

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/278032511>

# Challenges Associated with Management of Buruli Ulcer/Human Immunodeficiency Virus Coinfection in a Treatment Center in Ghana: A Case Series Study

Article in *The American journal of tropical medicine and hygiene* · June 2015

DOI: 10.4269/ajtmh.14-0571

CITATIONS

3

READS

102

12 authors, including:



**Joseph Tuffour**

Ga West Municipal Hospital, Accra , Ghana

2 PUBLICATIONS 3 CITATIONS

SEE PROFILE



**Marie Therese Ruf**

Swiss Tropical and Public Health Institute

56 PUBLICATIONS 386 CITATIONS

SEE PROFILE



**Sammy Yaw Aboagye-Domena**

Noguchi Memorial Institute for Medical Research

30 PUBLICATIONS 100 CITATIONS

SEE PROFILE



**Grace Kpeli**

University of Health and Allied Sciences

29 PUBLICATIONS 71 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



On the Origins of Mycobacterium Ulcerans [View project](#)



Undertsnading MU Transmission [View project](#)

## Challenges Associated with Management of Buruli Ulcer/Human Immunodeficiency Virus Coinfection in a Treatment Center in Ghana: A Case Series Study

Joseph Tuffour, Evelyn Owusu-Mireku, Marie-Therese Ruf, Samuel Aboagye, Grace Kpeli, Victor Akuoku, Janet Pereko, Albert Paintsil, Kofi Bonney, William Ampofo, Gerd Pluschke, and Dorothy Yeboah-Manu\*

Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana; Ga West Municipal Hospital, Ghana Health Service, Amasaman, Ghana; Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Basel, Switzerland; University of Basel, Basel, Switzerland; Reconstructive and Plastic Surgery Unit, Korle-BU Teaching Hospital, Accra, Ghana

**Abstract.** The synergy between *Mycobacterium tuberculosis* infection and human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome is well established but not so in Buruli ulcer (BU). We screened confirmed BU cases for HIV infection and followed seven BU/HIV-coinfected patients. Management of BU/HIV was based on the World Health Organization guidelines and patient condition. The HIV positivity among BU patients (8.2%; 11/134) was higher compared with that of general patients attending the facility (4.8%; 718/14,863;  $P = 0.07$ ) and that of pregnant women alone (2.5%; 279/11,125;  $P = 0.001$ ). All seven BU/HIV-coinfected cases enrolled in the study presented with very large (category III) lesions with four having multiple lesions compared with 54.5% of category III lesions among HIV-negative BU patients. During the recommended BU treatment with streptomycin and rifampicin (SR) all patients developed immune infiltrates including CD4 T cells in their lesions. However, one patient who received antiretroviral therapy (ART) 1 week after beginning SR treatment developed four additional lesions during antibiotic treatment, while two out of the four who did not receive ART died. Further evidence is required to ascertain the most appropriate time to commence ART in relation to SR treatment to minimize paradoxical reactions.

### INTRODUCTION

Buruli ulcer (BU), a disease caused by *Mycobacterium ulcerans*, leads mainly to the destruction of skin tissues.<sup>1</sup> The disease is rarely fatal, but delayed treatment often results in contracture deformities because of the massive skin destruction by the cytotoxic macrolide, mycolactone.<sup>2,3</sup> The disease presents in two main active clinical forms: non-ulcerative (papule, nodule, plaque, and edema) and ulcerative. Severe forms include osteomyelitis and disseminated (or multifocal) lesions.<sup>1,4</sup> BU lesions are categorized based on World Health Organization (WHO) classification into category I, which consist of lesions with a size of < 5 cm at the widest diameter; category II, which consist of lesions with a size between 5 and 15 cm at the widest diameter; and category III, which consist of lesions with a size > 15 cm at the widest diameter, lesions at critical sites and multiple lesions.<sup>1</sup> The WHO recommended first-line treatment of BU is daily injection with streptomycin (SR) and oral rifampicin for 8 weeks, and if necessary, surgery either to improve healing and/or to correct deformities.<sup>1,5,6</sup> The efficacy of this treatment regimen has been confirmed in several studies.<sup>7–9</sup> Active lesions present with large focal clusters of extracellular acid-fast bacilli (AFB) and only minor leukocyte infiltration.<sup>10,11</sup> Antimicrobial therapy leads to massive leukocyte infiltration, which culminates in the development of ectopic lymphoid structures in the lesions.<sup>12</sup>

Some studies have reported paradoxical reactions in BU patients, which is defined as an increase in lesion size of > 100% after initial improvement, and/or the appearance of a new lesion(s) following or during antimycobacterial treatment.<sup>13,14</sup> Currently, it is not clear whether immune reconstitution inflammatory syndrome (IRIS)-like mechanisms, secondary infections,<sup>15</sup> or other mechanisms are primarily

responsible for impaired wound healing and deterioration of lesions during and after SR treatment in some of the BU patients. A retrospective study conducted in Cameroon revealed that human immunodeficiency virus (HIV) infection may affect the clinical presentation and severity of BU disease with a reported increased incidence of multiple, larger, and ulcerated BU lesions.<sup>16–18</sup> Data available on the absorption of antituberculosis medications in tuberculosis (TB)/HIV coinfection compared with HIV-negative patients showed conflicting results.<sup>19,20</sup> Therefore, whether antimycobacterial combination treatment is less efficacious in persons with HIV infection is unknown and needs to be systematically studied.

The associations between HIV infection and some other infectious diseases are clearly defined, but that between BU and HIV is not fully understood.<sup>21</sup> It is well known that HIV infection is fueling the global TB epidemic and the convergence of the TB and the HIV epidemics pose new public health challenges.<sup>22</sup> The interaction between HIV and TB in coinfecting persons is bidirectional and synergistic; on one hand, HIV infection predisposes to the development of active TB, on the other hand the course of HIV-related immunodeficiency is worsened by active TB infection.<sup>22,23</sup> Although it is clearly known that HIV/acquired immunodeficiency syndrome (AIDS), which leads to reduced CD4 helper T-cell activity, is a risk factor for TB, immune protection mechanisms in BU disease are not fully understood.<sup>24</sup> However, like in other mycobacterial diseases, adaptive immune responses championed by CD4<sup>+</sup> activation of macrophages are presumably also crucial for protection against BU. Although large clusters of toxin-producing *M. ulcerans* bacteria are found in established BU lesions, there are indications that multiplication of the pathogen in phagocytes plays a role in the early steps of the infection.<sup>25,26</sup> Furthermore, BU disease leads to reduced interferon (IFN)- $\gamma$  release.<sup>27</sup>

Management of HIV/AIDS over the years has seen progressive improvement in drug therapy and clearer guidelines, which has dramatically decreased mortality and incidence of AIDS-defining opportunistic infections.<sup>28,29</sup> Despite this breakthrough

\*Address correspondence to Dorothy Yeboah-Manu, Bacteriology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, P.O. Box LG581, Legon, Accra, Ghana. E-mail: dyeboah-manu@noguchi.ug.edu.gh

in management, there is still the challenge of a paradoxical IRIS in HIV patients on antiretroviral therapy (ART), which is a phenomenon that has been defined to be a new type B or C AIDS-defining condition or emergence of a range of mucocutaneous or autoimmune conditions diagnosed within 180 days of starting ART with a corresponding CD4 response.<sup>30</sup> In the presence of a treated or ongoing opportunistic infection such as TB, such paradoxical IRIS could occur in which case it would be defined as 1) a new, worsening or recurrent sign or symptom consistent with an exaggerated or atypical inflammatory reaction to the previously diagnosed opportunistic infection, 2) the exclusion of medication toxicity or other disease processes as the cause of the abnormal event, and 3) a supportive evidence be it by some specialized imaging or histopathology.<sup>31</sup> The combined effect of the IRIS associated with HIV patients on ART<sup>30</sup> and the paradoxical reaction experienced in BU patients on antimicrobial treatment<sup>13,14</sup> could possibly pose a challenge to the management of BU/HIV coinfection.

Anemia is common among sub-Saharan Africans with BU.<sup>32</sup> Susceptibility to BU is associated with polymorphism in the gene for the iron transporter protein NRAMP1.<sup>33</sup> Models exist to explain iron deficiency anemia in mycobacterial diseases such as BU. Notable among them suggests sequestration of Fe<sup>2+</sup> from the body into phagosomes and the lack of NRAMP1 to export the iron back, as the possible cause of the anemia,<sup>34</sup> which could be worsened with an HIV coinfection depending on the clinical stage and state of immunity.<sup>35,36</sup>

This study compares the prevalence of HIV infection among confirmed BU patients at a district hospital in Ghana with the general population of patients in that same facility and also describes BU/HIV coinfection cases highlighting the challenges associated with the management of BU/HIV coinfection.

## MATERIALS AND METHODS

**Ethical statement.** Ethical clearance for the study was obtained from the institutional review board of the Noguchi Memorial Institute for Medical Research (NMIMR) (Federal-wide Assurance number FWA00001824). All study participants were well informed of the study objectives and written informed consent was obtained either from the patient or from the guardian of the patient. Sampling and laboratory confirmation for both *M. ulcerans* and HIV infection followed the national approved procedure. All confirmed cases were referred for appropriate treatment of BU and HIV/AIDS.

**Study participants.** The participants involved in the study were passively recruited from the Ga West Municipal Hospital in Amasaman, Ghana, from October 2009 to March 2013; one clinician at the health facility who made the final clinical diagnostic decision reviewed all patients in this study. A participant was included in this study if he/she met the WHO clinical definition for the different BU lesions and was positive for at least IS2404 polymerase chain reaction (PCR) as previously described.<sup>37</sup> Basic demographic data and clinical history of cases were recorded by adapting the BU01 form of the WHO. Lesions were categorized according to the WHO classification as previously described.<sup>1</sup> In addition lesions were classified either as ulcer, edema, nodule, or papule as well as single or multiple lesions.<sup>2</sup> Patients were screened for HIV infection by collecting blood samples after counseling and consent has been sought. Two immunochromatographic (lateral flow) strip-based rapid test kits, OraQuick (OraSure,

Bethlehem, PA) and/or First Response HIV 1-2.0 Card Test (PMC Medical Pvt. Ltd., Daman, India), were used for initial screening, and samples that tested positive were confirmed by the Inno-Lia HIV I/II immunoblot assay (Immunogenetics, Gent, Belgium).<sup>38</sup>

**BU confirmation.** Swabs were collected from the undermined edges of ulcerative lesions and fine needle aspirates were collected from cases with non-ulcerative lesions for bacteriological confirmation of BU disease.<sup>39,40</sup> All samples were analyzed by IS2404 quantitative PCR, microscopy and culture. For those patients presenting with multiple lesions, samples were collected from each lesion. For patients who underwent surgery, tissue biopsies were taken for further microbiological analyses at NMIMR and histopathology at Swiss Tropical and Public Health Institute.

**Treatment and monitoring.** The patients were treated with daily SR (15 mg/kg/day intramuscular injection) and rifampicin (10 mg/kg/day orally) according to the WHO protocol for duration of 8 weeks.<sup>8</sup>

Patients were questioned about side effects from the antibiotic treatment at each biweekly clinical assessment and asked to report any problems to the health center between periodic reviews. Surgical debridement was done 4 weeks after antibiotic treatment of BU for some of the lesions. Biopsy samples from the wound edges were sent for culture and histopathology. Any septic wound diagnosed clinically was confirmed by culture and sensitivity testing using swab samples and was treated accordingly. Biweekly wound assessment was done involving wound measurement and photography using a wound imaging, measurement, and documentation device, ARANZ Medical Silhouette Mobile™ (ARANZ Medical, Christchurch, New Zealand).

Standard moist wound management practices were conducted, which involved saline dressing and covering of the floor of the ulcers with Vaseline gauze.<sup>41</sup> Frequency of dressing change was individualized according to the characteristics of the ulcer. Following initial wound excision after antibiotic treatment, surgical debridement and skin grafting subsequently done was tailored to the need of each patient and on the discretion of the attending surgeon.

New lesions occurring during the course of therapy or follow-up period were closely examined and analyzed by culture and in certain cases by histopathology.<sup>13,14</sup>

**Laboratory and radiological investigations.** Baseline complete blood count, liver function test, blood urea electrolytes and creatinine, erythrocyte sedimentation rate, fasting blood sugar and sickling test with/without Hb electrophoresis as indicated were done for all patients. Unless clinically indicated earlier for a patient, complete blood count, liver function test, and blood urea electrolytes and creatinine tests were routinely repeated 8 weeks to ascertain any renal or hepatic toxicity and to assess the hemoglobin levels. Baseline CD4 count was done and repeated at 6-month intervals for only two patients until the wound was completely healed and patient discharged. X-rays of the limbs with the ulcers were done for all patients as part of baseline investigations to rule out osteomyelitis associated with BU/HIV coinfection. TB was ruled out in all the patients clinically and by means of normal chest X-rays. Computerized tomography scan of the head was done for one patient who developed some neurological deficits during wound management to rule out any space occupying lesions most especially cerebral toxoplasmosis.

**Histopathology.** Histopathological analysis was done for all surgical debridement and excisions. Surgically excised tissue samples were immediately fixed in 10% neutral-buffered formalin for 24 hours at room temperature to maintain tissue structures. Afterward samples were directly transferred to 70% ethanol for storage and transport. Tissue specimens were subsequently dehydrated, embedded into paraffin, and cut into 5  $\mu$ m sections. After deparaffinization and rehydration, sections were stained with Ziehl–Neelsen (ZN)/methylene blue, hematoxylin or by immunohistochemistry according to WHO standard protocols.<sup>42</sup> The following antibodies were used for T-cell staining (CD3, Dako; CD4, cell marque; CD8, Serotec) and B-cell staining (CD20, Dako). Tissue sections were analyzed with a Leica DM2500 Microscope (Wetzlar, Germany). Pictures were either taken with a Leica DFC 420C camera or with an Aperio ScanScope XT.

**Data analysis.** All data were entered into Microsoft Excel package and verified before exporting into the statistical package Stata (Release 12; Stata Corporation, College Station, TX) that was used to perform all the statistical analyses. The  $\chi^2$  tests at 95% confidence (CI) level was used to compare HIV prevalence among BU patients and HIV in the general hospital attendants and also among pregnant women screened at the same health facility.

## RESULTS

### Study participants and prevalence of HIV/BU coinfection.

All PCR-confirmed BU patients (67 males and females each) were screened for HIV at the Ga West Municipal Hospital. Their age range was between 3 and 86 years with a mean age of 33.8 years (standard deviation [SD]: 21.6); 36 (26.8%) were  $\leq$  15 years. Eleven (8.2%) of the cases (5 females and 6 males) were HIV positive. During the period, 14,863 individuals were tested for HIV infection in the same facility and the positivity rate was 4.8% (718/14,863) though lower was not significant,  $P = 0.070$ . On the other hand prevalence was significantly lower ( $P$  value = 0.001) among pregnant women using the preventing mother to child transmission (PMTCT) facility (2.5%; 279/11,125). The age range of the HIV-positive BU cases was between 12 and 65 years with a mean age of 37.1 years (SD: 13.1). Only one of the 11 HIV-positive BU cases was a child (aged 12), the remaining 10, were between 29 and 65 years of age. Seven out of the 11 HIV/BU coinfecting cases were followed during treatment and Table 1 shows the baseline characteristics of these seven study participants. The other four patients were lost to follow-up. All the seven patients went through some form of traditional or herbal treatment of the ulcers with duration of ulcers before antibiotic therapy ranging from 4 to 24 months. Four of them were males and three were females. The ages were from 12–46 years with a mean age of 33.1 years (SD: 11.7 years). All seven patients presented with category III lesions with six presenting with lower limb lesions and one presenting with an upper limb lesion. In comparison, 67/123 (54.47%) HIV-negative BU patients presented with category III lesions. One of the BU/HIV co-infected patients presented with an ulcer and a plaque not yet ulcerated at presentation, whereas the others had ulcerated forms only at presentation. Four had multiple lesions at presentation whereas three had single lesions. *M. ulcerans* infection of all seven patients was reconfirmed by IS2404 PCR. In addition, 6/7 were reconfirmed by microscopic

TABLE 1

Demographics, clinical features and diagnostic data of study participants with BU and HIV coinfection

Patients parameters	Values
Total no.	7
Sex (no. of males/females)	4/3
Median (range) age in years	33.7 (12–46)
No. (%) with indicated site of lesion	
Upper limb	1 (14.2)
Lower limb	6 (85.7)
No. (%) with indicated clinical form of lesion	
Ulcer	6 (85.7)
Plaque and ulcer	1 (14.2)
No. (%) with indicated category of lesion	
III	7 (100)
No. (%) with indicated number of lesions	
Single	3 (42.8)
Multiple	4 (57.1)
No. with indicated laboratory confirmation results	
<i>Mycobacterium ulcerans</i> culture ( $\pm$ )	3/4
PCR for IS2404 ( $\pm$ )	7/0

BU = Buruli ulcer; HIV = human immunodeficiency virus; PCR = polymerase chain reaction.

detection of AFBs after ZN staining and 3/7 were confirmed by positive *M. ulcerans* culture.

The mean hemoglobin level (Table 2) at baseline was 8.7 g/dL (SD: 2.5). One of the patients had a normal hemoglobin level at presentation but worsening anemia with new lesions formed. Median CD4 counts at baseline (Table 2) were 318 cells/mm<sup>3</sup> (interquartile range [IQR]: 265–750 cells/mm<sup>3</sup>). Two patients who were at WHO clinical stage 2 of HIV infection at baseline had 6 monthly CD4 count repeated until study end point. Of these two patients, one started ART 1 week after beginning of SR treatment and the other started ART 1 week after completing SR treatment. The CD4 counts (Table 2) measured after 6 months dropped to 185 cells/mm<sup>3</sup> from a baseline of 298 cells/mm<sup>3</sup> (for patient starting ART 1 week after SR started), which then improved to 586 cells/mm<sup>3</sup> at 1 year, whereas the second patient showed a steady rise in the CD4 count from baseline.

**Duration of SR treatment.** The duration of SR treatment was 8 weeks according to WHO protocol. However, three patients had treatment extended to 12 weeks upon the discretion of the clinician, whereas one patient had SR changed after 26 days to rifampicin–amikacin for an additional 30 days because of suspected adverse reaction to SR. One patient (case 5) died after 40 days on SR treatment.

**Time of starting ART in relation to SR treatment.** Of the three patients (cases 1, 2, and 3) who took ART during wound management, one (case 2) commenced 1 week after starting SR, another (case 3) started 1 week after completing SR, and the other (case 1) started 13 weeks after completing SR. The type of ART combination given is as shown in Table 3. Two (cases 4 and 5) of the remaining four patients died and the other two (cases 6 and 7) were referred to ART centers away from the center of the study after wounds had healed with no recurrence after 6 months of follow-up, because no ART center was established at the district hospital at the time of their management.

Figure 1 shows immune infiltrates observed in one patient (case 2) who developed four new lesions with excessive exudates at different sites of the same limb. One at the anteromedial aspect of the proximal third of the right limb, the other on the right lateral aspect of the thigh, and the other

TABLE 2  
CD4 counts and Hb level monitoring of the seven enrolled patients at 8-week intervals

BU case	WK 0		WK 8		WK 16		WK 24		WK 32		WK 40		WK 48	
	CD4	Hb (g/dL)	CD4	Hb (g/dL)	CD4	Hb (g/dL)	CD4	Hb	CD4	Hb (g/dL)	CD4	Hb (g/dL)	CD4	Hb (g/dL)
1	318	8.8	–	8.2	–	10.5	–	–	–	–	–	–	–	–
2	298	11.4	–	7.9	–	7.0	185	8.2	–	8.2	–	9.9	558	11
3	265	7	–	11.8	–	10.2	394	8.0	–	7.2	–	9.2	455	8.7
4	37	9.5	–	8.2	–	–	–	–	–	–	–	–	–	–
5	791	5.0	–	6.4	–	6.2	–	8.2	–	–	–	–	–	–
6	730	12.0	–	11.8	–	12.1	–	–	–	–	–	–	–	–
7	751	7.1	–	10.7	–	9.6	–	–	–	–	–	–	–	–

BU = Buruli ulcer; Hb = hemoglobin; WK = week.

two developed on the medial aspect of the knee and the dorsum of the right foot. The onset of the infiltration started about 2 weeks after starting the SR treatment. The other lesions developed following the administration of additional SR treatment after completion of the 8 weeks' standard regimen. The new lesions were managed by saline dressing and required no further antibiotic treatment.

**Wound healing rate.** We designated a wound healed when complete (100%) epithelialization of the ulcer had occurred. The study adopted an endpoint to healing at 52 weeks at which point all ulcers that were not 100% epithelialized were censored or classified as failing to heal.<sup>43</sup> The healing rate at the study endpoint was 42.9% (3/7) with a median time to healing of 37 weeks (IQR: 36–37) (Table 3). Two patients (cases 4 and 5) died within the study period and two (cases 2 and 3) had still not healed completely 56 and 64 weeks after start of treatment, respectively. The three patients (cases 1, 6, and 7) that healed had excision and skin grafting done. One of the nonhealing wounds (case 3) had the graft breaking down leading to a recurrent ulcer after 40 weeks of complete epithelialization and discharge from the hospital. *M. ulcerans* culture of tissue samples was negative ruling out relapse and the reopening was probably due to poor scar care.

**Development of immune infiltrates.** For histopathological analysis tissue samples were collected after completion of at least the standard 8 weeks of SR treatment. All samples still presented with BU typical histopathological features, such as epidermal hyperplasia, fat cell ghosts, and some remaining

tissue necrosis (Figure 2A, B, and E). In addition, all samples showed immune infiltration, blood vessel and granuloma formation (Figure 2A, C, and D), as is commonly observed in antibiotic-treated lesions of HIV-negative BU patients.<sup>12</sup> Mixed infiltrates containing large numbers of CD3 positive T cells were found mainly in the dermis and subcutaneous tissue layer. Granulomas and clusters of CD20 positive B cells were embedded in the mixed unstructured infiltrates. Granulomas were mainly formed by macrophages, giant cells, T cells, and B cells (Figure 2F–I). In all lesions the infiltration contained CD4 positive T cells, even when CD4 blood counts (Table 2) were low. However, in 4/6 patients analyzed, CD8<sup>+</sup> T cells outnumbered CD4<sup>+</sup> cells. Larger numbers of N-elastase-positive neutrophils were only observed in association with secondary bacterial infections. AFBs were detected in only 2/6 analyzed tissue samples as scattered beaded bacteria.

## DISCUSSION

Little is known about the impact of HIV infection on susceptibility to *M. ulcerans* infection and BU treatment outcomes such as cure, recurrence, long-term disability, and the incidence of paradoxical reactions secondary to antibiotic treatment. In a study conducted in 426 BU patients and 613 controls in southern Benin,<sup>44</sup> a significantly higher HIV prevalence was observed among BU patients than in controls (2.6% versus 0.3%;  $P = 0.003$ ). Although in this study, the HIV prevalence among BU patients (8.2%) was higher than that (4.8%)

TABLE 3  
Laboratory confirmation, HIV clinical stage, and treatment characteristics of the seven patients enrolled

BU case	BU confirmation	Retro serotype/HIV clinical stage	SR treatment	HIV treatment	Treatment outcome	Time to complete wound healing
1	PCR	HIV 1 Stage 2	SR 8	Tenofovir, lamivudine, efavirenz + co-trimoxazole	Wound healed with skin grafting	37 weeks
2	PCR, ZN	HIV 1 Stage 2	SR 12	Tenofovir, lamivudine, nevirapine + co-trimoxazole	Still dressing wound at 64 weeks of treatment	Failed to heal
3	PCR, ZN	HIV 1 and 2 Stage 2	SR 12	Tenofovir, lamivudine, efavirenz + co-trimoxazole	Wound reopened after 40 weeks of discharge still dressing wound at 52 weeks	Reopened leg ulcer
4	PCR, ZN	HIV 1 Stage 4	SR 8	Co-trimoxazole	Patient died of deterioration of disease	Censored
5	PCR, ZN	HIV 1 Stage 4	SR 8	Co-trimoxazole	Patient died of severe <i>Staphylococcus aureus</i> septicemia and severe anemia 26 weeks after completion of treatment	Censored
6	PCR, ZN	HIV 1 Stage 2	SR 8	Co-trimoxazole	Wound healed after skin grafting	37 weeks
7	PCR, ZN	HIV 1 Stage 2	SR 12	Co-trimoxazole	Wound healed after skin grafting	36 weeks

BU = Buruli ulcer; HIV = human immunodeficiency virus; PCR = polymerase chain reaction; SR = streptomycin; ZN = Ziehl-Neelsen.

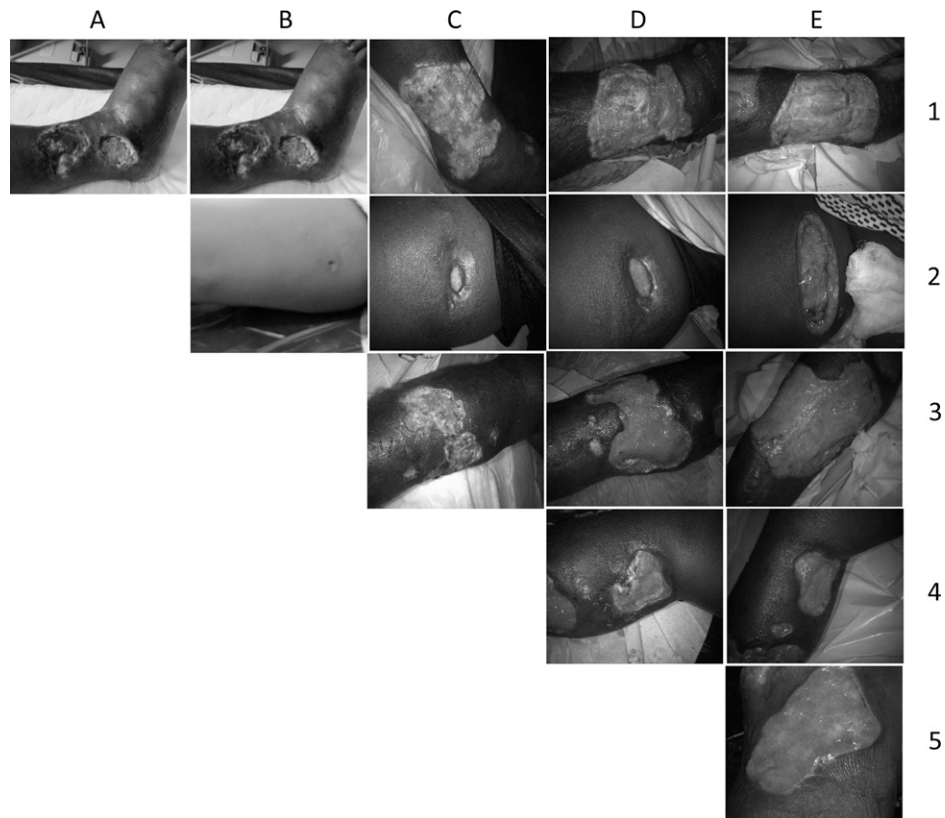


FIGURE 1. Emergence of new lesions during streptomycin (SR) treatment in one of the enrolled coinfecting patients (case 2). (A) Features of the first lesion before the start of SR treatment, (B) appearance of a new lesion after 2 weeks of antibiotic treatment, (C) appearance of a third lesion after 4 weeks of SR treatment, (D) appearance of a fourth lesion after 6 weeks of SR treatment, and (E) increase in wound sizes after surgical excision and appearance of a fifth lesion after start of the SR treatment. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

among general patients attending the same health facility, this difference was not statistically significant ( $P$  value = 0.070). In contrast, a significant difference ( $P$  value = 0.001) was found with the HIV prevalence of 2.5% among pregnant women attending the same health facility during the same period. The cases that were followed had varying disease presentation, responses to treatment as well as treatment outcomes. All seven BU/HIV coinfecting cases included in the study presented with category III lesions as compared with 54.47% of category III lesions in HIV-negative BU cases. Severity of BU disease did not necessarily reflect the level of underlying immune suppression especially when using CD4 as the marker, as a case with CD4 counts below 300 had no multifocal disease, while another case with CD4 counts above 500 developed multiple lesions. One of the BU/HIV coinfecting patients developed a chronic osteomyelitis (case 4). This patient was severely immunosuppressed, as evidenced by a CD4 count of 37 and clinical stage 4 HIV disease. Since osteomyelitis is also occurring in HIV-negative patients, further studies are required to establish whether a severe immune suppressed state increases the risk for developing osteomyelitis. Although CD4 blood counts were reduced in the six patients analyzed by histopathology (unfortunately no tissue sample became available from the patient with the very low CD4 blood count of 37), substantial numbers of CD4 positive T cells were found in mixed infiltrates and granulomas emerging in the treated BU lesions. However, CD8 T cells outnumbered CD4 T cells in 4/6 patients (Figure 2H and I).

The HIV coinfecting BU cases presented here, showed mild to moderate anemia at admission but severe anemia concomitant with new lesions in one patient (case 2). Anemia has been found to be present at varying degrees in HIV patients, correlating with the clinical stage and state of immunity of the patient.<sup>35,36</sup> However within the cases followed, we found severe anemia as defined by the CDC even though the CD4 count of the patient was above 500. Literature also supports that some mycobacteria such as *M. tuberculosis* and *M. avium* complex can cause peripheral blood cytopenia in HIV patients but no such documentation with *M. ulcerans* is currently available.<sup>45</sup> Persistent anemia resulting from a BU/HIV coinfection could account for the delayed healing of wounds (median time to wound healing being 37 weeks) of the cases presented here.

One of the BU-HIV coinfecting cases (case 2) developed new lesions 2 weeks after starting SR treatment, coinciding with 1 week after starting ART. Whether this occurrence was a result of the relative early onset of the ART with SR treatment is unclear. The appropriate time to start ART in HIV patients with opportunistic infection has always been a dilemma to clinicians since ART can trigger severe IRIS-like reactions when it is commenced early. On the other hand, delaying treatment could similarly lead to worsening of the disease condition and prognoses. With TB/HIV coinfection, it is recommended that ART should be started 2 weeks into antimicrobial treatment<sup>46</sup>; however, based on a large randomized controlled trial it is recommended to delay ART till

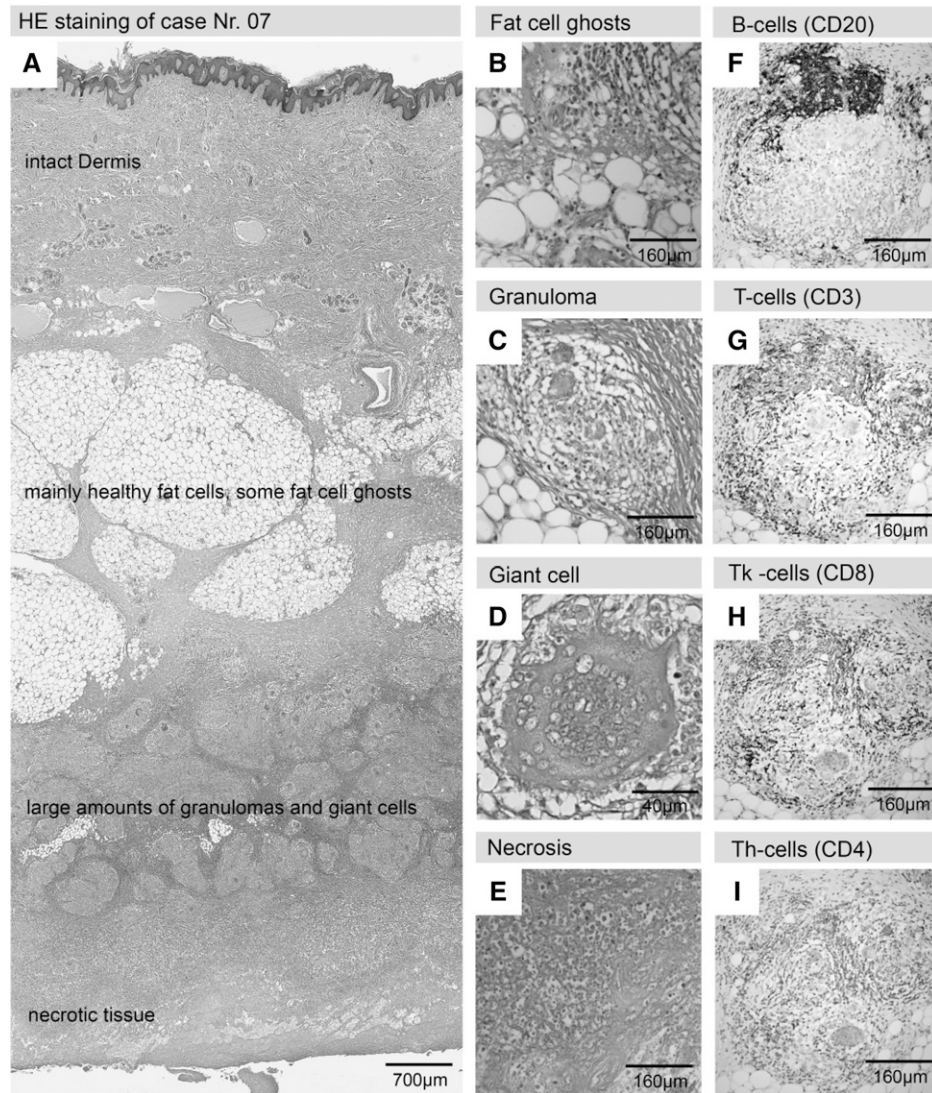


FIGURE 2. Emergence of immune infiltrates in the Buruli ulcer (BU) lesion during SR treatment in a representative patient (case 7). Histological sections were stained either with hematoxylin–eosin (A–E), or with antibodies against CD20 (F), CD3 (G), CD8 (H), or CD4 (I) (counterstain hematoxylin). (A) cross section through a BU lesion presenting with epidermal hyperplasia, an intact dermis, healthy fat cells as well as fat cell ghosts, a layer of numerous well-defined granulomas, and a necrotic area. Fat cell ghosts (B), granulomas (C), giant cells (D), and necrosis (E) are shown at higher magnification. Immunohistochemical analysis of the granulomas showed the presence of B-cell clusters (F) as well as the presence of large numbers of CD3, CD4, and CD8 positive T cells (G, H, and I). This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

5 weeks into antimicrobial treatment when managing HIV/*Cryptococcus* coinfection.<sup>47</sup> Hence, the optimal time to starting ART may depend on the specific coinfection and known interactions of some ART with antimicrobial agents. Studies have shown that taking ART containing nevirapine alongside SR treatment leads to a decreased  $C_{max}$  and  $C_{min}$  of nevirapine due to interaction with rifampicin.<sup>48–50</sup> This notwithstanding, early onset of ART and co-trimoxazole prophylaxis is highly recommended by WHO preliminary guidelines for management of HIV/BU coinfection<sup>51</sup> to build up immunity and to fight opportunistic infections associated with HIV as these could worsen the prognosis of the condition. Here 4/7 BU/HIV coinfecting patients did not start with ART treatment within the study period because of unavailability of an ART center within or close to the study center. Since HIV testing has become a standard element in BU management, access to an ART center should be secured as a part of BU care. The 2/7

study participants who died within the study period as a result of worsening disease were not started on ART. Possibly, such mortality could have been averted by early onset of ART.

The duration of SR treatment of 3/7 of the HIV/BU coinfecting cases studied here was extended based on the judgment of the responsible clinicians to 12 weeks, since deterioration of the lesions suggested *M. ulcerans* ongoing disease activity after completion of the standard 8 weeks of treatment. However, no laboratory confirmation for the presence of viable *M. ulcerans* bacteria before the extension of the antibiotic treatment was available.

## CONCLUSION

Despite the limitations of the small sample size, the difficulties in assessing the immunological statuses of some participants, as well as ascertaining the viral loads of the patients,

results of this study indicate that HIV coinfection could predispose BU patients to the development of more severe clinical forms (large and multiple lesions) and delayed wound healing. Although early onset of ART in BU/HIV coinfection is recommended, systematic studies are required to develop detailed guidelines for the management of BU/HIV coinfecting patients as there are for TB and *Cryptococcus*/HIV coinfections. Further studies would be required to determine the cumulative effect of the IRIS and paradoxical reactions in BU/HIV coinfecting patients on ART and SR treatments.

Received September 12, 2014. Accepted for publication March 13, 2015.

**Acknowledgments:** We acknowledge the nurses as the BU ward of the Ga-West Municipal Hospital and all study participants and caregivers for their time. We are also grateful to Kingsley Asiedu and Daniel O'Brien for reading through the article.

**Financial support:** The Stop Buruli Consortium funded by UBS Optimus Foundation provided funding for this study.

**Authors' addresses:** Joseph Tuffour, Victor Akuoku, and Janet Pereko, Ga West Municipal Hospital, Ghana Health Service, Buruli Ward, Amasaman, Ghana, E-mails: josephtuffour@rocketmail.com, vaksgh@yahoo.com, and jpereko@yahoo.com. Evelyn Owusu-Mireku, Samuel Aboagye, Grace Kpeli, Kofi Bonney, William Ampofo, and Dorothy Yeboah-Manu, Bacteriology Department, Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana, E-mails: nanaamaowusumireku@yahoo.com, saboagye@noguchi.ug.edu.gh, gkpelin@noguchi.ug.edu.gh, kbonney@noguchi.ug.edu.gh, wampofo@noguchi.og.edu.gh, and dyeboah-manu@noguchi.mimcom.org. Marie-Therese Ruf and Gerd Pluschke, Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland, E-mails: therese.ruf@unibas.ch and gerd.pluschke@unibas.ch. Albert Paintsil, Korle-BU Teaching Hospital, Reconstructive and Plastic Surgery Unit, Accra, Ghana, E-mail: albert@paintsil.com.

## REFERENCES

- Sizaire V, Nackers F, Comte E, Portaels F, 2006. *Mycobacterium ulcerans* infection: control, diagnosis and treatment. *Lancet Infect Dis* 6: 288–296.
- Van der Werf TS, Van der Graaf WTA, Tappero JW, Asiedu K, 1999. *Mycobacterium ulcerans* infection. *Lancet* 353: 1013–1101.
- Agbenorku P, 2014. Buruli ulcer disability in Ghana: the problems and solutions. *BJMMR* 4: 1355–1365.
- Pszolla N, Sarkar RM, Strecker W, Kern P, Kinzl L, 2003. Buruli ulcer: a systemic disease. *Clin Infect Dis* 37: 78–82.
- Converse PJ, Nuermberger EL, Almeida DV, Grosset JH, 2011. Treating *Mycobacterium ulcerans* disease (Buruli ulcer): from surgery to antibiotics, is the pill mightier than the knife? *Future Microbiol* 6: 1185–1198.
- O'Brien DP, Comte E, Serafi M, Ehounou G, Antierens A, 2013. The urgent need for clinical, diagnostic, and operational research for management of Buruli ulcer in Africa. *Lancet Infect Dis* 14: 435–440.
- Etuaful S, Carbone B, Lucas S, Horsefield C, Philips R, 2005. Efficacy of the combination rifampin-streptomycin in preventing growth of *Mycobacterium ulcerans* in early lesions of Buruli ulcer in humans. *Antimicrob Agents Chemother* 49: 3182–3186.
- Chauty A, Ardant MF, Adeye A, Euverte H, Guedenon A, 2007. Promising clinical efficacy of streptomycin-rifampin combination for treatment of Buruli ulcer (*Mycobacterium ulcerans* disease). *Antimicrob Agents Chemother* 51: 4029–4035.
- Sarfo FS, Philips R, Asiedu K, Ampadu E, Bobi N, 2010. Clinical efficacy of rifampin and streptomycin for treatment of *Mycobacterium ulcerans* disease. *Antimicrob Agents Chemother* 54: 3678–3685.
- Adusumilli S, Mve-Obiang A, Sparer T, Meyers W, Hayman J, 2005. *Mycobacterium ulcerans* toxic macrolide, mycolactone modulates the host immune response and cellular location of *M. ulcerans* in vitro and in vivo. *Cell Microbiol* 7: 1295–1304.
- Peduzzi E, Groeper C, Schütte D, Zajac P, Rondini S, 2007. Local activation of the innate immune system in Buruli ulcer lesions. *J Invest Dermatol* 12: 638–645.
- Schütte D, Um-Boock A, Mensah-Quainoo E, Itin P, Schmid P, 2007. Development of highly organized lymphoid structures in Buruli ulcer lesions after treatment with rifampicin and streptomycin. *PLoS Negl Trop Dis* 1: e2.
- Ruf MT, Chauty A, Adeye A, Ardant MF, Koussemou H, 2011. Secondary Buruli ulcer skin lesions emerging several months after completion of chemotherapy: paradoxical reaction or evidence of immune protection? *PLoS Negl Trop Dis* 5: e1252.
- Wanda F, Nkemenang P, Ehounou G, Tchaton M, Comte E, 2014. Clinical features and management of a severe paradoxical reaction associated with combined treatment of Buruli ulcer and HIV co-infection. *BMC Infect Dis* 14: 423.
- Yeboah-Manu D, Kpeli GS, Ruf M-T, Asan-Ampah K, Quenin-Fosu K, 2013. Secondary bacterial infections of Buruli ulcer lesions before and after chemotherapy with streptomycin and rifampicin. *PLoS Negl Trop Dis* 7: e2191.
- Christinet V, Comte E, Ciaffi L, Odermatt P, Serafini M, Antierens A, Rossel L, Nomo AB, Nkemenang P, Tsoungui A, Delhumeau C, Calmy A, 2014. Impact of HIV on the severity of Buruli ulcer disease: results of a retrospective study in Cameroon. *Open Forum Infect Dis* 1: ofu021.
- Toll A, Gallardo F, Ferran M, Gilaberte M, Iglesias M, 2005. Aggressive multifocal Buruli ulcer with associated osteomyelitis in an HIV-positive patient. *Clin Exp Dermatol* 30: 649–651.
- Komenan K, Elidjé EJ, Ildevert GP, Yao KI, Kanga K, Kouame KA, Abdoulaye S, Hamdam KS, Yao YP, Jean-Marie K, 2013. Multifocal Buruli ulcer associated with secondary infection in HIV positive patient. *Case Rep Med* 2013: 348628.
- Peloquin CA, Nitta AT, Burman WJ, Brudney KF, Miranda-Massari JR, McGuinness MF, Berning SE, Gerana GT, 1996. Low antituberculosis drug concentrations in patients with AIDS. *Ann Pharmacother* 30: 919–925.
- Taylor J, Smith PJ, 1998. Does AIDS impair the absorption of antituberculosis agents? *Int J Tuberc Lung Dis* 2: 670–675.
- Girma M, Teshome W, Petros B, Endeshaw T, 2014. Cryptosporidiosis and isosporiasis among HIV-positive individuals in south Ethiopia: a cross sectional study. *BMC Infect Dis* 14: 100.
- Shastri S, Naik B, Anita Shet A, Bharat Rewari B, De Costa A, 2013. TB treatment outcomes among TB-HIV co-infections in Karnataka, India: how do these compare with non-HIV tuberculosis outcomes in the province? *BMC Public Health* 13: 838.
- Havir DV, Getahun H, Sanne I, Nunn P, 2008. Opportunities and challenges for HIV care in overlapping HIV and TB epidemics. *JAMA* 300: 423–430.
- Jones BE, Young SM, Antoniskis D, Davidson PT, Kramer F, Barnes PF, 1993. Relationship of the manifestations of tuberculosis to CD4 cell counts in patients with human immunodeficiency virus infection. *Am Rev Respir Dis* 148: 1292–1297.
- Torrado E, Fraga AG, Castro AG, Stragier P, Mayers WM, 2006. Evidence for intramacrophage growth phase of *Mycobacterium ulcerans*. *Infect Immun* 75: 977–987.
- Oliveira MS, Fraga AG, Torrado E, Castro AG, Pereira JP, 2005. Infection with *Mycobacterium ulcerans* induces persistent inflammatory response in mice. *Infect Immun* 73: 6299–6310.
- Yeboah-manu D, Peduzzi E, Mensah-quainoo E, Asante-poku A, Ofori-adeje D, 2006. Systemic suppression of interferon- $\gamma$  responses in Buruli ulcer patients resolves after surgical excision of the lesions caused by the extracellular pathogen *Mycobacterium ulcerans*. *J Leukoc Biol* 79: 1150–1156.
- Palella FJ Jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Glen A, Satten GA, Aschman DA, Holmberg SD, 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Out-patient Study Investigators. *N Engl J Med* 338: 853–860.
- Mocroft A, Ledergerber B, Katlama C, Kirk O, Reiss P, d'Arminio Monforte A, Knysz B, Dietrich M, Philips AN, Lundgren JD, 2003. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. *Lancet* 362: 22–29.
- Meintjes G, Boule A, 2012. Immune reconstitution inflammatory syndrome in a large multicenter cohort study: case definition and comparability. *Expert Rev Anti Infect Ther* 10: 737–741.

31. Achenbach CJ, Harrington RD, Dhanireddy S, Crane HM, Casper C, Kitahata MM, 2012. Paradoxical immune reconstitution inflammatory syndrome in HIV-infected patients treated with combined antiretroviral therapy after AIDS-defining opportunistic infections. *Clin Infect Dis* 54: 424–433.
32. Hercberg S, Chauliac M, Galan P, Devanlay M, Zohoun I, Agboton Y, Soustre Y, Auvert B, Masse-Raimbault AM, Dupin H, 1988. Prevalence of iron deficiency and iron-deficiency anaemia in Benin. *Public Health* 102: 73–83.
33. Stienstra Y, van der Werf TS, Oosterom E, Nolte IM, van der Graaf WT, Etuaful S, Raghunathan PL, Whitney EA, Ampadu EO, Asamoah K, Klutse EY, te Meerman GJ, Tappero JW, Ashford DA, van der Steege G, 2006. Susceptibility to Buruli ulcer is associated with the SLC11A1 (NRAMP1) D543N polymorphism. *Genes Immun* 7: 185–189.
34. Van Zandt KE, Sow FB, Florence WC, Zwilling BS, Satoskar AR, Schlesinger LS, Lafuse WP, 2008. The iron export protein ferroportin 1 is differentially expressed in mouse macrophage populations and is present in the mycobacterial-containing phagosome. *J Leukoc Biol* 84: 689–700.
35. Lundgren JD, Mocroft A, 2003. Anemia and survival in human immunodeficiency virus. *Clin Infect Dis* 37: S297–S303.
36. Ferede G, Wondimeneh Y, 2013. Prevalence and related factors of anemia in HAART-naive HIV positive patients at Gondor University Hospital, northwest Ethiopia. *BMC Haematol* 13: 205.
37. Mensah-Quainoo E, Yeboah-Manu D, Asebi C, Patafuor F, Ofori-Adjei D, Junghans T, Pluschke G, 2008. Diagnosis of *Mycobacterium ulcerans* infection (Buruli ulcer) at a treatment centre in Ghana: a retrospective analysis of laboratory results of clinically diagnosed cases. *Trop Med Int Health* 13: 191–198.
38. O'Connell RJ, Merritt TM, Malia JA, VanCott TC, Dolan MJ, 2003. Performance of the OraQuick rapid antibody test for diagnosis of human immunodeficiency virus type 1 infection in patients with various levels of exposure to highly active antiretroviral therapy. *J Clin Microbiol* 41: 2153–2155.
39. Yeboah-Manu D, Asante-Poku A, Asan-Ampah K, Ampadu ED, Pluschke G, 2011. Combining PCR with microscopy to reduce costs of laboratory diagnosis of Buruli ulcer. *Am J Trop Med Hyg* 85: 900–904.
40. Yeboah-Manu D, Konadu E, Asan-Ampah K, Asante-Poku A, Nakobu Z, Pluschke G, 2011. Isolation of *Mycobacterium ulcerans* from swab and fine-needle-aspiration specimens. *J Clin Microbiol* 49: 1997–1999.
41. Myers BA, 2011. *Wound Management: Principles and Practices*, 3rd edition. Upper Saddle River, NJ: Prentice Hall.
42. WHO, 2001. *Buruli Ulcer: Diagnosis of Mycobacterium ulcerans Disease*. Portaels F, Johnson P, and Meyers WM, eds. Geneva, Switzerland: World Health Organization.
43. Nienhuis WA, Stienstra Y, Thompson WA, Awuah PC, Abass KM, Tuah W, Awuah-Boateng NY, Ampadu EO, Slegmund V, Schouten JP, Adjei O, Bretzel G, van der Werf TS, 2010. Antimicrobial treatment for early, limited *Mycobacterium ulcerans* infection: a randomised controlled trial. *Lancet* 375: 664–672.
44. Johnson RC, Nackers F, Glynn JR, de Biurrun Bakedano E, Zinsou C, Aguiar J, Tonglet R, Portaels F, 2008. Association of HIV infection and *Mycobacterium ulcerans* disease in Benin. *AIDS* 22: 901–903.
45. Opie J, 2012. Haematological complications of HIV infection. *S Afr Med J* 102: 465–468.
46. Blanc FX, Sok T, Laureillard D, Borand L, Rekeciewicz C, Nerrienet E, Madec Y, Marcy O, Chan S, Prak N, Kim C, Lak KK, Hak C, Dim B, Sin CI, Sun S, Guillard B, Sar B, Vong S, Fernandez M, Fox L, Deifraissy JF, Goldfeld AE, 2011. Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. *N Engl J Med* 365: 1471–1481.
47. Boulware DR, Meya DB, Muzoora C, Rolfes MA, Hullsiek KH, Musubire A, Taseera K, Nabeta HW, Schutz C, Williams DA, Rajasingham R, Rhein J, Thienemann F, Lo MW, Nielsen K, Bergemann TL, Kambugu A, Manabe YC, Janoff EN, Bohjanen PR, Meintjes G; COAT Trial Team, 2014. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. *N Engl J Med* 370: 2487–2498.
48. Ribera E, Pou L, Lopez RM, Crispo M, Falvo V, 2001. Pharmacokinetic interaction between nevirapine and rifampicin in HIV-infected patients with tuberculosis. *J Acquir Immune Defic Syndr* 28: 450–453.
49. Kadima JN, Mukanyangezi MF, Uwizeye CB, 2014. Effectiveness and safety of concurrent use of first line antiretroviral and antituberculous drugs in Rwanda. *J Trop Med* 2014: 9.
50. Pillay P, Ford N, Shubber Z, Ferrand RA, 2013. Outcomes of efavirenz versus nevirapine-containing regimens for treatment of HIV-1 infection: a systematic review and meta-analysis. *PLoS One* 8: E68995.
51. O'Brien DPO, Ford N, Vitoria M, Christinet V, Comte E, Calmy A, Stienstra Y, Eholie S, Asiedu K, 2014. Management of HIV-BU co-infection. *Trop Med Int Health* 19: 1040–1047.