recurrence. The $C_{\text{max}}$ of clarithromycin was 0.4 mg/L and the area under the concentration-time curve was 3.9 mg *h/L. Compared to standard CLA dosed as 7.5 mg/kg in a previous study, the extended release clarithromycin resulted in a 58% decrease in $C_{\text{max}}$ and a 30% increase in area under the concentration-time curve. Clarithromycin co-administration did not alter the pharmacokinetics of rifampicin. These data suggest that extended release clarithromycin suffers more from rifampicin induced metabolism than regular clarithromycin. Therefore, the benefits of extended release clarithromycin over immediate release are questionable. As CLA is known to accumulate in tissues, microdialysis fluid or punch biopsy samples at the site of infection might yield high CLA concentrations that reflect its antimicrobial activity more accurately.

Among the other oral alternatives to streptomycin, moxifloxacin has shown good results in vitro and in vivo. In chapter 5, we discuss some of the advantages and
disadvantages of its use in Buruli ulcer. Some authors have suggested that it is a better choice than clarithromycin, as there is no interaction with rifampicin, and as it also has activity against *Mycobacterium tuberculosis*, and thus might be well suited to treat a *M. tuberculosis* and *M. ulcerans* co-infected patient. While it is true that it does not suffer from rifampicin induced CYP3A4 metabolism, it is relatively contra-indicated in children, which is the largest group of Buruli ulcer patients in West-Africa. In addition, as active tuberculosis is a predominantly urban disease, and Buruli ulcer a predominantly rural one, their co-occurrence is yet to be documented. We believe moxifloxacin should remain a second line drug, and that in the rare event of BU-TB co-infection, both diseases should be treated in concordance with local and international management guidelines.

During antimicrobial therapy for Buruli ulcer a paradoxical increase of the size of the lesion may occur. These paradoxical reactions are believed to be a sign of immune reconstitution following antimicrobial induced inhibition of mycolactone production. In chapter 6 we studied paradoxical reactions and their clinical and genetic associations. We found that in our cohort of Buruli ulcer patients from Ghana and Benin, paradoxical reactions occurred in 22% of patients during antimicrobial therapy. A larger lesion appears to increase the risk of a paradoxical reaction, as does the 3’UTR INS/INS polymorphism in the SLC11A1 gene, a gene associated with innate immunity to mycobacterial infections. Even though paradoxical reactions were frequent, all patients healed without additional interventions. These findings contrast the often advocated need for additional antibiotics, corticosteroids or surgery.

**Wound care**

As most Buruli ulcer patients in West-Africa have large, slow-healing ulcers, a high standard of wound care is essential. The WHO has issued guidelines on wound care for Buruli ulcer, but little is known about the implementation of these guidelines in current practice. In chapter 7, we assessed the current state of Buruli ulcer wound care in Ghana and Benin. We visited 6 hospitals and 8 health care centers in Ghana and Benin. There, we observed the wound care process, and interviewed 31 health care workers that regularly performed Buruli ulcer wound care. There appeared to be a general understanding of wound assessment, but formal assessment was not observed. A large variety of different topical antiseptics was used, even though a proper indication for their use was missing. Moderate pressure irrigation of the wound, as advised by the WHO, was never reported. With a few exceptions, gauze was the only available dressing type, and bleeding and pain were observed frequently upon its removal. The standard of wound care differed considerably between health care workers and between institutions. In addition to more expensive modern dressing materials, several low-cost interventions were identified that can be made to enhance the standard of wound care: using a simple system for classify-
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ing and monitoring the wound based on size and color, washing the area around the wound before dressing, using a syringe or pierced water bottle for moderate pressure irrigation of the wound, and avoiding the routine use topical antiseptics.

In chapter 8 we describe a pilot project where modern dressings were used for Buruli ulcer wound care. For this pilot, we chose HydroTac (Hartmann), a hydrofoam dressing. As it is a dressing that aims at maintaining a moist wound bed by being highly absorbent for exsudative wounds, and creating a moist environment through its gel component for dry red wounds, it appeared to be well suited for the various stages of Buruli ulcer. In addition, it was hypothesized that it could remain on the wound for a longer period of time than plain gauze, reducing staff and patient burden. A total of 13 patients were dressed for an average of 4 weeks with HydroTac, and the wounds were observed, photographed and measured during dressing changes. After four weeks, patients were also asked to rate both the gauze and the HydroTac and to comment on them. We found that the absorbent capacity of these dressings was too limited for highly exsudative Buruli ulcers, and dressings still had to be changed quite frequently. However, the dressings yielded clean and healing wounds, and they prevented the pain and bleeding associated with gauze dressings. In addition, they were very much preferred by the patients. Future studies with modern dressings in Buruli ulcer should select dressings with high absorbent capacity. In the context of the currently ongoing drug trial in Ghana and Benin, Drawtex (Beier), a highly absorbent fiber mesh dressing is being used.

In spite of good wound care, Buruli ulcers can become infected with other pathogens next to Mycobacterium ulcerans. Chapter 9 describes a study on these secondary infections. First, charts of 51 former Buruli ulcer patients in Ghana and Benin were studied and the occurrence of secondary infections, and the prescription of additional antibiotics next to rifampicin and clarithromycin was recorded. Forty out of 51 patients admitted to the hospital received additional antibiotic courses, in most cases as prophylaxis for secondary infections following surgical interventions. Only 12 patients received antibiotics for an actual suspected secondary infection. The second part of the study consisted of superficial cultures and antibiograms. Approximately 30% of wounds were culture negative. The bacteria most commonly found to have contaminated the wounds were S. aureus (of which 38% was MRSA), P. aeruginosa, and enterobacteriaceae. Based on these findings, we proposed a guideline for rational antibiotic treatment for suspected secondary infections or prophylaxis. In summary, this guideline proposes not to use prophylactic antibiotics following surgical procedures in BU other than skin grafts, where a single or double dose of flucloxacillin is the first regimen of choice. In addition, it proposes to only use antibiotics in patients with a suspected secondary infection that display systemic signs of illness, where a combination of amoxicilline/clavulanic acid with a fluoroquinolone is the first regimen of choice. Adherence to this guideline can have a major impact on antibiotic use in hospitalized Buruli ulcer patients and can lead to a reduction of costs, toxicity, and development of antimicrobial resistance.
Public health aspects

In chapter 10, we studied the impact of a community-based surveillance volunteer program on the early reporting of Buruli ulcer lesions. Although Buruli ulcer usually begins with a small and painless nodule, patients often present to the hospital with large ulcers, which puts them at risk for prolonged hospitalization, surgery, and persisting functional limitations. One potential approach to ensure that patients report to the hospital early is to establish a network of community-based surveillance volunteers. We studied the aspects and impact of a community-based surveillance volunteer program in a highly endemic district in Ghana. The program consisted of 44 volunteers, that received basic training in Buruli ulcer diagnosis, and that were provided with a small financial incentive for each laboratory confirmed Buruli ulcer case that they referred to the hospital. Between 2009 and 2013, 451 Buruli ulcer cases were seen at the clinic. Volunteers referred 203 (45%) of these cases, more cases than any other referral source. In addition, they referred significantly more cases in the early stages of the disease than any other referral source. As a result, a total of 70% percent of cases reported in the earliest – WHO Category I - stage of the disease, potentially minimizing the need for hospitalization or surgery.

In chapter 11, we reported the first data on therapy compliance in Buruli ulcer. We analyzed the Buruli ulcer case report forms at a Buruli ulcer treatment center in an endemic region in Ghana. These showed that therapy non-compliance was widespread, and that only 46% of patients completed the WHO-recommended 56 dosages of streptomycin and rifampicin. Non-compliance was significantly associated with self-referral, female gender, smaller lesions, and travel time. We attempted to follow-up on these non-compliers, and were able to locate 57 former BU patients. When asked for their reasons of defaulting, 35% mentioned travel costs, 19% stopped coming when their ulcer got healed, and 14% defaulted due to the ototoxic adverse effects of streptomycin. The future introduction of oral treatment for Buruli ulcer might reduce certain barriers to therapy compliance such as travel costs, toxicity, and discomfort. However, patients will no longer be required to report to a health center on a daily basis for streptomycin injections, and this might represent a challenge to therapy compliance in its own right. Therefore, when oral therapy is implemented, we argue that compliance should be systematically monitored.

Although it is often reported that Buruli ulcers can have serious sequelae, the quality of life of former Buruli ulcer patients has not been studied before. In chapter 12, we assessed the quality of life of healed Buruli ulcer patients that took part in a drug trial between 2006 and 2009, which only included small and early lesions. A total of 127 out of 151 patients were available for follow-up. All former patients aged 16 or older completed the Dermatology Life Quality Index, and the abbreviated World Health Organization Quality of Life scale (WHOQOL-BREF). The WHOQOL-BREF was administered to 82 matched healthy controls as well. Those younger than 16 completed the Children’s Dermatology
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Life Quality Index (CDLQI) only. Eighty-five percent of patients reported only a small or no effect of the disease on their current life according to the Dermatology Life Quality Index. In addition, good Quality of Life was also reported on the World Health Organisation Quality of Life Scale. On follow-up, we found that most scars were small and functional limitations were rare, and that both general and skin specific quality of life was good. We believe that these results demonstrate the potential of the combination of early detection and proper antibiotic treatment of Buruli ulcer. Public health efforts aimed at diagnosing the disease in its early stage and providing standardized treatment in endemic areas should therefore be intensified.