The Use of Point-of-Care Bacterial Autofluorescence Imaging in the Management of Diabetic Foot Ulcers: A Pilot Randomized Controlled Trial

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OBJECTIVE

To estimate comparative healing rates and decision-making associated with the use of bacterial autofluorescence imaging in the management of diabetic foot ulcers (DFUs).

RESEARCH DESIGN AND METHODS

This is a single-center (multidisciplinary outpatient clinic), prospective pilot, randomized controlled trial (RCT) in patients with an active DFU and no suspected clinical infection. Consenting patients were randomly assigned 1:1 to either treatment as usual informed by autofluorescence imaging (intervention), or treatment as usual alone (control). The primary outcome was the proportion of ulcers healed at 12 weeks by blinded assessment. Secondary outcomes included wound area reduction at 4 and 12 weeks, patient quality of life, and change in management decisions after autofluorescence imaging.

RESULTS

Between November 2017 and November 2019, 56 patients were randomly assigned to the control or intervention group. The proportion of ulcers healed at 12 weeks in the autofluorescence arm was 45% ($n = 13$ of 29) vs. 22% ($n = 6$ of 27) in the control arm. Wound area reduction was 40.4% (autofluorescence) vs. 38.6% (control) at 4 weeks and 91.3% (autofluorescence) vs. 72.8% (control) at 12 weeks. Wound debridement was the most common intervention in wounds with positive autofluorescence imaging. There was a stepwise trend in healing favoring those with negative autofluorescence imaging, followed by those with positive autofluorescence who had intervention, and finally those with positive autofluorescence with no intervention.

CONCLUSIONS

In the first RCT, to our knowledge, assessing the use of autofluorescence imaging in DFU management, our results suggest that a powered RCT is feasible and justified. Autofluorescence may be valuable in addition to standard care in the management of DFU.

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Worldwide, ~537 million adults are living with diabetes and this number is predicted to increase to 783 million by 2045 (1). Up to 25% of those with diabetes will develop a diabetic foot ulcer (DFU) in their lifetime, and up to 70% of those whose wound(s) heal will develop ulcer recurrence over a 5-year period (2,3). Chronic wounds cost Medicare an estimated $28.1–96.8 billion per year (4).

Timely healing in patients with active ulceration is imperative, because impediments increase the risk of adverse events such as infection, hospitalization, and amputation. DFUs present for >30 days have fivefold increased odds of infection compared with those that heal, and infection increases odds of hospitalization 55 fold and odds of amputation 154-fold when compared with noninfected DFUs (5). Adverse events affect patient quality of life (QoL) and increase costs, with two-thirds of the total cost of wound care attributed to the 30% of wounds that fail to heal (6). Despite guidance on the optimal care of DFUs (7), only 48.7% of the 33,155 DFUs included in the U.K. National Diabetic Foot Care Audit were healed at 12 weeks (8)

Nonhealing wounds are biologically characterized by prolonged inflammation, defective re-epithelialization, and impaired matrix remodeling (9). There is increasing evidence to suggest that bacteria play a key role in wound chronicity. Up to 80% of chronic wounds contain bacteria in a biofilm state (10). Biofilm acts through multiple mechanisms to prevent the usual sequence of wound healing, preventing transformation from the inflammatory to proliferative phase and promoting a chronic inflammatory state (11). Xu et al. (12) reported a 44% delay in DFU healing for each $\log_{10}$ increase in CFU/mL DFU wound fluid, with static or increased wound size over 28 days in those wounds with $>10^4$ CFU/mL. However, the sessile phenotype of bacteria within biofilms is often nonresponsive to antibiotics (13) and, therefore, alternative strategies for treatment are required.

Living bacteria have porphyrins and pyoverdines in the cell wall that cause natural autofluorescence to appropriate stimuli. At a controlled ambient light and distance from the wound, the MolecuLight i:X device has a 95–100% positive predictive value for detection of wound bacteria at $\geq 10^4$ CFU/g (14–17). The first-in-man study examining its use in guiding chronic wound treatment showed an association with reduction in wound area in the 13 chronic wounds enrolled, 83% of which were DFUs. However, the decision-making leading to these improvements was not documented (14). In this pilot randomized controlled trial (RCT), we aimed to estimate comparative healing rates and the decision-making associated with the use of a point-of-care wound bacterial autofluorescence imaging device, MolecuLight i:X, in the management of DFUs to inform a definitive RCT.

RESEARCH DESIGN AND METHODS

Trial Design, Setting, and Participants

This was a single-center, open, parallel-group, prospective pilot RCT with patients with a DFU, with blinded outcome assessments. Participants were assessed for eligibility and recruited from an acute care multidisciplinary (MDT) outpatient clinic, Leeds Teaching Hospitals NHS Trust, Leeds, U.K. Participants were followed at 4-week intervals over 12 weeks. The trial was run in accordance with the ethical principles of the Declaration of Helsinki and recommendations for Good Clinical Practice. The study was approved by the UK National Research Ethics Committee (reference 17/YH/0349) and registered with ClinicalTrials.gov (identifier NCT03270904).

Participants were assenting adults aged ≥18 years who had a diagnosis of diabetes (according to World Health Organization criteria) (18) with active ulceration of the foot below the malleoli; were expected to comply with treatment strategies and follow-up schedule; and consented to foot and wound photography. Patients were excluded if they had a suspected clinical infection of their DFU (per Infectious Diseases Society of America guidelines) (19); significant renal impairment with estimated glomerular filtration rate <20 mL/min/1.73 cm^2; severe ischemia with ankle brachial pressure index <0.5 or opening toe pressure <30 mmHg; received immunosuppressive therapy (planned or previous treatment with corticosteroids to an equivalent dose of prednisolone >10 mg/day or other immunosuppressive therapy) within 4 weeks prior to randomization; evidence of connective tissue disease or dermatological disorder as cause of ulceration; had previously been enrolled in the study; lacked mental capacity and/or were unable to provide informed consent. Written or witnessed verbal informed consent was obtained from all participants. For participants who were deemed capable of giving informed consent but were physically unable to complete the written aspect of the consent form, witnessed consent was obtained. An appropriate witness was a participant's family member, friend, or a member of their health care team who was not directly involved in the trial. Baseline characteristics are listed in Table 1.

Randomization and Blinding

Randomization was performed by sequential allocation of sealed opaque envelopes in a 1:1 treatment allocation to one of two arms: *1*) treatment as usual, guided by bacterial autofluorescence imaging (intervention group) and *2*) treatment as usual alone (control group). Randomization was stratified according to DFU etiology (neuropathic versus neuro-ischemic) and anatomical site (forefoot versus midfoot or hindfoot) to ensure groups were well balanced for these characteristics. Neuro-ischemia was classified by absence of a palpable pedal pulse or multiphasic, handheld Doppler signal. Forefoot was defined as an ulcer distal to the tarsometatarsal joints.

The primary outcome assessments were completed by an independent clinical assessor who had no previous involvement with or knowledge of the participants' index ulcer treatment and, as such, was blind to the randomized strategy. The blinded assessor carried out wound measurements using acetate tracing, photography for digital planimetry using MolecuLight i:X, and photography of the foot when healing of the index ulcer was reported. The randomized treatment strategy was applied to the index ulcer on the day of randomization, and treatment of any other ulcers continued per the treating clinician's discretion. In participants with more than one ulcer, the largest ulcer was deemed the index ulcer.

Table 1—Baseline characteristics

Characteristic	Intervention (n = 29)	Control (n = 27)	All (N = 56)
Age, years			
Mean ± SD	68.3 ± 11.7	66.6 ± 11.4	67.6 ± 11.5
Range	47–93	45–84	45–93
Sex, *n* (%)			
Male	23 (79.3)	17 (63.0)	40 (71.4)
Female	6 (20.7)	10 (37)	16 (28.6)
Ethnicity, *n* (%)			
White	28 (96.6)	25 (92.6)	53 (94.6)
Asian	1 (3.4)	0 (0)	1 (1.8)
Black	0 (0)	2 (7.4)	2 (3.6)
Diabetes			
Type 2 diabetes, *n* (%)	23 (79.3)	24 (88.9)	47 (83.9)
Duration (IQR), years	15 (8–20)	14 (6.5–19.25)	14 (7.5–20)
HbA_{1c} (IQR), mmol/mol	57 (46–73)	64 (53–78)	61 (48–76)
Ulcer etiology, *n* (%)			
Neuropathic	14 (48.3)	14 (51.9)	28 (50)
Neuro-ischemic	15 (51.7)	13 (48.1)	28 (50)
Ulcer location, *n* (%)			
Forefoot	23 (79.3)	21 (77.8)	44 (78.6)
Mid/hindfoot	6 (20.7)	6 (22.2)	12 (21.4)
SINBAD score (IQR)	2 (1–3)	2 (1–3)	2 (1–3)
Wound			
Area (IQR), cm^2	0.37 (0.24–2.21)	0.54 (0.25–1.18)	0.55 (0.20–1.17)
Duration (IQR), weeks	20 (10.5–30)	15 (8–35)	19.5 (9–30.5)
Offloading, *n* (%)			
Use of below-knee walker or TCC	10 (34.5)	6 (22.2)	16 (28.6)
Comorbidities, *n* (%)			
Hypertension	14 (48.3)	14 (51.9)	28 (50)
IHD	9 (31.0)	2 (7.4)	11 (19.6)
COPD	1 (3.4)	3 (11.1)	4 (7.2)
CKD	4 (13.7)	1 (3.7)	5 (8.9)
CCF	3 (10.3)	1 (3.7)	4 (7.2)
Stroke	2 (6.8)	0	2 (3.6)

CCF, congestive cardiac failure; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; IHD, ischemic heart disease; SINBAD, site, ischemia, neuropathy, bacterial infection, depth; TCC, total contact cast.

Treatment as Usual

Treatment as usual comprised of care provided in line with the National Institute for Health and Care Excellence guidance NG19 (7).This included review at the MDT clinic staffed by specialist podiatrists, diabetes physicians, vascular surgeons, and orthotists, supported by microbiologists and radiologists, for assessments of wound healing; sharp, nonsurgical debridement of callus and nonviable tissue; review of off-loading; identification and treatment of infection; and assessment of perfusion with revascularization when clinically indicated. For plantar forefoot ulcers, off-loading with a below-knee walker boot or total contact cast was encouraged when patient balance and mobility allowed. Other assessments and treatments to optimize diabetes management, as well as community specialist podiatrist and nurse visits for wound assessments and treatment between MDT appointments, formed part of standard care.

Bacterial Autofluorescence

MolecuLight i:X is a handheld device with a built-in iPod nano that emits 405 nm (violet) wavelength light with dual-band filter (590–690 nm). Under light stimulus from the device, porphyrin-producing bacteria emit red fluorescence signals, pyoverdines produced by *Pseudomonas* species emit cyan fluorescence signals, and collagen and elastin within the soft tissue emit green fluorescence signals (14,20). Components within the wound are excited up to a depth of 1.5 mm. All 10 clinicians involved in the trial were given training in image interpretation. Imaging was performed after treatment as usual sharp, nonsurgical debridement at each trial follow-up visit (every 4 weeks). If patients attended the MDT clinic between trial visits, autofluorescence imaging was not used.

The room was darkened as much as possible, and dark drapes were used if the ambient light level was above the threshold for the device. Wounds were imaged at an optimal distance of 8–12 cm from the wound and the image captured using the built-in camera. A positive imaging result was defined as the presence of red or cyan autofluorescence

signals within the wound bed or periphery. Clinical interpretation of the imaging results and the influence on DFU treatment, including choice of any additional debridement or change in wound management plan, was at the discretion of the treating clinician. After debridement, repeated autofluorescence imaging to assess response was permitted to guide need for additional wound management.

Wound Measurement

Follow up data collection was undertaken by an independent assessor blind to the randomized allocation, using a paper case-report form. Prior to follow up assessments, participants received treatment as usual by the attending clinical team, and the index ulcer area was measured after treatment as usual sharp, nonsurgical debridement. Measurement of the index ulcer was performed using two methods. First, acetate tracing was done, which was the primary measurement for outcomes. Wound area was calculated using ImageJ software; the detailed methodology having been described elsewhere (21). Second, MolecuLight i:X digital planimetry imaging was performed, which was used as a back-up in case of any issues with the acetate. MolecuLight i:X digital planimetry imaging performed using the standard image mode of the device, whereby the autofluorescence function was not in use, at normal, ambient light levels. These images were not shared with the attending clinician. Measuring stickers were applied to edges of the wound, facilitating automated calculation of wound area. After data collection, the attending clinical team completed the treatment as usual visit; in the intervention group, this included autofluorescence imaging.

Outcomes

All participants were followed up at intervals 4 weeks apart for a total of 12 weeks (including those in whom healing of the index ulcer had been confirmed), or 14 weeks if healing was first reported at week 12 of follow up.

The primary outcome measure was healing at 12 weeks. Healing was defined as the complete closure of the wound surface with 100% epithelialization with the absence of drainage maintained for 2 weeks (22), confirmed by the blinded assessment of healing at two consecutive assessments.

The secondary outcome measures were wound-area reduction of the index ulcer at 4 and 12 weeks after randomization; the clinical management decision for the index ulcer after autofluorescence imaging; the health-related QoL as measured by EQ-5D-5 L and Diabetic Foot Ulcer Scale–Short Form at 4 and 12 weeks after randomization; and adverse events (namely, infection, hospitalization and amputation).

Statistical Analysis

This was a pilot study of a novel diagnostic technology; therefore, the sample size was based on current recommendation for pilot studies informing RCTs: 30 participants per group (23). The study was reported using the Consolidated Standards of Reporting Trials guidelines on pragmatic randomized controlled trials (24).

The intention-to-treat analysis population was used. Because this was a pilot study, analyses methods primarily focused on descriptive and exploratory statistics. Baseline data were described, and outcome measures were summarized with mean and 95% CIs for parametric data or median and interquartile ranges for nonparametric data. To inform patterns, exploratory statistical analyses were carried out. The Shapiro-Wilk test was used to determine the normality of distribution. The Clopper-Pearson interval was calculated for 95% CIs around point proportions for the primary outcome. Missing outcome data were imputed using last observation carried forward. Sensitivity analysis for the primary outcome was performed using worst-case scenario, whereby participants in the autofluorescence arm were deemed unhealed if data were missing and those in the control deemed to have their ulcer healed at week 12 of follow-up (25). Statistical tests were performed using SPSS, version 26.

RESULTS

Baseline Characteristics

From November 2017 to November 2019, of 304 people with diabetes and a DFU who were assessed for eligibility, 149 (49%) were eligible. Of these, 71 (47.7%) were not randomly assigned because the stratification group was closed, 22 (14.8%) refused to participate, and 56 (37.5%) consented to participate in the trial and were randomly assigned to a trial arm (Fig. 1).

The most common reasons for ineligibility were active infection ($n = 42$) and severe ischemia ($n = 36$) (Fig. 1). The majority of patients had neuropathic forefoot ulcers; therefore, this group was closed to recruitment earlier than the others.

Of the 56 patients randomly assigned into the trial, 29 were allocated to the autofluorescence group and 27 to the control group (Fig. 1). The groups were well matched for demographics and factors thought to affect the healing of DFU, including ulcer etiology, size, anatomical location, SINBAD (site, ischemia, neuropathy, bacterial infection, and depth) score, chronic obstructive pulmonary disease and congestive heart failure (Table 1). The mean age of participants was 67.6 (SD 11.5) years (range, 45–93 years), 40 (71.4%) were male, and 53 (94.6%) were of White British ethnicity (26). Overall, participants had a median duration of diabetes of 14 (interquartile range [IQR] = 7.5–20) years, a median HbA_{1C} of 61 (IQR = 48–76) mmol/mol, and 50 participants (89%) had type 2 diabetes. There was an equal number of neuropathic 28 (50%) and neuro-ischemic 28 (50%) ulcers, and the majority were on the forefoot ($n = 44$; 78.6%). The median ulcer size was 0.55 (IQR = 0.19–1.17) cm^2 at baseline, with a median duration of 19.5 (IQR = 9–30.5) weeks. Overall, the patient demographics and wound size are representative of the overall diabetic foot population seen by our service (27). Use of a below-knee walker boot or total contact cast was more common in the autofluorescence arm (34.5% vs. 22.2%). Two participants in the intervention group did not complete the study (one was lost to follow-up; one died), and four in the control group did not complete the study (three were lost to follow-up; one withdrew).

Primary Outcome

For the ITT analysis population, 13 of 29 ulcers (45%, 95% CI 26–64%) had healed

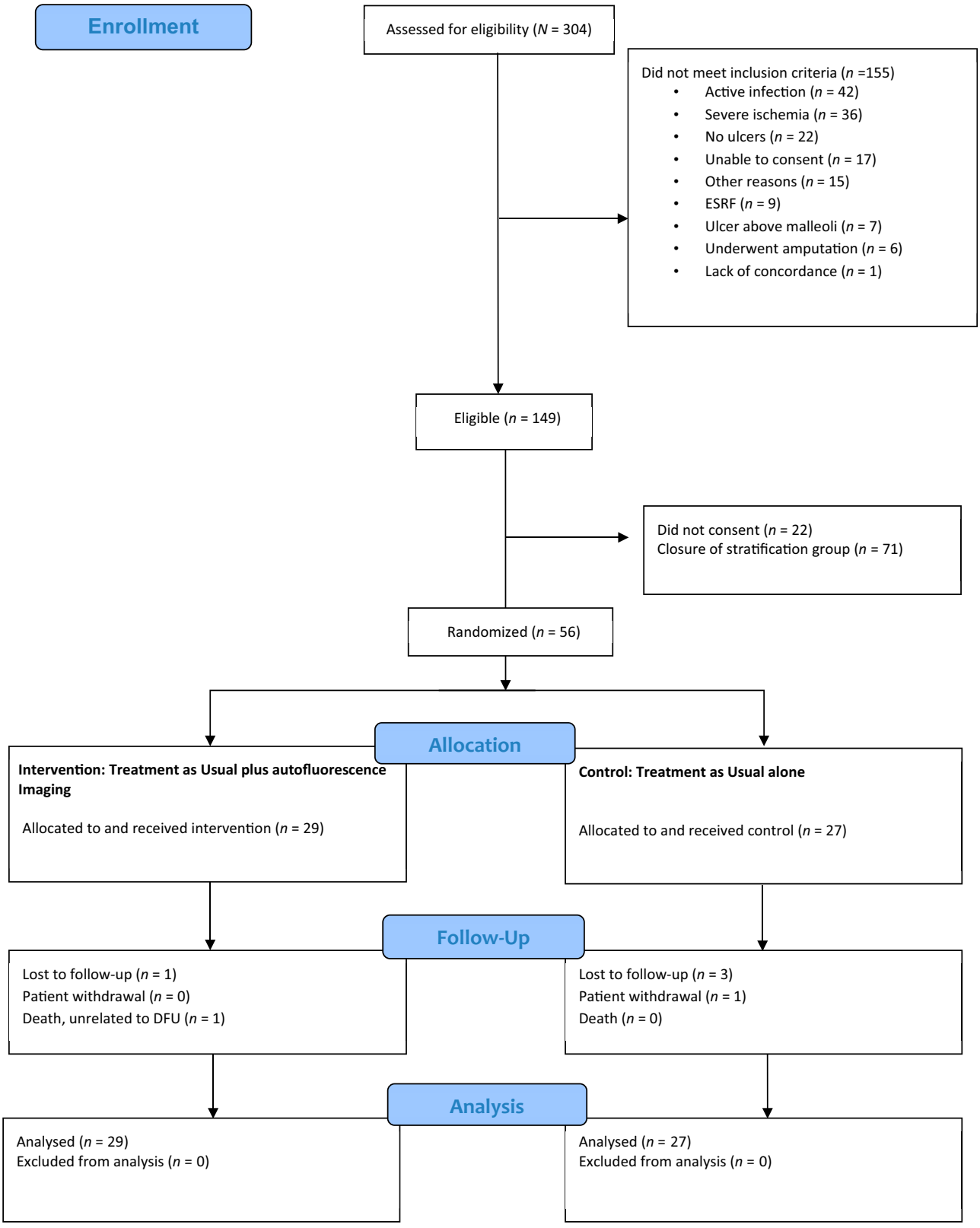

Figure 1—Consolidated Standards of Reporting Trials diagram. ESRF, end stage renal failure.

by 12 weeks in the autofluorescence arm versus 6 of 27 (22.2%, 95% CI 9–42%) in control arm.

Sensitivity analysis was performed using worst-case scenario ITT analysis. For the sensitivity analysis, 13 ulcers (45%, 95% CI 26–64%) had healed by 12 weeks in the autofluorescence arm versus 10 (37%, 95% CI 19–58%) in the control arm.

Secondary Outcomes

Wound-Area Reduction

The median percentage wound-area reduction at 4 weeks after randomization was 40.7% (IQR = 0.6–61.0%) in the autofluorescence arm and 38.6% (IQR = 0.5–53.1%) in the control arm. At 12 weeks after randomization, the median percentage was 91.3% (IQR = 47.3–100%) in the autofluorescence arm versus 72.8% (−22.3% to 100%) in the control arm.

Management Decision After Autofluorescence Imaging

At baseline, 16 of 29 participants (55.2%) in the autofluorescence arm had positive autofluorescence imaging and 13 of 29 (44.8%) had a negative result. The proportion of patients with positive imaging at baseline who progressed to ulcer healing at 12 weeks was 37.5% (n = 6 of 16) vs. 53.9% (n = 7 of 13) in those with negative imaging.

Throughout the follow-up period, of the 16 participants with positive baseline autofluorescence imaging, 10 remained positive at further clinic visits; 2 had a negative result; 3 did not have further autofluorescence imaging, due to ulcer healing; and 1 was lost to follow-up. Of the 13 DFUs that had negative autofluorescence imaging at baseline, 7 remained negative at further clinic visits; 2 had a positive result; 3 did not have autofluorescence imaging, due to ulcer healing; and 1 patient died.

At baseline, 9 of the 16 participants (56.3%) with positive autofluorescence imaging had a change in their management plan as a direct result of autofluorescence imaging, with further wound debridement. At week 4 follow-up, 5 of the 10 participants (50%) with positive imaging had a change in management plan; 4 underwent further wound debridement and 1 had their wound dressing changed to an antimicrobial dressing. At week 8 follow up, 3 of 11 participants (27.3%) with positive imaging had a change in management plan, with further wound debridement.

Participants who had negative baseline autofluorescence imaging had a median wound-area reduction of 100% (IQR = 80.8–100%) at week 12. Those with positive imaging and an associated change in their management plan had a median wound-area reduction of 84.2% (IQR = 38.1–100%) at week 12. Those with positive imaging but no change in their management plan had a median wound-area reduction of 56.1% (IQR = 7.6–100%) at week 12. Participants in the control group had a median wound-area reduction of 72.8% (IQR = −22.3–100%) at week 12 (Fig. 2).

Health-Related QoL

At baseline, no clinically meaningful or statistically significant differences were observed between groups with respect to the health-related QoL global score, as measured by either the generic EQ-5D-5 L or the disease-specific Diabetic Foot Ulcer Scale–Short Form. There was a modest increase in disease-specific QoL in the autofluorescence arm, most pronounced at 4 weeks, and similar decrease in the control arm, most pronounced at 12 weeks (Table 2).

Adverse Events

Overall, the number of adverse events was low, occurring in 4 participants (13.8%) in the intervention arm and 6 (22.2%) in the control arm (Table 3). The death in the autofluorescence arm was not related to the DFU. All adverse events noted were unrelated to the device.

CONCLUSIONS

This prospective, pilot, randomized controlled trial with blinded outcome assessment is, to our knowledge, the first RCT assessing the use of autofluorescence in wound healing. The proportion of wounds healed by 12 weeks was increased when autofluorescence imaging was added to standard care: 45% (95% CI 26–64%) vs. 22% (9–42%), respectively. There was a similar trend in greater median wound-area reduction at 12 weeks in the intervention group, but not at 4 weeks. Positive autofluorescence imaging led to further wound debridement in 40.9% of occasions in total, falling from 56.3% at baseline to 27.3% at week 8 review. The improvements in healing in the autofluorescence arm were associated with a modest improvement in disease-specific QoL compared with the control arm at 12 weeks, similar to those previously reported with DFU healing using the Diabetic Foot Ulcer Scale–Short Form score (28).

The strengths of the study include that this is the first RCT of autofluorescence technology, to our knowledge, and we adhered to the standards required of trials in this disease cohort (29). We did not think it was possible to blind the clinician, because the decision-making and interventions based on the results are integral to the efficacy of the technology. Furthermore, it was also not possible to blind the patient, because *1*) they may receive additional therapy after autofluorescence imaging (e.g., further debridement or dressing changes), and verbal consent for additional treatment after imaging would alert them to the randomization strategy; and *2*) autofluorescence imaging can be used as part of a patient education

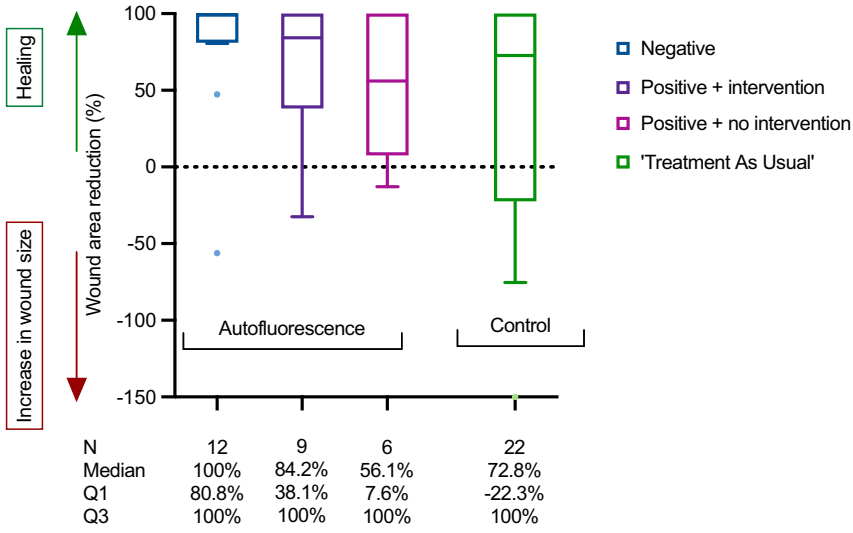

Figure 2—Boxplot of baseline autofluorescence imaging result and week 12 wound-area reduction. Q, quartile.

Table 2—Health-related QoL as measured by EQ-5D-5 L index score and Diabetic Foot Ulcer Scale–Short Form global score at baseline and follow-up time points

	Intervention		Control	
	n	Mean ± SD	*n*	Mean ± SD
EQ-5D-5L				
Baseline	26	0.56 ± 0.32	27	0.55 ± 0.26
Week 4	24	0.61 ± 0.27	23	0.56 ± 0.32
Week 12	20	0.49 ± 0.36	15	0.57 ± 0.26
Change between baseline and week 4	22	0.07 ± 0.38	23	−0.01 ± 0.29
Change between baseline and week 12	20	−0.10 ± 0.43	15	−0.05 ± 0.41
Diabetic Foot Ulcer Scale–Short Form				
Baseline	28	51.8 ± 25.7	27	50.6 ± 26.3
Week 4	24	61.6 ± 22.9	23	54 ± 28.3
Week 12	21	54.6 ± 22	16	44.9 ± 31.9
Change between baseline and week 4	23	5.2 ± 17.7	23	2.4 ± 18.3
Change between baseline and week 12	21	1.4 ± 18.8	16	−3.9 ± 24.9

Scores normally distributed.

package for wound cleansing and hygiene, and we did not want to remove this option from clinicians if they felt it appropriate as part of improving patient care. However, outcome assessments were performed by an assessor blinded to both the treatment allocation and clinical care provided, thereby reducing the risk of detection bias in the assessment of wound healing and measurement of wound area. Finally, using stratified randomization, we controlled for differences in the proportions of mid and hindfoot ulcers and neuro-ischemic ulcers, which are confounding variables in ulcer healing, with ischemia being the variable having the greatest influence on being alive and ulcer free at 12 weeks in the U.K. National Diabetic Foot Care Audit (8).

There are a number of limitations to this study. First, it was a pilot study and, therefore, was not powered to show a difference in the primary outcome. Although the results suggest an improvement in the primary outcome measure in the autofluorescence arm, the results must be viewed with some caution, and a fully powered study is required to determine whether there is definitive evidence that the use of autofluorescence to guide standard care is superior to standard care alone. Second, the randomization was performed using serial opaque envelopes by stratification group. Although this method of concealment may present a greater risk of selection bias compared with having a central and independent randomization service, this was the optimum approach given the limited funding available for a single-center study. Finally, although all attempts were made to minimize differences in baseline characteristics and the provision of standard care between the randomization groups, there may have been differences in the treatment received and patient concordance based on knowledge of randomization strategy. For example, there was a higher proportion of patients in the intervention group who were treated with below-knee walker boots or total contact casts than in the control group (34.5% vs. 22.2%, respectively).

The healing rates in the control arm of the study were lower than may have been anticipated given the inclusion and exclusion criteria. However, the rates do reflect outcomes from other trials of hard-to-heal DFUs (30,31). The median ulcer duration of those included in this trial at randomization was 19.5 weeks, defining the ulcers as hard to heal, with half being neuro-ischemic. Furthermore, only 56.3% of patients at baseline and 42.2% of autofluorescence positive images overall had an additional intervention, with these interventions being 4 weeks apart. Despite that, a larger proportion of DFUs healed at 12 weeks when autofluorescence imaging was used to guide the provision of standard care.

Healing rates for DFUs were low, with only 48.7% of the 33,155 patients included in the U.K. National Diabetic Foot Care Audit alive and ulcer free at 12 weeks (8). It is increasingly recognized that bacterial load is a major contributor to delayed healing, prolonging the chronic inflammatory response and preventing progression to the proliferative phase of wound healing (11). The bacterial bioburden, both in terms of prevalent species and concentration of bacteria, has been shown to be important, with bacterial concentrations of $>10^4$ CFU/mL associated with nonhealing of DFUs (12). MolecuLight i:X has a 95–100% positive predictive value for bacteria at this concentration, allowing real-time identification and image-guided intervention. This contrasts with the Clinical Symptoms and Signs Checklist components (32) that have a poor sensitivity for wound bacterial burden (33,34)

Table 3—Adverse events

	Intervention (n = 29)	Control (n = 9)
Soft-tissue infection	1	2
Osteomyelitis	0	1
Admission (other)	2	1
Death	1	0
New ulcer	0	1
Amputation	0	1

The role of debridement in the acceleration of wound healing is well established (35–37): it converts the wound bed from the state of being stalled in the inflammation phase to one in the acute phase of healing (38). Debridement is now also recommended as a mainstay of biofilm treatment (39), providing a 24- to 48-h window of reduced bacterial load to allow topical antiseptic and antibiotic therapies to be applied in a biofilm-free environment. Most studies report that bacterial autofluorescence is most prevalent in the periwound tissue (40,41) rather than the wound bed itself, and that targeted debridement is able to reduce or eliminate this fluorescence (41). Use of autofluorescence has a better sensitivity for wound bacterial load than do the Clinical Symptoms and Signs Checklist components alone (61.0% vs. 15.3%) with similar specificity (84%) (34), improves clinician confidence in wound assessment, improves antimicrobial stewardship by reducing the inappropriate prescribing of antibiotics, and guides intervention. In a recent case series, bacterial autofluorescence was present in 10 of 11 DFUs with a median duration of 16 weeks. Autofluorescence-targeted weekly debridement led to healing in 6 of 11 wounds over 12 weeks of follow-up, with a median healing time of 6.3 weeks from enrollment. Negative autofluorescence was associated with a 27.7% median, weekly wound-area reduction in comparison with a 6.5% increase in those with residual positive imaging (40).

In this study, positive autofluorescence was evident in more than half of the participants who had their ulcer management informed by the use autofluorescence imaging. In these patients, a change in clinical management occurred in 56% at first assessment and in 42% across all assessments, most commonly with additional wound debridement. No systemic antibiotics were prescribed on the basis of positive imaging. The most favorable healing rates were in participants with a negative autofluorescence imaging result, followed by those with a positive result who underwent additional intervention and, finally, those with a positive result and a clinical decision was not to intervene further (Fig. 2). These findings support the importance of serial DFU debridement within management guidelines, but they highlight the potential benefit of more aggressive, image-guided, targeted debridement in those with high bacterial load. It is important to note that in this study, autofluorescence imaging was performed after initial cleansing and debridement; therefore, it is likely that the true prevalence of critical colonization was higher than identified in our results.

The majority of participants with a negative autofluorescence imaging result continued to have a negative result throughout the follow-up period. In contrast, those with a positive result often remained positive despite further debridement, perhaps due to biofilm regrowth in the absence of an appropriate topical therapy between visits. Therefore, autofluorescence imaging may also have the potential to be used as a triage tool to identify wounds early that are negative for autofluorescence imaging and possibly more likely to heal without requiring advanced interventions, aiding early decision-making for adjuvant therapies.

DFUs have a significant impact on patients' QoL, with the literature showing similar QoL to that of persons with amputation (42). The changes in disease-specific QoL seen between groups at 12 weeks may reflect the larger proportion of patients with healed ulcer in the autofluorescence group and are similar in extent to differences previously described between groups with healed and unhealed ulcers (28).

The results of this pilot RCT demonstrate that a powered study of autofluorescence imaging is feasible. More research to fully assess the use of autofluorescence imaging in addition to standard care in reducing time to healing is warranted, given these positive signals. However, in future trials, researchers should consider shorter intervals between assessments, suggest debridement is performed in all ulcers with positive autofluorescence, and consider the use of topical antiseptic and antibiotic therapy as an adjunct between visits after debridement.

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