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CASE STUDY

Instantly evaluating bacterial infections on skin ulcers in an Asian population using a fluorescence-emitting device

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Abstract

MolecuLight i:X® is a handy instrument capable of visualizing the bacterial adhesion over 10,000 CFU/g by recognizing porphyrin and pyoverdine as fluorescence. We took a total of 55 clinical photographs and fluorescence images (20 cases) from May 2021 to December 2021, after which the correlation between fluorescence observation and culture results was investigated. In addition, the course of fluorescent and ulcer status was shown in representative cases. The results suppose that MolecuLight i:X® is in real-time use and would be helpful in determining the range of collection of bacterial cultures as well as in judging therapeutic necessity for intractable skin ulcers.

KEYWORDS
dermatological practice management, fluorescence, infectious diseases, MolecuLight, wounds

1 | INTRODUCTION

The instruments utilizing fluorescent dyes in cutaneous disorders include Wood's ramp for vitiligo and indocyanine green fluorescence for sentinel lymph node detection.[1,2] MolecuLight i:X® (MolecuLight Inc.) makes it possible to visualize the adhesion of bacteria over 10,000 CFU/g by recognizing porphyrin produced by staphylococci, etc., as red fluorescence and pyoverdine produced by *Pseudomonas aeruginosa* as cyan fluorescence, with a peak excitation light of 405 nm[3]. Cyan fluorescence has a wavelength of 501–542.5 nm (±1.5 nm), while red fluorescence has a wavelength of 601–664 nm (±1.5 nm).[3] It is believed that using fluorescence image observation can estimate the adhesion of bacteria through non-invasive and real-time observation, while appropriately setting the range of debridement improved the healing rate of ulcers.[4,5] In this study, we investigated clinical utility and accuracy of MolecuLight i:X® to evaluate bacterial clustering on intractable ulcers in Asian patients.

2 | CASE REPORT

From a total of 20 cases, 55 photographs were taken from May to December 2021 in this study. Table 1 summarizes the enrolled patients and ulcer profile. Aggravation/improvement of ulcers and changes in fluorescence over time were collated. Furthermore, the usefulness of MolecuLight i:X® as a non-invasive imaging tool was analyzed by collating the results of wound cultures and fluorescence, then calculating the sensitivity and specificity of fluorescence against the wound cultures.

3 | THE CONSISTENCY OF FLUORESCENCE AND CULTURE NOT INCLUDING COMBINED INFECTION CASES

Excluding mixed infections of porphyrin-producing bacteria and *Pseudomonas aeruginosa*, 36 fluorescence photographs were

TABLE 1 Patients' profile enrolled in this study

Patients (N = 20)	Type	Number
Sex	Male	8
	Female	12
Age	Youngest	10 years old
	Oldest	86 years old
Preceding diseases	Epidermolysis bullosa (EB)	4
	Squamous cell carcinoma caused by EB	1
	Lichen sclerosis	1
	Systemic sclerosis	1
	Arteriosclerosis obliterans	2
	Mycosis fungoides	1
	Sarcoidosis	1
	Ulceration after skin grafting	3
	Stasis dermatitis	3
	Skin damage induced by immunotherapy	1
	Radio dermatitis	1
	Malignant melanoma	1

TABLE 2 The consistency of fluorescence and culture not including combined infection cases

	Culture (+)	Culture (−)	Sum
Fluorescence (+)	14	5	19
Fluorescence (−)	5	12	17
Sum	19	17	36

obtained, with a fluorescence sensitivity of 74% and a specificity of 71% (true positive: 14 cases, false positive: 5 cases, true negative: 12 cases, and false negative: 5 cases) (Table 2). For example, when MRSA was detected via a bacterial culture from the ulcer site and red fluorescence was observed, it was deemed to be true positive.

4 | THE CONSISTENCY OF FLUORESCENCE AND CULTURE INCLUDING COMBINED INFECTION CASES

Fifty-five fluorescence photographs were obtained in cases with a mixed infection of porphyrin-producing bacteria and *P. aeruginosa*. If partial consistency was also considered as correct, 87% of sensitivity and 71% of specificity were archived (true positive: 33 cases, false positive: 5 cases, true negative: 12 cases, and false negative: 5 cases) (Table 3). On the other hand, if only complete consistency was considered as correct, the sensitivity was decreased to 63% and 71% of specificity was comparable (true positive: 24 cases, false positive: 5 cases, true negative, 12 cases, and false negative: 14 cases) (Table 4). For example, when MRSA and *P. aeruginosa* were detected in a bacterial culture and only red fluorescence was observed, it was deemed to be a true positive or false negative.

5 | REPRESENTATIVE CASES

5.1 | Case 1 wound cure along with decreased fluorescence

A 40s female. The underlying disease causing her ulcer was systemic scleroderma and her left second and third toes were observed. Cellulitis and abscess formation in the left 2nd and 3rd toes were found upon a second observation of the fluorescence image, so toe amputation was performed. *Candida* sp, *Enterococcus faecalis*, and *Prevotella bivia* were detected in the bacterial culture at that time, with MolecuLight i:X® detecting red fluorescence in the area indicated by the arrow. After the ulcer was improved by antibiotic treatment with meropenem, vancomycin, and topical isodine sugar, the wound culture turned to be negative along with sequentially decreased fluorescence (Figure 1A–G).

5.2 | Case 2 wound worsening with tumor progression along with maintained fluorescence

A 40s female. The underlying disease causing her ulcer was squamous cell carcinoma due to epidermolysis bullosa, with the ulcer and a tumor observed on her back. MRSA, *Corinebacterium* sp, and *P. aeruginosa* were detected from the bacterial culture at the time of initial observation, with MolecuLight i:X® indicating red and cyan fluorescence in the area enclosed by the dotted line. Although chemotherapy and topical azunol ointment were applied, the size of the tumor and ulcer did not improve along with remained bacteria fluorescence. The tumor persisted, the infection did not improve, and the fluorescence identified by MolecuLight i:X® was maintained (Figure 2A,B).

6 | DISCUSSION

Anderson reported that the fluorescence from MolecuLight i:X® decreased as the ulcer improved[6] and Le et al.[7] reported that 68.9% of cases undergoing ulcer treatment changed the treatment method based on the results of fluorescence observation. Because fluorescence changes were consistent with ulcer aggravation or

TABLE 3 The partial consistency of fluorescence and culture including combined infection cases

	Culture (+)	Culture (−)	Sum
Fluorescence (+)	33	5	38
Fluorescence (−)	5	12	17
Sum	38	17	55

improvement in this study as in previous literature, we believe that semi-quantitative fluorescence comparisons would help determine long-term therapeutic efficacy in ulcer treatment and assist in deciding whether to change the treatment regimens.

With regard to the usefulness of fluorescence imaging in ulcer treatment, Ottolino-Perry et al.[8] reported that in 31 cases of diabetic foot ulcers, they were able to collect bacteria more accurately when bacterial cultures were obtained under the guidance of fluorescence imaging, compared with bacterial cultures obtained without the use of fluorescence imaging. Price[5] reported a 23% increase in wound healing within 12 weeks when using fluorescence imaging to determine areas of debridement. Although the sensitivity was 74% and the specificity was 88%, as a result of an integrated analysis of 613 cases in 10 references,[9] the concordance between fluorescence detection and culture results in this study indicated a sensitivity of 87% and a specificity of 71%, which was higher than the results shown in the past literature.[9] Based on this result, it was assumed that the probability of detecting bacteria on the ulcer surface in real time was higher, thus making it useful for determining the site for the collection of wound cultures and the site for debridement based on fluorescence images. Since the specificity of this study was lower than that of past studies,[9] it is required to consider a comprehensive judgment including clinical findings and culture test in cases without no fluorescence.

Because 28 out of 32 species commonly emitted red fluorescence,[10] we needed a sensitivity test to determine the type of antibiotics. Furthermore, in this study, it was difficult to distinguish between the cyan fluorescence emitted by *P. aeruginosa* and the

TABLE 4 The complete consistency of fluorescence and culture including combined infection cases

	Culture (+)	Culture (−)	Sum
Fluorescence (+)	24	5	29
Fluorescence (−)	14	12	26
Sum	38	17	55

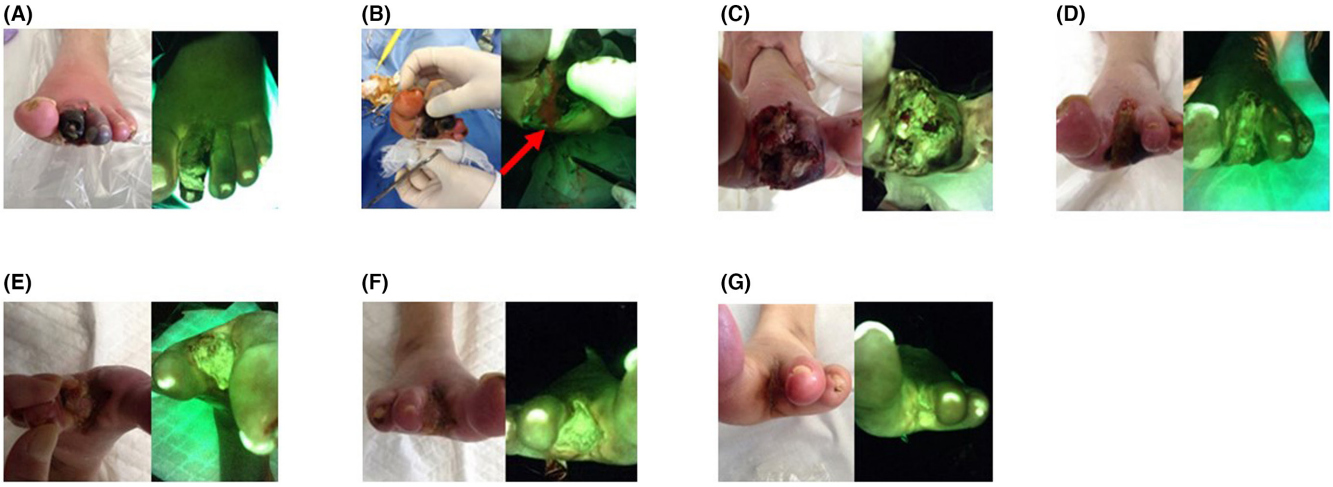

FIGURE 1 Sequential view of visual and fluorescent images of the acral gangrene in case 1. (A–G) Images at the 1st to 7th assessments. The red fluorescence supposed bacterial infection in B (red arrow).

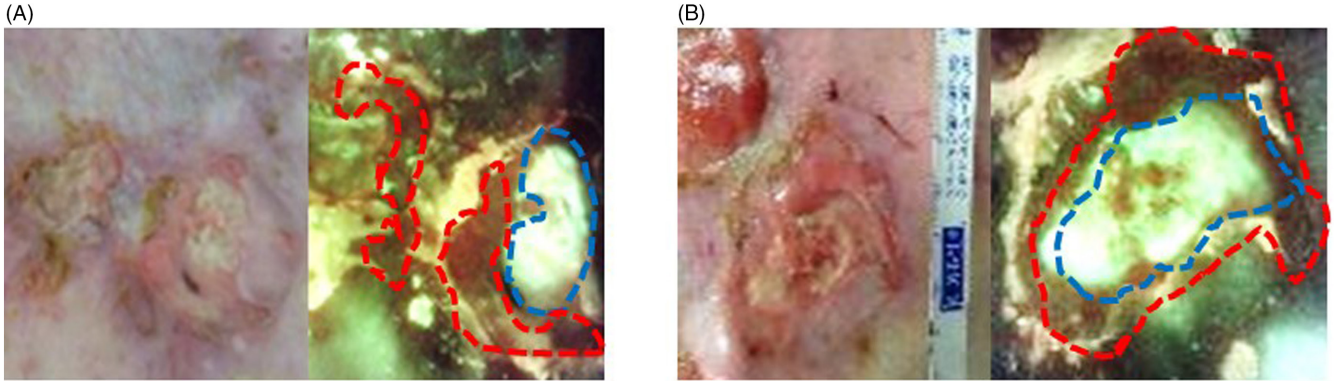

FIGURE 2 Sequential view of visual and fluorescent images of the lower leg ulcers in case 2. Red and cyan fluorescence are indicated by red and blue dot circles, respectively. (A) Taken at the 1st assessment. (B) Taken at the 2nd assessment.

fluorescence emitted by surrounding structures such as scabs, thus making it difficult to determine whether or not bacteria existed in the scab, based only on fluorescence images.

In conclusion, this study evaluated the usefulness of MolecuLight i:X® as a real-time observation tool of bacteria adhering to ulcers in Asian patients. MolecuLight i:X® is handy and easy to use for serial evaluation, as it simultaneously captures and stores brightfield and fluorescence photographs. Further, it is required to accumulate the more cases and examine the significance of MolecuLight i:X® in clinical practice.

CONFLICT OF INTEREST

Dr Manabu Fujimoto is the Editor in Chief for the Journal of Cutaneous Immunology and Allergy. Management of the peer review process, and all editorial decision-making, for this article was undertaken by an Associate Editor.

ETHICS STATEMENT

Approval of the research protocol: N/A.
Informed Consent: N/A.
Registry and the Registration No. of the study/trial: N/A.
Animal Studies: N/A.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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