

# Impact of Human Immunodeficiency Virus on the Severity of Buruli Ulcer Disease: Results of a Retrospective Study in Cameroon

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**Background.** Buruli ulcer is the third most common mycobacterial disease after tuberculosis and leprosy and is particularly frequent in rural West and Central Africa. However, the impact of HIV infection on BU severity and prevalence remains unclear.

**Methods.** This was a retrospective study of data collected at the Akonolinga District Hospital, Cameroon, from January 1, 2002 to March 27, 2013. Human immunodeficiency virus prevalence among BU patients was compared with regional HIV prevalence. Baseline characteristics of BU patients were compared between HIV-negative and HIV-positive patients and according to CD4 cell count strata in the latter group. Buruli ulcer time-to-healing was assessed in different CD4 count strata, and factors associated with BU main lesion size at baseline were identified.

**Results.** Human immunodeficiency virus prevalence among BU patients was significantly higher than the regional estimated prevalence in each group (children, 4.00% vs 0.68% [ $P < .001$ ]; men, 17.0% vs 4.7% [ $P < .001$ ]; women, 36.0% vs 8.0% [ $P < .001$ ]). Individuals who were HIV positive had a more severe form of BU, with an increased severity in those with a higher level of immunosuppression. Low CD4 cell count was significantly associated with a larger main lesion size ( $\beta$ -coefficient,  $-0.50$ ;  $P = .015$ ; 95% confidence interval [CI],  $-0.91$ – $0.10$ ). Buruli ulcer time-to-healing was more than double in patients with a CD4 cell count below 500 cell/mm<sup>3</sup> (hazard ratio, 2.39;  $P = .001$ ; 95% CI, 1.44–3.98).

**Conclusion.** Patients who are HIV positive are at higher risk for BU. Human immunodeficiency virus-induced immunosuppression seems to have an impact on BU clinical presentation and disease evolution.

**Keywords.** Buruli ulcer; Cameroon; HIV; *Mycobacterium ulcerans* disease.

*Mycobacterium ulcerans* disease, also known as Buruli ulcer (BU), is a tropical disease that can lead to devastating tissue and bone destruction. The virulence of *M. ulcerans* is mainly attributed to mycolactone, a toxin with cytotoxic and immunomodulation properties [1]. The disease can be localized or disseminated [2]. It is

rarely life threatening, but it may lead to severe esthetic sequelae, functional impairment, and long-lasting handicap [3]. Buruli ulcer is the third most common mycobacterial disease in the world after tuberculosis and leprosy [4]. It is typically present in humid rural areas from tropical and subtropical regions, and the highest incidence is observed in West and Central Africa [5,6]. Children are mostly affected by *M. ulcerans* and the median age of patients is 15 years [7,8].

The clinical diagnosis of BU performed by skilled practitioners in resource-poor settings is usually good [8]. Although it can be confirmed by different laboratory methods, such as acid-fast bacilli (AFB) direct examination with Ziehl-Neelsen staining (ZN), polymerase chain reaction (PCR), and culture or histopathology, these techniques are not always easily accessible in remote rural settings [10,11]. Historically, surgery was

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the only available treatment. Since 2004, the World Health Organization (WHO) recommends (1) the addition of antimycobacterial treatment with rifampicin and streptomycin [12] or (2) a complete oral regimen associating rifampicin with clarithromycin [13]. Complementary debridement, excision, skin grafting, or, rarely, amputation may be necessary, depending on the type and size of lesion and the affected zone [14].

It is unclear how human immunodeficiency virus (HIV) infection affects BU disease progression. Unlike *M tuberculosis*, which is the most prevalent opportunistic infection [15], the role of HIV in the occurrence and clinical manifestations of BU remains inconclusive. Two case-control studies have addressed the role of HIV as a risk factor for BU. Johnson et al [16] demonstrated a higher HIV prevalence among BU cases compared with controls in their unmatched case-control study. In their matched case-control study, Raghunathan et al [9] found also a higher HIV prevalence when comparing HIV prevalence among BU patients and non-BU controls, but the difference did not reach statistical significance. Of note, both studies were conducted in low HIV prevalence settings.

Cases of severe disseminated BU have been reported among individuals infected with HIV (Figure 1) [17–20], but similar manifestations have also been described in individuals who were HIV negative [21]. Portaels et al [22] analyzed 73 cases of BU-associated osteomyelitis. Human immunodeficiency virus was 1 of 4 factors significantly predicting bone involvement. Furthermore, HIV was considered as a risk factor for disseminated disease in a BU case series [23]. Hypoproteinemia, anemia, genetic factors, a paradoxical reaction to antimycobacterial treatment, and the use of traditional medicine have also been suggested to be associated with severe and disseminated disease [21, 24, 25].

Immunity seems to play an important role in *M ulcerans* clinical manifestations [2, 26–32], but the level of immunodeficiency as measured by CD4 cell count has only been reported for a few

cases. Individuals infected with HIV with a profound immunosuppression have been described with disseminated overwhelming BU disease [20, 33]. Moreover, it has been suggested that the clinical picture of BU may be similar in individuals who are HIV negative and HIV positive with preserved CD4 cell counts [34]. Consequently, we conducted a retrospective analysis of data collected in a district hospital in Cameroon to investigate the impact of HIV infection on BU severity and prevalence.

## METHODS

### Study Setting and Design

We conducted a retrospective analysis of data routinely collected in a district hospital managing BU patients in Akonolinga, Cameroon, supported by the non-governmental organization Doctors without Borders/Médecins Sans Frontières (MSF). Akonolinga lies on the Nyong river basin in the central province of Cameroon where BU is known to be highly prevalent [29, 35]. Human immunodeficiency virus prevalence in the central province is estimated to be 6.1% (women, 6.9%; men, 5.3%) [36].

Patient baseline characteristics and BU clinical specificities have been collected in a dedicated database since the beginning of the MSF-supported BU project in 2002.

### Patient Recruitment

Most patients come self-referred to the MSF BU pavilion located in the hospital, but some patients are referred from other facilities or by the MSF BU early detection team in surrounding localities. Patients with a suspected BU were seen at least once at the hospital for registration purposes. Once registered, swab samples from the undermined edges of ulcerated lesions or biopsies from nonulcerated lesions were taken by a skilled health professional. Buruli ulcer diagnosis relied on the clinical lesion aspect and direct smear examination of AFB with ZN staining. Polymerase chain reaction and culture were also performed at the Pasteur Centre, Yaoundé; histopathology was conducted in unclear cases. For clinically confirmed BU, HIV testing and CD4 cell count have been performed since 2002 based on the clinical decision (systematically among adults since 2008 and among children since 2010). Human immunodeficiency virus diagnosis was made according to national guidelines by 2 different rapid tests performed at the Akonolinga district hospital laboratory.

### Statistical Analyses

Human immunodeficiency virus prevalence among BU patients was calculated and stratified by gender and age and compared with the local estimated HIV prevalence. Human immunodeficiency virus prevalence was calculated for all cases and for PCR-confirmed BU cases only.

A comparative analysis assessing the impact of a positive HIV status on BU clinical manifestations was conducted on patients enrolled since 2008. Other comparative analyses assessing the



**Figure 1.** A typical Buruli ulcer lesion of a human immunodeficiency virus-immunosuppressed patient.

impact of low CD4 cell count (immunosuppression) on BU clinical manifestations were conducted on the whole cohort of patients enrolled since 2002. Fisher's exact test was used to compare categorical variables, and the Kruskal-Wallis test was used for numerical variables.

Time-needed-to-heal BU, defined as the time of diagnosis until complete wound closure, was analyzed with Kaplan-Meier curves and a log-rank test. Univariable and multivariable analyses were performed using a Cox model. The significance level of a *P* value below 2 was used to select explanatory variables in the multivariable model. All patients with a registered exit status in the program database (defined as written information on an event terminating the follow-up process) were included in the analyses. Patients were considered healed if their registered exit program status was defined as healed with or without sequelae. Patients lost-to-follow-up, deceased, or transferred elsewhere were censored (considered not yet healed) at the time of the event. For patients still under treatment, the censoring date corresponded to the time of analysis. Forty patients with an unregistered exit program status were excluded from the analysis. Factors associated with the size of the BU main lesion, defined as the largest diameter of the main lesion, were analyzed by regression analysis. A logarithmic transformation of the main lesion largest diameter

value (in centimeters) was performed to obtain a normal distribution fitting the model.

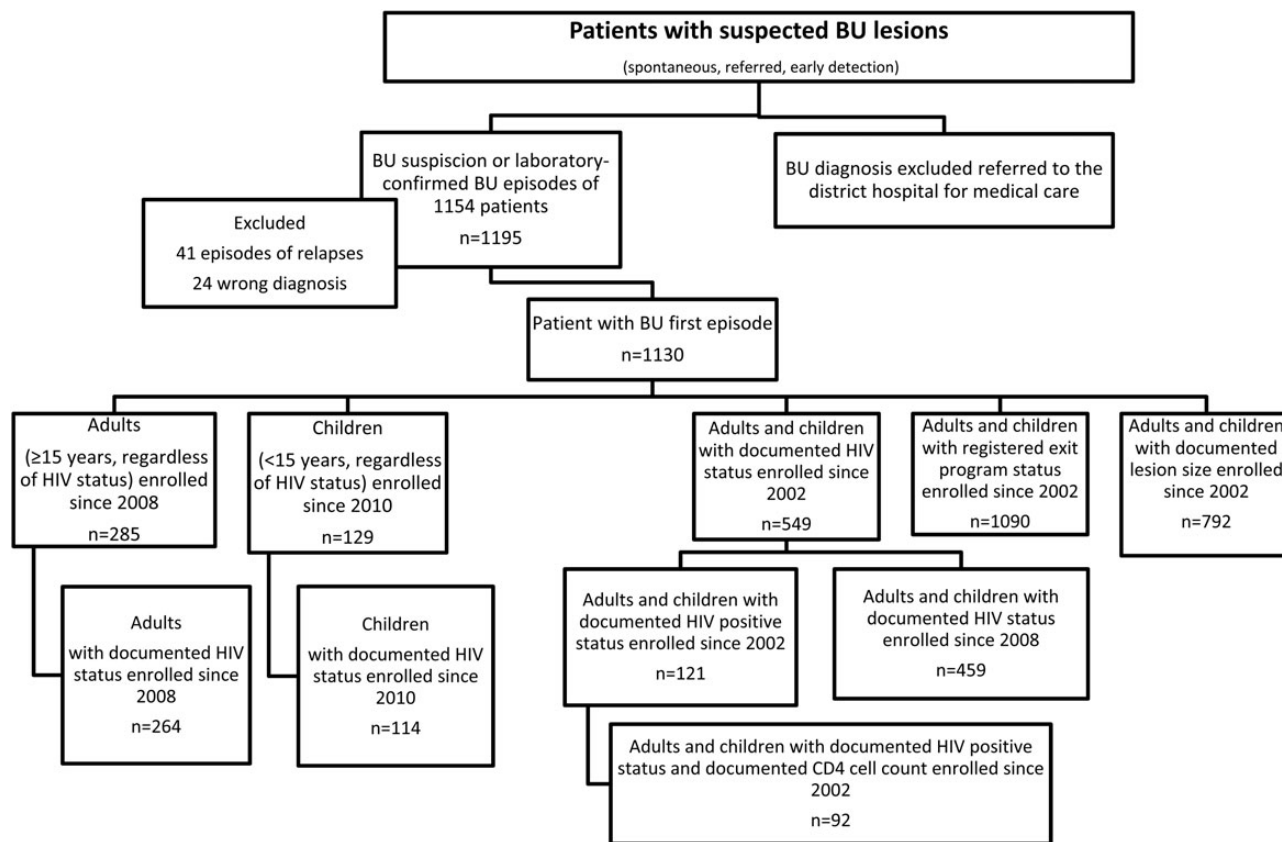
CD4 cell counts were stratified either at 2 levels (below or above 500 cell/mm<sup>3</sup>) or at 3 levels (below 201, between 201 and 500, or above 500 cell/mm<sup>3</sup>). Data analysis was conducted using Stata, version 12.1 (Stata Corp., College Station, TX).

### Ethical Considerations

Data were collected as routine monitoring data. Data analyses were performed on an anonymized database. The Humanitarian Committee of the University of Geneva Hospitals approved the project (proposal 76, 2007).

## RESULTS

From January 1, 2002 until March 27, 2013, 1195 episodes of BU concerning 1154 patients were registered in the database (Figure 2). Two patients had been treated 3 times and 37 patients had been treated twice. Twenty-four patients were finally considered not to have BU and were excluded from the analyses. The final database included 1130 patients diagnosed with a first BU episode.



**Figure 2.** Flowchart of the study populations selected for each analysis. BU, Buruli ulcer; HIV, human immunodeficiency virus; +, positive.

**Table 1. HIV Prevalence Among Patients Treated for BU (and PCR-Confirmed BU) in Akonolinga District Hospital Compared With the Regional Estimated HIV Prevalence**

|  | Females With BU ≥15 Years (Akonolinga District Hospital) | Males With BU ≥15 Years (Akonolinga District Hospital) | Children With BU <15 Years (Akonolinga District Hospital) |
|--|--|--|---|
| HIV prevalence among patients with BU (95% CI)                   | <b>37% [29–45]<sup>a</sup></b>                           | <b>20% [13–27]<sup>a</sup></b>                         | <b>4% [2–10]<sup>c</sup></b>                              |
| HIV prevalence among patients with PCR-confirmed BU (95% CI)     | <b>39% [30–50]</b>                                       | <b>17% [11–26]</b>                                     | <b>5% [2–13]</b>  |
| Regional estimated prevalence                                    | 6.9% [5–9] <sup>b</sup>                                  | 5.3% [4–7] <sup>b</sup>                                | 0.68% [.36–.98] <sup>d</sup>                              |
| BU population compared with reference population <i>P</i> -value | <.001  | <.001  | <.001   |

Abbreviations: BU, Buruli ulcer; CI, confidence interval; HIV, human immunodeficiency virus; PCR, polymerase chain reaction.

<sup>a</sup> HIV prevalence among adults has been calculated over a 5-year period (2008–2013).

<sup>b</sup> Female and male (15–49 years) HIV prevalence in Cameroon Central province [37].

<sup>c</sup> HIV prevalence among children has been calculated over a 3-year period (2010–2013).

<sup>d</sup> National HIV prevalence among 0–14 years children in 2009 (<http://www.unicef.org/sowc2012/pdfs/SOWC-2012-TABLE-4-HIV-AIDS.pdf>;<http://esa.un.org/unpd/wpp/index.htm>).

### HIV Prevalence Among Patients With BU

From January 1, 2008 until March 27, 2013, 93% (264 of 285) of adults (≥15 years old) treated for BU were tested for HIV; 29% were HIV-positive (women, 37%; men, 20%) (Table 1). From January 1 2010 to March 27, 2013, 88% of children (114 of 129) treated for BU were tested for HIV and 4% were found to be HIV-positive. The HIV prevalence calculation was performed among PCR-confirmed BU cases and showed similar results. The prevalence of HIV infection in each population was significantly higher than the regional estimated HIV prevalence (Table 1).

### Comparison of BU Baseline Characteristics With and Without HIV Coinfection

Baseline characteristics of 83 HIV-infected patients (adults and children) included between 2008 and 2013 were compared with 376 HIV-negative patients consecutively admitted to Akonolinga District Hospital with a BU diagnosis (Table 2). Patients who were HIV positive had significantly more multiple BU lesions at

the time of diagnosis compared with individuals who were HIV negative (24% vs 11%, respectively;  $P = .004$ ). Patients with an HIV infection also tended to have larger BU lesions, defined as the larger diameter of the main lesion (5.5 cm [interquartile range (IQR), 3–12 cm] vs 5 cm [IQR, 2–9.5 cm];  $P = .119$ ; Figure 3). A higher proportion of HIV-positive patients had ulcerated lesions (93% vs 86%, respectively;  $P = .120$ ) (Figure 4), despite a shorter time to BU diagnosis (35 weeks [IQR, 25–56 weeks] vs 42 weeks [IQR, 25–57 weeks];  $P = .493$ ) (Table 2). Time to BU diagnosis was defined as the duration of the BU episode before enrollment in care.

There were significantly more females among HIV-infected BU patients compared with the HIV-negative group (66% vs 46%;  $P = .001$ ). Enrollment date, body mass index (BMI), residence, time to BU diagnosis, use of traditional treatment, and the number of PCR-confirmed cases were comparable (Table 2).

### Baseline HIV Characteristics of BU-HIV Coinfected Patients

Only 1 patient was known to be HIV-positive prior to routine testing for HIV and under antiretroviral treatment before BU diagnosis. The median CD4 cell count among 67 HIV-positive patients enrolled since 2008 with CD4 data available (81%) was 414 cell/mm<sup>3</sup> (IQR, 258–629 cell/mm<sup>3</sup>) (Table 2).

### Impact of Immunosuppression in HIV Coinfected Patients

From January 1, 2002, a total of 121 patients treated for BU tested positive for HIV. Ninety-two (76%) had baseline CD4 data available: 28 patients (30%) had CD4 >500 cell/mm<sup>3</sup>; 44 (48%) between 201 and 500 cell/mm<sup>3</sup>; and 20 (22%) below the threshold of 201 cell/mm<sup>3</sup>. The proportion of patients with multiple lesions tended to be lower in the highest CD4 strata, but this did not increase according to immunosuppression status (14%, 30%, and 20%, respectively, from highest to lowest CD4 strata;  $P = .3$ ). The main lesion size was significantly increased with decreasing CD4 cell counts (4.3 cm [IQR, 3–7 cm]; 6.5 cm [IQR, 4–15 cm]; and 7 cm [IQR, 4–20 cm], respectively;  $P = .02$ ; Figure 3). The proportion of patients with ulcerated lesions tended to increase with decreasing CD4 cell counts (85%, 95%, and 100%;  $P = .102$ ; Figure 4).

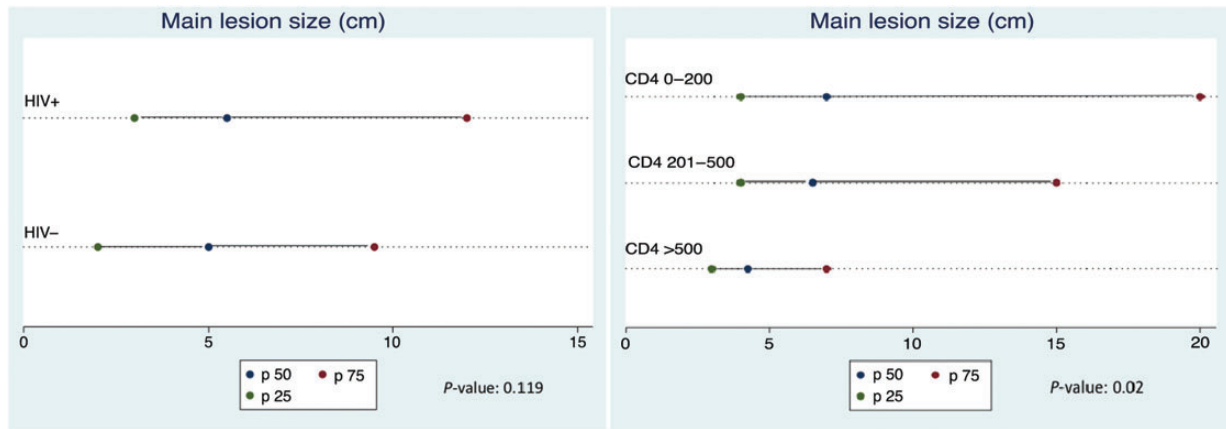
### Factors Associated With Time-to-Heal of BU Lesions

We assessed baseline characteristics potentially associated with the time needed to heal BU by univariable and multivariable analyses. Human immunodeficiency virus-positive status ( $n = 547$ ; hazard ratio [HR], 2.35;  $P = .011$ ; 95% confidence interval [CI], 1.41–3.94), presence of multiple lesions at baseline ( $n = 1107$ ; HR, 0.42;  $P < .001$ ; 95% CI, 0.32–0.55), adulthood ( $n = 1112$ ; HR, 0.64;  $P < .001$ ; 95% CI, 0.55–0.74), place of residence more than 70 km from the district hospital ( $n = 1110$ ; HR, 0.76;  $P = .022$ ; 95% CI, 0.61–0.96), main lesion size more than 5 cm at baseline ( $n = 785$ ; HR, 0.46;  $P < .001$ ; 95% CI, 0.39–0.55), use of traditional medicine ( $n = 1113$ ; HR, 0.71;  $P < .001$ ; 95% CI,

**Table 2. Comparison of Baseline Characteristics, Time-to-Heal BU, Antimycobacterial Therapy, Surgery, and Mortality Among HIV-Positive and -Negative Adults and Children Presenting With BU Lesions Since the Introduction of HIV Systematic Testing in 2008**

|  | HIV+ (2008–2013) (n = 83) | HIV– (2008–2013) (n = 376) | P Value |
|--|---------------------------|----------------------------|---------|
| Date of Patient Enrollment                               |                           |                            |         |
| Median   | 11 Oct 2010               | 22 Jul 2010                | .2      |
| IQR  | (13 Oct 2009–22 Aug 2011) | (11 Jun 2009–22 Jun 2011)  |         |
| Time to BU diagnosis (weeks)                             |                           |                            |         |
| Median   | 35                        | 41.5                       | .5      |
| IQR  | (25–56)                   | (25–57)                    |         |
| Missing  | 1                         | 12                         |         |
| Used traditional/herbal treatment                        | 37%                       | 32%                        | .4      |
| Female proportion  | 66%                       | 46%                        | .001    |
| Housewife status among adults                            | <b>n = 76</b>             | <b>n = 188</b>             |         |
| Proportion   | 34%                       | 16%                        | .001    |
| <b>Age at baseline among adults (years)</b>              | <b>n = 76</b>             | <b>n = 188</b>             |         |
| Median   | 36                        | 33                         | .3      |
| IQR  | (27–49.5)                 | (19–58)                    |         |
| <b>Age at baseline among children (years)</b>            | <b>n = 7</b>              | <b>n = 188</b>             |         |
| Median   | 12                        | 8                          | .8      |
| IQR  | (3–13)                    | (5–11)                     |         |
| <b>BMI at baseline among adults (kg/m<sup>2</sup>)</b>   | <b>n = 76</b>             | <b>n = 188</b>             |         |
| Median   | 20.8                      | 21.8                       | .1      |
| IQR  | (19.7–23.1)               | (19.3–24.7)                |         |
| Missing  | 3                         | 11                         |         |
| <b>BMI at baseline among children (kg/m<sup>2</sup>)</b> | <b>n = 7</b>              | <b>n = 188</b>             |         |
| Median   | 16.5                      | 15.4                       | .1      |
| IQR  | (15.2–18.1)               | (13.9–16.8)                |         |
| Missing  | 2                         | 15                         |         |
| Resident within 70 km from the district hospital         | 86%                       | 85%                        | 1       |
| ZN Akonolinga  |                           |                            |         |
| Positive   | 75%                       | 58%                        | .007    |
| Missing  | 4                         | 33                         |         |
| ZN Pasteur Yaoundé                                       |                           |                            |         |
| Positive   | 59%                       | 48%                        | .1      |
| Missing  | 19                        | 88                         |         |
| PCR  |                           |                            |         |
| Positive   | 77%                       | 76%                        | .9      |
| Missing  | 13                        | 57                         |         |
| Baseline CD4 cell count/mm <sup>3</sup>                  |                           |                            |         |
| Median   | 414                       | N/A                        |         |
| IQR  | (258–629)                 | N/A                        |         |
| Missing results  | 16                        | N/A                        |         |
| Patients with multiple lesions (>1 lesion)               | 24%                       | 11%                        | .004    |
| Main lesion large diameter (cm)                          |                           |                            |         |
| Median   | 5.5                       | 5                          | .1      |
| IQR  | (3–12)                    | (2–9.5)                    |         |
| Missing  | 3                         | 19                         |         |
| Ulcerative lesion  |                           |                            |         |
| Proportion   | 93%                       | 86%                        | .1      |
| Missing  | 8                         | 43                         |         |
| Antimycobacterial therapy                                | 77%                       | 78%                        | .9      |
| Undergone surgery  | 43%                       | 40%                        | .5      |
| Time-to-heal BU (days)                                   |                           |                            |         |
| Median   | 161                       | 148                        | .5      |
| IQR  | (91–290)                  | (80–242)                   |         |
| Missing  | 10                        | 33                         |         |
| Deceased (%)   | 11% (8)                   | 1% (5)                     | <.001   |

Abbreviations: BMI, body mass index; BU, Buruli ulcer; HIV, human immunodeficiency virus; IQR, interquartile range; N/A, not applicable; PCR, polymerase chain reaction; ZN, Ziehl-Neelsen staining.



**Figure 3.** Main lesion size (median and interquartile range in cm) according to human immunodeficiency virus (HIV) status (left) and immunosuppression level (right).

0.61–0.83), or at least 1 positive laboratory confirmation (Z<sub>N</sub> or PCR) ( $n = 1113$ ; HR, 0.80;  $P = .006$ ; 95% CI, 0.68–0.94) were all significantly associated with a longer healing time of the BU lesion.

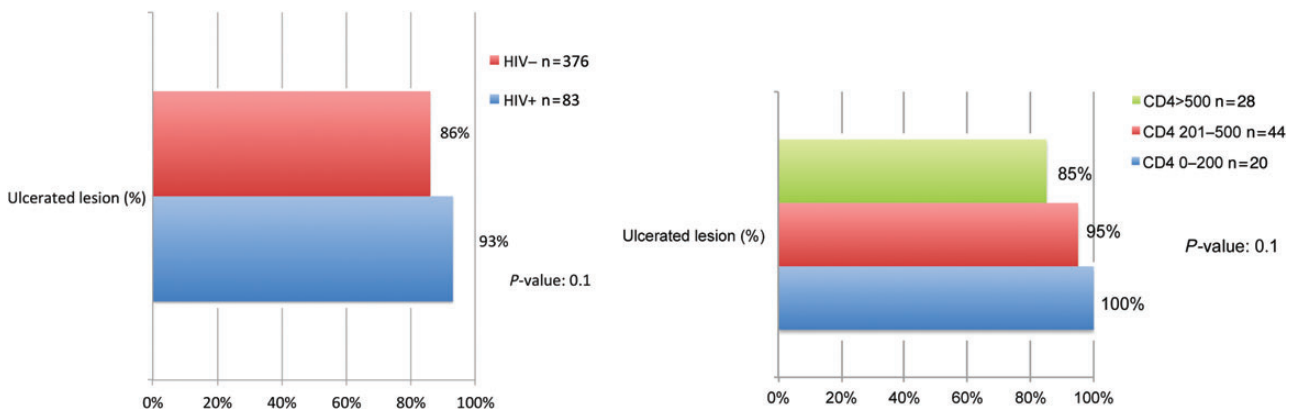
By contrast, a hemoglobin level above 11 g/L ( $n = 344$ ;  $P = .946$ ), gender ( $n = 1113$ ;  $P = .059$ ), a Bacillus Calmette-Guérin scar ( $n = 947$ ;  $P = .055$ ), an ulcerative lesion ( $n = 1024$ ;  $P = .376$ ), time to BU diagnosis ( $n = 1076$ ;  $P = .114$ ), and BMI at baseline ( $n = 920$ ;  $P = .061$ ) were not associated with a BU lesion time-to-heal. In the multivariable analysis, the role of HIV was no longer significantly associated with healing time. However, when CD4 cell strata were entered into the model ( $n = 92$ ), a CD4 above the threshold of 500 cell/mm<sup>3</sup> was the only remaining significant factor associated with the time needed for BU lesion resolution (HR, 2.39;  $P = .001$ ; 95% CI, 1.44–3.98). Kaplan-Meier analysis showed that a CD4 above 500 cell/mm<sup>3</sup> was significantly associated with halving the time needed to BU lesion resolution (Figure 5).

### Factors Associated With Increased BU Lesion Size

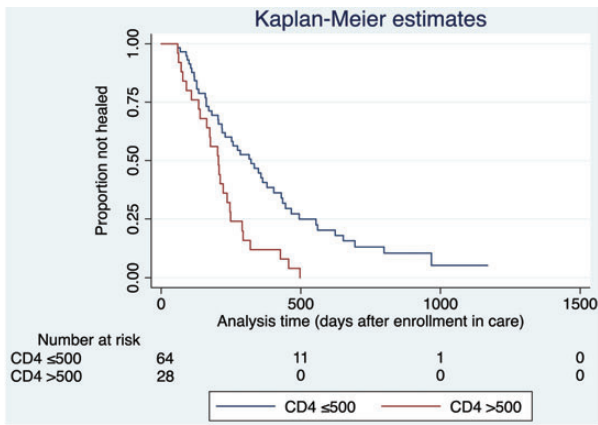
Results of risk factor analysis for an increased lesion size are presented in Table 3. In the multivariable analysis, an HIV-positive status is significantly and independently associated with an increased BU lesion size after adjustment for other influencing factors, such as the hemoglobin level, use of traditional treatment, and place of residence (Table 3). When CD4 data were entered into the multivariable analysis, a CD4 above the threshold of 500 cell/mm<sup>3</sup> was significantly associated with a smaller baseline lesion size ( $\beta$ -coefficient,  $-0.5$ ;  $P = .015$ ; 95% CI,  $-0.91$ – $-0.10$ ) when adjusted for the use of traditional medicine, which was the other remaining factor associated with the baseline lesion.

### Mortality Among HIV-Positive and -Negative Patients

During follow-up, the proportion of deceased patients was significantly higher among HIV-positive than HIV-negative BU patients (11% vs 1%;  $P < .001$ ) (Table 2). The median CD4 cell count among the 8 deceased patients was 228.5 cell/mm<sup>3</sup>



**Figure 4.** Proportion of ulcerated Buruli ulcer lesion according to human immunodeficiency virus (HIV) status and immunosuppression level.



**Figure 5.** Kaplan-Meier analysis of Buruli ulcer time-to-heal according to 0–500/>500 CD4 cell count strata. Log rank test: relative ratio, 2.38;  $P < .0006$ ; 95% confidence interval, 1.43–3.96.

(IQR, 98–378). None was under antiretroviral therapy (ART). The median duration of time to death was 41.5 days (IQR, 16.5–56.5).

## DISCUSSION

We assessed the impact of HIV coinfection on *M. ulcerans* disease in a district hospital in Cameroon. We observed a higher than expected HIV prevalence among patients admitted for BU disease when stratified by gender and age groups. These results are consistent with 2 case-control studies conducted in Benin and Ghana, which showed a higher proportion of HIV-positive patients among BU cases than among controls [9, 16] and support the hypothesis of HIV as a risk factor for BU development. Because HIV immunodeficiency is a strong risk factor for developing *Mycobacterium tuberculosis* disease and ART is strongly associated with a reduction in the incidence of tuberculosis across all CD4 count strata [37], our results suggest a similar pattern of HIV disease in inducing BU overt disease. In addition, we showed an association between HIV-induced immunosuppression (CD4 cell counts) and the clinical severity of BU.

Our data provide further evidence that BU's clinical manifestations are more severe in the presence of HIV-induced immunosuppression. Indeed, HIV-positive patients frequently present with more multiple and larger lesions compared with HIV-negative individuals. The fact that the proportion of ulcerated lesions is higher among HIV-positive patients across all immunosuppression strata, together with a shorter time to BU diagnosis, suggests an accelerated necrotizing process. Females were overrepresented among HIV-infected patients, reflecting the local HIV epidemiology in Akonolinga, Cameroon [36].

Buruli ulcer treatment consists of surgery and antibiotic treatment. Wound closure is a good marker of cure and

**Table 3. Univariable and Multivariable Analyses of BU Main Lesion Size (Documented Main Lesion of Large Diameter [ $n = 792$ ])**

| Main Lesion Large Diameter in cm (log)  | $\beta$ -Coefficient | $P$ Value | 95% CI       | No. of Observations |
|---|----------------------|-----------|--------------|---------------------|
| <b>Univariable Analysis</b>   |                      |           |              |                     |
| HIV-positive status   | 0.22                 | .040      | 0.01, 0.43   | 504                 |
| Age $\geq 15$ years   | 0.26                 | .000      | 0.13, 0.39   | 791                 |
| Hb $\geq 11$ g/L  | -0.29                | .010      | -0.52, -0.07 | 330                 |
| CD4 >500  | -0.58                | .004      | -0.98, -0.19 | 88                  |
| CD4 0–200/<br>201–500/<br>>500  | -0.39                | .003      | -0.64, -0.13 | 88                  |
| Multiple lesions (>1 lesion)  | 0.41                 | .000      | 0.20, 0.62   | 789                 |
| Enrollment date >2007   | -0.18                | .012      | -0.03, -0.04 | 791                 |
| Used traditional/herbal treatment   | 0.56                 | .000      | 0.43, 0.69   | 791                 |
| Resident within 70 km of the district hospital                                    | -0.38                | .000      | -0.58, -0.19 | 791                 |
| BU laboratory confirmation (1 or more)  | 0.09                 | .235      | -0.06, 0.24  | 791                 |
| BMI >19   | 0.13                 | .068      | -0.01, 0.27  | 717                 |
| Female gender   | -0.05                | .444      | -0.18, 0.08  | 791                 |
| BCG scar  | -0.03                | .739      | -0.17, 0.12  | 652                 |
| Time to BU diagnosis >12 weeks  | -0.01                | .882      | -0.18, 0.15  | 775                 |
| <b>Multivariable Analysis With HIV Status (<math>n = 302</math>)</b>              |                      |           |              |                     |
| HIV-positive status   | 0.37                 | .007      | 0.10, 0.64   |                     |
| Hb $\geq 11$ g/L  | -0.25                | .036      | -0.48, -0.02 |                     |
| Used traditional/herbal treatment   | 0.47                 | .000      | 0.24, 0.70   |                     |
| Resident within 70 km of the district hospital                                    | -0.31                | .048      | -0.62, -0.00 |                     |
| <b>Multivariable Analysis With CD4 Strata 0–500/&gt;500 (<math>n = 88</math>)</b> |                      |           |              |                     |
| CD4 0–500/>500  | -0.50                | .015      | -0.91, -0.10 |                     |
| Used traditional/herbal treatment   | 0.40                 | .038      | 0.02, 0.77   |                     |

Abbreviations: BCG, Bacillus Calmette-Guérin; BMI, body mass index; BU, Buruli ulcer; CI, confidence interval; Hb, hemoglobin; HIV, human immunodeficiency virus.

correlates with the follow-up time. Patients who were HIV positive were less likely to have a timely cure of BU, and wound

closure time was negatively associated with CD4 cell counts. We also observed a strong association between the level of immunodeficiency and the baseline size of the main lesion, which might partly explain the longer time-to-heal in patients with low CD4 cell counts. The level of CD4 T-cell counts remained systematically and independently associated with a longer time-to-heal and to main lesion size, despite adjustment for disease severity factors.

The use of traditional medicine, which can consist in local, general, or spiritual interventions [38], is often cited as an aggravating factor of the lesion and was also observed in our analyses. In our study, the use of traditional medicine had an even a stronger impact on the severity of the BU lesion than the delay of time of access to diagnosis and care. Laboratory-confirmed cases seemed to have a more severe clinical course. One explanation may be that the probability of having a positive ZN sample is higher among large and multiple BU lesions with more excision material. Indeed, patients who were HIV positive had a significantly higher proportion of positive ZN compared with individuals who were HIV negative. Socioeconomic status was not assessed in our analyses, although some studies suggested that it was a risk factor for overt BU disease [39].

Our study has some limitations. Observational studies suffer the inherent drawback of uncontrolled bias. Bone involvement, surgical care, antimycobacterial treatment, and ART can all be confounders in the time-to-heal BU analysis. However, we were unable to include these in the analysis due to a lack of data or because they were time- and physician-dependent variables and would have required other statistical models. No conclusion can be drawn on the effect of ART on BU, including possible worsening of BU lesions due to its initiation (immune reconstitution inflammatory syndrome). Finally, not all BU cases could be confirmed with a second diagnostic method as recommended by WHO [11]. However, the number of misdiagnosed BU is most probably very low given the high proportion of PCR-confirmed cases and the clear clinical picture of most of our patients.

Overall, our analyses suggest that HIV-associated immunosuppression affects BU disease. The CD4 level is not only an important determinant of BU baseline severity, but patients with moderate immunosuppression are also at risk for developing severe BU disease. In July 2013, WHO issued new recommendations in which all patients with CD4 cell counts below 500 should be offered treatment. We welcome these recommendations, which will allow clinicians to place patients with moderate immunosuppression on antiretroviral drugs. However, 2 questions need further investigation. (1) What is the best timing for ART initiation in eligible patients? Would it be beneficial to offer a rapid introduction of ART within the first weeks of BU (and HIV coinfection) diagnosis? (2) Should patients with CD4 cells over the threshold of 500 be offered ART, similar to patients with tuberculosis?

We conclude that it will be critical to gather prospective data in BU/HIV coinfecting patients regarding the impact of ART on the evolution of BU disease, including immune reconstitution inflammatory syndrome risks, and suggest that guidance on how to use and monitor antiretroviral use should be further strengthened. It is becoming increasingly obvious that HIV and its accompanying immunosuppression have an effect on BU incidence and clinical presentation, similar to tuberculosis, another mycobacterial infection. Thus, we recommend testing all BU patients for HIV infection, initiating ART as soon as possible (within 2–4 weeks) in all patients with a CD4 cell count below 500, and to consider ART according to WHO criteria in patients with CD4 cells above this threshold after the completion of antimycobacterial treatment.

## Supplementary Data

Supplementary material are available online at *Open Forum Infectious Diseases* online (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

## Notes

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**Author contributions.** V. C., A. C., and E. C. were responsible for concept and design. V. C. and A. C. were responsible for drafting the article. V. C. was responsible for analysis and interpretation of data. The MSF and District hospital team were responsible for field work. L. R. and A.-B. N. were responsible for data management. V. C. was responsible for patient enrollment, collection of samples, and data assembly. All authors were responsible for critical revision for important intellectual content and final approval of article.

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