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SHORT COMMUNICATION

Severe Buruli Ulcer Treated with Minimal Surgical Excision after Prior Biopsy Mapping

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Buruli ulcer is the third most common mycobacterial infection next to tuberculosis and leprosy caused by *Mycobacterium ulcerans* affecting the skin, subcutaneous tissues, and sometimes bone (1). Early excision of small pre-ulcerative lesions, including papules and nodules, may be curable; however, extensive lesions, including plaques and edema, require adequate surgical treatment since residual necrotic tissue can cause persistent subcutaneous infection (2). Such treatment entails the excision of all dead tissues with a 3–4 cm margin (3), which can lead to secondary functional limitations. We reported previously a case of Buruli ulcer where the extent of surgical excision was determined by retrospective examination (4). We present here a case of Buruli ulcer caused by *M. ulcerans* subspecies *shinshuense*, which was treated successfully with minimal surgical excision as determined by preoperative mapping biopsy procedure.

CASE REPORT

A 20-year-old Japanese male noticed redness and swelling around his left elbow in February 2014. He was diagnosed with cellulitis and treated by a local doctor with oral antibacterial agents, including cefdinir, azithromycin, and levofloxacin, which showed no apparent improvement. The redness and swelling gradually spread to the forearm in a month, and an ulcer measuring about 10 mm developed at the center of the erythematous lesion. Ziehl-Neelsen (ZN) staining for mycobacterium smear examination from the ulcer revealed multiple copies of banded acid-fast bacilli (AFB). Therefore, he was referred to our hospital in March 2014. Physical findings revealed diffuse erythema, swelling, and necrotic ulcer on his left arm with tenderness around the ulcer (Fig. 1A). He had no prior history of autoimmune disease or malignancy. Laboratory investigations were normal, except for elevated levels of white blood cells (10,300/mm^3) and C-reactive protein (1.78 mg/dl; normal < 0.3 mg/dl). No growth in the Ogawa egg medium was observed; however, the PCR analysis targeting the insertion sequence, *IS2404*, and 16S rRNA gene sequences of the cutaneous biopsy specimen from the erythema revealed Buruli ulcer caused by *M. ulcerans* subspecies *shinshuense* (5). We initiated treatment with oral clarithromycin 800 mg/day, levofloxacin 500 mg/day, and rifampicin 450 mg/day.

We performed the first debridement 10 days after the initiation of the combined anti-mycobacterial treatment. In order to minimize the extent of surgical excision, preoperative mapping biopsies were performed as described in our previous report (4) using a 3 mm punch from 9 sites at the edge and one site at the center of the erythematous lesion on his left arm (Fig. 1B). Granuloma and diffuse inflammatory cell infiltration from the dermis to the subcutaneous tissues were observed, to a greater or lesser extent, on all sites (Fig. 1C). Therefore, we decided the extent of surgical excision within the erythematous lesion by lining the mapping sites 1–9 that show granulomatous reactions, although necrosis was observed on 5 sites (Fig. 1D). The ZN staining revealed many AFB in the subcutaneous tissues of some parts of the resected specimen obtained from the first debridement (Fig. 1E). Two weeks later, the erythema persisted on the peripheral skin near the biopsied sites of 4 and 5 and on the ulnar skin near the biopsied sites of 8

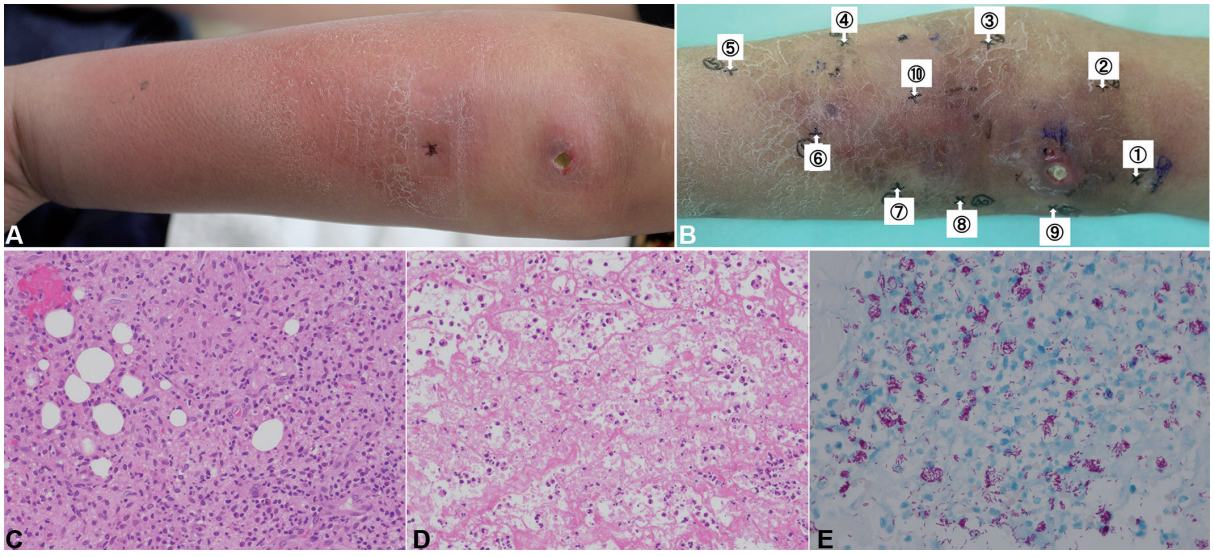

Fig. 1. (A) Initial clinical presentation. Diffuse edematous erythema with necrotic ulcer was observed on the left arm. (B) Preoperative mapping biopsies were performed from 9 sites at the edge and one site at the center of the erythematous lesion on the left arm. (C) Histopathological examination showed granuloma in all samples of the mapping biopsy and (D) necrosis in 5 samples of the mapping biopsy (H-E × 200). (E) Ziehl-Neelsen staining revealed many acid-fast bacilli in the subcutaneous tissue of the resected specimen obtained from the first debridement (× 400).

and 9. We performed additionally a second debridement and skin grafting. Although histopathological findings of the resected specimens demonstrated both granuloma and necrosis, ZN staining was negative. The erythematous lesion disappeared after the second debridement. Oral antibacterial agents were administrated for 8 weeks. No recurrence has been observed for 13 months.

DISCUSSION

Although Buruli ulcer is present in around 30 countries (6, 7), there are no established guidelines of treatment for the disease. In Japan, *M. ulcerans* subspecies *shinshuense* have been isolated in all cases, usually affecting the upper limb and presenting multiple lesions. Children are less frequent affected in Japan compared to Australia and Africa (8, 9).

Almost half of the patients with severe Buruli ulcer have functional limitations after the treatment of larger lesions (3). In 2004 the WHO recommended a dual antibiotic therapy with rifampicin and streptomycin rather than a purely surgical treatment (6).

In this study, we performed the preoperative mapping biopsy on 10 sites before the first debridement; the analytic results are summarised in Table I. Since improvement was achieved with marginal excision of the erythematous lesions on sites showing granuloma without necrosis, we avoided unnecessary radical surgery using this mapping biopsy procedure as proposed in our previous report (4). The erythema persisted after the first debridement, but significant improvement was observed with additional debridement for the peripheral and ulnar lesions. Although no AFB were observed on all sites with the ZN staining, the PCR revealed positive for the *IS2404* in 80% of the sites showing necrosis (see Table I). Thus, we think that we should debride sites showing necrosis, even though granuloma formation was observed already. We could not determine the extent of excision on sites showing necrosis. Additional mapping biopsy is probably needed to evaluate the spread of necrosis similar to the method used for extramammary Paget's disease (10, 11), where we obtained specimens at 1, 2, and 3 cm sites beyond the clinical border (11).

Histopathological findings demonstrated both necrosis and granuloma in some specimens in this case, which is different from our previous report (4). We speculate that the difference is associated with the interval until the initiation of antibacterial agents, duration of therapy, and phase of wound healing. Ruf et al. (6) pointed out that the histopathological findings of Buruli ulcer changed with time from the large regions presenting massive coagulative necrosis without inflammatory infiltration to the inflammatory infiltration mainly forming granuloma. Antibacterial treatment leads to the reduction of the amount of bacilli and concentration of lipidic exotoxin, mycolactone, secreted by the bacilli, which induce a normal immune response. Since we considered that the formation of granuloma represented a normal immune response to AFB, we decided the surgical margin within the erythematous lesion lined by mapping sites showing granuloma despite the presence of necrosis. However, it seems that debridement of the sites showing necrosis is needed. This case healed without functional limitations or recurrences in spite of the persistence of other erythematous lesion with granuloma and without necrosis.

Table I. *Mapping biopsy evaluated by histopathological findings, and the results of Ziehl-Neelsen staining and PCR analysis*

	Mapping site									
Analysis type	1	2	3	4	5	6	7	8	9	10
Granuloma (H-E)	+	+	+	+	+	+	+	+	+	+
Necrosis (H-E)	–	–	+	+	+	–	–	+	+	+
Ziehl-Neelsen	–	–	–	–	–	–	–	–	–	–
PCR (*IS2404*)	–	–	+	+	–	–	+	+	+	–

Paradoxical reactions, recognized as a reversal of an immune-inhibitory state induced by mycolactone, may deteriorate the response after initial treatment, mostly with surgery (12). Avoidance of further surgical resection for the lesions showing paradoxical reactions is recently emphasized (13). Such lesions show inflammatory findings including giant cells, while lesions before antibiotics show necrosis (12, 13). However, the histological distinction between paradoxical reactions and treatment failure is not established. Although some sites of mapping biopsy in this case might be regarded histologically as paradoxical reaction, additional debridement was needed at the sites presenting granuloma and necrosis.

Although further investigation is needed, the mapping biopsy procedure seems to be helpful to determine the extent of surgical excision in treating Buruli ulcer, which contributes to avoidance of unnecessary debridement and complications.

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