Clinical epidemiology of laboratory-confirmed Buruli ulcer in Benin: a cohort study

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Summary

Background Buruli ulcer, caused by *Mycobacterium ulcerans*, was identified as a neglected emerging infectious disease by WHO in 1998. Although Buruli ulcer is the third most common mycobacterial disease worldwide, understanding of the disease is incomplete. We analysed a large cohort of laboratory-confirmed cases of Buruli ulcer from Pobè, Benin, to provide a comprehensive description of the clinical presentation of the disease, its variation with age and sex, and its effect on the occurrence of permanent functional sequelae.

Methods Between Jan 1, 2005, and Dec 31, 2011, we prospectively collected clinical and laboratory data from all patients with Buruli ulcer diagnosed at the Centre de Dépistage et de Traitement de l'Ulcère de Buruli in Pobè, Benin. We followed up patients to assess the frequency of permanent functional sequelae. All analyses were done on cases that were laboratory confirmed.

Findings 1227 cases of laboratory-confirmed Buruli ulcer were included in the analysis. Typically, patients with Buruli ulcer were children (median age at diagnosis 12 years) presenting with a unique (1172 [96%]) large (≥15 cm, 444 [36%]) ulcerative (805 [66%]) lesion of the lower limb (733 [60%]). Atypical clinical presentation of Buruli ulcer included Buruli ulcer osteomyelitis with no identifiable present or past Buruli ulcer skin lesions, which was recorded in at least 14 patients. The sex ratio of Buruli ulcer widely varied with age, with male patients accounting for 57% (n=427) of patients aged 15 years and younger, but only 33% (n=158) of those older than 15 years (odds ratio [OR] 2·59, 95% CI 2·04–3·30). Clinical presentation of Buruli ulcer was significantly dependent on age and sex. 54 (9%) male patients had Buruli ulcer osteomyelitis, whereas only 28 (4%) of female patients did (OR 2·21, 95% CI 1·39–3·59). 1 year after treatment, 229 (22% of 1043 with follow-up information) patients presented with permanent functional sequelae. Presentation with oedema, osteomyelitis, or large (≥15 cm in diameter), or multifocal lesions was significantly associated with occurrence of permanent functional sequelae (OR 7·64, 95% CI 5·29–11·31) and operationally defines severe Buruli ulcer.

Interpretation Our findings have important clinical implications for daily practice, including enhanced surveillance for early detection of osteomyelitis in boys; systematic search for *M ulcerans* in osteomyelitis cases of non-specific aspect in areas endemic for Buruli ulcer; and specific disability prevention for patients presenting with osteomyelitis, oedema, or multifocal or large lesions. Our findings also suggest a crucial underestimation of the burden of Buruli ulcer in Africa and raise key questions about the contribution of environmental and physiopathological factors to the recorded heterogeneity of the clinical presentation of Buruli ulcer.

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Introduction

Buruli ulcer, caused by *Mycobacterium ulcerans*, is the third most common mycobacteriosis worldwide, after tuberculosis and leprosy.[1] It mostly affects rural areas of tropical countries. Although no official estimate of global incidence is available at present, west Africa is the main endemic zone, with more than 4000 new cases reported by Côte d'Ivoire, Ghana, and Benin in 2010.[2] Buruli ulcer is a devastating necrotising skin infection classically characterised by preulcerative lesions (nodules, plaques, oedematous infiltration), which develop into deep ulcers with undermined edges that can spread to an entire limb and disseminate to the bone. Osteomyelitis occurs in 5–10% of patients with Buruli ulcer and is usually concomitant to a skin lesion.[3] In some patients, most of whom are children, Buruli ulcer causes lifelong functional sequelae.

This disease was first reported in the late 19th century, but became a public health problem in the 1980s. WHO formally identified Buruli ulcer as a neglected emerging tropical disease with the launch of its global initiative against Buruli ulcer in 1998.[4] The increase in the number of cases was too large to be attributed exclusively to a previous lack of awareness and has been explained in several instances by local environmental events, such as changes in forest cover, flooding, the building of dams,

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or irrigation.[5] Several risk factors for Buruli ulcer have been repeatedly identified and include proximity to stagnant or slow-flowing bodies of water, poor wound care, and not wearing protective clothing (eg, long-sleeved shirts or shoes).[6–9] The exact mode of transmission is unclear, and might involve direct transmission of the bacterium from the environment to wounded skin or indirect transmission mediated by a biting vector insect.[10–12]

The first comprehensive epidemiological description of Buruli ulcer dates from 1971, with the seminal report on the prospective follow-up of epidemics in the Kinyara settlement, in which about 10% of 2500 initially unaffected refugees developed the disease after moving into this zone of endemic Buruli ulcer in Uganda.[13] This study remains unique in its almost experimental setting and it led to the first analysis of the incubation period for Buruli ulcer, the age-specific and sex-specific incidence of Buruli ulcer, and the effect of the distance between the patients' dwelling and the water source on risk of Buruli ulcer. The 1999 Ghana national case search[14] included the largest number of patients (>5000) studied worldwide so far and provided information about the age and sex of patients and the site of lesions. The Beninese Zagnanado study assessed 1630–2399 cases, but did not clearly specify the proportion of these cases confirmed by laboratory tests.[3,15,16] A short report described the distribution of age, sex, site, and type of lesions for 2598 patients attending four centres in Benin, including Pobè, in 2003–05.[17] Finally, a series of 750 cases focused on the site of Buruli ulcer lesions.[18] Other studies have provided valuable descriptions of smaller case series (100–300 patients; appendix).

See Online for appendix

Although of great interest, each of these studies had substantial limitations, such as the use of a purely descriptive approach (ie, no measurement of association), a large proportion of diagnoses being retrospective and scar-based or prospective but only clinical, or the inclusion of a large proportion of individuals for whom key data were missing. WHO insists on laboratory confirmation of clinically suspected Buruli ulcer, with possible differential diagnoses including other tropical ulcers, skin fungal infections, or cutaneous tuberculosis.[1] The most sensitive and specific test is the detection of *M ulcerans* DNA by PCR on fine-needle aspiration, or biopsy or swab samples.[1,19] Direct smear examination after Ziehl-Neelsen staining, culture, or histopathology can also be used. In the four largest epidemiological studies of Buruli ulcer (ie, ≥750 individuals), laboratory confirmation of diagnosis was available for about 50% of cases at best.[13–15,18] In this study, we analyse a large cohort of laboratory-confirmed cases of Buruli ulcer from Pobè, Benin, to provide a robust and comprehensive description of the clinical presentation of Buruli ulcer, of its variation with age and sex, and its effect on the frequency of permanent functional sequelae as assessed after systematic follow-up.

Methods

Participants and study design

Between Jan 1, 2005, and Dec 31, 2011, we prospectively collected clinical and laboratory data from all consecutive patients treated for Buruli ulcer at the Centre de Dépistage et de Traitement de l'Ulcère de Buruli (CDTUB) in Pobè, Benin. We prospectively recorded age at diagnosis, sex, geographic origin of the patient, clinical form (nodule, plaque, oedema, ulcer, osteomyelitis), WHO lesion size category (maximum diameter <5 cm, 5–15 cm, ≥15 cm), and site (right or left, upper or lower limb, thorax, abdomen, head, perineum), laboratory confirmation test (culture, Ziehl-Neelsen staining or highly specific IS2404 PCR), HIV status, medical or surgical treatment, and time to healing. Notably, a patient with Buruli ulcer might have lesions at one or more sites (defining multifocality—eg, right and left arms), but also several forms at one site (eg, an ulcerated plaque).

We followed up the patients of our cohort to assess the frequency of permanent functional sequelae, ranging from the loss of ten or more degrees of joint mobility to the amputation of a whole limb. Access to the registry

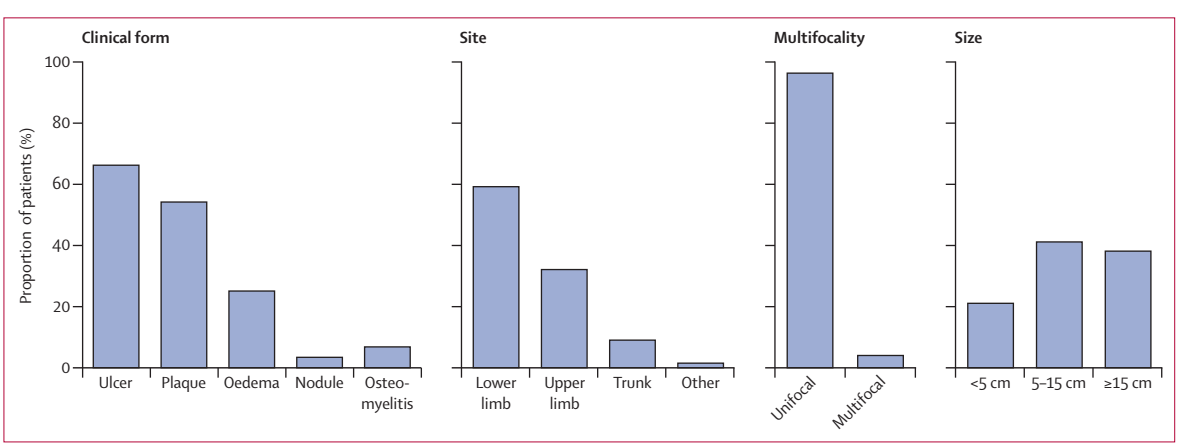

Figure 1: **Clinical presentation of Buruli ulcer (Pobè, Benin, laboratory-confirmed cases, 2005–11)**
Note that a patient with Buruli ulcer might have lesions at one or more sites (defining multifocality—eg, right and left arms), but also several forms at a single site (eg, an ulcerated plaque).

was approved by the institutional review board of the CDTUB and the national Buruli ulcer control authorities.

In accordance with WHO recommendations for diagnosis of Buruli ulcer (ie, confirmation of diagnosis by at least one laboratory test; appendix), we focused our descriptive and analytical studies on laboratory-confirmed cases only. We described the clinical presentation of Buruli ulcer and analysed the effect of age at diagnosis and sex on this presentation. Ten elements of clinical presentation and outcome were assessed: male sex; localisation of a lesion on the upper body; presence of a nodule, plaque, ulcer, oedema, or osteomyelitis; largest lesion with a maximum diameter exceeding 15 cm; presence of multifocal lesions; and the frequency of permanent functional sequelae. The upper body was defined as the head, thorax, abdomen, and arms, and the lower body as the perineum and legs.

Statistical analysis

We systematically fitted three logistic regression models, one with age, one with sex, and a third with both age and sex as explanatory variables for each of the ten elements of Buruli ulcer clinical presentation and outcome. We then screened each element of clinical presentation of Buruli ulcer for their effect on the frequency of permanent functional sequelae in both univariable and multivariable models. We operationally defined severe Buruli ulcer as presenting with at least one clinical element at higher risk for permanent functional sequelae. We further validated our definition by assessing the effect of Buruli ulcer severity on time to healing (Wilcoxon test). Age was analysed as a continuous variable and modelled by means of multiple fractional polynomials (with corresponding p values referred to as MFP-p).[20] For ease of interpretation, odds ratios (OR) for dichotomised age with a cutoff point at 15 years were also reported (with corresponding OR referred to as OR_{15}). Parameter estimation and significance testing were done within the classical maximum likelihood framework. The analyses were done with R statistical analysis software (version 3.1.0, *glm* functions), together with the additional *mfp* (version 1.4.9) and *ggplot2* (version 0.9.3.1) packages.[21–23]

Role of the funding source

The funders of the study had no role in study design; data collection, data analysis, data interpretation; writing of the report or in the decision to submit the paper for publication. AAl had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

1511 patients with Buruli ulcer were treated between Jan 1, 2005, and Dec 31, 2011, at the CDTUB, Pobè, Benin. The appendix shows the distribution of cases according to year of diagnosis, month of diagnosis, and management characteristics (antibiotic therapy, surgery, hospital admission, and time to healing). 1251 (83%) cases were confirmed by one or more laboratory tests. PCR results were positive in 1177 (78%) cases, Ziehl-Neelsen staining was positive in 842 (56%) cases, and culture was positive in 214 (14%) cases (appendix). To ensure maximum robustness and homogeneity in our dataset, we focused

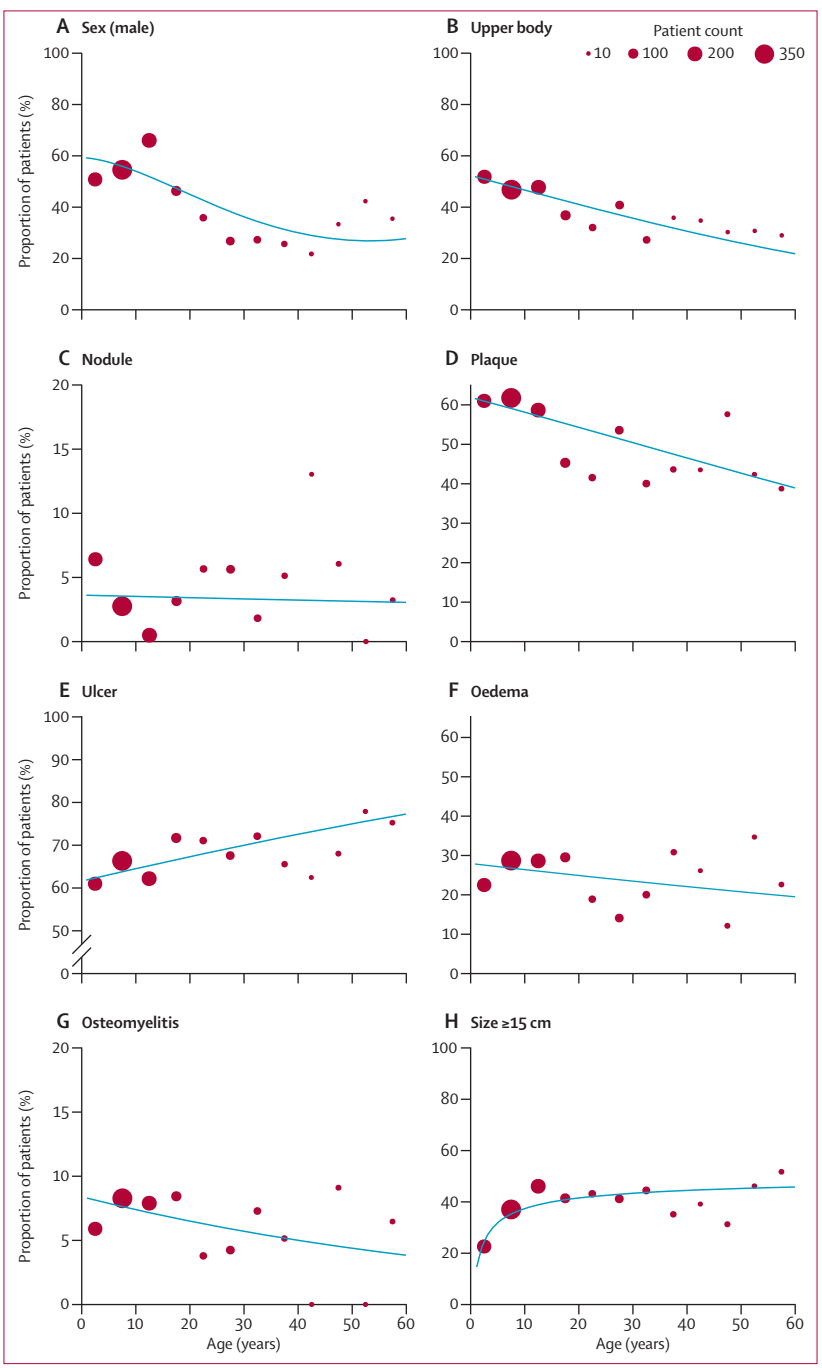

Figure 2: **Clinical presentation of Buruli ulcer as a function of age (Pobè, Benin, laboratory-confirmed cases, 2005–11)**

Red circles show the percentage of individuals with the clinical characteristic by age groups with a width of 5 years. The area of circles are scaled to the total number of individuals in the age group (ranging from about 20 to 350). The blue lines show the prediction of the logistic regression model, as fitted by the fractional polynomial method. Total counts of individuals in each regression model were 1163 for size and 1224 for all other analyses.

our analyses on 1227 laboratory-confirmed cases without HIV co-infection. We describe Buruli ulcer clinical presentation, analyse the effect of age and sex on Buruli ulcer clinical presentation, and assess the relation between clinical presentation and the occurrence of permanent functional sequelae, incidentally leading to the proposal of an operational definition of severe Buruli ulcer.

	Patients, n (%)	Univariable*		Bivariable†	
		Crude OR (95% CI)	p value‡	Adjusted OR (95% CI)	p value
Male sex					
Age					
Continuous	..	..	<0·0001	..	..
>15 years	158 (34%)	1	..	..	..
≤15 years	427 (57%)	2·59 (2·04–3·30)	<0·0001	..	..
Upper body lesion					
Age					
Continuous	..	..	<0·0001	..	<0·0001
>15 years	150 (32%)	1	..	1	..
≤15 years	364 (48%)	2·00 (1·58–2·55)	<0·0001	2·13 (1·67–2·74)	<0·0001
Sex					
Female	275 (43%)	1	..	1	..
Male	239 (41%)	0·91 (0·73–1·14)	0·4241	0·78 (0·61–0·98)	0·0366
Nodule					
Age					
Continuous	..	..	0·7496	..	0·4886
>15 years	19 (4%)	1	..	1	..
≤15 years	23 (3%)	0·75 (0·40–1·41)	0·3639	0·85 (0·45–1·62)	0·6247
Sex					
Female	28 (4%)	1	..	1	..
Male	14 (2%)	0·53 (0·27–1·01)	0·0528	0·51 (0·26–0·99)	0·0427
Plaque					
Age					
Continuous	..	..	<0·0001	..	<0·0001
>15 years	211 (45%)	1	..	1	..
≤15 years	457 (61%)	1·90 (1·51–2·40)	<0·0001	2·06 (1·62–2·63)	<0·0001
Sex					
Female	361 (56%)	1	..	1	..
Male	307 (52%)	0·85 (0·68–1·06)	0·1491	0·75 (0·60–0·95)	0·0167
Ulcer					
Age					
Continuous	..	..	0·0004	..	<0·0001
>15 years	333 (71%)	1	..	1	..
≤15 years	469 (62%)	0·68 (0·53–0·88)	0·0024	0·64 (0·49–0·82)	0·0004
Sex					
Female	405 (63%)	1	..	1	..
Male	400 (68%)	1·24 (0·98–1·57)	0·0731	1·36 (1·07–1·74)	0·0120
Oedema					
Age					
Continuous	..	..	0·0445	..	0·0413
>15 years	103 (22%)	1	..	1	..
≤15 years	204 (27%)	1·33 (1·01–1·75)	0·0390	1·35 (1·02–1·78)	0·0349
Sex					
Female	160 (25%)	1	..	1	..
Male	147 (25%)	1·00 (0·77–1·30)	0·9863	0·95 (0·73–1·24)	0·7214

(Table 1 continues on next page)

Patients with typical Buruli ulcer were children presenting with one large ulcerative lesion of the lower limb, as detailed below (figure 1). The median age at diagnosis was 12 years (IQR 7–28 years; mean 19·3 years). Comparison with the median age of the Beninese population (18 years in 2010[24]) shows that Buruli ulcer is over-represented in children. 1172 (96%) patients presented with one localisation and 444 (36%) patients presented with a lesion of more than 15 cm in diameter (figure 1). 733 (60%) patients presented with a lesion on the lower limb. 805 (66%) patients had ulcers, 668 (54%) had plaques, 307 (25%) had oedema, and 42 (3%) had nodules. 82 (7%) patients had osteomyelitis (figure 1), of whom at least 14 (17%) presented with no identifiable present or past Buruli ulcer skin lesions (notably, several of these patients had fistulisation of the bone infection to the skin, as commonly recorded in osteomyelitis).

Although the overall sex ratio of the patients was balanced (640 [52%] women *vs* 587 [48%] men, p=0·13), a major distortion of the sex ratio was recorded as a function of age, with males being predominant in younger patients and females in older patients (OR_{15} 2·59, 95% CI 2·04–3.30, MFP-p<0·0001, figure 2A). Specifically, male patients accounted for 427 (57%) of the patients younger than 15 years, but only 158 (33%) of those older than 15 years (table 1). This effect cannot be accounted for by the demography of the country because the Beninese population shows a balanced sex ratio both in patients younger than 15 years and in those older than 15 years.[24] Thus, age at diagnosis was substantially different between the male and female patients with Buruli ulcer: the median age at diagnosis was 10 years (mean 16 years) for male patients and 15 years (22·5 years) for female patients (p<0·0001).

Table 1 and figure 2 show the complete set of results regarding clinical presentation as a function of age and sex. 54 (9%) men had osteomyelitis, but only 28 (4%) women did (OR 2·21, 95% CI 1·39–3·59; p=0·0007; table 1). Age was significantly associated with the frequency of clinical forms such as developing lesions on the upper body, presenting with a plaque, and presenting with an ulcer (table 1). Younger patients were prone to developing lesions on the upper body (OR_{15} 2·00, 95% CI 1·58–2·55, MFP-p<0·0001; figure 2) and to present with a plaque (1·90, 1·51–2·40; MFP-p<0·0001; figure 2D). Conversely, older patients were more likely to present with ulcers (OR_{15} 0·68, 95% CI 0·53–0·88; MFP-p=0·0004; figure 2E). Additionally, we noted a number of borderline significant associations; nodules were more frequent in female patients (OR 0·53, 95% CI 0·27–1·01, p=0·05; table 1) than in male patients, and osteomyelitis was more frequent in younger patients than in older patients (OR_{15} 1·46, 0·91–2·41, MFP-p=0·06; figure 2G).

Follow-up information was available for a median of 359 days for 1043 (85%) patients, 229 (22%) of whom

presented with permanent functional sequelae, including ten amputations (<1%; five on the upper limb, five on the lower limb, ranging from one finger to a whole limb). Age and sex were not significantly associated with the frequency of permanent functional sequelae (table 1), whereas patients presenting with oedema, bone lesions, large lesions (more than 15 cm of diameter), or multifocal lesions were at significantly increased risk of permanent functional sequelae (table 2). On the basis of this finding, we propose an operational definition of a severe case of Buruli ulcer as a patient presenting with at least one of these clinical characteristics. 616 (50%) patients had severe Buruli ulcer. 192 (37%) patients with severe Buruli ulcer developed permanent functional sequelae compared with 37 (7%) patients with non-severe Buruli ulcer (OR 7·64, 95% CI 5·29–11·31, p<0·0001; figure 3). A multivariable regression analysis showed some dependencies between the four clinical components of severity: only osteomyelitis and lesion size had independent significant effects on sequelae (osteomyelitis, OR 6·48, 95% CI 2·27–20·9, p=0·0004; lesion size, 6·95, 4·70–10·4, p<0·0001). In further validation of the relevance of our definition of severity, the median time to healing increased from 81 days in patients with non-severe Buruli ulcer to 107 days in patients with severe Buruli ulcer (p<0·0001).

	Patients, n (%)	Univariable*		Bivariable†	
		Crude OR (95% CI)	p value‡	Adjusted OR (95% CI)	p value
(Continued from previous page)					
Osteomyelitis					
Age					
Continuous	..	..	0·0560	..	0·1993
>15 years	25 (5%)	1	..	1	..
≤15 years	57 (8%)	1·46 (0·91–2·41)	0·1181	1·23 (0·76–2·05)	0·4106
Sex					
Female	28 (4%)	1	..	1	..
Male	54 (9%)	2·21 (1·39–3·59)	0·0007	2·09 (1·30–3·38)	0·0019
Lesion size ≥15 cm					
Age					
Continuous	..	..	<0·0001	..	<0·0001
>15 years	189 (42%)	1	..	1	..
≤15 years	255 (36%)	0·78 (0·61–0·99)	0·0422	0·74 (0·57–0·94)	0·0157
Sex					
Female	226 (36%)	1	..	1	..
Male	218 (40%)	1·18 (0·93–1·49)	0·1746	1·28 (1·00–1·63)	0·0466
Multifocal lesions					
Age					
Continuous	..	..	0·1292	..	0·1308
>15 years	18 (4%)	1	..	1	..
≤15 years	35 (5%)	1·23 (0·69–2·24)	0·4878	1·22 (0·68–2·26)	0·5020
Sex					
Female	27 (4%)	1	..	1	..
Male	26 (4%)	1·05 (0·60–1·82)	0·8654	0·97 (0·55–1·70)	0·9114
Severe form					
Age					
Continuous	..	..	0·0003	..	0·0002
>15 years	245 (52%)	1	..	1	..
≤15 years	371 (49%)	0·90 (0·71–1·13)	0·3537	0·85 (0·67–1·08)	0·1736
Sex					
Female	306 (48%)	1	..	1	..
Male	310 (53%)	1·22 (0·97–1·53)	0·0842	1·27 (1·01–1·59)	0·0416
Permanent sequelae					
Age					
Continuous	..	..	0·1098	..	0·2006
>15 years	79 (20%)	1	..	1	..
≤15 years	150 (23%)	1·20 (0·88–1·63)	0·2479	1·13 (0·83–1·55)	0·4425
Sex					
Female	109 (20%)	1	..	1	..
Male	120 (24%)	1·30 (0·97–1·75)	0·0783	1·26 (0·93–1·70)	0·1289

OR=odds ratio· *Univariable analysis of each clinical characteristic as a function of the predictors age and sex. †Bivariable analysis of each clinical characteristic as a function of the predictors age and sex. ‡Age was analysed both as a continuous variable modelled with multiple fractional polynomials (MFP) and as a categorical variable with a cutoff point at 15 years (figure 2 shows MFP visualisation and appendix shows MFP formulas).

Table 1: **Effect of age at diagnosis and sex on clinical presentation and outcome of Buruli ulcer (laboratory-confirmed cases, 2005–11, Pobè, Benin): univariable and bivariable analysis**

Discussion

Despite its discovery more than a century ago and its rapid progression since the 1980s, Buruli ulcer remains one of the most neglected infectious diseases. With our unique collection of data recorded prospectively during 7 years at a leading treatment centre for Buruli ulcer (appendix), we have generated a comprehensive epidemiological description of Buruli ulcer in a west African setting, including systematic follow-up for permanent functional sequelae. This work provides insight into the clinical presentation and outcome of Buruli ulcer and proposes guidelines for future research efforts (panel). By contrast with other studies, this study was based on a large number of prospectively diagnosed laboratory-confirmed cases (95% of which by PCR) with a very low proportion of missing data throughout the analyses. Several characteristics of the disease consistently reported in previous studies were confirmed (appendix), a strong support to the generalisability of our findings: Buruli ulcer being mainly a paediatric disease (median age of 12 years at diagnosis); the preponderance of unifocal lesions (>90%); the predominance of lesions on the lower limbs (about 60%); ulcer as the most frequent clinical presentation (about 70%); a not unsubstantial proportion of patients presenting with osteomyelitis (>5%); and a high frequency of permanent functional sequelae (>20%). Of the many additional findings reported in this study, five are of particular importance and further discussed below: the variation of Buruli ulcer sex ratio with age; the over-representation of boys among patients with Buruli ulcer osteomyelitis; the existence of Buruli ulcer osteomyelitis patients with no identifiable present or past Buruli ulcer skin lesions; the strong association between clinical presentation and the

	n (%) with permanent sequelae	Univariable*		Multivariable†	
		Crude OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Upper body					
Unaffected	124 (21%)	1	..	1	..
Affected	105 (24%)	1·22 (0·91–1·63)	0·1930	1·59 (1·10–2·30)	0·0128
Nodule					
Absence	227 (23%)	1	..	1	..
Presence	2 (5%)	0·20 (0·03–0·65)	0·0045	0·47 (0·07–1·73)	0·2829
Plaque					
Absence	143 (31%)	1	..	1	..
Presence	86 (15%)	0·40 (0·29–0·54)	<0·0001	0·64 (0·45–0·92)	0·0156
Ulcer					
Absence	69 (20%)	1	..	1	..
Presence	160 (23%)	1·24 (0·91–1·71)	0·1758	2·07 (1·33–3·28)	0·0012
Oedema					
Absence	146 (19%)	1	..	1	..
Presence	83 (31%)	1·89 (1·38–2·59)	<0·0001	1·39 (0·90–2·15)	0·1347
Osteomyelitis					
Absence	185 (19%)	1	..	1	..
Presence	44 (66%)	8·18 (4·87–14·1)	<0·0001	6·48 (2·27–20·9)	0·0004
Size					
<15 cm	47 (8%)	1	..	1	..
≥15 cm	153 (41%)	8·30 (5·82–12·0)	<0·0001	6·95 (4·70–10·4)	<0·0001
Multifocal					
No	210 (21%)	1	..	1	..
Yes	19 (49%)	3·59 (1·87–6·87)	0·0002	0·91 (0·37–2·18)	0·8328
Severe form					
No	37 (7%)	1	..	..	..
Yes	192 (37%)	7·64 (5·29–11·3)	<0·0001	..	..

OR=odds ratio. *Univariable analysis of permanent functional sequelae as a function of each of the nine predictors. †Multivariable analysis of permanent functional sequelae was done as a function of all clinical characteristics (except severity).

Table 2: **Effect of clinical presentation of Buruli ulcer on permanent functional sequelae (laboratory-confirmed cases, 2005–11, Pobè, Benin)**

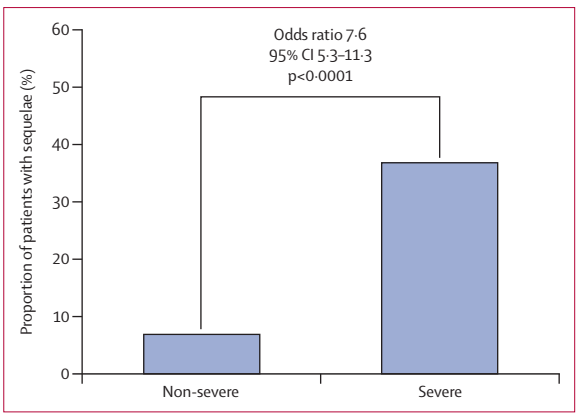

Figure 3: **Frequency of permanent functional sequelae in non-severe Buruli ulcer versus severe Buruli ulcer (Pobè, Benin, laboratory-confirmed cases, 2005–11)**
Severe Buruli ulcer was defined as presenting with at least one of the following clinical elements: oedema, osteomyelitis, multifocal lesions, or large lesions (≥15 cm).

development of permanent functional sequelae; and the underestimation of the burden of Buruli ulcer in Africa.

Although overall balanced, the sex ratio varied widely with age. Male patients accounted for significantly more than half of the patients diagnosed younger than 15 years, but only a third of those diagnosed older than 15 years. Such sex ratio variation with age has been previously reported,[7,13,14,25,26] but surprisingly has always been disregarded or even denied in review papers.[1,6,19,27–29] Sexual dimorphism is frequently recorded in human infectious diseases.[31] In the context of human mycobacteriosis, rare infections with *Mycobacterium marinum* or *Mycobacterium avium intracellulare* and common infections with *Mycobacterium leprae* and *Mycobacterium tuberculosis* are more frequent in adult men than they are in adult women.[31–35] Explanatory hypotheses include differential exposure, differential host response, or both, according to sex. Experimental studies comparing the inoculation of intact male mice, female mice, and castrated male mice have shown that high testosterone concentrations impair resistance to *M marinum*.[36] In our observational study in natura, infection with *M ulcerans* followed the opposite pattern in adults, showing that despite its close relation to *M marinum*, the toxin-secreting *M ulcerans* has a distinctive physiopathology. This information is of particular interest and substantiates the need for specific studies that aim to understand the mechanisms underlying the age-dependent dynamics of the sex ratio in Buruli ulcer.

The risk of developing Buruli ulcer osteomyelitis was significantly greater in male patients than in female patients (OR 2·21, 95% CI 1·39–3·59), and we also recorded borderline significant association with younger age (figure 2G, table 1). No effects of age and sex on the occurrence of Buruli ulcer osteomyelitis were detected in a previous study of BCG efficacy in Buruli ulcer.[37] In view of our results, this difference could mean that the previous study had insufficient power to detect these effects—the power to detect an OR of 2·2 in a sample of 186 male patients and 187 female patients, assuming a type I error of 0·05, is less than 40%.[37] Because of its crucial clinical and physiopathological relevance, this result needs to be confirmed in independent well powered studies of Buruli ulcer, but such an excess of males has also been reported in non-Buruli ulcer osteomyelitis.[38] Close monitoring of boys with Buruli ulcer should be undertaken to detect osteomyelitis as early as possible. Understanding why boys are prone to Buruli ulcer osteomyelitis (eg, hormonal or host genetics components) is of major physiopathological interest and, again, should be the focus of future studies.

We report for the first time, to our knowledge, cases of Buruli ulcer osteomyelitis with no identifiable present or past Buruli ulcer skin lesions and estimated that these so-called exclusive Buruli ulcer osteomyelitis cases represent

about 20% of all Buruli ulcer osteomyelitis cases—a lower bound estimate in view of the stringent criteria that we applied. These patients presented with clinical osteomyelitis that was not specific to Buruli ulcer in appearance, with *M ulcerans* identified only through systematic laboratory investigation. Arguably, recall bias could have resulted in previous small skin lesions being overlooked in some of these cases, but very likely not in all of them. Even with this oversight, this finding would remain of fundamental clinical, public health, and physiopathological relevance. The clinical relevance is obvious: *M ulcerans* causes osteomyelitis in zones endemic with Buruli ulcer, which implies that laboratory testing for the bacterium should be done and the antibiotic regimen should be adapted. With regard to physiopathology, these exclusive cases are a proof of concept that osteomyelitis in Buruli ulcer is not necessarily the result of an uncontrolled multiplication of the bacterium in its elective tissue (skin). At least some individuals are susceptible to bone invasion by a small load of bacteria without contiguous tissue destruction. Importantly, we do not question the route of inoculation of *M ulcerans* in humans—ie, we do not imply that these patients were not bitten by a vector insect or did not have a bruise that came into contact with contaminated water. However, we suggest that some patients with Buruli ulcer can develop bone lesions without an overwhelming skin reservoir for the mycobacterium. In view of the homogeneity of *M ulcerans* strains at this local scale,[39] this suggestion, in turn, questions the source of the interindividual variability of the human immune response to this infectious agent. We deem this is an important point to be urgently investigated through ad-hoc studies—eg, human genetic studies. This observation also supports the possible haematogenous transport of the bacterium from the site of inoculation to the bone in some patients with Buruli ulcer.

For the first time, we systematically assessed the effect of clinical presentation of Buruli ulcer on the frequency of permanent functional sequelae. Four elements of clinical presentation were associated with a longer time to healing and an increased risk of permanent functional sequelae. We thus propose an operational definition of severe Buruli ulcer as a clinical course including oedema, osteomyelitis, multifocal, or large (≥15 cm) lesions. The estimated OR of 7·6 for the development of permanent functional sequelae in patients with severe forms of Buruli ulcer is compelling and has straightforward clinical implications. Patients presenting with any of these four types of lesions should benefit from specific clinical care, such as enhanced monitoring, intensive physiotherapy, and timely reconstruction surgery. Our finding also raises the question as to why some patients with Buruli ulcer develop severe disease. Because the delay to diagnosis was unknown in our study and previous studies, we can only conjecture that longer delay to diagnosis would account for the severity of the clinical presentation in a proportion of patients. However, we surmise that some patients have severe Buruli ulcer despite rapid diagnosis. Identification of such patients would be of special interest to investigate variations in the host susceptibility to Buruli ulcer.

Several findings in our study suggest the probable underestimation of the burden of Buruli ulcer in Africa. The incidental observation of patients coming from Nigeria to be treated at the Pobè CDTUB (located in Benin) shed light on this phenomenon; although the annual number of cases declared by Nigeria to the WHO is consistently less than ten (eg, seven in 2010),[40] about 20 Nigerian cases have been treated every year in Pobè for the past 7 years. Because Nigeria is much larger than Benin, the official figures for Nigeria are therefore likely to be a severe underestimation. Additionally, so far, the failure to diagnose Buruli ulcer with atypical clinical presentation, such as exclusive Buruli ulcer osteomyelitis, also contributes to the underestimation of the burden of Buruli ulcer, because these cases are not usually tested for the presence of *M ulcerans*.

Our results raise several questions that should be addressed by specific studies in the future, most of

***Panel:* Research in context**

Systematic review

On March 15, 2010, we searched PubMed with the search terms "buruli OR ulcerans NOT corynebacterium" and identified about 600 publications on Buruli ulcer, including many isolated case reports. Of papers with epidemiological relevance, roughly 80 were review articles, roughly 20 were case-control studies of risk factors of Buruli ulcers, and roughly 20 were cross-sectional studies or case series of more than ten patients with Buruli ulcer. This small number of heterogeneous studies prompted us to undertake a large-scale epidemiological study in Benin to present characteristics never found together in published epidemiological studies of Buruli ulcer. Our study was representative of Buruli ulcer in west Africa, where most cases arise and the number of cases is large; all cases were prospectively diagnosed and laboratory-confirmed; follow-up for permanent functional sequelae was systematic; and the clinical course of the disease was carefully recorded resulting in sparse missing data. Taking advantage of such a setting, we were able to comprehensively describe the clinical presentation of Buruli ulcer to provide a robust and powerful analysis of its variation with age and sex and to assess its effect on the occurrence of permanent functional sequelae, leading to the operational definition of severe Buruli ulcer.

Interpretation

Our findings are of key public health, clinical, and physiopathological relevance. With respect to public health, several of our findings suggest a crucial underestimation of the burden of Buruli ulcer in west Africa. We describe a new form of clinical presentation for Buruli ulcer, in which patients presented with osteomyelitis with no identifiable present or past Buruli ulcer skin lesions. We propose several clinical implications of our study: enhanced surveillance for the early detection of osteomyelitis in boys; systematic search for *Mycobacterium ulcerans* in osteomyelitis cases of unspecific aspect in areas endemic with Buruli ulcer; and specific disability prevention for patients presenting with oedema, osteomyelitis, multifocal or large lesions (ie, severe Buruli ulcer). Major questions were raised with regard to the environmental or biological processes underlying the recorded heterogeneity of clinical presentation of Buruli ulcer. Further studies would be of particular interest to decipher the relative contributions of the environment, exposure, lifestyle, health-care systems, microbial strains, and host genetics to the dynamics and natural history of Buruli ulcer in natural conditions.

which are in agreement with a recent review.[41] Studies of the dynamics of the healing of Buruli ulcer should be undertaken, with prospective collection of information designed to disentangle several situations that we could not directly differentiate in our study—namely, recurrence (a short-term infectious clinical relapse mediated by live *M ulcerans* that was incompletely eradicated), paradoxical reaction (a non-infectious clinical relapse mediated by an immunological event), and reinfection (an independent Buruli ulcer infectious episode). In several instances, we recorded new Buruli ulcer lesions that occurred several years after healing of the initial episode (appendix). By analogy with tuberculosis, this finding suggests reinfection or perhaps long-term reactivation of the contained infection. We believe that further characterisation of these patients would be particularly relevant to better our understanding of the human immune response to *M ulcerans*, especially in the prospects of vaccine development (appendix). Finally, although we ensured the homogeneity of the dataset, the monocentric design of our study restricts the number of patients for some important aspects of the disease (eg, Buruli ulcer–HIV co-infection), calling for further research. The monocentric design of our study also raises the important question of the generalisability of our findings. We believe our study to be representative of Buruli ulcer in tropical zones, where almost all Buruli ulcer cases arise. However, large series of confirmed cases from non-tropical zones, such as Australia or Japan, will be of particular interest to decipher the relative contributions of the environment, exposure, lifestyle, health-care systems, microbial strains, and host genetics to the development and dynamics of Buruli ulcer in humans.

Contributors

All authors were responsible for the study concept and design, and the analysis and interpretation of data. M-FA, AAd, AG, J-PSA, DA, CJ, LM, and AC collected the data. M-FA, AAd, AG, J-PSA, JC, MK, LM, and AC did or confirmed diagnostics. QV, MFA, DA, CJ, AC, and AAl acquired the data. QV, M-FA, LA, and AAl did the statistical analysis. QV, M-FA, AC, LM, LA, and AAl drafted the report. QV, LM, AC, and AAl obtained the funding.

Declaration of interests

We declare no competing interests.

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