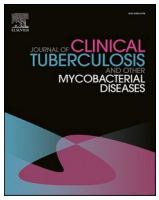
Journal of Clinical Tuberculosis and Other Mycobacterial Diseases 34 (2024) 100415

Contents lists available at ScienceDirect

Journal of Clinical Tuberculosis and Other Mycobacterial Diseases

journal homepage: www.elsevier.com/locate/jctube



Clinical and microbiological predictors of healing in Buruli ulcer disease

Bernadette Agbavor [a,b], Abigail Agbanyo [a], Aloysius Dzigbordi Loglo [a], Philemon Boasiako Antwi [a], Nancy Ackam [a,b], Jonathan Adjei [a,c], Venus Frimpong [a], Kwadwo Boampong [b], Michael Frimpong [a,c], Matthew Glover Addo [b], Mark Wansbrough-Jones [d], Yaw Ampem Amoako [a,e,*], Richard Odame Phillips [a,e]

[a] Kumasi Centre for Collaborative Research into Tropical Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[b] Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[c] Department of Molecular Medicine, School of Medicine and Dentistry, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[d] Institute of Infection and Immunity, St George's University of London, United Kingdom
[e] Department of Medicine, School of Medicine and Dentistry, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

ARTICLE INFO

Keywords:
Buruli ulcer
Wound measurements
Rate of healing
Linear method
Ghana

ABSTRACT

Introduction: Wound measurements are relevant in monitoring the rate of healing (RoH) and may predict time to healing. Predicting the time to healing can help improve the management of Buruli ulcer. We examine three methods for the determination of RoH and their use as predictors of time to healing.

Methods: Lesion measurements of Buruli ulcer patients treated from 2007 to 2022 were obtained with acetate sheet tracings (2D) or Aranz software (3D) fortnightly. RoH was determined using the absolute area, percentage area reduction and linear methods at 4 weeks post onset of antibiotic treatment. Predicted time to healing was compared to the actual healing time. Baseline characteristics were assessed for associations with healing.

Results: All three methods for calculating the RoH significantly distinguished between fast and slow healers ($p < 0.0001$). The predicted healing time using the linear method was comparable to the actual healing time for fast healers ($p = 0.34$). The RoH was influenced by the form of lesion, with plaques [OR 2.19 5 %CI (1.2–3.6), $p = 0.009$], and oedemas [OR 8.5; 95 %CI (1.9—36.9), $p = 0.004$] being associated with delayed healing. The proportion of patients with paradoxical reactions 16 % vs 3 %, $p < 0.0001$), higher baseline bacterial load (75/104;72 % vs 21/47;45 %, $p = 0.001$) and delayed clearance of viable organisms (71/104;68 % vs 9/47;19 %, $p < 0.0001$) was higher in the slow healers than the fast healers.

Conclusion: Predicted healing rates were comparatively lower for slow healers than fast healers. Baseline characteristics associated with healing can be explored for an improved disease management plan to reduce patient and caregiver anxiety.

1. Introduction

Buruli ulcer (BU), a necrotizing skin disease caused by *Mycobacterium ulcerans* is common in west Africa [1,2]. It typically presents as a painless nodule or plaque, characterized by a raised firm and discoloured portion of the skin or non-pitting oedema that can affect the whole limb or surround an ulcer. Subsequently, the lesion enlarges and ulcerates, with a characteristic necrotic base and cottonlike yellow slough [3,4].

Current treatment is with combination of rifampicin and clarithromycin [5,6]. Additionally, good wound care with normal saline for cleansing, covering with vaseline gauze, and short-stretch bandaging to reduce surrounding oedema are essential. Skin grafting may be needed for larger lesions, and physiotherapy can reduce disability, especially if joints are affected. Time to complete wound closure is a crucial endpoint for clinicians managing BU wounds. Buruli lesions treated with antibiotics may heal rapidly or enlarge due to breakdown of necrotic tissue, experience a paradoxical reaction, or develop secondary bacterial infection; these can delay wound healing [3,7–11]. Establishing the time to healing or rate of healing at patient presentation can be a challenge highlighting the need for tools to guide critical care decisions.

Wound measurements using tools including ruler, acetate tracings,

* Corresponding author at: Kumasi Centre for Collaborative Research, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
E-mail address: yamoako2002@yahoo.co.uk (Y.A. Amoako).

https://doi.org/10.1016/j.jctube.2024.100415

Available online 6 January 2024

digital planimetry, and structured light devices, can determine wound size, surface area, volume, tissue analysis, and healing scores. Tracking changes in wound size is commonly used to compute the rate of healing [12–15] while tissue analysis can help detect signs of infection and complications [16].

Determining the rate of healing (RoH) in BU is crucial for assessing effectiveness, predicting complications, and guiding treatment decisions. Real-time feedback on healing progress helps providers decide whether to continue current treatment, consider early surgical intervention, or adjust for better clinical outcomes [17,18].

The RoH in BU is not well understood and varies among individuals. Delayed healing can be frustrating for patients and caregivers. Predicting healing time through wound measurements could greatly aid BU management. In this study, we examined slow and fast healing in Buruli ulcers to identify associated factors and explore the potential of wound measurements in guiding patient management in Ghana.

2. Methods

2.1. Ethical considerations

The Committee on Human Research, Publications, and Ethics (CHRPE) of the Kwame Nkrumah University of Science and Technology (CHRPE/AP/335/19) approved the study. Written informed consent was obtained from participants, and ethical principles of the Declaration of Helsinki were followed [19].

2.2. Study setting

Individuals with confirmed BU between 2007 and 2022 from Agogo Presbyterian, Tepa, Toase and Dunkwa Hospitals were included. Wound care was provided in health posts, health centres, and Community-based Health Planning Services (CHPS) compounds. Clinical and demographic data was collected using standardized WHO BU01 and case report forms.

2.3. Study design and population

In this prospective observational study, individuals with PCR confirmed BU who consented were included, while those with BU-negative lesions were excluded.

2.4. Study procedures

Fine needle aspirates from non-ulcerative lesions and swab samples from undermined edges of ulcerated lesions were collected for laboratory procedures.

2.4.1. Laboratory procedures

Samples were transported in appropriate transport media and processed immediately upon arrival at the Kumasi Centre for Collaborative Research (KCCR). All tests and molecular assays were performed using established methods including smear microscopy for acid fast bacilli, Culture on Lowenstein-Jensen medium and IS*2404* qPCR [20,21].

2.4.2. Combined 16S rRNA reverse transcriptase / IS2404 qPCR assay

Samples were transported in RNA Protect bacterial solution, and DNA and RNA were extracted simultaneously using the Qiagen AllPrep DNA/RNA kit (Qiagen, Hilden Germany). The extracts were then subjected to 16S rRNA and IS*2404* qPCR for quantification and detection of viable organisms [3,22].

2.4.3. Treatment for BU

Antibiotic therapy comprised of either rifampicin (10 mg/kg) with streptomycin (15 mg/kg) (RS8) or clarithromycin 12.5 mg/kg (RC8) daily for 8 weeks. Some received treatment with rifampicin (10 mg/kg) and streptomycin (15 mg/kg) for 2 weeks followed by rifampicin(10 mg/kg) and clarithromycin daily for 6 weeks (RS2RC6).

2.4.4. Wound care procedure and follow up

Wounds were dressed daily or on alternate days using standard procedures, including cleaning with normal saline, covering with vaseline gauze or nitric oxide-releasing dressings. Large wounds were treated with sterile gauze sponge (Drawtex®) to absorb exudates. Short stretch bandages were applied to reduce surrounding oedema. Trained health workers performed wound care during clinic visits, while in the communities, trained community-based surveillance volunteers (CBSVs) or affected individuals and caregivers provided self-care at home with monitoring by nurses or CBSVs. This decentralised approach aligns with national and global objectives [23]. Participants were reviewed fortnightly by experienced clinicians during the 8 weeks of antibiotic treatment and monthly thereafter for 1 year.

2.4.5. Wound measurements

Wound measurements were recorded at each clinic visit using acetate sheets or Aranz software. Acetate sheets were placed on the lesion, and its outline was traced and measured. Silhouette 3-dimensional imaging (ARANZ Medical, Christchurch, New Zealand) was also used for automatic computation of wound dimensions, including width, length, perimeter, surface area, depth, and volume. Measurements were recorded in millimeters, and digital photographs were taken at each visit (Fig. 1).

2.4.6. Clinical data

The time to healing (complete epithelization), WHO reporting details obtained on BU01 forms, and microbiological/molecular characteristics of lesions (time to clearance of *M. ulcerans*, baseline bacteria load, and *M. ulcerans* culture) were documented in a Microsoft Excel database.

2.5. Data management

Data was managed with Microsoft Excel database. RoH was calculated for each wound using the Absolute Area (AA), Percentage Area Reduction (PAR), and Linear methods (Linear advancement method, LM) as proposed by Gilman (Table 1) [13]. The three methods were compared to determine the most reliable for computing healing rates. Calculations were based on measurements recorded at baseline (week 0) and week 4. Calculated wound healing rates were used to predict

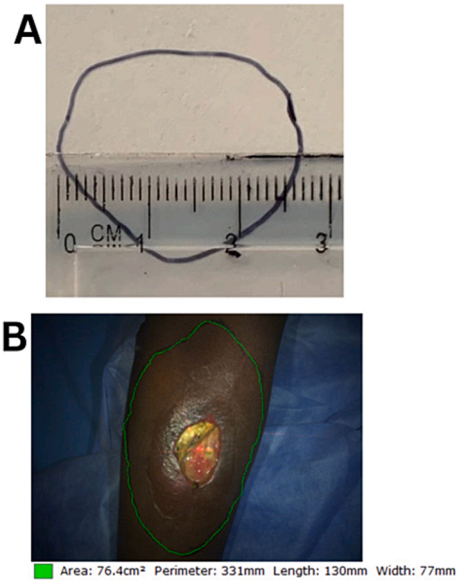


Fig. 1. Wound measurements using acetate sheet (A) and Silhouette 3-dimensional imaging/ Aranz software (B).

Table 1
Methods for calculating wound measurements.

Measurement method	Formula used	Unit of measurement
Absolute Area (AA)	HR = ΔA/t	mm^2 /week
Healing Rate (HR)	Where $\Delta A = A_0 - A_4$	Weeks
Predicted Healing Time (PHT)	t = 4PHT= $(A_0*t)/\Delta A$	
Percentage Area Reduction (PAR)	HR = $\Delta A/(A_0 * t)$	% /week
Healing Rate (HR)	Where $\Delta A = A_0 - A_4$	
PredictedHealing Time (PHT)	t = 4 PHT = median % $\Delta A/A_0$	
Linear method (LM)	HR = d/t	mm / week
Healing Rate (HR)	Where $d = \Delta A/P_{avg}$	Weeks
Predicted Healing Time (PHT)	t = 4 $\Delta A = A_0 - A_4$ $P_{avg} = P_0 + P_4$ / 2PHT = D_{max} *t/d $D_{max} = W_o/2$ t = 4 $d = \Delta A/P_{avg}$	

t-time; A_o-Area at week 0; A_4 -Area at week 4; P_o -Perimeter at week 0; P_4-Perimeter a week 4; P_{avg}-Average perimeter; W_o-longest diameter at week 0.

healing times and compared to actual healing times.

2.6. Data analysis

Data was analysed using GraphPad Prism 9 (GraphPad Prism, San Diego, California USA) and Stata 17 (Stata Corps USA). Data were compared using the Fisher exact, Chi-square or the non-parametric Mann-Whitney test as appropriate. A logistic regression was performed to find the association between baseline characteristics and the RoH. Percent wound area progress was calculated by expressing the change in area as a percentage of the initial size. Wound trajectories were plotted using the average area progress per week (up to 10 weeks) for each healing category. To identify the set of clinical variables that best predicted delayed wound healing, a logistic regression analysis with backward selection based on likelihood ratios was performed. The variance of the model was assessed using Nagelkerke R squared and we determined the goodness-of-fit by the Hosmer-Lemeshow test. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Participant characteristics

Table 2 shows the clinical characteristics of 536 participants with PCR-confirmed BU. The median age was 15 years [IQR (9,30)] with a median time to healing of 19 weeks [IQR (8,28)]. There was a marginal preponderance of females [276 (51.5 %)] but this did not have any influence on the time to healing.

Lesions comprised 157 (29 %) nodules, 114 (21 %) plaques, 31 (6 %) oedema and 234 (44 %) ulcers located principally on the upper limbs 217 (40 %), lower limbs 257 (48 %) or other sites 62 (12 %). Lesion categories were 286 (53 %) category I, 203 (38 %) category II and 47 (9 %) category III. All participants completed 8 weeks of antibiotic therapy and were monitored until complete healing.

One hundred and sixty one (30 %) of participants had complete lesion healing by week 8 post initiation of antibiotic treatment and were classified as fast healers and 375 (70 %) whose lesions healed after week 8 post initiation of antibiotic treatment were classified as slow healers.

3.2. Clinical characteristics of slow and fast healers

There were statistically significant differences between Buruli ulcers that healed fast with respect to clinical forms ($p = 0.0011$) and lesion category ($p < 0.0001$). There was a higher proportion of participants with oedema (94 %) and plaque lesions (78 %) in the slow healers than there were in the fast healer groups. Age of participants ($p = 0.76$), gender ($p = 0.45$) and lesion site ($p = 0.37$) were comparable between fast and slow healers. The type of antibiotic therapy had no influence on the time to healing. There were significantly more paradoxical reactions among slow healers 61 (16 %) compared to fast healers 5 (3 %), ($p < 0.0001$) (Table 2).

Table 2
Baseline characteristics of study participants.

		Healing Category		
Characteristic	**All, n = 536**	**Slow Healers n = 375**	**Fast Healers n = 161**	**p value**
Age, median (IQR), years	15(9,30)	14(9,28)	16(10,31)	0.76
Gender, n (%)				
Male	260(48.5)	186(49.6)	74(46.0)	0.45
Female	276(51.5)	189(50.4)	87(54.0)	
Clinical forms, n (%)				
Nodule	157(29.0)	99(26.0)	58(36.0)	**0.0011**
Plaque	114(21.0)	89(24.0)	25(16.0)	
Oedema	31(6.0)	29(8.0)	2(1.0)	
Ulcer	234(44.0)	158(42.0)	76(47.0)	
WHO category, n (%)				
I (<5cm)	286(53.0)	164(44.0)	122(76.0)	**<0.0001**
II (5–15 cm)	203(38.0)	165(44.0)	38(24.0)	
III (>15 cm)	47(9.0)	46(12.0)	1(1.0)	
Location of lesion, n (%)				
Lower limb (LL)	257(48.0)	174(46.4)	83(52.0)	0.37
Upper limb (UL)	217(40.0)	159(42.4)	58(36.0)	
Other locations	62(12.0)	42(11.2)	20(12.0)	
Antibiotic Treatment type, n (%)				
CR8	140(25.0)	94(25.0)	46(29.0)	0.66
SR2CR6	34(6.7)	25(6.7)	9(6.0)	
SR8	362(68.3)	256(68.3)	106(66.0)	
Study site, n (%)				**<0.0001**
Agogo	345(64.0)	226(65.0)	119(35.0)	
Tepa	133(25.0)	107(80.0)	26(20.0)	
Dunkwa	30(6.0)	17(57.0)	13(43.0)	
Nkawie	28(5)	25(89.0)	3(11.0)	
Microscopy, AFBs positivity, ratio (%)	136/261 (52.0)	121/197 (61.0)	15/64 (23.0)	**<0.0001**
Bacteria Culture, confirm growth, ratio (%)	60/161 (37.0)	52/112(46.0)	8/49(16.0)	**0.0003**
IS*2404*, median cps/ml (IQR)	500 (500,1000)	500 (500,1750)	500(500, 500)	**0.038**
Baseline Mu 16S rRNA, median cps/ml (IQR)	500 (0,1000)	500 (250,2000)	0(0,500)	**0.003**
Week 4 Mu 16S rRNA, median cps/ml (IQR)	0(0,500)	500(0,1500)	0(0,0)	**<0.0001**
Time to clearance of Mu 16S rRNA, *median (weeks) (IQR)*	8(0, 12)	12(5,12)	0(0,4)	**<0.0001**
Time to healing, median (IQR), weeks	19(8,28)	24(16,33)	6(4,8)	**<0.0001**
Development of paradoxical reaction, n (%)	66(12.0)	61(16.0)	5(3.0)	**<0.0001**

3.3. Microbiological and molecular characteristics of slow and fast healers

Microbiological parameters such as AFB positivity, *M. ulcerans* culture positivity and molecular parameters such as baseline *M. ulcerans* 16S rRNA and IS*2404* quantity in copies/ml were significantly different in the fast healer and slow healers.

The proportion of participants with positive *M. ulcerans* culture (52/112 (46 %) vs 8/49 (16 %), p = 0.0003) and the proportion with positive microscopy results (121/197 (61 %) vs 15/64 (23 %), p < 0.0001) before initiation of treatment were significantly higher in slow than fast healers (Table 2). The time to healing was shorter for fast healers than the slow healers [median (IQR) 6 (4, 12) vs 24 (20, 33) weeks; p < 0.0001]. Similarly, slow healers had a significantly higher bacterial load expressed in IS*2404* copy numbers at baseline, [median (IQR) 500 (500, 1750) vs 500 (250, 2000) cps/ml, (p = 0.038)] and viable *M ulcerans 16srRNA* [median (IQR) 500 (500, 500) vs 0 (0, 500) cps/ml, (p = 0.003)] than fast healers. The median (IQR) time to clearance of viable *M. ulcerans* for slow healers [12 (5, 12) weeks] was longer than that of the fast healers [0, (0, 4) weeks].

3.4. Comparison of rate of healing using different wound measurement methods

Table 3 shows the comparison of rate of healing at 4 weeks as assessed by AA, PAR or LM in slow and fast-healing Buruli ulcers. The median (IQR) rate of healing assessed by the AA [117.4 (-19.66, 659.0)], PAR [0.075 (-0.24, 0.032)], and the LM [0.60 (0.47, 1.51)] were significantly lower for the slow healers compared to the AA [165.2 (297.3, 551.6)], PAR [0.25 (0.19, 0.22)] and LM [2.79 (2.95, 4.03)] for fast healers (p < 0.0001).

Lesion sizes increased in some slow healers, including those with paradoxical reactions. Excluding individuals with paradoxical reactions eliminated the negative healing rates as indicated by the IQR in Table 3 and supplementary Table S1 for slow healers. Furthermore, the median rate of healing as assessed using all three methods was still lower for slow healers than fast healers when individuals with paradoxical reactions were excluded from the analysis (supplementary Table S1).

The rate of healing was compared between the method of recording the lesion measurement: acetate method (2D) and the silhouette software from Aranz (3D). The rates of healing using 2D and 3D measurements were comparable when computed using AA (p = 0.893) and PAR (p = 0.489), but a significant difference was observed in healing rates obtained using the linear method (p = 0.0012) (Table 3).

Figs. 2a and 2b shows wound trajectory curves obtained using the average area progress per week for fast and slow healers constructed using pooled, percent area progress data for both fast and slow healers. Fast healers approached 100 % wound healing indicated by complete closure quicker than slow healers. The wound trajectory for fast healers was clearly different from the trajectory for slow healers right from the start of treatment. By week 4, the median percent area progress of the two trajectories was significantly different (72 % vs. 31 %, p < 0.0001).

3.5. Predictability of time to healing using different measurement methods

The actual time to healing and predicted healing time were compared for slow and fast healing Buruli ulcers based on the AA and the LM for measuring healing rate at 4 weeks (Table 4.). The LM successfully predicted healing time in fast healers but not in slow healers. However, the actual and predicted healing times were significantly different for AA (p < 0.0001) and linear (p < 0.0001) methods for slow healers while for fast healers, there was a difference between actual healing time and predicted healing time using the AA method (p = 0.02).

3.6. Association of baseline characteristics with healing

In a logistic regression model, slow healing was strongly associated with clinical markers such as larger lesions; category II [OR 3.2; 95 %CI (2.1–4.9), p < 0.0001], category III [OR 34.2; 95 %CI (4.7–252.1), p = 0.001], plaques [OR 2.195 %CI (1.2–3.6), p = 0.009], and oedematous lesions [OR 8.5; 95 %CI (1.9–36.9), p = 0.004]. Lesion location was not associated with healing status. Development of paradoxical reactions [OR 6.1, 95 %CI (2.4–15.4p < 0.0001] and the late clearance of viable organisms [OR, 10.8,95 %CI (4.5–25.6), p < 0.0001] were also strongly associated with slow healing (Table 5).

Microbiological and molecular parameters such as positive microscopy for AFB [OR 5.2 (2.7–9.9), p < 0.0001], positive *M. ulcerans* culture [OR 4.4 (1.9–10.4), p = 0.001], 16S rRNA positivity at baseline [OR 3.9 (1.92–8.12), p < 0.0001) and after week 4 [OR 10.8 (4.5–25.6) p < 0.0001] were associated with slow healing.

To identify the set of clinical variables that best predicted delayed wound healing, a logistic regression analysis with backward selection based on likelihood ratios was performed. Variables included were lesion site, form of lesion, category of lesion, treatment type, study site and the development of paradoxical reaction. The most appropriate model predicting delayed healing in the study population included lesion category, form of lesion, study site and the development of paradoxical reaction ($R^2 = 0.15$, Hosmer-Lemeshow goodness-of-fit test, p = 0.97). Development of paradoxical reaction and category III lesions produced a larger effect on the model (Supplementary Table S3).

4. Discussion

This study sought to investigate the occurrence of slow and fast healing in Buruli ulcers and to determine early predictors of complete wound healing. Here, we show that 30 % of Buruli ulcer lesions healed in less than 8 weeks (fast healers) compared with 70 % who healed after 8 weeks (slow healers) following initiation of antibiotic therapy. Clinical parameters associated with slow healing were plaque forms (2-fold), oedema (8-fold), category II lesion (3-fold), category III lesion (34-fold), and development of paradoxical reactions (6-fold). Interestingly microbiological factors such as a positive AFB result, higher bacterial load, and a positive *M. ulcerans* culture result that were recently shown to be associated with the development of paradoxical reaction [3] were also strongly associated with slow healing. A positive baseline microscopy result for AFB (5-fold), positive culture (4-fold), a positive *M. ulcerans* 16S rRNA result at baseline (4-fold), and a positive *M. ulcerans* 16S rRNA at 4 weeks (10-fold) increased likelihood of slow healing. Efforts made to identify such predictors are likely to lead to better care for Buruli ulcer patients.

Although antibiotics have transformed the outlook for patients with BU, the current oral regimen of rifampicin with clarithromycin for 8 weeks is far from ideal [5,6]. While the disease can be cured in most patients who adhere to this regimen, healing rates are highly variable

Table 3
Comparison of rate of healing using different wound measurement approaches.

	Healing category			Measurement recording method		
Method	**Slow healers (n = 375)**	**Fast healers (n = 161)**	***p value***	**2D (n = 260)**	**3D (n = 276)**	***p value***
Absolute area						
Median, (IQR)	117.40(-19.66,659.00)	165.20(297.30,551.60)	*<0.0011*	127.70(23.32,426.10)	150.00(12.50,376.00)	*0.89*
Percentage Area Reduction						
Median, IQR	0.07(-0.24,0.03)	0.25(0.19,0.22)	*<0.0001*	0.13(0.03,0.21)	0.13(0.01,0.23)	*0.49*
Linear Model						
Median, IQR	0.60(0.47,1.51)	2.79(2.95,4.03)	*<0.0001*	0.50(0.02,2.63)	1.84(0.16,3.61)	*0.001*

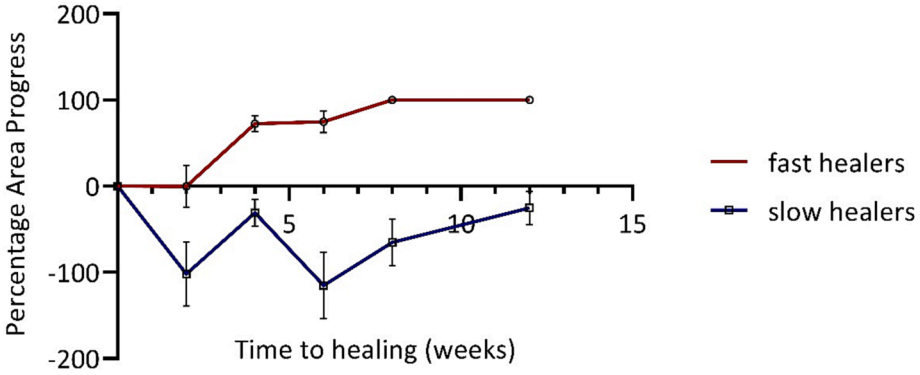


Fig. 2a. Wound trajectory for fast and slow healers (individuals who developed paradoxical reactions included) *Error bars represent ± standard error of mean for each time point.*

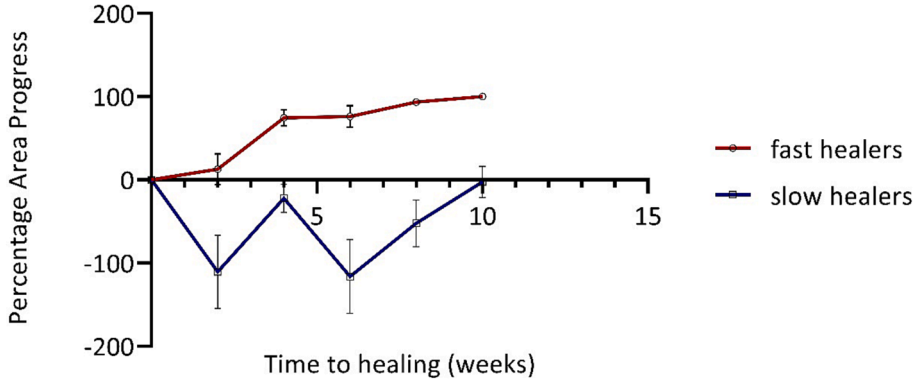


Fig. 2b. Wound trajectory for fast and slow healers (individuals who developed paradoxical reactions excluded) *Error bars represent ± standard error of mean for each time point.*

Table 4
Predictability of time to healing by different wound measurement methods.

Methods	Slow Healers			Fast healers		
	Actual healing time	Predicted Healing time	P value	Actual healing time	Predicted Healing time	p value
Absolute Area						
Median (IQR)	24(16,33)	5.82(-1.13,11.61)	<0.0001	6(4,8)	4(4,4.70)	0.02
Linear Model						
Median (IQR)	24(16,33)	8.98(-3.52,21)	<0.0001	6(4,8)	4(4,5.88)	**0.34**

p values were computed using the Mann Whitney *t*-test; healing times were measured by weeks.

even in patients with seemingly similar lesions. Nienhuis *et al* reported median healing times for category I lesions of 18 weeks and 30 weeks for category II and III lesions [24]. Sarfo *et al* specified median healing times for nodules of 8 weeks, 12 weeks for ulcers, and 2–48 weeks for oedema [25]. Phillips *et al* described median healing times of 14 weeks (RS8) and 16 weeks (RS2RC6) [26]. Vincent *et al* observed median healing times of 12.6 weeks [27]. In a recent cohort that established oral antibiotic regimen, the median time to healing was 24 weeks (IQR 8–28) in the RS8 group, and 16 weeks (IQR 8–25) in the RC8 group [5]. Our findings that two-thirds of patients were slow healers indicate that urgent measures need to be implemented to identify such patients early.

To improve outcomes in BU, several measures are proposed. Patients could be triaged to ensure that those with a likelihood of healing slowly are provided with better monitoring. Plaque and oedema forms, large lesions, a positive AFB at baseline could be triaged as severe forms for better care. Development of paradoxical reactions, with renewed inflammation, despite progressive bacterial killing by antibiotics [3] should be a red flag for slow healing. The median time to complete healing for patients with paradoxical reaction was 28 weeks compared to 16 weeks for those with no paradoxical reaction [3]. Presence of viable *M. ulcerans* assessed with 16S rRNA at baseline and at week 4 would provide a strong indication of likelihood of slow healing. Hospitalization for close monitoring, early skin grafting offered as an option after antibiotic completion at week 8, improved nutrition [28–30] or consideration for extended antibiotic treatment in patients with viable *M. ulcerans* may result in improved outcomes.

Here we investigated simple wound measurement tools that have the capability of predicting the slow or fast healing of Buruli wounds to allow clinicians to properly plan patient care. Measurement of wound size is important in monitoring the healing process and in the evaluation of the effects of treatment. The results of this study suggest that clinicians could effectively use either of the three measuring approaches. The median healing rates computed by all three methods were significantly lower for slow healers than fast healers. The LM resulted in comparable actual and predicted healing times in fast healers than the AA or PAR methods. None of the three methods produced comparable actual

Table 5
Logistic regression analysis of the association of baseline characteristics with the rate of healing.

Characteristics	Number (%) in Cohort	Number (%) in slow healers	Number (%) in fast healers	Unadjusted		Adjusted	
				OR (95 % CI)	p value	OR (95 % CI)	p value
Clinical Forms n (%) Nodule	157(29)	99(63)	58(37)	1		1	
Plaque	114(21)	89(78)	25(22)	2.1(1.2–3.6)	**0.009**	1.0(0.5–1.9)	0.99
Oedema	31(6)	29(94)	2(6)	8.5(1.9–36.9)	**0.004**	1.1(0.2–5.1)	0.89
Ulcer	234(44)	158(68)	76(32)	1.2(0.8–1.9)	0.36	0.6(0.4–1.1)	0.09
WHO Category, n (%)							
I (<=5cm)	286(53)	164(57)	122(43)	1		1	
II (5–15 cm)	203(38)	165(81)	38(19)	3.3(2.1–4.9)	**<0.001**	3.1(1.9–5.1)	**<0.0001**
III (>15 cm)	47(9)	46(98)	1(2)	34. 2 (4.6–252.1)	**0.001**	26.3 (2.9–235.5)	**0.003**
Location of lesion, n (%)							
Lower limb (LL)	257(48)	174(67)	83(33)	1		1	
Upper limb (UL)	217(41)	159(73)	58(27)	1.3(0.9–1.9)	0.17	1.4(0.8–2.2)	0.21
Other locations	62(12)	42(68)	20(32)	1.0(0.6–1.9)	0.93	1.3(0.7–2.4)	0.46
Study site							
Agogo	345(64)	226(65)	119(35)	1		1	
Tepa	133(25)	107(80)	26(20)	2.12(1.3–3.5)	**0.002**	2.16 (1.18–3.60)	**0.01**
Dunkwa	30(6)	17(57)	13(43)	0.67(0.3–1.5)	0.33	0.7(0.3–1.6)	0.37
Nkawie	28(5)	25(89)	3(11)	4.3(1.3–14.8)	**0.017**	4.2(1.1–16.0)	**0.03**
Microscopy, AFBs positivity, ratio (%)							
Negative	125/261(48)	76(61)	49(39)	1		1	
Positive	136/261(52)	121 (89)	15 (11)	5.2(2.7–9.9)	**<0.0001**	3.9(1.9–7.7)	**<0.0001**
Bacteria culture, confirm growth, ratio (%)							
Negative	101/161(63)	60(59)	41(41)	1		1	
positive	60/161(37)	52(87)	8(13)	4.4(1.9–10.4)	**0.001**	4.9(2.0–11.7)	**<0.0001**
Baseline Mu 16S rRNA, Positivity							
Negative	58/152(38)	29(50)	29(00)	1		1	
Positive	94/152(62)	75(80)	19(20)	3.9(1.9–8.1)	**<0.0001**	1.9(0.8–4.3)	0.142
Wk 4 Mu 16S rRNA, Positivity							
Negative	82/152(54)	41(50)	41(50)	1		1	
Positive	70/152(46)	63(90)	7(10)	8.9(3.7–22.0)	**<0.0001**	6.8 (2.56–2.19)	**<0.0001**
Time to clearance of Mu 16S rRNA, median (weeks) (IQR)							
<week 4	73/152(48)	33(45)	40(55)	1		1	
>week 4	79/152(52)	71(90)	8(10)	10.86 (4.5–25.6)	**<0.0001**	4.9(1.8–3.8)	**0.002**
Development of paradoxical reaction, n (%)							
No	470(88)	314(84)	156(97)	1		1	
Yes	66(12)	61(16)	5(3)	6.1(2.4–15.4)	**<0.0001**	5.0(1.9–12.9)	**0.001**

Multivariate logistics regressions reporting odds ratios to test associations with baseline characteristics. Adjustment was performed for all characteristics. Abbreviations: WHO, World Health Organization; AFB, Acid-Fast Bacilli; OR, Odds Ratio; CI, Confidence Interval.

healing times and predicted healing times in slow healers. Gilman [13] recommends linear measurements as the most useful and valid measurements for clinical practice but contrary suggestions have been proposed [31–33]. In many clinical situations, there is a need for wound measurement methods that do not require cameras, computers, or a lot of data manipulation. Wound trajectories for lesions were clearly discriminatory by treatment week 4 for fast and slow healers suggesting their potential usefulness as a simple clinical tool in determining the course of a BU lesion [15,31,34].

In our study wound healing rates computed using measurements from acetate tracings (2D) or using the Silhouette Camera (3D) applying AA and PAR were comparable suggesting that they could be used in the same clinic. On the contrary healing rates were not comparable when the linear advancement method was employed for 2D and 3D measurements suggesting that if employed in clinical settings for BU, either should be used consistently to prevent errors in wound monitoring. Although, it is well known that shallow wounds are not planar but are 3-dimensional (3D), methods for obtaining quantitative 3D wound measurements are not always convenient as the 2D methods [35–37]. However, the newer digital tool (silhouette camera) employed in this study was convenient to use but cost implications need to be considered.

5. Conclusion

The rate of healing for slow healers using all methods was lower compared to fast healers. The linear method could be used to predict fast healers while the percentage area reduction method could be used to predict an approximate time to healing. High baseline bacterial load and delayed clearance of viable *M ulcerans* are associated with slow healing in BU disease. The clinical and microbiological characteristics associated with healing in BU should be considered in the development of improved disease management plans and reducing patient and caregiver anxiety.

Continuous variables were compared using Mann-Whitney *U* test. All Proportions were compared using chi-square tests for categorical data and Fisher's exact test. Abbreviations: WHO, World Health Organisation; cps, copies; IQR, Interquartile Range; AFB, Acid-Fast Bacilli; SR8, Streptomycin+.

Funding statement

This work was funded on the BuruliNox study which is part of the EDCTP2 programme supported by the European Union (101897

BuruliNox TMA 2016 SF-1509). The funder had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

CRediT authorship contribution statement

Bernadette Agbavor: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Writing – original draft, Writing – review & editing. **Abigail Agbanyo:** Data curation, Investigation, Project administration, Visualization, Writing – review & editing. **Aloysius Dzigbordi Loglo:** . **Philemon Boasiako Antwi:** . **Nancy Ackam:** Investigation, Validation, Visualization, Writing – review & editing. **Jonathan Adjei:** Investigation, Validation, Visualization, Writing – review & editing. **Venus Frimpong:** Investigation, Visualization, Writing – review & editing. **Kwadwo Boampong:** Supervision, Validation, Writing – review & editing. **Michael Frimpong:** Investigation, Supervision, Validation, Writing – review & editing. **Matthew Glover Addo:** . **Mark Wansbrough-Jones:** Conceptualization, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Yaw Ampem Amoako:** Conceptualization, Methodology, Supervision, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Richard Odame Phillips:** Conceptualization, Methodology, Supervision, Validation, Resources, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank all participants of this study.

Ethical statement.

The Committee on Human Research, Publications, and Ethics (CHRPE) of the Kwame Nkrumah University of Science and Technology (CHRPE/AP/335/19) approved the study. Written informed consent was obtained from participants, and ethical principles of the Declaration of Helsinki were followed.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jctube.2024.100415.

References

[1] Röltgen K, Pluschke G. Buruli ulcer: history and disease burden. Buruli ulcer: Mycobacterium ulcerans disease; 2019. p. 1–41.
[2] Phillips, RO, Saunderson, P, Simpson, H, Deribe, K, and Tabah, EN, *Mapping the global distribution of Buruli ulcer: a systematic review with evidence consensus.* The Lancet Global Health, 2019. **7**(7): p. e912-e922.10.1016/S2214-109X(19)30171-8.
[3] Frimpong M, Agbavor B, Duah MS, Loglo A, Sarpong FN, et al. Paradoxical reactions in Buruli ulcer after initiation of antibiotic therapy: Relationship to bacterial load. PLoS Negl Trop Dis 2019;13(8):e0007689.
[4] Portaels Fo, Johnson P, Meyers WM, Organization WH, Initiative GBU. Buruli ulcer: diagnosis of Mycobacterium ulcerans disease: a manual for health care providers. World Health Organization; 2001.
[5] Phillips, RO, Robert, J, Abass, KM, Thompson, W, Sarfo, FS, et al., Randomised trial to compare clarithromycin (extended release)-rifampicin and streptomycin-rifampicin for early, limited lesions of M. ulcerans infection. The Lancet, 2020. **395** (10232): p. 1259-1267.10.1016/S0140-6736(20)30047-7.
[6] Organization, WH, *Treatment of Mycobacterium ulcerans disease (Buruli ulcer): guidance for health workers*. 2012: World Health Organisation.
[7] Barogui YT, Klis S-A, Johnson RC, Phillips RO, van der Veer E, et al. Genetic susceptibility and predictors of paradoxical reactions in Buruli ulcer. PLoS Negl Trop Dis 2016;10(4):e0004594.
[8] Ruf M-T, Chauty A, Adeye A, Ardant M-F, Koussemou H, et al. Secondary Buruli ulcer skin lesions emerging several months after completion of chemotherapy: paradoxical reaction or evidence for immune protection? PLoS Negl Trop Dis 2011; 5(8):e1252.
[9] Aboagye SY, Kpeli G, Tuffour J, Yeboah-Manu D. Challenges associated with the treatment of Buruli ulcer. J Leukoc Biol 2019;105(2):233–42.
[10] Gyamfi E, Narh CA, Quaye C, Abbass A, Dzudzor B, et al. Microbiology of secondary infections in Buruli ulcer lesions; implications for therapeutic interventions. BMC Microbiol 2021;21(1):1–12.
[11] Yeboah-Manu D, Kpeli GS, Ruf M-T, Asan-Ampah K, Quenin-Fosu K, et al. Secondary bacterial infections of buruli ulcer lesions before and after chemotherapy with streptomycin and rifampicin. PLoS Negl Trop Dis 2013;7(5): e2191.
[12] Cardinal M, Eisenbud DE, Phillips T, Harding K. Early healing rates and wound area measurements are reliable predictors of later complete wound closure. Wound Repair Regen 2008;16(1):19–22. https://doi.org/10.1111/j.1524-475X.2007.00328.x.
[13] Gilman T. Wound outcomes: the utility of surface measures. Int J Low Extrem Wounds 2004;3(3):125–32. https://doi.org/10.1177/1534734604264419.
[14] Cukjati D, Reberšek S, Miklavčič D. A reliable method of determining wound healing rate. Med Biol Eng Compu 2001;39:263–71.
[15] Franz MG, Kuhn MA, Wright TE, Wachtel TL, Robson MC. Use of the wound healing trajectory as an outcome determinant for acute wound healing. Wound Repair Regen 2000;8(6):511–6.
[16] FrykbergRobert G. Challenges in the treatment of chronic wounds. Adv Wound Care 2015.
[17] Rogers LC, Bevilacqua NJ, Armstrong DG, Andros G. Digital planimetry results in more accurate wound measurements: a comparison to standard ruler measurements. J Diabetes Sci Technol 2010;4(4):799–802. https://doi.org/10.1177/193229681000400405.
[18] Nichols E. Wound assessment part 1: how to measure a wound. Wound Essentials 2015;10(2):51–5.
[19] Williams JR. The Declaration of Helsinki and public health. Bull World Health Organ 2008;86:650–2.
[20] Portaels, F and Organization, WH, *Laboratory diagnosis of buruli ulcer: a manual for health care providers*. 2014, World Health Organization.
[21] Beissner M, Herbinger K-H, Bretzel G. Laboratory diagnosis of Buruli ulcer disease. Future Microbiol 2010;5(3):363–70.
[22] Sarpong-Duah M, Frimpong M, Beissner M, Saar M, Laing K, et al. Clearance of viable Mycobacterium ulcerans from Buruli ulcer lesions during antibiotic treatment as determined by combined 16S rRNA reverse transcriptase/IS 2404 qPCR assay. PLoS Negl Trop Dis 2017;11(7):e0005695.
[23] WHO. *WHO. Promoting the integrated approach to skin-related neglected tropical diseases: https://www.who.int/activities/promoting-the-integrated-approach-to-skin-related-neglected-tropical-diseases#*. 2022.
[24] Nienhuis WA, Stienstra Y, Thompson WA, Awuah PC, Abass KM, et al. Antimicrobial treatment for early, limited Mycobacterium ulcerans infection: a randomised controlled trial. Lancet 2010;375(9715):664–72.
[25] Sarfo FS, Phillips R, Asiedu K, Ampadu E, Bobi N, et al. Clinical efficacy of combination of rifampin and streptomycin for treatment of Mycobacterium ulcerans disease. Antimicrob Agents Chemother 2010;54(9):3678–85.
[26] Phillips RO, Sarfo FS, Abass MK, Abotsi J, Wilson T, et al. Clinical and bacteriological efficacy of rifampin-streptomycin combination for two weeks followed by rifampin and clarithromycin for six weeks for treatment of Mycobacterium ulcerans disease. Antimicrob Agents Chemother 2014;58(2): 1161–6.
[27] Vincent, QB, Ardant, M-F, Adeye, A, Goundote, A, Saint-André, J-P, et al., *Clinical epidemiology of laboratory-confirmed Buruli ulcer in Benin: a cohort study.* The Lancet Global Health, 2014. **2**(7): p. e422-e430.
[28] Arnold M, Barbul A. Nutrition and Wound Healing. Plast Reconstr Surg 2006;117 (7S):42S–58S. https://doi.org/10.1097/01.prs.0000225432.17501.6c.
[29] Russell, L, *The importance of patients' nutritional status in wound healing.* British Journal of Nursing, 2001. **10**(Sup1): p. S42-S49.
[30] Shields BE, Diet in Wound Care: Can Nutrition Impact Healing? Current,. Psychiatry 2021;20(12).
[31] Santamaria N, Ogce F, Gorelik A. Healing rate calculation in the diabetic foot ulcer: comparing different methods. Wound Repair Regen 2012;20(5):786–9.
[32] Saiko G. The impact of the wound shape on wound healing dynamics: Is it time to revisit wound healing measures? Bioimaging 2021.
[33] Gorin DR, Cordts PR, LaMorte WW, Menzoian JO. The influence of wound geometry on the measurement of wound healing rates in clinical trials. J Vasc Surg 1996;23(3):524–8.
[34] Robson MC, Hill DP, Woodske ME, Steed DL. Wound healing trajectories as predictors of effectiveness of therapeutic agents. Arch Surg 2000;135(7):773–7.
[35] Jørgensen LB, Sørensen JA, Jemec GB, Yderstræde KB. Methods to assess area and volume of wounds–a systematic review. Int Wound J 2016;13(4):540–53.
[36] Jørgensen LB, Halekoh U, Jemec GB, Sørensen JA, Yderstræde KB. Monitoring wound healing of diabetic foot ulcers using two-dimensional and three-dimensional wound measurement techniques: a prospective cohort study. Adv Wound Care 2020;9(10):553–63.
[37] Shah A, Wollak C, Shah J. Wound measurement techniques: comparing the use of ruler method, 2D imaging and 3D scanner. J Am College Clinical Wound Specialists 2013;5(3):52–7.