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Use of Fine-Needle Aspiration for Diagnosis of *Mycobacterium ulcerans* Infection▽

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Noninvasive methods for the bacteriological diagnosis of early-stage *Mycobacterium ulcerans* infection are not available. It was recently shown that fine-needle aspiration (FNA) could be used for diagnosing *M. ulcerans* infection in ulcerative lesions. We report that FNA is an appropriate sampling method for diagnosing *M. ulcerans* infection in nonulcerative lesions.

Mycobacterium ulcerans infection (Buruli ulcer) is one of the 13 most neglected tropical diseases (9) and the third most common mycobacterial infection after tuberculosis and leprosy in immunocompetent humans (2, 6, 13–14). In general, this skin disease initially manifests as a painless nodule or papule, plaque, or edema (2). Without early intervention, these symptoms evolve into painless ulcers with undermined edges. The epidemiological, scientific, and management aspects of this disease have been well described (12). Over recent years, the management of Buruli ulcer patients has considerably changed with advances in antibiotherapy (3, 5).

Laboratory diagnosis of this mycobacterial infection is based on detection of acid-fast bacilli (AFB) through the direct examination of samples, isolation of mycobacteria by culture, histological analysis, and detection of *M. ulcerans* DNA by PCR (12). Ulcerative lesion specimens are collected using swabs (12). Swabbing from the undermined edges of ulcers may sometimes be difficult and painful. Collecting specimens from patients with nonulcerative lesions necessitates invasive procedures, such as incisional, excisional, or punch biopsies, which require hospital infrastructure not available in remote rural areas in Africa where *M. ulcerans* infection is endemic. Two studies recently reported that fine-needle aspirates could be used to diagnose *M. ulcerans* infection in ulcerative lesions. In both studies, the number of patients enrolled was not large enough to draw conclusions on the effectiveness of this technique in diagnosing nonulcerative forms.

First, we compared the diagnostic sensitivities of fine-needle aspiration (FNA) and swabbing in 64 patients with ulcerative lesions. These patients had skin lesions consistent with active *M. ulcerans* infection, based on the clinical definition of the World Health Organization (12). For each patient with ulcerative lesions, two swab samples were taken from beneath the undermined edges of the ulcers and one FNA sample was taken from the edge of the lesion. The FNA procedure was similar to that described previously (4, 11); however, we used 20-gauge, 25-mm needles (attached to 5-ml syringes) instead of the 21-gauge and 23-gauge needles used in other studies. All samples were placed in sterile Venosafe tubes (Terumo) and sent, at room temperature, to the bacteriology unit of Angers University Hospital, France, within 7 days of collection for processing.

Significant differences were observed in the efficacies of the two sampling methods. PCR using FNA samples detected *M. ulcerans* DNA in 56 of the 71 patients (diagnostic sensitivity of 79%), and PCR using swab samples detected *M. ulcerans* DNA in 68 of 71 patients (sensitivity of 95%) (Table 1). Chi-square tests showed that the number of positive smears (direct smear examination) was significantly different ($P < 0.0001$) between swab (50.7%) and FNA (9.9%) samples. The number of pos-

TABLE 1. Results of direct smear examination and *M. ulcerans* DNA detection from swabs and fine-needle aspirations from ulcerative and nonulcerative lesions

Lesion specimen and sampling method	No. (%) of specimens with indicated result:			
	DSE[a]		PCR	
	Positive	Negative	Positive	Negative
Ulcerative ($n = 71$)				
FNA	7 (9.9)	64 (90.1)	56 (78.9)	15 (21.1)
Swabbing	36 (50.7)	35 (49.3)	68 (95.8)	3 (4.2)
Nonulcerative, FNA ($n = 64$)	16 (25.0)	48 (75.0)	57 (89.0)	7 (11.0)

[a] DSE, direct smear examination.

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Faces pain scale						
Analog pain scale	**0**	**2**	**4**	**6**	**8**	**10**
Swab						
Adults (n=10)	0	0	0	3	7	0
Children (n=10)	0	0	0	0	6	4
FNA						
Adults (n=10)	0	6	4	0	0	0
Children (n=10)	0	5	5	0	0	0

FIG. 1. Pain assessment during swabbing and FNA.

itive FNA PCR results was not significantly lower than those for swab samples ($P < 0.46$). However, there was a significant difference in the number of negative PCR results between fine-needle aspiration (21.1%) and swab (4%) samples ($P < 0.007$). Overall, these comparisons showed that PCR analysis of swab samples was more accurate than that of FNA samples for diagnosing ulcerative forms. For each swab collection and FNA, the patient's response to pain was assessed according to standard pain assessment methods (15). Twenty adults (aged 15 to 35 years) and 20 children (aged 5 to 12 years) presenting ulcerative lesions (5 to 15 cm in diameter) localized on right or left limbs were enrolled. The analysis of results clearly demonstrated that FNA was less painful and thus more comfortable for the patient than swabbing (Fig. 1).

Second, 64 patients were recruited to evaluate the diagnostic sensitivity of FNA from nonulcerative lesions. FNA was used to collect a unique sample from the estimated center of the lesion. We detected AFB in 25% of these samples (16/64 samples) through direct smear examination after Ziehl-Neelsen staining. PCR was positive for *M. ulcerans* DNA in 89% of the samples (57/64) (Table 1). Therefore, the sensitivity of PCR in FNA samples from nonulcerative forms was 89%. Seven cases were initially negative for *M. ulcerans* DNA detection. Repeating the sampling a few days later resulted in positive PCR results for four of these seven patients; the remaining three patients were diagnosed with lymphoma through differential diagnosis. Therefore, the sensitivity of the diagnosis from FNA samples was calculated to be 93.4% (57/61). The rate of positive smears from ulcerative and nonulcerative lesions in our study was lower than those obtained with other sampling methods (1, 8, 10). Thus, *M. ulcerans* DNA not associated with bacilli may be detected in aspirate liquid. *M. ulcerans* secretes vesicles containing mycolactone (7). Indeed, mass spectrometry analysis demonstrated the presence of mycolactone A/B in FNA liquid in samples collected from confirmed (PCR) Buruli ulcer patients. Moreover, we demonstrated that the vesicles contain *M. ulcerans* DNA (unpublished results). Therefore, we hypothesize that many vesicles and few bacilli (which are localized in tissues) are collected during FNA, explaining the high rate of positive PCR results compared to the low rate of positive smears.

To conclude, FNA is a simple, fast, accurate, painless, and inexpensive method of sampling which may be used for diagnosing *M. ulcerans* infection by PCR, particularly in patients presenting early-stage nonulcerative lesions.

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