Buruli Ulcer and HIV Coinfection: Cases in Togo

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Abstract

Background: In Togo, as in all sub-Saharan countries, the burden of HIV infection remains high. The registration of new cases of Buruli ulcer every year also remains a major public health problem. Buruli ulcer (BU) is a disabling disease and the presentation of lesions is frequently severe. A feature of BU and HIV coinfection is the rarity of cases, which makes its study difficult, but, nevertheless, important to study its seroprevalence, biological data, risk factors and genetic diversity. The purpose of this study is to explore the comorbidity of Buruli ulcer and HIV by evaluating HIV seroprevalence in BU patients, assessing demographic data, reviewing biological data including CD4+ T cell count, hemoglobin levels, and viral loads, and evaluating clinical and therapeutic data. Methods: This is a cross-sectional study including only BU patients confirmed by Ziehl Neelsen staining and IS 2404 PCR. The patients were hospitalized in the National Reference Center for Tsévie. They were recovered patients and patients undergoing outpatient treatment in the Gati and Tchekpo Deve treatment centers, respectively, within the Sanitary Districts of Zio and Yoto of the Maritime Region during the period from August 2015 to March 2017. Results: The number of HIV-positive BU patients is 4 out of a total of 83 BU patients. All patients are HIV-1 positive. HIV prevalence among BU patients is 4.8% compared to 2.5% nationally and 3% at regional level. Three BU patients are seropositive out of a total of 46 female patients while one patient under 15 years is seropositive out of a total of 37 male BU patients. There are a greater proportion of female patients with BU/HIV coinfections. Half of the BU/HIV positive patients (BU/HIV+) have a CD4+ TL of fewer than 500 cells/μl and the difference is significant between those of the BU HIV− and those of the BU/HIV+ patients. Two patients have unde-
tectable viral loads while the other two have more than 1000 copies/ml (33,000 and 1,100,000 copies/ml). Anemia is significantly present in BU/HIV+ patients with a p-value = 0.003. Half of BU patients have primary education, while three-quarters of BU/HIV+ patients have no education. All patients are either in stage I or stage II of the AIDS WHO classification. All patients are on first line ARV therapy and only ARV nucleoside reverse transcriptase inhibitors (NRTIs) are used. **Conclusion:** In Togo, the prevalence of HIV in BU patients, although higher, is not significantly different from that of national and regional. The relatively high CD4+ LT levels of relatively high BU HIV+ patients, undetectable viral loads, and AIDS WHO stages I and II indicate good quality management. **Author Summary:** Buruli ulcer disease (BUD) is a mycobacterial skin disease that leads to extensive ulcerations and causes disabilities in approximately 25% of the patients. Co-infection with HIV is described by the authors through the prism of risk factors and the severity of ulcerations. Healing time is described as longer than in BU/HIV− patients. The scarcity of cases seems to be an obstacle for further study. Noteworthy are the study of cases in Benin and the study of cohort cases in Cameroon. However, no study appears to be based on the seroprevalence of this morbid association, the biological data and the antiretroviral regimens. These regimens, if poorly instituted, conflict with antimycobacterial drugs against Buruli ulcer. This study, although confronted with the particular configuration of Togo, a country with a low HIV prevalence of 2.8% national prevalence and an average of 55 cases of Buruli ulcer per year, is studying the biological aspects of co-infection HIV/BU, including seroprevalence of HIV, CD4+ LT levels, patient viral load and hemoglobin levels and ARV regimens. This study shows the need for future studies, including the study of the genetic diversity of circulating *Mycobacterium ulcerans* strains in Togo and the study of Buruli ulcer co-infection/HIV and tuberculosis.

**Keywords**
Buruli Ulcer, Coinfection Buruli Ulcer and HIV, HIV Seroprevalence, *Mycobacterium ulcerans*, TOGO

1. Introduction

Despite the various sustained efforts through National HIV/AIDS Programs (NACPs) in sub-Saharan African countries, particularly in West Africa, the incidence of HIV infection in the population remains a real public health problem given the morbidity of the disease. Buruli ulcer is a real public health challenge, considering the virulence of the disease caused by the production of mycolactone, a toxin with cytotoxic activity, immunomodulatory [1] and the necrotizing nature of dermal tissues or even bone, and remains the third most common tropical mycobacterial disease after tuberculosis and leprosy [2]. Buruli ulcer may be localized or disseminated [3], rarely chronic but often induces aesthetic,
functional and life-threatening sequelae [4]. BU patients are often diagnosed by the clinic with or without biological confirmation according to WHO recommendations [5]. The biological diagnosis is made either by the Ziehl Neelsen staining method for acid-fast bacilli (AFB), by histopathology, or by the Polymerase chain reaction (PCR) method. The bacteriological culture is not often used for diagnosis. In sub-Saharan Africa, only Ziehl Neelsen staining is often available in the sites for BU confirmation [6]. WHO recommends at least two methods for biological confirmation of BU cases. Most cases of BU present as dermal ulcerations. The most severe cases, in particular osteomyelitis, often become complicated, leading either to amputations, or vicious retractions of skin leading to the loss of movement and these severe cases are described more frequently with co-infection with HIV, as well as the disseminated cases of BU [7].

The combination of HIV and BU thus becomes a morbid pairing whose severity of symptoms constitutes a real problem that deserves a study in order to appreciate the different proponents. The aim of this study was to evaluate HIV seroprevalence in BU patients and to study biological data, including CD4+ T lymphocytes cell count, anemia, viral loads, and antiretroviral therapy (ARV) introduced. This study aims to study the comorbidity of BU and HIV. Specifically, the objectives are to determine the prevalence of HIV in BU patients in Togo, to study demographic data, to evaluate biological data such as HIV type, assay hemoglobin, CD4+ T lymphocytes, measurement of viral loads, and, finally, to evaluate the clinical and therapeutic data.

2. Methods

Study Setting and Design

In Togo, the National Reference Center for Buruli Ulcer patients in Tsevie has the highest number of cases per year and the Zio and Yoto districts are the largest providers of cases. This is a cross-sectional study that included 83 patients. Patients are those hospitalized in the National Reference Center for BU patients in Tsevie, patients being treated in the Gati and Tchekpo Deve outpatient treatment centers with high prevalence of BU patients, respectively, in the health districts of Zio and Yoto located in the Maritime Region. And, finally, the recovered patients in these two health districts during the period from 15 August 2015 to 31 March 2017. These patients were selected in order to have an overview of the prevalence of HIV among BU patients in Togo and to study biological data.

Patient Recruitment

All enrolled cases are those who had biological confirmation through the identification of AFB from a lesion sample by the Ziehl-Neelsen technique at CHR-Tsevie and/or by PCR IS2404 classical technique of the Mastercycler type EPPENDORF, 53322K058 at the National Institute of Hygiene (INH) of Lomé.

HIV testing was performed on blood samples from patients who were counseled and gave consent. The HIV test is carried out according to the WHO strategy-based national algorithm in which an initial screening test is carried out, and
if the latter is found to be positive, a second specific test is carried out for the confirmation and the type of HIV [8]. In this study, the first test was "Determine ALERE HIV 1/2 0 (Alere Medical Co. Ltd., Chiba, Japan) or ELISA MUREX TM (DiaSorin SpA, Dartford, UK), the second test was HIV Tri Dot TM (Mitra & Co. Pvt. Ltd., New Delhi, India).

CD4+ LT counting is performed using the BD FACSCount (BD Biosciences, San Jose, CA, USA) flow immunocytometry technique with BD FACSCount TM reagents CD4 Reagent absolute value/reference percentage class number 339,010 (BD Biosciences, San José, CA, USA) of the CHR Tsevie laboratory, which is the regional reference laboratory for HIV biology.

According to the national guidelines, ARV treatment is conditioned, inter alia, at the WHO clinical stage and at a CD4+ LT level of less than 500 cells/μl of blood for stage I and II patients [8]. Viral loads are analyzed in the Laboratory of Molecular Biology and Immunology of the Faculty of Health Sciences of Lomé (BIOILIM) for HIV positive patients. The technique used is the measurement of plasma RNA according to the NASBA technique on NucliSENS EasyQ equipment with NucliSENS HIV-1 reagents version 2.0.

**Statistical Analyses**

The prevalences and rates were compared using Fisher’s exact test with a 95% confidence interval.

**Ethical Considerations**

The clinical and epidemiological information of patients enrolled from August 2015 to March 2017 is available on the BUO1 database (Access 2003, Microsoft Cooperation, Redmond, WA). The information was recorded using an elaborated fact sheet including demographic data and biological data. The data are anonymous. Informed consent was signed by all enrolled persons or their legal guardian after being well informed of the objectives of the study. The National Program for the Control of Buruli Ulcer, Leprosy and Lean approved the study project (N° 159/2015/MS/DGS/DSSP/PNLUB-LP) as well as the Bioethics Committee for Health Research (CBRS) (Avis N° 30/2017/CBRS of October 9, 2017). All confirmed cases of HIV have been referred to the Maritime Region HIV Therapeutics Committee and the Buruli Ulcer Management Center.

### 3. Results

From August 15, 2015 to March 31, 2017, 83 cases of BU patients hospitalized, being treated as outpatients, or recovered were enrolled for HIV testing. Four cases were positive for HIV 1: three cases among females between 35 and 50 years, 1 male case 12 years of age.

Comparison of HIV prevalence among BU patients in Togo is shown in Table 1.

No BU patient group has an HIV prevalence statistically different from national prevalence. National prevalence is included in the CI 95% of the respective prevalence of the UB patient groups.

Evaluation of the different demographic, biological, clinical and therapeutic data of patients with BU/HIV− and BU/HIV+ (Table 2).
**Table 1.** Comparative table of HIV prevalence among BU patients.

|                        | Children under 15 years with BU (n = 48) | Children under 15 years | Women > 15 years with BU (n = 26) | Women > 15 years | Men > 15 years with BU (n = 9) | All BU patients (n = 83) | National prevalence | Regional prevalence |
|------------------------|------------------------------------------|-------------------------|-----------------------------------|------------------|-------------------------------|-----------------------|-------------------|-------------------|
| **Prevalence du VIH (%)** | 2.1%                                     | 5.6%                    | 12.5%                             | 3.1%             | 0.0%                          | 1.7%                  | 4.8%              | 2.5%              | 3.0%              |
| **95% CI**             | [0.0 - 10.4]                             | [2.7 - 32.4]            | [0.0 - 36.9]                      |                  |                               | [13.0 - 11.9]         |                   |                   |

**Table 2.** Comparative table of different demographic data, biological, clinical and therapeutic of patients BU/HIV− and of BU/HIV+ patients.

|                                   | Total (%)       | Patients UB/HIV− (%) | Patients UB/HIV+ (%) | p value |
|-----------------------------------|-----------------|----------------------|----------------------|---------|
| Number                            | 83 (100.0)      | 79 (95.2)            | 4 (4.8)              |         |
| **Gender**                        |                 |                      |                      |         |
| Male                              | 37 (100.0)      | 36 (97.3)            | 1 (2.7)              | p = 0.62|
| Female                            | 46 (100.0)      | 43 (93.5)            | 3 (6.5)              |         |
| **Age**                           |                 |                      |                      |         |
| 0 - 14 years                      | 46 (100.0)      | 45 (97.8)            | 1 (2.2)              | p = 0.32|
| ≥ 15 years                        | 37 (100.0)      | 34 (91.9)            | 3 (8.9)              |         |
| **Educational level**             |                 |                      |                      |         |
| No education                      | 19 (100.0)      | 16 (84.2)            | 3 (15.8)             |         |
| Primary studies                   | 58 (100.0)      | 57 (98.3)            | 1 (1.7)              | p = 0.035|
| Secondary studies                 | 5 (100.0)       | 5 (100.0)            | 0 (0.0)              | RR = 10.11 |
| Tertiary studies                  | 1 (100.0)       | 1 (100.0)            | 0 (0.0)              | [1.11 - 92.6]|
| **HIV screening**                 |                 |                      |                      |         |
| VIH type                          |                 |                      |                      |         |
| Old infection                     | 2               |                      |                      |         |
| Patients newly screened           | 2               |                      |                      |         |
| **Hemoglobin rate**               | 12.02  [11.77 - 12.28] | 10.15 [8.55 - 11.74] | p = 0.003 |         |
| **CD4+ LT rate**                  |                 |                      |                      |         |
| [0 - 500] cells/µl               | 1               | 2                    | p = 0.006            |         |
| ≥ 500 cells/µl                   | 73              | 2                    |                      |         |
| Cells average/µl IC95%            | 1059            | 539                  | p = 0.01             |         |
| [970 - 1148]                      |                 | [133 - 945]          |                      |         |
| **HIV viral load**                |                 |                      |                      |         |
| Undetectable                      | 2               |                      |                      |         |
| [40 - 1 000] copies/ml           | 0               |                      |                      |         |
| ≥ 1 000 copies/ml                | 2               |                      |                      |         |
Females are the most represented and the most infected by HIV. Patients above 15 years old are more infected. The risk of HIV positive in uneducated patients equals 10 times the risk in educated patients and this difference is statistically significant with $p = 0.035$. All the patients are infected by HIV1. Hemoglobin and CD4+ LT levels in HIV positive patients were on average significantly lower than those in HIV negative patients ($p = 0.003$, $p = 0.01$). Half of the patients had undetectable viral load. All of the patients are in stage 1 or 2 of AIDS WHO categorization. All of the patients were placed under first-line ARVs.

### 4. Discussion

This study was conducted in a context of countries with low HIV prevalence with an average of 55 cases of Buruli ulcer per year. Analysis of the epidemiological situation of HIV/AIDS in Togo showed the generalized nature of the epidemic with an average prevalence of 2.5% in the general population aged 15 - 49 [8] (EDST III). It also confirmed the predominance in women with 3.1% in women compared with 1.7% in men. In this study, the prevalence of HIV in BU patients is higher at 4.8%, although this is not statistically different from the EDST III prevalence, which is included in the 95% CI of the prevalence of BU patient groups. There is also a trend towards feminization, that is, 12.5% against 0% for the male sex. In this study, the prevalence of HIV in BU patients is almost the double of that found by RC Johnson et al. in Benin in 2008 [9], who in their case control study, found a prevalence of 2.6%.

Few data are available on BU and HIV coinfection, but according to O’Brien et al. in 2014 [10], African countries endemic to Buruli ulcer are also HIV-infected, with a proportion of 1% HIV to 5% BU-HIV coinfection. In the studies in Cameroon, HIV prevalence in BU patients is three to six times higher than in the general population, *i.e.* 37% against 7% for women, 20% against 5% for men and 4% for women 0.7% for children [11]. In Ghana, HIV prevalence is five times higher among BU patients, *i.e.* 5% compared with 0.9% [12]. This corroborates the results of this study. The only bias could be the reduced number of our sample.

According to the results of this study, the most infected BU/HIV patients were...
those without education and those with only primary education. In Togo women are also generally less educated than men. Although few studies seem to dwell on this aspect, the risk of HIV positivity in poorly educated patients is 10 times higher than the risk in educated patients and this difference is statistically significant with \( p = 0.035 \).

The type of HIV detected in our study is 100% HIV 1. This corresponds to the national data in which HIV 1 is detected in a proportion of 99.1% for HIV 1, 0.4% for HIV 2 and 0.5% for HIV 1/HIV 2 co-infection [8].

All patients are in stages I and II of Buruli ulcer WHO lesions categories. These are patients with simple ulcerations in total remission. It is therefore necessary to set up systematic screening of HIV as soon as patients are registered with BU in order to study the future the impact of HIV on the severity of cases of Buruli ulcer, in particular the disseminated forms and bone lesions often associated with HIV-BU co-infections, and thus to define more precisely the healing times of these patients [13], since the authors’ time seems to be longer.

The results of blood analysis reveal anemia in BU/HIV patients, but is more pronounced in BU/HIV+ patients with a significant difference (\( p = 0.003 \)). These results confirm the work of Hercberg S et al. [14] that anemia is specific to sub-Saharan African BU patients. This predisposition is associated with the polymorphism of the NRAMP1 iron transporter gene [15]. This is an iron deficiency anemia present during mycobacterial diseases due to sequestration in the phagosomes of the ferrous ion (Fe\(^{2+}\)) of the organism, and the inability of the NRAMP1 to recover it [16]. This disease state would be aggravated by HIV coinfection [17], serological status and AIDS WHO stage.

On the other hand, the results of the hemoglobin levels reveal a good general state of the BU patients which corroborate the results of Connor DH et al. [18] which explains this by the mycolactone inhibitory action on the production of tumor necrosis factor (TNF), usually responsible for fevers and catechesis [19].

The immune mechanism for HIV co-infection and other diseases is clearly defined and studied. Examples include HIV/tuberculosis co-infection, another mycobacterial disease, where immunity is bidirectional and synergistic with a strong predisposition to CD4+ T-cell immunodeficiency which corresponds to a risk factor. On the other hand, the immune mechanism of protection for Buruli ulcer remains poorly understood [20]. The results of this study show that the CD4+ LT level of BU/HIV+ patients is lower than that of BU/HIV negative patients—both for levels below 500 cells/μl (133 - 945) and above 500 cells/μl (970 - 1148). Two findings emerge from these results: with regard to BU/HIV co-infection, immunity based on helper T lymphocytes is reduced, which could constitute a real risk factor for severity of BU according to Christinet et al. [11]. However, Tuffour J et al. [21] state that the level of CD4+ LT is not always correlated with the severity of ulcer disease, as confirmed by the results, since for BU/HIV negative immunity is preserved with high levels of CD4+ LT of up to 2000 cells/μl.

According to Shastri S et al. [22], the immune response to mycobacterial dis-
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...ases is mediated by the activation of CD4+ LT by macrophages and is thus probably crucial for Buruli ulcer. Especially since large phagosomes produced by *Mycobacterium ulcerans* are indications of the multiplication of the pathogen in the phagocytes. This plays an important role in the early hours of the infection, which leads to a reduction in the release of interferon gamma (IFNgamma) [23], whose synergistic action with TNF potentiates the production of oxide metabolites nitric acid [24] to eliminate mycobacteria, hence the importance of CD4+ LT levels as much for the follow-up of UB HIV positive patients as for BU HIV negative patients.

The results of viral loads yielded undetectable results for two patients and viral loads above 1000 copies/ml (33,000 and 1,100,000 copies/ml). Since Togo is a country with similar care settings as in resource-constrained countries, it is often confronted with the difficulty of systematically measuring the viral load of all the patients detected and placing patients under ARV. The dosage of CD4+ LT remains a mandatory passage for treatment, but according to the work of Hoffmann CJ et al. in 2016 [25] only the viral load is the best argument for deciding on ARV treatment and for monitoring all the patients. It is therefore crucial that the viral load is systematic for vulnerable patients including BU patients.

The patients in this study were placed under first-line ARVs: AZT + 3TC + EFF, TDF + 3TC + EFV, AZT + 3TC + EFV and ABC + 3TC + EFV for patients under 15 years of age. This corroborates the WHO recommendations [26] for whom due to the interactions between rifampicin and certain ARVs, the NNRTI component of the regimen should be changed by prescribing EFV instead of NVP and, if inhibitors of Protease would be prescribe the guidelines for tuberculosis/HIV [27] should be consulted for the proposed management.

This is all the more true as the onset of ARV treatment is specific in the case of co-infection for interactions with antimycobacterial treatments since nevirapine concomitantly with streptomycin would reduce Cmax and Cmin due to interaction with rifampicin. While concomitant use with cotrimoxazole would establish immunity and combat opportunistic infections. In this study, the patients were treated mainly with nucleoside and non-nucleoside inhibitors with only flat effavirenz recognized as a molecule potentiating the adverse effects of antimycobacterial treatments in particular claythromycin [28] often not used in treatments in Togo. It is therefore essential to provide routine screening of tuberculosis to all patients co-infected with BU/HIV, since this is a significant player in the ARV regimen.

**5. Conclusion**

The BU and HIV coinfection is a real public health problem that deserves more study. In Togo, the prevalence of HIV in BU patients, although higher, is not significantly different from that of national and regional. The HIV biological, clinical and therapeutical data indicate good quality management. The problem of HIV coinfection and tuberculosis is still evident, since it often resides in coun-

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tries endemic to tuberculosis, the treatment of which could lead to resistance to ARV. This study therefore raises the need for future studies on the management of the association of Buruli ulcer, HIV and tuberculosis and the integration of the efforts of the various national control programs concerned.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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