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Case Report: Severe Multifocal Form of Buruli Ulcer after Streptomycin and Rifampin Treatment: Comments on Possible Dissemination Mechanisms

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Abstract. Buruli ulcer (BU), a disease caused by *Mycobacterium ulcerans*, leads to the destruction of skin and sometimes bone. Here, we report a case of severe multifocal BU with osteomyelitis in a 6-year-old human immunodeficiency virus (HIV)-negative boy. Such disseminated forms are poorly documented and generally occur in patients with HIV coinfection. The advent of antibiotic treatment with streptomycin (S) and rifampin (R) raised hope that these multifocal BU cases could be reduced. The present case raises two relevant points about multifocal BU: the mechanism of dissemination that leads to the development of multiple foci and the difficulties of treatment of multifocal forms of BU. Biochemical (hypoproteinemia), hematological (anemia), clinical (traditional treatment), and genetic factors are discussed as possible risk factors for dissemination.

INTRODUCTION

Buruli ulcer (BU), a disease caused by *Mycobacterium ulcerans*, leads to the destruction of skin and sometimes bone.[1,2] Buruli ulcer is rarely fatal, but poorly treated cases often result in contracture deformities, which are the main complication of BU. The disease presents in two main active clinical forms: non-ulcerative (papule, nodule, plaque, and edema) and ulcerative.[3] Other forms have also been described, namely the osteomyelitis and disseminated (or multifocal) forms.[4–6]

The multifocal forms, though rarely described in the literature, have been associated with osteomyelitis and human immunodeficiency virus (HIV) coinfection.[1,2,5–9] However, the dissemination process behind these forms is not fully understood. Here, we present the case of a young boy who was admitted to the hospital with a bifocal form of BU that disseminated during treatment, generating several new foci.

CASE REPORT

A 6-year-old boy (DR) from Adjohoun (Oueme department, south Benin) was admitted to the BU treatment center of Allada (Atlantique department, south Benin) on May 18, 2007, 3 months after the appearance of the first lesion (according to his parents). His medical history included Bacille Calmet-Guerin (BCG) immunization and two cousins who had been diagnosed with BU in the past, one of whom had died of the disseminated form 2 years prior. DR's condition at admission was very poor, with moderate to severe malnutrition and the following Z scores: height for age = –2.90, weight for height = – 1.67, and weight for age = –2.87 (normal ranges = –1 to +1). He presented with two different clinical lesions (mixed form) at two different sites (bifocal form), with plaques and ulcers on the right foot and the left hand (Figure 1). According to his parents, the initial lesion was edema of a finger (left hand) that evolved into a plaque after a few days. He was treated first with herbs by a traditional healer in his village, but 2 weeks later, a new edematous lesion appeared on the right toe, and this lesion also evolved to a plaque that covered the whole foot. No other lesions were detected at the time of his admission. Microbiological examinations of biopsies and fine needle aspirations (FNA) were performed, as previously described.[3,10] At admission, IS*2404* polymerase chain reaction (PCR) and direct smear examination (DSE) after Ziehl-Neelsen (ZN) stain were positive, but cultures on Löwenstein-Jensen media were negative. Histopathology revealed lesions consistent with BU (Figures 2 and 3). All of these epidemiological, clinical, and microbiological features confirmed a category 3 BU case, according to World Health Organization (WHO) definitions.[3,11,12] In addition, radiography revealed focal osteomyelitis under the two lesions. Other biological examinations showed moderate anemia (hemoglobin = 11 g/dL; normal range [NR] = 12–16 g/dL), leukocytosis (white blood cells [WBC] = 35,000/dL; NR = 6000–8000/dL), and eosinophilia (8%, NR = 0–2%). He also had hypoproteinemia (protein rate = 66.5 g/dL; NR = 70–80 g/dL). The human immunodeficiency virus (HIV) serology was negative and creatinine, transaminases, and blood sugar levels were normal.

Four days after admission, on May 22, 2007, streptomycin (S) and rifampin (R) therapy (S+R) was started according to WHO protocol.[11] An assessment was conducted every 2 weeks (measurement of lesions, photography, and biopsy when possible). Because the clinical evolution of the lesions after 4 weeks was unfavorable (deterioration of the lesions and worsening of the patient's general condition), surgery was performed (excision and amputation of two fingers), and the patient completed 4 more weeks of S+R treatment.

Even though treatment compliance was excellent during the 8 weeks of S+R therapy (56 doses administered over 56 days), DR developed several episodes of dissemination. The various clinical features and evolution after completion of S+R therapy are summarized in Table 1 and illustrated in Figure 4A–H.

We noted that the emergence of each new disseminated lesion was always preceded by an episode of fever over 39°C and a high white blood cell (WBC) count, as well as eosinophilia ranging between 0% and 10%. Anemia was also present, with a hemoglobin concentration ranging between 6.2 and 10.1 g/dL (Table 2). Fine needle aspiration (FNA) and

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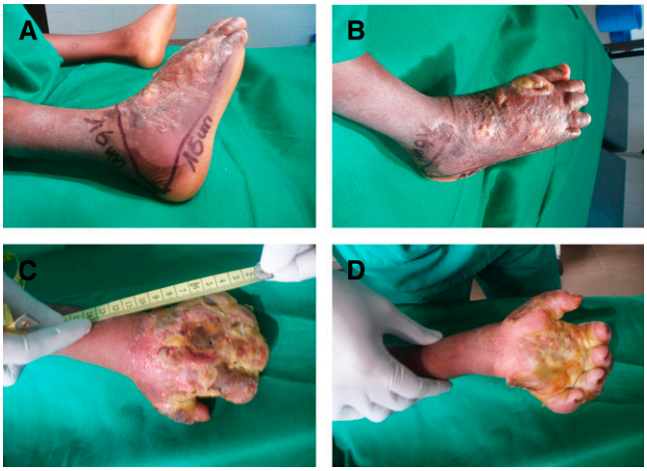

FIGURE 1. Patient DR at admission (May 18, 2007), with bifocal lesions on (**A**, **B**) the right foot and (**C**, **D**) the left hand. This figure appears in color at www.ajtmh.org.

biopsies from these new lesions were DSE or PCR positive, but cultures remained negative. Histopathologic examination revealed coagulative necrosis, eosinophilic infiltration, neutrophil-containing inflammatory infiltration, vasculitis, and vascular thrombosis. The microbiologic and histopathologic results are presented in Table 1. Stool examinations for parasites, including cysts and eggs, were negative.

All new lesions were excised, and the skin was grafted as soon as possible. During surgery, bone involvement was detected under each new lesion. Thus, bone surgery was also performed in addition to the excision of infected soft tissue (Table 1). During fever episodes, large spectrum antibiotics were administered several times without success. These antibiotics included ceftriaxon (50 mg/kg/day) or a combined therapy of amoxicillin (100 mg/kg/day), metronidazole (30 mg/kg/day), and gentamicin (3 mg/kg/day) for at least 10 days.

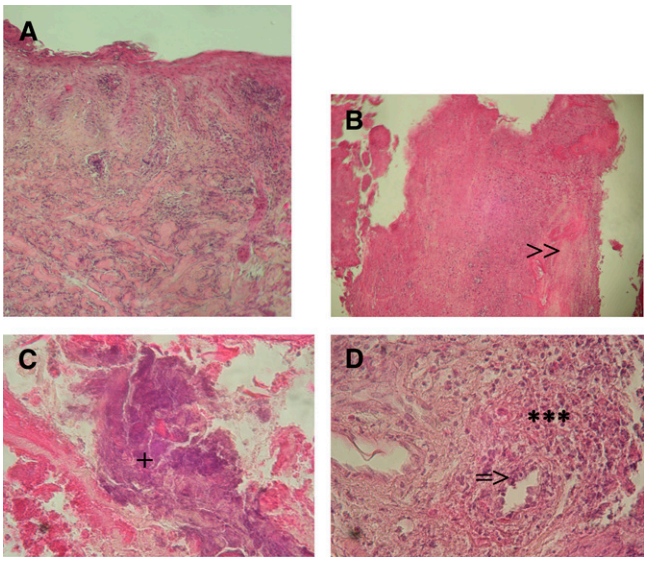

FIGURE 2. Histopathologic appearance of lesions at admission. **Type of specimen**: skin (epidermis, dermis, hypodermis). **Lesions**: psoriasiform hyperplasia of the skin (**A**); coagulative necrosis (**B**, >>) of the hypodermis with calcification foci (**C**, +) and inflammatory infiltration of mild to moderate intensity (neutrophils, lymphocytes) (**D**, ***). Images of vasculitis (**D**, = >). **Diagnosis**: consistent with BU. Hematoxylin and eosin staining (HE). This figure appears in color at www.ajtmh.org.

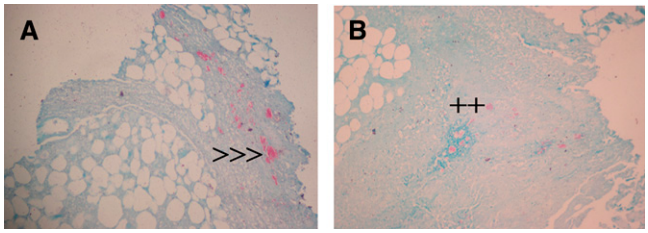

FIGURE 3. Histopathologic appearance of lesions after 4 weeks of S+R therapy (June 2007). Ziehl-Neelsen (ZN) staining showing coagulative necrosis, steatonecrosis, many clusters of bacilli in the interlobular wall (**A**) and few clusters of bacilli in the coagulative necrosis area (**B**). Magnifications: **A** and **B**: ZN ×100. This figure appears in color at www.ajtmh.org.

A blood sample was collected in April 2009 to study the immune status of the patient. The results indicated that the lymphocyte differential count (T, B, and natural killer [NK] cells) was normal for a child of his age. The young patient had 2,587 $CD4^+$ T cells (45%) per µL and 1,438 $CD8^+$ T cells per µL (25%), as well as a normal $CD4^+$:$CD8^+$ ratio of 1.8. The number of NK cells per µL was 512 (8.9%), and the number of B cells per µL was 978 (17%). No excessive T cell activation was observed; 8.4% of the $CD4^+$ T cells and 0.6% of the $CD8^+$ T cells expressed the cell membrane activation marker CD38 on their memory subsets ($CD45RO^+$ cells), indicating a normal activation state.

All lesions healed, and the patient was cured after 18 months of hospitalization and 15 surgical interventions but with severe sequelae (amputation of the left hand and the second right toe; functional limitation of elbows, the right wrist, the right knee, and both ankles).

DISCUSSION

Two relevant aspects of this case should be considered: the mechanism of dissemination leading to the development of multiple foci and the difficulties of treatment of the multifocal forms of BU. The rare published cases of disseminated forms of BU are often associated with bone involvement[1,2,5,8,9] and/or HIV co-infection.[7,8] The first large series of BU with laboratory-confirmed bone involvement focused on 73 cases from 18 months to 45 years of age with a median age of 14.5 years.[2] Thirty out of the 73 selected cases had a multifocal form at admission. Some of those patients continued to develop new lesions during hospitalization or after cure of all initial lesions. Nine of them were tested for HIV, of whom four (44.4%) were positive, confirming the association between the BU multifocal form, bone involvement, and HIV co-infection. Most BU osteomyelitis and disseminated lesions are localized to limbs, at distal joints, and on small bones.[1,2,6,9] The number of foci typically range between two and seven,[1,9] and our case is in accordance with these observations. This case also shows that apparently small or non-ulcerated lesions may hide bone involvement. Thus, practitioners should consider obtaining radiographic images in the presence of known risk factors for osteomyelitis, such as multifocal BU or HIV co-infection.[13,14]

No previous publication has addressed the general clinical symptoms or biochemical and hematological parameters in

TABLE 1

Chronology of clinical features, dissemination episodes of the patient, and summary of the microbiological and histopathological results

Date (W no. or M no. after the beginning of S+R therapy)	Description of lesion	Illustration	DSE	PCR	Histopathology	Surgery date (act*)
May 2007 Start S+R therapy	• Admission to hospital		Positive	Positive	Consistent with BU	
	• Plaque + ulcer + bone lesion on the right foot	Figure 1A and B				
	• Plaque + ulcers + bone lesion on the left hand	Figure 1C and D				
June 2007 (W4)	• Worsening of the initial lesions (plaque + ulcer + bone lesion on the right foot and the left hand)		Positive	Negative	Consistent with BU	June 20, 2007 (excision and amputation of a toe and two fingers) June 30, 2007 (amputation of the left hand)
July 2007 (W8)	• End of S+R therapy					
July 2007 (W10)	• 1st dissemination		Positive	Positive		August 2, 2007 (excision and bone surgery)
	• Swelling + bone lesion on the left elbow	Figure 4A				
	• Swelling + bone lesion on the right middle finger	Figure 4B				
August 2007 (W12)	• 2nd dissemination		Negative	Positive	Inflammatory infiltrate, not specific to BU	August 17, 2007 (excision)
	• Swelling on the left foot	Figure 4C				
	• Plaque + small ulcer and bone lesion on the right elbow	Figure 4D	Positive	Positive		August 27, 2007 (excision and bone surgery)
October 2007 (M5)	• 3rd dissemination: plaque + ulcer + bone lesion on the left elbow		Negative	Positive		October 18, 2007 (excision) November 15, 2007 (bone surgery)
December 2007 (M7)	• 4th dissemination: swelling on the right wrist	Figure 4E	Positive	Not done	Vasculitis and vascular thrombosis	January 31, 2008 (excision)
January 2008 (M8)	• 5th dissemination: swelling + bone lesion on the left foot	Figure 4F	Positive	Positive		February 28, 2008 (excision and bone surgery)
March 2008 (M10)	• 6th dissemination: ulcer (less than 5 cm in diameter) + bone lesion on the right knee	Figure 4G	Positive	Not done		May 5, 2008 (bone surgery)
November 2008 (M18)	• End of hospitalization					
January 2009 (M20)	• 7th dissemination: swelling on the lateral aspect of the right leg	Figure 4H				

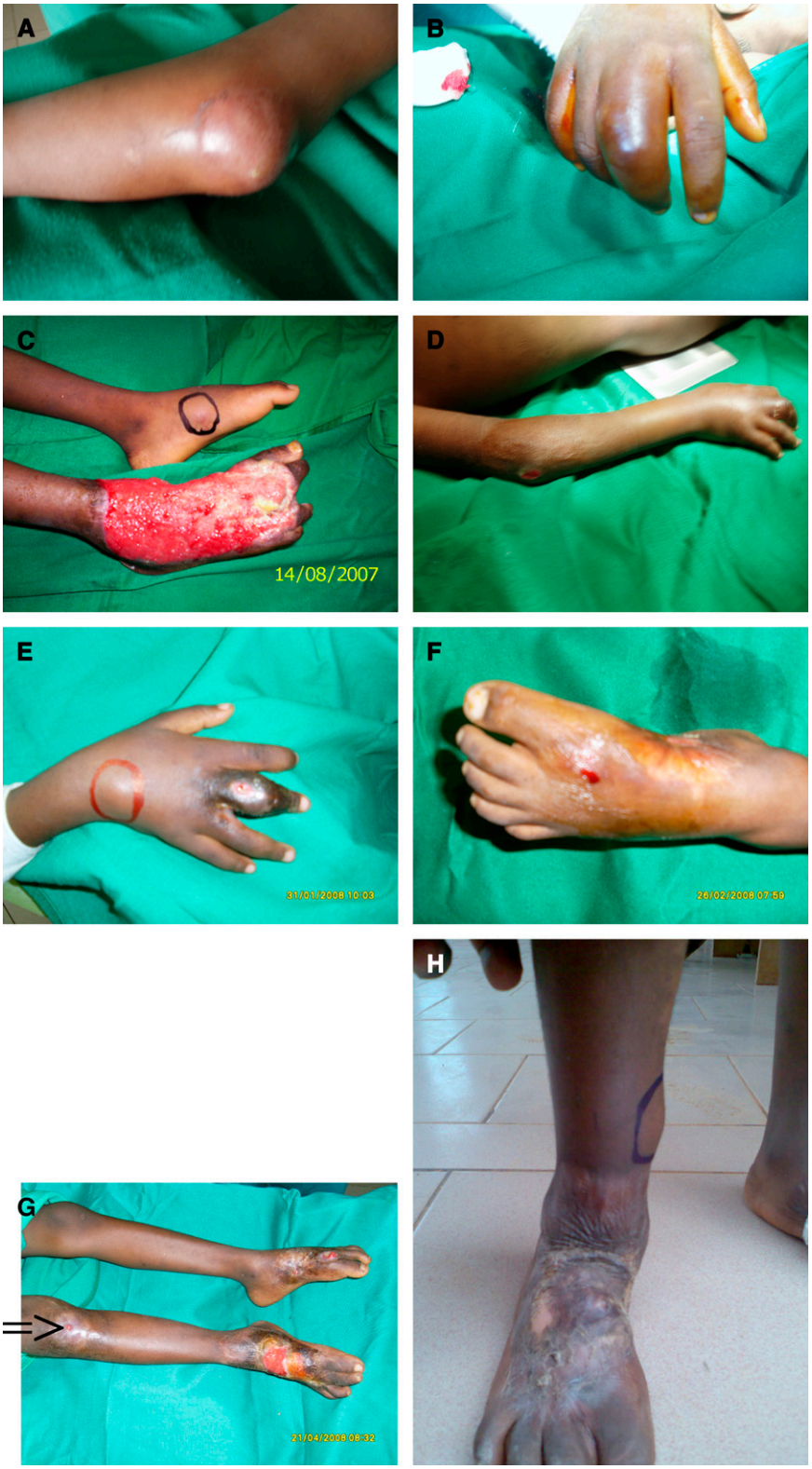

FIGURE 4. Patient DR dissemination steps after the beginning of S+R therapy: July 2007 (W10): (**A**) first dissemination with swelling of the left elbow and (**B**) right hand; August 2007 (W12): (**C**) second dissemination with swelling of the left foot and (**D**) a plaque with a small ulcer on the right elbow; December 2007 (M7) and January 2008 (M8): (**E**) fourth and fifth disseminations with swelling of the right wrist and (**F**) the right foot; March 2008 (M10): (**G**) sixth dissemination with ulcer on the right knee; January 2009 (M20): (**H**) seventh dissemination with swelling on the lateral aspect of the right leg. This figure appears in color at www.ajtmh.org.

TABLE 2
Summary of hematology and biochemical results*

Period after beginning of S+R therapy	Hb (g/dL)	Htc (%)	WBC (no./dL)	PN (%)	Eo (%)	L (%)	M (%)	Proteinemia (g/L)	Albumin/globulin Fraction	Albumin (g/L)	Alpha 1 (g/L)	Alpha 2 (g/L)	Beta 1 (g/L)	Beta 2 (g/L)	Gamma (g/L)
Normal values	**12–16**	**37–43**	**6000–8000**	**60–65**	**2**	**30-35**	**5–8**	**70–80**		**42–50**	**0.7–2**	**5–9**	**2–6**	**4–8**	**2–12**
W1	**10.1**↓	32↓	**22,700**↑	50↓	**10**↑	30	10↑	**66.5**↓	0.73	**28.0**↓	**5.7**↑	9.6	5.9	4.3	13.0↑
W4	**8.56**↓	23↓	**13,900**↑	74↑	0	20↓	6	**55.0**↓	0.82	**24.8**↓	**4.2**↑	8.2	5.8	2.9↓	9.1
W10	**6.2**↓	22↓	**19,400**↑	68	6↑	28↓	0	104.1↑	0.70	42.9	**5.9**↑	**13.8**↑	**9.7**↑	3.0↓	**28.8**↑
W12	**7.64**↓	22↓	**11,000**↑	56↓	2	42↑	0								
M5	**8.63**↓	28↓	**15,300**↑	56↓	4↑	40↑	0								
M10	**7.82**↓	24↓	**14,100**↑	44↓	8↑	46↑	2								

*↑ = increase of the value compared with the normal range; ↓ = decrease of the value compared with the normal range; Hb = hemoglobin; Htc = hematocrit; WBC = white blood cells; PN = polynuclear neutrophils; Eo = eosinophils; L = lymphocytes.

disseminated BU cases. In this case, and in many other cases treated in the BU treatment centers of Allada and Zagnanado in Benin (Aguiar J, personal communication), fever and an increase in WBCs occurred before the development of each new lesion, despite the administration of large-spectrum antibiotics, even in patients treated with surgery alone. Secondary infections or other bacteria sepsis may explain these phenomena. Unfortunately, blood cultures are not available in our setting. However, the lack of response to antibiotics allows us to discard these hypotheses (secondary infections or other bacteria sepsis) and suggest a probable bacteremia with extracellular or intraphagocytic *M. ulcerans*. *Mycobacterium ulcerans* is known to be present within phagocytes in BU lesions.[15,16] Accordingly, the presence of the bacteria in the new lesions was confirmed by the positive DSE or PCR results (Table 1) and the presence of acid-fast bacilli (AFB) on histopathologic slides. In addition, the distributions of these AFB indicated that the lesions were new, active ones. Indeed, numerous extracellular AFB were seen in the interlobular wall in the subcutaneous tissue (Figure 3A) and very few in the areas of coagulative necrosis (Figure 3B). A high eosinophil count during BU disease has never been described. It is unlikely that eosinophilia, both in the blood and in tissue, is related to intestinal parasitic infection, as the stool examinations were negative. A skin parasitic co-infection could be speculated here but cannot be proved. However, the presence of eosinophilia in mycobacterial diseases has been reported.[17–19] The high eosinophil count could also be linked to the antibiotics received by the patient (streptomycin and amoxicillin) or a possible immunoglobulin E (IgE)-dependent hypersensitivity.[19]

The emergence of new lesions could be linked to an excessive specific immune response. The histopathologic results revealed neutrophil-containing inflammatory infiltrates, vasculitis and vascular thrombosis, indicating that there was an intensive inflammatory response. Several reports have described a similar excessive immune response following effective antimycobacterial therapy.[20–22] This phenomenon, called "paradoxical reaction"[22] or "immune reconstitution inflammatory syndrome (IRIS)," has also been described in patients with HIV who are coinfected with other mycobacteria, such as *Mycobacterium tuberculosis* or *Mycobacterium leprae*.[23,24] Although our patient was HIV-negative, the IRIS is highly supported by the clinical characteristics (fever, swelling, or abscess) previously described,[20,24–26] the increase in the WBC count, the alpha-1, alpha-2, and gamma globulin values (Table 2), and the histopathologic results. Because of technical constraints, the immune status of this patient was not extensively assessed at admission. Even though he was HIV-negative and the limited immunological evaluation did not reveal immunodeficiency in April 2009, other immunosuppressive factors were observed at admission, namely hypoproteinemia. Fock and others[27] and Rodriguez and others[28] showed that protein-energy malnutrition increases the production of immunosuppressive interleukin in response to lipopolysaccharide (LPS) stimulation. This impairs immune responses, increasing susceptibility to infections.[29] Similarly, other authors have described disseminated tuberculosis in patients with weight loss,[30] or disseminated leprosy in rats submitted to a protein-free diet.[31] In our case, infected tissues were removed surgically, which contributed, in addition to protein supplements, to the improvement of the general status of the patient. Subsequently, at the time of the first dissemination, protein levels normalized

(Table 2). Therefore, it is possible that the IRIS in this BU case was simply caused by the correction of immunosuppressive factors, namely hypoproteinemia.

Anemia could also be a factor that promotes dissemination. Our patient had moderate anemia at admission but severe anemia concomitant with each new lesion (Table 2). Pszolla and others[14] suggested that low tissue oxygen levels could promote the hematogenous spreading of *M. ulcerans*. This low tissue oxygenation could have several etiologies, including anemia and vascular thrombosis. The role of hypoproteinemia and anemia as possible risk factors in the dissemination of BU lesions may have an important impact on treatment. Clinicians might consider anemia correction and protein supplementation as adjuvant treatment of BU.

Stienstra and others[32] have demonstrated the role of the SLC11A1 (NRAMP1) gene in susceptibility to BU, as in tuberculosis and leprosy. Our patient had two cases of BU in his family history, suggesting the possible involvement of genetic factors. However, additional studies are necessary to explore the possible link between genetic factors and the occurrence of severe forms of BU. Other factors that could be explored are the virulence of the bacteria and the herbal treatment sought by most patients in Benin (including DR) as a first treatment choice before presenting to a hospital.[33]

Before 2004, BU treatment consisted of wide excision of the affected and healthy surrounding tissue.[12] Thus, the number of surgical interventions that patients with disseminated BU endured was quite significant (up to 32).[2] The current WHO protocol combines S+R, surgery, and physiotherapy, depending on the category of the lesion.[11,12] With this new protocol, the hope was to reduce the risk of dissemination for category 3 lesions. Despite strict adherence to these recommendations, in our case, new lesions still appeared 20 months after initial therapy. Such disseminated lesions during antimycobacterial treatment associated with surgery have been described elsewhere[13,14,34] and emphasize the difficulties of treatment of such cases even with the combination of antibiotherapy and surgery. Because of the comprehensive clinical, biological, and treatment data available in our case, several factors that may contribute to the observed dissemination have been highlighted. However, many questions remain to be clarified concerning the mechanisms involved in the dissemination of *M. ulcerans* in BU patients and the occurrence of paradoxical reactions during antibiotic treatment.

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