Buruli ulcer disease: rethinking diagnostic techniques

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Background

- Buruli ulcer (BU), caused by *Mycobacterium ulcerans*, is a progressive necrotizing skin infection endemic to rural and deforested areas in 33 countries, mostly in Africa.[1] In 2022, there were 2,121 new cases reported to WHO, yet BU is considered to be vastly underreported.[1]
- Pathogenesis linked to production of polyketide toxin known as mycolactone which is cytotoxic and has immunomodulatory properties that induce debilitating lesions, and not from growth of bacteria like in other cutaneous non-tuberculous mycobacterial diseases.[2]
- Diagnosis is crucial for timely treatment and prevention of permanent disability, but there are no suitable point-of-care diagnostic tests.[1,3]
- Mycolactone detection may be an early diagnostic metric; efforts are underway to develop a point-of-care test based on its detection.[2,4]

Figure 1. Clinical course of Buruli ulcer. Ulcerations develop 2-3 months after the initial lesion. Consent obtained for all photographs.

Table 1. Currently available diagnostic tests for Buruli ulcer and their limitations.

Type	Details	Gaps
PCR	Identification of *IS2404*	- High rate of false negatives - Access to lab facilities
Histopathology	Necrosis in subcutaneous adipose tissue, acid-fast bacilli (+) with Ziehl-Neelsen stain	- Invasive to patients - Access to lab facilities
Smear test	Acid-fast bacilli (+) with Ziehl-Neelsen stain	- Access to lab facilities
Mycobacterial culture	Culture in mycobacterial media (Löwenstein-Jensen medium, Brown and Buckle or Ogawa medium) at 29-33 °C (*M. tuberculosis* grows at 37 °C)	- Access to lab facilities - Long incubation period (months)

Study Design

- **Objective:** To explore an alternative diagnostic approach using mycolactone levels measured via enzyme-linked immunosorbent assay (ELISA)
- **Study population:** 32 individuals with suspected Buruli ulcer disease in Côte d'Ivoire who tested positive for mycolactone by ELISA
- **Methods:**
  - 6 wound swabs were collected from each patient and sent to Institut Pasteur de Côte d'Ivoire and to Swiss Tropical And Public Health Institute (STPH) for laboratory analyses
  - PCR and ELISA were performed according to protocols[5] described by Warryn et al. 2020
  - Likelihood of BU based on clinical signs was independently assessed by 2 dermatology experts

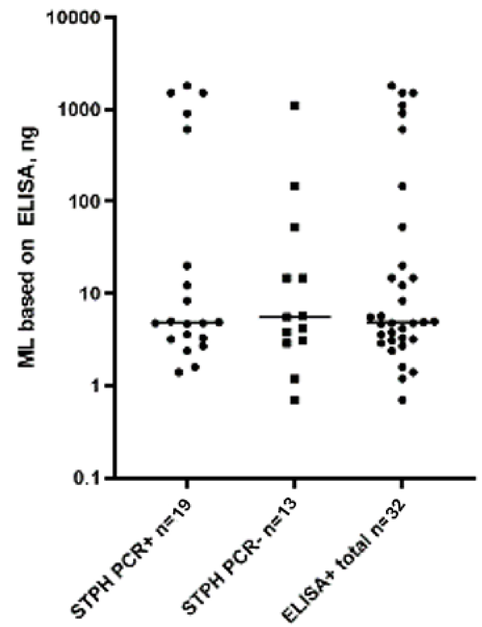
Figure 2. Collection of swabs from Buruli ulcer wounds.

Results

Key Findings

- Median (range) age: 25 years (1 – 91 years)
- Sex assigned at birth: 15/32 (47%) Female
- Median disease duration: 8 months (Range: 7 days – 14 years)
- *Morphological presentation:*
  - Ulcers were most common lesion type, present in 27/32 (84%)
  - 2 participants had nodules,1 had plaques, and 29 had overlying edema
  - 14/32 (44%) exhibited necrotic tissue
- Median wound area: 30 cm^2
- Based on clinical presentation alone, 22/32 (69%) were scored to have very high likelihood of having BU before any diagnostic tests were performed.
- 8/32 (25%) had negative PCR and culture results.
- Mycolactone levels captured by ELISA exhibited a wide range of values across participants.
  - Median = 4.9 ng [Range: 0.7-1810.9 ng]
  - Nine samples had > 20 ng and 4 samples had > 1000 ng
  - Importantly, no significant differences in sex, age, lesion type, and clinical BU likelihood score among those with > 1000 ng compared against all others

Figure 2. Distribution of mycolactone amounts detected by ELISA in the 32 samples that were ELISA-positive, regardless of PCR+ or PCR- status.



Conclusions

- Results support hypothesis that mycolactone detection can be used as target marker for effective new diagnostic tool in BU, in addition to or potentially to replace PCR
- Observed discrepancies within any patient may be due to sample-to-sample variations, efficiency of sample collection, and nature of sequential sampling of 6 swabs per lesion. Low concentration of mycolactone detected in some samples may be due to degradation between field sites and laboratory facilities.
- Although PCR is current gold standard, many challenges with false-negative and false-positive findings have been documented[4,6]
- An increased understanding of the relationship between mycolactone levels and clinical presentation of BU can aid in early detection and management, going towards efforts to reduce burden of this neglected cutaneous disease

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