

Paper

Point-of-care fluorescence imaging reveals extent of bacterial load in diabetic foot ulcers

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The MolecuLight *i:X*® and DX™ are FDA-cleared to produce a fluorescence image in skin wounds that, "when used in combination with CSS, has been shown to increase the likelihood that clinicians can identify wounds containing bacterial loads $>10^4$ CFU per gram as compared to examination of clinical signs and symptoms alone." This improved ability to detect elevated bacterial loads may enhance clinical decision making. This research publication evaluated the impact of the MolecuLight *i:X*'s imaging on clinical decision making. The paper explores some uses of the *i:X*® / DX™ that are outside the FDA clearance just quoted. The paper has not been evaluated by FDA.

ORIGINAL ARTICLE

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Abstract

Elevated levels of bacteria, including biofilm, increase the risk of chronic wound infection and inhibit healing. Addressing asymptomatic high bacterial loads is challenged by a lack of clinical terminology and diagnostic tools. This post-hoc multicenter clinical trial analysis of 138 diabetic foot ulcers investigates fluorescence (FL)-imaging role in detecting biofilm-encased and planktonic bacteria in wounds at high loads. The sensitivity and specificity of clinical assessment and FL-imaging were compared across bacterial loads of concern (10^4 – 10^9 CFU/g). Quantitative tissue culture confirmed the total loads. Bacterial presence was confirmed in 131/138 ulcers. Of these, 93.9% had loads $>10^4$ CFU/g. In those wounds, symptoms of infection were largely absent and did not correlate with, or increase proportionately with, bacterial loads at any threshold. FL-imaging increased sensitivity for the detection of bacteria across loads 10^4 – 10^9 ($P < .0001$), peaking at 92.6% for $>10^8$ CFU/g. Imaging further showed that 84.2% of ulcers contained high loads in the periwound region. New terminology, chronic inhibitory bacterial load (CIBL), describes frequently asymptomatic, high bacterial loads in diabetic ulcers and periwound tissues, which require clinical intervention to prevent sequelae of infection. We anticipate this will spark a paradigm shift in assessment and management, enabling earlier intervention along the bacterial-infection continuum and supporting improved wound outcomes.

KEYWORDS

bacterial load, diabetic foot, fluorescence imaging, infection, wound healing

Key Messages

- high levels of bacteria, including biofilm, increase infection risk, and inhibit healing in chronic wounds
- we performed post-hoc analysis of 138 diabetic foot ulcers from a multicenter clinical trial to investigate the role of fluorescence imaging in detecting bacterial loads

- most ulcers did not exhibit symptoms of bacteria and infection despite harbouring clinically concerning bacterial loads in the wound bed and peri-wound regions; fluorescence imaging increased detection sensitivity across all bacterial loads
- new clinical terminology, chronic inhibitory bacterial load (CIBL), describes the presence of bacteria in wounds with or without clinical symptoms that must be detected and addressed to prevent further tissue damage

1 | INTRODUCTION

Despite numerous guidelines on foot ulcer management in diabetic patients^{1,2} and the increasing availability of advanced therapies, up to 60% of diabetic foot ulcers (DFUs) will experience infection at some point in their care.³ Infections contribute to low DFU healing rates,⁴ increased cost of patient care,⁵ and increasing minor lower extremity amputation rates.⁶ Out of the nearly half a billion people who have diabetes worldwide, an estimated 1 in 3 will develop a DFU,⁶ with an associated 1.89 increased mortality risk.⁷ These alarming numbers, along with the breadth of complications associated with diabetes, have led the World Health Organization to declare the diabetes epidemic a public health concern.

Multiple lines of evidence suggest a deleterious relationship between an unaddressed bacterial burden and DFU healing. Bacterial endotoxins have been shown to disrupt all stages of wound healing;⁸ they attract immune cells (e.g., neutrophils, proinflammatory cytokines), which heighten and maintain inflammation,⁹ and at high concentrations, inhibit fibroblast proliferation, and collagen production, which reduces the early development of tensile strength¹⁰ and tissue reepithelisation.¹¹ Bacteria impede wound healing to a varying extent depending on the bacterial load and virulence,¹²⁻¹⁴ the species present,¹⁴ and biofilm interactions.¹⁵ Biofilms, which are present in 68%–100% of DFUs,¹⁶ stimulate inflammation and perpetuate the chronic wound cycle.¹⁵ Furthermore, chronic wound exudate is rich with inflammatory cytokines, which degrade periwound tissues (e.g., maceration)¹⁷ into an environment where bacteria can thrive. Accordingly, Xu et al. report that neuropathic DFUs with bacterial loads of at least 10^4 CFU/g increase in size or have no significant decrease in wound area over 28 days.¹² Additional studies suggest that a threshold of 10^4 CFU/g is critical for delayed healing¹⁴ and that the degree of wound healing inhibition is proportional to the bacterial load.¹⁸

There are few accurate and reliable diagnostic tools to directly detect the presence of bacteria in wounds, most of which do not provide immediate results. Retrospective analyses indicate that early detection and appropriate infection management can reduce the risk of amputation

among patients with DFUs by up to 72%,¹⁹ highlighting the relevance of time-efficiency in their management and detection of potential complications. In the absence of assessment metrics specific to bacterial loads, the assessment of clinical signs and symptoms (CSS) of infection is the principal strategy for the bedside diagnosis of clinically significant bacterial burden in DFUs. But, this approach may not always yield accurate, timely, and actionable information, particularly in the chronically ill and immunocompromised diabetic population who often have peripheral artery disease and concomitant dialysis.²⁰ Diabetes also hinders the ability of the host to mount a normal immune response, which increases susceptibility to infection and can attenuate CSS. Despite the presence of high bacterial loads, most DFUs do not present with overt CSS,²¹ and reliance solely on CSS could lead to a delay in therapy and haphazard antimicrobial prescribing.²²

Current guidelines advise that wound sampling and microbiological analysis be performed *only* if an infection is suspected.^{1,2} This enables antibiotic therapy to be targeted; however, microbiological investigations are thwarted by the prevalent use of superficial sampling methods, unreliable semi-quantitative wound cultures,²³ challenges in culturing anaerobes and biofilm-encased bacteria (especially when antibiotics are already administered), and delays in receiving microbiological culture results. In some patients, subclinical infection (i.e., infection with minimal or no overt CSS) could hamper wound healing.¹⁴ Accurate, dependable, and rapid microbiological diagnostic tools could help clinicians signal the need for hygiene-based or antimicrobial intervention before CSS develops.

Point-of-care fluorescence imaging (FL-imaging) of bacteria has emerged as a biotechnology positioned to fill this gap. This objective and sensitive method of identifying high bacterial loads in wounds^{24,25} harnesses endogenous FL signals produced by bacterial metabolites and virulence factors.²⁶ Clinicians have adopted FL-imaging into their wound care regimes²⁷ to enable non-invasive localization of the presence of most live bacterial species (including Gram-positive, Gram-negative, aerobic, and anaerobic bacteria) at clinically-relevant levels ($>10^4$ CFU/g).²⁶ This technology can identify the presence of planktonic bacteria as well as biofilm-encased bacteria,²⁶ which is

critical considering the prevalence and pathogenicity of biofilm in DFUs.¹⁶

We propose new terminology, *chronic inhibitory bacterial load (CIBL)*, to define the presence of bacteria at high loads that is distinct from infection but may inhibit wound healing, therefore calling for clinical intervention (Table 1). This is in light of literature evidence and our own findings, which suggest the existence of this opportunity for pre-infection intervention, if identified in a timely manner.

2 | STUDY OBJECTIVES

This post-hoc clinical trial analysis of 138 DFUs aimed to:

1. Evaluate the prevalence of high bacterial loads in DFUs, with or without CSS.
2. Understand the spatial distribution of bacteria in and around DFUs.
3. Evaluate whether FL-imaging could be reliably used to detect high bacterial loads in DFUs.

TABLE 1 Key terminologies used in this paper

Term	Definition (for the purpose of this work)
Infection	Invasion of proliferating microorganisms into viable tissue surrounding a wound that damages body tissues and elicits a host defensive inflammatory response. ^a
Inflammation	A set of complex and dynamic host responses to tissue injury primarily caused by toxins, some environmental agents, trauma, overuse, or infection.
High bacterial load	Defined in this study as greater than 10 ⁴ colony forming units (CFU) per gram of tissue.
Periwound	Tissue surrounding a wound, defined as the 2 cm radius extending out from the wound edge.
Chronic inhibitory bacterial load (CIBL)	Chronic presence of bacterial microorganisms in a wound or its surrounding tissue at loads which can damage tissues and be inhibitory to healing, as well as requires clinical intervention, with or without presence of clinical symptoms.

^aBased on International Wound Infection Institute 2016 guidelines.²⁸

3 | MATERIALS AND METHODS

3.1 | Participants and study design

In this post-hoc analysis, we evaluated a subset of 138 DFUs from the FL-Imaging Assessment and Guidance (FLAAG) clinical trial, a prospective, single-blind, multi-centre cross-sectional clinical trial of 350 adults (>18 years) presenting with wounds of unknown infection status ([clinicaltrials.gov#NCT03540004](https://clinicaltrials.gov/ct2/show/study?term=NCT03540004)).²⁴ The aim of the FLAAG trial was to compare the performance of standard CSS assessment using International Wound Infection Institute (IWII) guidelines versus CSS in combination with FL-imaging to detect clinically relevant bacterial loads. In the FLAAG trial, patients were recruited from 14 outpatient wound care centres across the U.S. between May 2018 and April 2019; exclusion criteria were limited to treatment with an investigational drug within the last month, a recent wound biopsy (<30 days), inability to consent, or an anatomical location that was unable to be imaged. All DFUs assessed in the FLAAG trial were included in this analysis. An independent third party (Ironstone Product Development, Toronto, ON) was used to control for bias and ensure appropriate blinding. The study was conducted in accordance with Health Insurance Portability and Accountability Act guidelines, adhered to the tenets of the International Conference on Harmonisation E6 Good Clinical Practice (ICH GCP), and the Declaration of Helsinki, and was approved by an external institutional review board (Veritas IRB, Montreal, QC). The SerenaGroup[®] research foundation received funding from MolecuLight, Inc. to cover the conduct of the study and data collection.

3.2 | Assessment of bioburden based on clinical signs and symptoms and fluorescence imaging

Clinicians were provided with training on the use of the device, image interpretation, good clinical practice, and trial procedures prior to commencing the study. Clinicians reviewed the patient's history and inspected the wounds for all signs and symptoms of covert, overt, and spreading infection identified by the IWII 2016 guidelines.²⁸ Each of these signs and symptoms were recorded when detected, including delayed healing beyond expectations. This was identified if the wound area had not reduced by at least 30% during the prior 4 weeks of care, per the reporting standard of the clinical organisation conducting and managing the trial. In this post-hoc analysis, we reviewed the recorded CSS to identify wounds that fulfilled the International Working Group of the

TABLE 2 Significance of various colour signals on fluorescence images

Source of fluorescence	Signal	Indication
Bacteria ^a	Red	Porphyrin-producing bacteria (most Gram positive, Gram negative, aerobic, and anaerobic species) ²⁶
	Blush red/pink/orange/yellow	Subsurface porphyrin-producing bacteria ²⁹
	Cyan (with glowing white centre)	<i>Pseudomonas aeruginosa</i> ²⁵
Tissue	Light to dark green	Extracellular matrix components in tissue and slough; colour range is because of skin tone and/or presence of flaky tissue ²⁹
	Bright green to glowing white	Dense, collagen rich tissue structures such as tendon, bone, and nail ²⁹
	Black/maroon	Necrotic or highly vascular (e.g., granulation) tissue ²⁹
Blood	Black/maroon	Haemoglobin ²⁹
Callus	Bright green	Callused skin
	Yellow/orange	Bacteria below callused skin can appear yellow because of overlap of bacteria (red) and skin (green) signals

^aAt loads $>10^4$ CFU/g³⁰

Diabetic Foot (IWGDF) criteria for infection. Based on the IWGDF classification, a wound was considered positive for CSS based on the detection of at least two of the following signs and symptoms: swelling, erythema, local pain or tenderness, increased warmth, and purulent discharge.¹ Immediately following clinical assessment, standard and FL images were captured using the FL-imaging device (MolecuLight i:X, Toronto, Canada). This advanced imaging technology creates a map of high bacterial loads in and around wounds^{24,29} without using any contrast agents. Clinicians participating in the trial underwent didactic and hands-on training on use of the device and image interpretation and were required to pass an image interpretation certification test with a score of $>80\%$. Red FL on images indicates the presence of endogenously produced porphyrins from most common wound pathogens,²⁶ while cyan FL indicates pyoverdine virulence factors from *Pseudomonas aeruginosa*,²⁵ both at loads $>10^4$ CFU/g (Table 2). Prior studies have validated with wound tissue biopsies the high ($>93\%$) positive predictive value (PPV) of these red or cyan FL signals corresponding to the presence and location of most bacterial species (with the exception of non-porphyrin producing bacteria, including those from the *Streptococcus*, *Enterococcus*, and *Finnegoldia spp.* genres) at loads $>10^4$ CFU/g.^{25,30}

3.3 | Total bacterial load of diabetic foot ulcers

Up to three punch biopsies (6 mm diameter, trimmed to 2 mm depth) were collected under local anaesthetic from

each DFU for quantitative analysis of the total bacterial load. After cleansing the wound with saline and gauze to clear away debris or surface contamination, a punch biopsy was taken from the centre of the wound, as per standard of care. An additional one or two biopsies could have been collected if: (1) the clinician identified an area of interest based on CSS detected outside of the wound centre and/or (2) red or cyan FL was detected outside of the wound centre. Each biopsy was transported ambiently using the Remel A.C.T. II culture transport system to a CLIA-certified central laboratory (Eurofins Central Laboratory, Lancaster, PA); transport time ranged between 24–48 h. Use of a non-nutritive medium provided a protective environment that preserved the specimens while preventing bacterial growth during transportation. The laboratory used gold standard, aseptic techniques for analysis of load and species. Quantitative culture was performed as previously described,²⁴ with every effort made to provide optimal conditions for bacteria that are challenging to culture. Diluted biopsy samples were cultured on various agars in conditions to support both aerobic and anaerobic growth.^{23,24} Matrix-assisted laser desorption ionisation-time of flight mass spectrometry (Bruker Daltonics) was used to identify bacterial species. Microbiologists were blinded to the results of the CSS assessment and FL-imaging.

3.4 | Statistical analysis

Statistical analyses calculating and comparing sensitivity and specificity were conducted using MedCalc© Version

19.1.5. The data were compiled in 2×2 tables to calculate sensitivity and specificity with 95% confidence intervals (exact Clopper-Pearson). Tables were generated with positive-case cut-off thresholds of 10^4 , 10^5 , 10^6 , 10^7 , and 10^8 CFU/g, for IWGDF criteria alone compared with IWGDF+FL. Two-sided exact McNemar tests were used to compare sensitivity and specificity at each threshold ($>10^4$ – $>10^8$ CFU/g) between the two tests, with two-sided *P*-values based on the cumulative binomial distribution. Ninety five percent confidence intervals were calculated according to Sheskin.³¹ To compare bacterial loads between the wound centre and periwound biopsies, the bacterial load data was log transformed, found to be normally distributed, and compared using a 2-sided paired student *t*-test.

4 | RESULTS

Most participants recruited over the 11-month study period had a DFU for >3 months duration, with additional characteristics reported in Table 3. Participants spanned the full range of the Fitzpatrick scale for skin tone (I to VI); this is an important consideration when evaluating an imaging diagnostic for skin and wounds. Of the 138 DFUs examined in this study, seven did not have bacterial loads (0 CFU/g). A total of 131 DFUs had

some bacterial presence, of which 6.1% (8/131) had bacterial loads below 10^4 CFU/g, 93.9% (123/131) had bacterial loads exceeding 10^4 CFU/g, and 83.2% (109/131) had bacterial loads exceeding 10^5 CFU/g. The average bacterial load of DFUs with confirmed bacterial presence was 1.44×10^8 CFU/g (range: 5.70×10^2 – 7.79×10^9 CFU/g). The average number of bacterial species per biopsy was 2.74, with some wounds having up to 8 species present. The most common species detected was *Staphylococcus aureus* (52.4%).

Table 4 reports the frequency of IWGDF criteria detected in DFUs for each bacterial threshold of concern exceeding 10^4 CFU/g and up to $>10^8$ CFU/g. Swelling was the only IWGDF criteria detected in DFUs with bacterial loads of 10^4 to 10^5 CFU/g. Among DFUs with the highest levels of bacteria ($>10^8$ CFU/g), swelling (11.5%), erythema (15.4%), pain (11.5%), and local warmth (11.5%) were the most common IWGDF criteria detected. Purulent discharge was the least common IWGDF criteria observed, and this was consistent across all bacterial load thresholds. Red and/or cyan FL was detected in the majority of DFUs (Figure 1A). As the bacterial load increased, the proportion of DFUs with FL indicating bacterial loads also increased. In DFUs with the highest loads of bacteria ($>10^8$ CFU/g), red or cyan FL was detected 92.3% of the time. IWGDF criteria were largely absent in wounds with the highest bacterial loads (Table 4).

Although not a criterion included in the IWGDF classification system, delayed wound healing beyond expectation was the most common clinical sign detected (Figure 1A). Delayed healing was observed in 52.0% (64/123) of DFUs with loads $>10^4$ CFU/g; as bacterial load increased up to 10^8 CFU/g, the frequency of DFUs with delayed healing increased, peaking at 64.7% of wounds with 10^7 – 10^8 CFU/g (Figure 1A). Of those DFUs experiencing delayed healing, the majority were FL positive (70.0%–95.5%); this also peaked at the bacterial threshold of 10^7 – 10^8 CFU/g (Figure 1B).

The receiver operator characteristic (ROC) curves of the five CSS in the IWGDF classification (new or increasing pain, erythema, local warmth, local swelling, and purulent discharge), and delayed healing are plotted individually in Figure 2A. Each data point represents a minimal threshold of clinically significant bacterial loads, ranging from 10^4 – $>10^8$ CFU/g. Each of the five CSS in the IWGDF classification did no better than chance at predicting wounds with bacterial loads of 10^4 CFU/g or greater. In three out of the five IWGDF criteria, sensitivity did not improve with increasing bacterial loads; pain and purulent discharge were the only criteria for which sensitivity correspondingly increased to higher bacteria loads, but with a prevalence of only 11.5% and 7.7%,

TABLE 3 Characteristics of patients with diabetic foot ulcers

	No.	%
Total number of DFUs	138	
Mean age (SD)	58 (10.44)	
Gender		
Female	36	26
Male	102	74
Prior antibiotic use ^a		
Yes	38	28
No	100	72
Fitzpatrick score (skin tone)		
I or II	72	52
III or IV	52	38
V or VI	14	10
Wound duration		
< 3 months	38	28
3–6 months	28	20
6–12 months	19	14
12+ months	53	38

^aUse of topical, oral or intravenous antibiotics within 2 weeks prior to study enrollment.

TABLE 4 Prevalence of clinical signs and symptoms of infection in diabetic foot ulcers per the IWGDF criteria¹

Bacterial load (CFU/g) (n)	Swelling	Erythema	Pain	Warmth	Purulent discharge	Delayed healing ^a	≥ 2 Criteria (CSS+)
10 ⁴ –10 ⁵ (14)	21.4%	0%	0%	0%	0%	28.6%	0%
10 ⁵ –10 ⁶ (25)	20.0%	12.0%	0%	12.0%	0%	40.0%	12.0%
10 ⁶ –10 ⁷ (24)	16.7%	4.2%	4.2%	0%	0%	50.0%	8.3%
10 ⁷ –10 ⁸ (34)	20.6%	14.7%	8.8%	8.8%	2.9%	64.7%	11.8%
>10 ⁸ (26)	11.5%	15.4%	11.5%	11.5%	7.7%	61.5%	11.5%

Note: Values represent the percentage of wounds corresponding to each bacterial load threshold. DFUs with loads <10⁴ CFU/g (n = 15) are not shown.

Abbreviations: CFU/g, colony forming units per gram; CSS, clinical signs and symptoms of infection.

^aDelayed healing beyond expectation is not one of the IWGDF infection criteria.

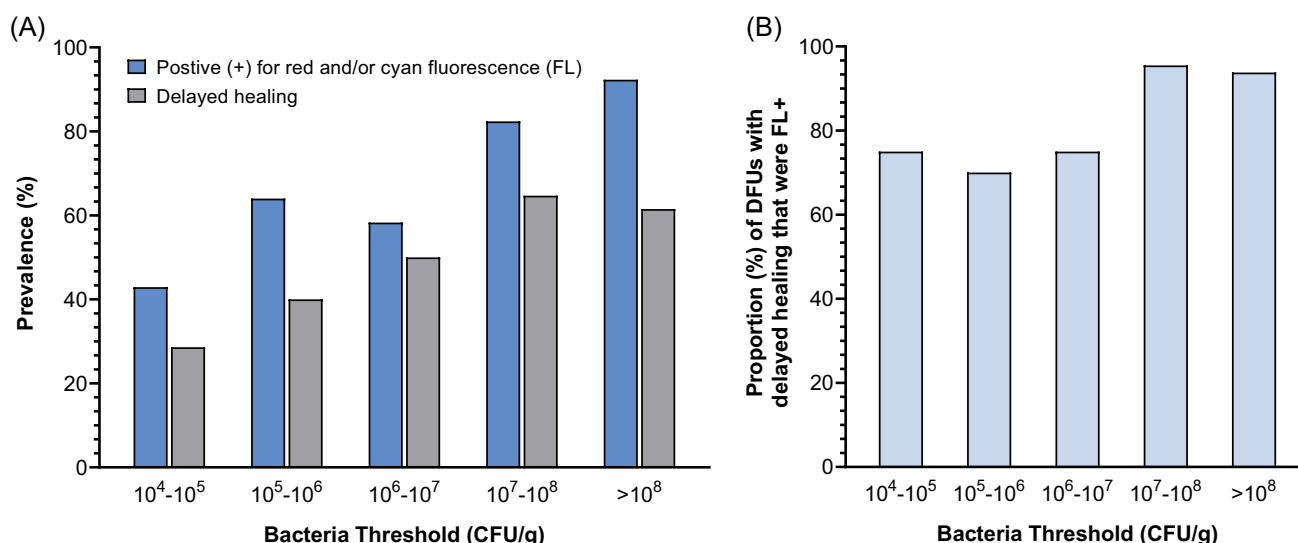


FIGURE 1 (A) Prevalence of red and/or cyan fluorescence and delayed healing at each log increase in bacterial load (10⁴–>10⁸ CFU/g). (B) Proportion of DFUs exhibiting delayed healing that were positive for red and/or cyan fluorescence at each bacterial threshold. Delayed healing = delayed healing beyond expectation, identified if the wound area had not reduced by at least 30% during the prior 4 weeks of care

respectively, in wounds with >10⁸ CFU/g. The sensitivity of erythema, warmth, and swelling did not increase with higher bacterial loads. The low sensitivity and 1-specificity values observed here suggest that all 5 IWGDF criteria were poor predictors of high bacterial load. Sensitivity of delayed healing beyond expectation was high across all bacterial thresholds, but specificity was lower than IWGDF criteria. Figure 2B shows ROC curves of combined IWGDF criteria (IWGDF; 2 or more criteria considered positive for infection) compared with FL-imaging alone (FL) or FL-imaging used together with the IWGDF criteria (IWGDF+FL). When detecting high bacterial loads using only IWGDF criteria, sensitivity ranged from 9.8% to 11.7% (Table 5). Adding FL-imaging to IWGDF, the sensitivity to detect DFUs with bacterial loads increased across all thresholds ($P < .0001$) (Table 5). The sensitivity of two or more IWGDF+FL was significantly higher than IWGDF alone at each

subsequent increase in bacterial thresholds, peaking at 92.6% (Table 5).

Of the DFUs that displayed red or cyan bacterial FL, 84.2% (80/95) had FL indicating bacteria outside of the wound bed, which was mostly confined to the ring of callused tissue in the periwound (2 cm radius extending out from the wound edge³²). Examples of DFUs with endogenous FL from bacteria are reported in Figure 3, and the significance of the colours observed on FL images is described in Table 2 (Methods). Red FL signals within the callused region were usually blushing to orange in hue because of the bacteria being subsurface (e.g., Figure 3C–E). However, bright red FL signals were also observed and attributed to presence of higher bacterial loads at or near the callus surface (e.g., Figure 3F). Where an additional biopsy was indicated by a region of red or cyan FL outside of the wound bed (the wound centre was biopsied for all wounds), we compared the relative

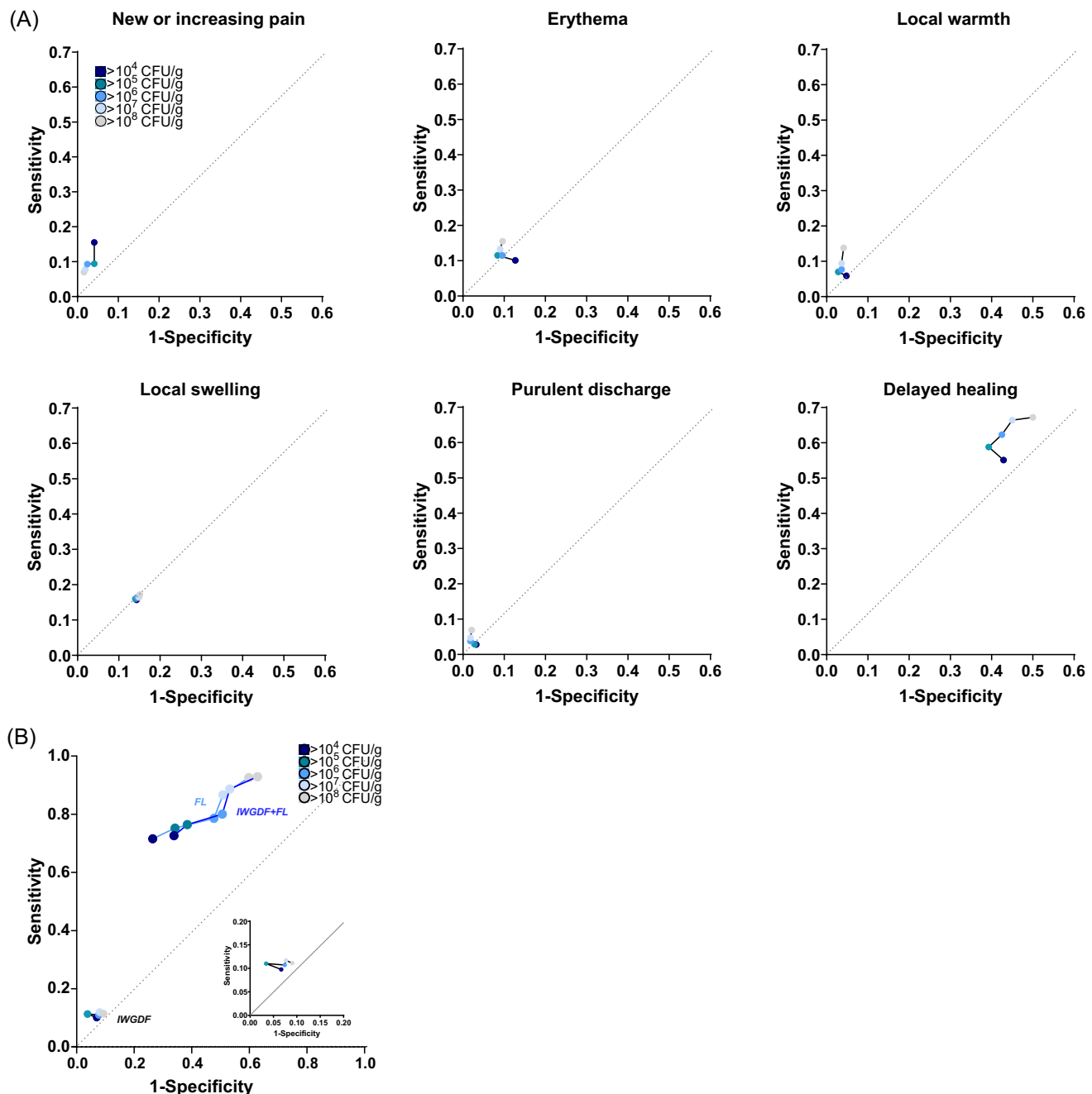


FIGURE 2 (A) Receiver operator characteristic (ROC) curves for each CSS in the IWGDF diabetic foot infection classification (pain, erythema, local warmth, local swelling, and purulent discharge) and delayed healing beyond expectation plotted across each of the bacterial load thresholds assessed ($>10^4$ – $>10^8$ CFU/g). (B) ROC curve of combined IWGDF criteria (2 or more criteria considered positive for infection) compared with combined IWGDF+FL and FL alone at $>10^4$ CFU/g. Inset shows combined (2 or more) IWGDF ROC curve. Diagonal grey dotted line denotes 'line of chance'

number of species and bacterial load within and outside the wound bed. The mean bacterial load from regions of red or cyan FL in the periwound region was significantly higher than the mean bacterial load of biopsies collected from the wound centre (log (7.13) versus log (5.97), $P = .000439$).

5 | DISCUSSION

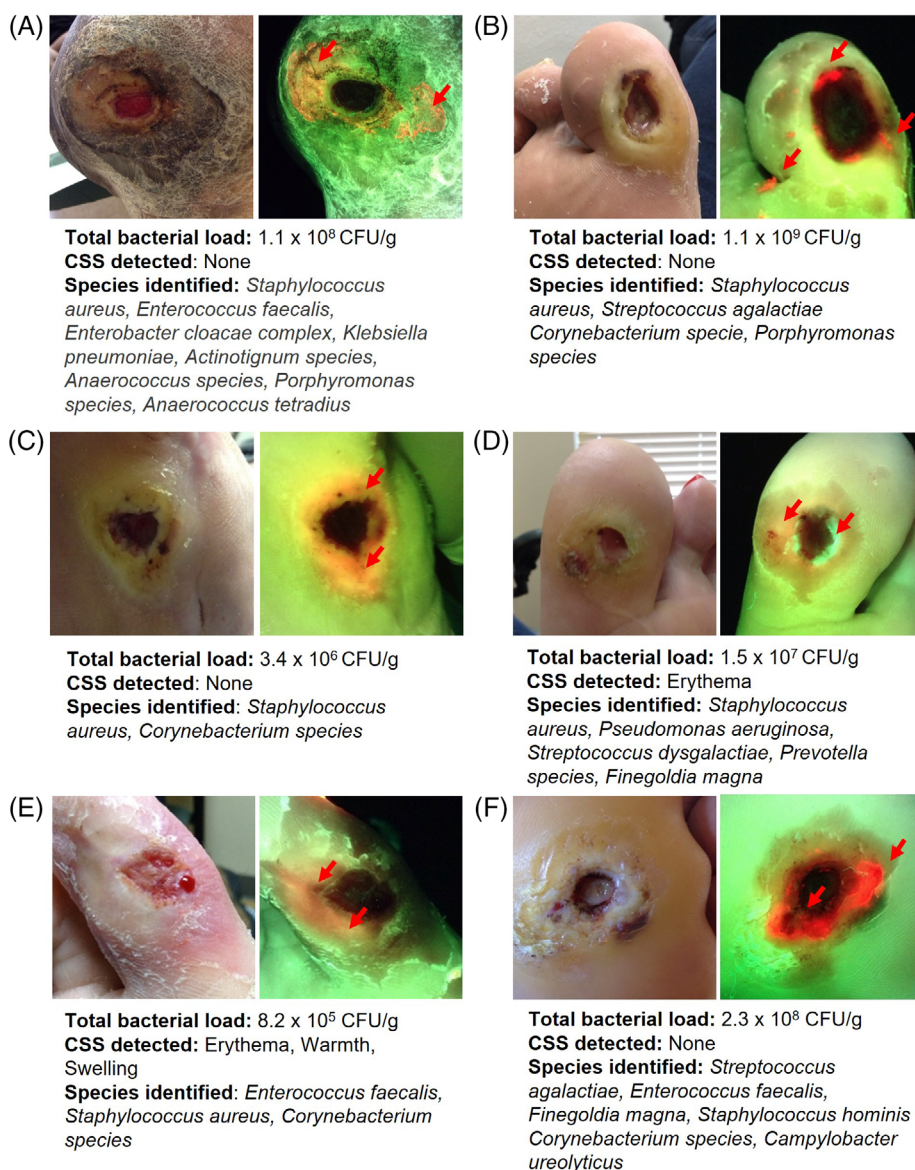
This post-hoc analysis of 138 biopsied and imaged DFUs found that 89.1% (123/138) harboured bacterial loads greater than 10^4 CFU/g. The CSS of infection in these DFUs were largely absent, and ROC curves showed that

Bacterial load (CFU/g)	n	CSS sensitivity (%)	CSS + FL sensitivity (%)	P-value
10^4 – 10^5	14	9.8	71.6	$P < .0001$
10^5 – 10^6	25	11.0	75.2	$P < .0001$
10^6 – 10^7	24	10.7	78.6	$P < .0001$
10^7 – 10^8	34	11.7	86.7	$P < .0001$
$>10^8$	26	11.1	92.6	$P < .0001$

Note: See Figure 2 for specificity data.

TABLE 5 Sensitivity to detect bacterial loads using clinical signs and symptoms of infection (CSS; per the IWGDF criteria¹) alone versus CSS in combination with fluorescence imaging (FL)

FIGURE 3 Fluorescence imaging of DFUs shows high prevalence of asymptomatic bacterial loads and high prevalence of bacterial loads in the periwound area. Clinicians classified wounds as positive (CSS+) or negative (CSS-) for clinical signs and symptoms of infection.¹ Bacterial loads in all cases shown exceeded 10^5 CFU/g (confirmed by microbiological analysis). In each of these cases, red or cyan fluorescence indicative of elevated bacterial load ($>10^4$ CFU/g) was detected (red arrows)



signs and symptoms (2 or more IWGDF criteria) were no better than chance at predicting DFUs with bacterial loads ranging from 10^4 – $>10^8$ CFU/g. Of the DFUs with bacterial loads $>10^4$ CFU/g, 52.0% experienced delayed healing beyond expectations, which increased

proportionally with every log increase in bacterial load up to 10^8 CFU/g. These findings prompted us to propose new clinical terminology, CIBL, to highlight this CSS-independent, healing-inhibitory state. With the addition of FL-imaging, the sensitivity to detect DFUs with high

bacterial loads increased by 3-fold or more, depending on the bacterial threshold (range: 10^4 – 10^9 CFU/g). The sensitivity of FL peaked at $>10^8$ CFU/g (92.6% vs. 11.1% for IWGDF CSS). The DFUs in this study experiencing delayed healing were likely to be FL-positive, regardless of the bacterial threshold. Most DFUs that were FL-positive had signals indicating bacteria in the peri-wound region (84.2%).

The accumulation of bacteria perpetuates a pathogenic state, which increases the risk of infection and can inhibit or delay wound healing.^{8,12,14,15} For the diabetic foot, there is no destructive force greater than infection. The International Wound Infection Institute (IWII) 2022 consensus update on wound infection in clinical practice describes wound infection as a continuum that spans bacterial contamination through colonisation, local infection, spreading infection, and finally systemic infection.²⁰ The presence of pathogenic bacteria must precede infection, and whether a colonised wound progresses to infection depends on microbial factors^{20,33,34} (e.g., virulence, biofilm formation, antimicrobial resistance), host factors^{20,34} (e.g., humoral and cell-mediated immunity, nutritional status, comorbidities), and environmental factors^{20,35} (e.g., wound hygiene). Although some have reported high infection risk when bacterial loads reach 10^5 CFU/g,^{33,36} this is host dependent. There is no one bacterial load threshold that triggers infection. In this study, we report that bacterial loads $>10^4$ CFU/g are highly prevalent in DFUs (89.1%, 123/138), with the mean (1.44×10^8 CFU/g) being three-orders of magnitude above the level historically associated with wound infection.^{33,36}

As evidenced in this study, clinical assessment is unable to detect a high bacterial load or infection in the absence of signs and symptoms (e.g., immunocompromised patients such as those with diabetes). CSS did not correlate with high bacterial loads, as found by quantitative methods. In fact, aside from delayed healing beyond expectations, CSS were largely absent. IWGDF criteria—swelling, erythema, pain, local warmth, and purulent discharge—were infrequently detected (7.7%–15.4%) in DFUs with the highest levels of bacteria ($>10^8$ CFU/g). Accordingly, the ROC curves in this study show that IWGDF CSS in the presence of elevated bacterial loads were rare, and their sensitivity for detecting elevated bacterial load did not increase at even the highest bacterial threshold ($>10^8$ CFU/g; Table 5). Similarly, Gardner et al.²¹ reported that the signs and symptoms in the IDSA classification were no better than chance at predicting DFUs with high bacterial loads (defined as $>10^6$ CFU/g in that study). Other clinical trials have reported similar findings across DFUs, VLU, pressure injuries, and surgical sites.^{24,37} In contrast, delayed wound healing was observed in a large proportion of DFUs in this

study, with its frequency increasing as bacterial load thresholds increased from 10^6 to 10^8 CFU/g (41.7%–57.7%). There are many factors aside from bacterial presence which contribute to delayed wound healing. However, this observed correlation between bacterial load threshold and frequency of delayed healing in DFUs, together with clinical evidence in other wound types (e.g., VLU, PU, surgical wounds),^{12,14,18,24} supports conceptual notions that bacteria at high loads impacts time and ability to heal.^{8–15}

Clearly, CSS-based criteria alone cannot serve as a proxy for detecting high bacterial loads in DFUs. And given its high prevalence, clinicians need terminology which recognises high bacterial load as a unique clinical concern for healing disruption and tissue damage, distinct from infection, but putting the wound at higher infection risk.

Through these data and supporting evidence in the literature, we found it pertinent to describe and illustrate this important pathogenic state through the term ‘CIBL.’ This terminology is akin to the redefinition of critical limb ischemia as chronic limb threatening ischemia (CLTI), which introduced mild/moderate ischemia as a precursor to critical ischemia on the perfusion continuum.³⁸ Similarly, CIBL is not tied to any set bacterial threshold, but rather occurs when the persistent presence of an elevated bacterial load causes pathology, such as detriment to healing, regardless of wound infection status. Just as biofilm spans the IWII wound infection continuum and is therefore addressed using step-down/step-up biofilm-based wound care,²⁰ CIBL may exist in a wound at any point beyond contamination and can be addressed with bacteria-based wound care (e.g., debridement, cleansing) (Figure 4). CIBL is a concept that naturally fits into holistic wound infection prevention and management paradigms. Furthermore, CIBL both acknowledges and is inclusive of the role of biofilm in wound chronicity. In this study, we found that 52.0% of DFUs with bacterial loads $>10^4$ CFU/g experienced delayed healing beyond expectation; this further emphasises that CIBL can be a key indicator for clinical intervention before the onset of infection.

Given that there is no correlation between bacterial load and CSS of infection, how can CIBL be diagnosed in a wound? Owing to the very definition of infection, current practices are unable to respond to asymptomatic but potentially pathogenic bacterial loads. The current study highlights FL-imaging as an objective solution to address this diagnostic gap by visualising high bacterial loads synchronously across the entire wound/periwound. Consistent with other trials,^{24,25,37,39–41} the data herein show a high PPV of FL-imaging and sensitivity well above that of the CSS of infection. Advancing on past works, we also demonstrate that the sensitivity of FL-imaging increases

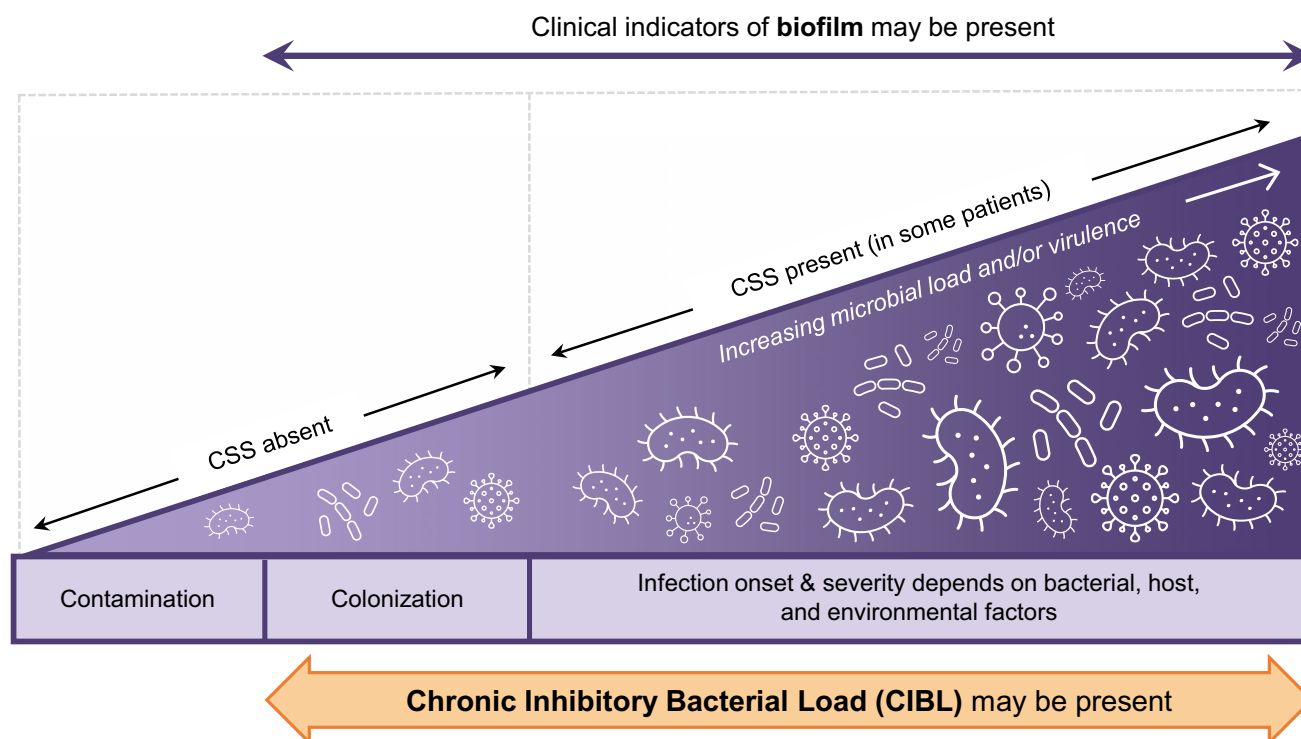


FIGURE 4 Chronic inhibitory bacterial load on the bacterial-infection continuum. Based on the International Wound Infection Institute (IWII) 2022 wound infection continuum.²⁰ ‘CSS’ denotes clinical signs and symptoms of chronic wound infection, as identified by the IWII (e.g., delayed healing, hypergranulation, erythema, local warmth, bleeding, swelling)²⁸

proportionally with bacterial load, while the sensitivity of CSS does not.

FL-imaging also provides critically important information on the distribution of CIBL. DFUs often present with macerated tissue, which contributes to delayed healing^{17,42} and is an ideal environment for the colonisation of bacteria that thrive in a moist environment (e.g., *Pseudomonas aeruginosa*, *Staphylococcus aureus*).⁴² In this study, FL-imaging showed that 84.2% of DFUs contain elevated bacterial loads in the periwound region (including those with and without macerated tissue) and in callus tissue. Given the depth to which bacteria can extend in periwound tissues,^{13,32,43} aggressive wound hygiene strategies (e.g., sharp debridement) may be warranted when CIBL is identified in the DFU periwound.^{32,43} Going forward, this region should be recognised as a frequent harbour for subsurface CIBL.

The potential clinical implications of removing CIBL using point-of-care imaging information are extensive. DFU healing outcomes are shown to improve when more bacteria are removed through biofilm disruption⁴⁴ and with more frequent and/or more aggressive debridement methods (e.g., sharp debridement).⁴⁵ However, recent evidence demonstrates that many bacteria are left behind in chronic wounds after using these ‘best practice’ methods.^{13,25,43,46} We therefore anticipate potential utility

for FL-imaging in several clinical applications within diabetic foot care:

1. *Monitoring treatment effectiveness.* Post-treatment FL-imaging shows whether bacteria have been effectively removed through debridement and other methods,^{13,25,43,46} and can indicate the potential necessity of additional wound management strategies.³² Other studies have used this technology to monitor the effectiveness of negative pressure wound therapy (NPWT) and improve cellular tissue product (CTP) integration by confirming the absence of bacteria prior to placement.²⁷
2. *Antimicrobial stewardship.* Approximately 70% of DFUs are prescribed antibiotics at some point during their care, and over 80% are prescribed antimicrobial dressings,⁴⁷ often in a haphazard manner.²² Diagnostic uncertainty has been listed as a key factor in antibiotic overuse in wound care.²² FL signals as a real-time imaging biomarker of CIBL could enable clinicians to more effectively leverage hygiene-based strategies to remove bacteria rather than resorting to antibiotics.^{22,24,27,48} Indeed, retrospective data from Price showed that after adopting FL-imaging, antibiotic and antimicrobial dressing prescriptions decreased by 33% and 49%, respectively, alongside improvements in 12-week healing rates.⁴⁷

3. **Wound healing outcomes.** The first randomised controlled trial evaluating FL informed care in DFUs was reported in 2022 by Rahma et al.⁴⁹ and demonstrated a doubling of 12-week wound healing rates over the control arm (45% vs. 22%). A stepwise increase in wound area reduction at 12-weeks was observed, which favoured patients with negative baseline FL, followed by patients with positive baseline FL who received additional care (primarily further debridement), and finally patients with positive baseline FL who did not receive additional care. Overall, DFU healing improved when clinicians had objective information on bacterial load and location(s) to consider in their treatment plans. A 229-DFU retrospective study reported increased (+23%) 12-week foot ulcer healing rates after incorporation of FL-imaging, attributed to earlier awareness and removal of the bacterial load.⁴⁷ A smaller prospective longitudinal study correlated the elimination of CIBL FL signals with improved wound area reduction rates,¹³ and a prospective observational study of 55 perineal wounds found that the presence of CIBL FL signals reduced the odds of wound healing within 4 weeks by 79%.⁵⁰

5.1 | Strengths and limitations

As this was a single time point study, we did not assess the clinical impacts of improved CIBL detection, although there are other published works on this topic.^{47,49} However, we did fortify the FL-imaging diagnostic accuracy measures herein by conducting wound biopsy and quantitative culture analysis to confirm bacterial loads. Furthermore, these results are generalizable to the overall DFU population because of the minimal participant exclusion criteria, the heterogeneous sample of patients (138 DFUs, spanning all skin tones on the Fitzpatrick scale), and recruitment from numerous clinical sites and clinicians from a range of wound specialties. However, there were limitations to these methodologies. Clinicians had limited experience using FL-imaging in a clinical context before the study; this may have lowered the sensitivity of FL-imaging to detect bacteria at loads $>10^4$ CFU/g (sensitivity previously reported to range from 72% to 100%^{37,39-41}). A recent study on FL-imaging of surgical sites demonstrated that sensitivity significantly increased when images were read by clinicians more experienced with the technology,³⁷ as is the case with most other diagnostic imaging modalities. Limitations of the imaging technology described include a limited (1.5 mm) depth of excitation²⁹ and the inability to detect non-porphyrin-producing bacteria, including all species from the *Streptococcus*, *Enterococcus*, and *Finexgoldia*

genres,²⁶ although these rarely occur monomicrobially in chronic wounds. This study focused primarily on high bacterial load as a contributor to wound pathogenicity, but there are additional systemic factors which delay DFU healing and increase infection risk (e.g., peripheral artery disease, poor glycemic control, neuropathy). Finally, as the number of datapoints for each bacterial load threshold ranges from $n = 14$ to 34, these results should be interpreted with caution.

6 | CONCLUSIONS

The prevalence and distribution of bacterial burden in DFUs have historically been underappreciated, with its pathogenicity uncertain. This underappreciation is likely because of a lack of reliable methods for identifying the presence and locations of bacteria in wounds, along with the absence of a clinical definition for such a finding in asymptomatic patients. We anticipate that the definition of CIBL will spark a paradigm shift in DFU wound assessment and management that encourages and enables earlier intervention along the bacterial-infection continuum, thereby preventing sequelae of infection and supporting improved DFU outcomes. FL-imaging of bacterial burden has enormous potential for facilitating early bacterial intervention, monitoring treatment effectiveness during and after debridement, aiding antimicrobial stewardship to limit antibiotic and antimicrobial dressing prescriptions, and improving wound healing outcomes.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

- Lipsky BA, Senneville E, Abbas ZG, et al. Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). *Diabetes Metab Res Rev*. 2020;36:e3280. doi:10.1002/dmrr.3280
- Lipsky BA, Berendt AR, Cornia PB, et al. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin Infect Dis*. 2012;54(12):e132-e173. doi:10.1093/cid/cis346
- Jia L, Parker CN, Parker TJ, et al. Incidence and risk factors for developing infection in patients presenting with uninfected diabetic foot ulcers. *PLoS One*. 2017;12(5):e0177916. doi:10.1371/journal.pone.0177916
- Fife CE, Eckert KA, Carter MJ. Publicly reported wound healing rates: the fantasy and the reality. *Adv Wound Care (New Rochelle)*. 2018;7(3):77-94. doi:10.1089/wound.2017.0743
- Stockl K, Vanderplas A, Tafesse E, Chang E. Costs of lower-extremity ulcers among patients with diabetes. *Diabetes Care*. 2004;27(9):2129-2134. doi:10.2337/diacare.27.9.2129
- Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. *N Engl J Med*. 2017;376(24):2367-2375. doi:10.1056/NEJMr1615439
- Brownrigg JR, Davey J, Holt PJ, et al. The association of ulceration of the foot with cardiovascular and all-cause mortality in patients with diabetes: a meta-analysis. *Diabetologia*. 2012;55(11):2906-2912. doi:10.1007/s00125-012-2673-3
- Rippon MG, Westgate S, Rogers AA. Implications of endotoxins in wound healing: a narrative review. *J Wound Care*. 2022;31(5):380-392. doi:10.12968/jowc.2022.31.5.380
- Phillipson M, Kubes P. The healing power of neutrophils. *Trends Immunol*. 2019;40(7):635-647. doi:10.1016/j.it.2019.05.001
- Metzger Z, Nitzan D, Pitaru S, Brosh T, Teicher S. The effect of bacterial endotoxin on the early tensile strength of healing surgical wounds. *J Endod*. 2002;28(1):30-33. doi:10.1097/00004770-200201000-00007
- Crompton R, Williams H, Ansell D, et al. Oestrogen promotes healing in a bacterial LPS model of delayed cutaneous wound repair. *Lab Invest*. 2016;96(4):439-449. doi:10.1038/labinvest.2015.160
- Xu L, McLennan SV, Lo L, et al. Bacterial load predicts healing rate in neuropathic diabetic foot ulcers. *Diabetes Care*. 2007;30(2):378-380. doi:10.2337/dc06-1383
- Cole W, Coe S. Use of a bacterial fluorescence imaging system to target wound debridement and accelerate healing: a pilot study. *J Wound Care*. 2020;29:S44-S52. doi:10.12968/jowc.2020.29.Sup7.S4
- Lantis JC 2nd, Marston WA, Farber A, et al. The influence of patient and wound variables on healing of venous leg ulcers in a randomized controlled trial of growth-arrested allogeneic keratinocytes and fibroblasts. *J Vasc Surg*. 2013;58(2):433-439. doi:10.1016/j.jvs.2012.12.055
- Goldberg S, Diegelmann R. What makes wounds chronic. *Surg Clin North Am*. 2020;100(4):681-693. doi:10.1016/j.suc.2020.05.001
- Johani K, Malone M, Jensen S, et al. Microscopy visualisation confirms multi-species biofilms are ubiquitous in diabetic foot ulcers. *Int Wound J*. 2017;14(6):1160-1169. doi:10.1111/iwj.12777
- Rodgers A, Watret L. Maceration and its effect on periwound margins. *Diabetic Foot*. 2003;6(3):S2.
- Robson MC, Heggers JP. Delayed wound closure based on bacterial counts. *J Surg Oncol*. 1970;2(4):379-383. doi:10.1002/jso.2930020410
- Alvarsson A, Sandgren B, Wendel C, Alvarsson M, Brismar K. A retrospective analysis of amputation rates in diabetic patients: can lower extremity amputations be further prevented? *Cardiovasc Diabetol*. 2012;11:18. doi:10.1186/1475-2840-11-18
- Wounds International (2022) International Consensus Update 2022 International Wound Infection Institute (IWII) Wound Infection in Clinical Practice: Principles of best practice. Available from <https://woundinfection-institute.com/>. Accessed June 22, 2022.
- Gardner SE, Hillis SL, Frantz RA. Clinical signs of infection in diabetic foot ulcers with high microbial load. *Biol Res Nurs*. 2009;11(2):119-128. doi:10.1177/1099800408326169
- Serena TE, Gould L, Ousey K, Kirsner RS. Reliance on clinical signs and symptoms assessment leads to misuse of antimicrobials: post hoc analysis of 350 chronic wounds. *Adv Wound Care (New Rochelle)*. 2021;11:639-649. doi:10.1089/wound.2021.0146
- Serena TE, Bowler PG, Schultz GS, D'Souza A, Rennie MY. Are semi-quantitative clinical cultures inadequate? Comparison to quantitative analysis of 1053 bacterial isolates from 350 wounds. *Diagnostics (Basel)*. 2021;11(7):1239. doi:10.3390/diagnostics11071239
- Le L, Baer M, Briggs P, et al. Diagnostic accuracy of point-of-care fluorescence imaging for the detection of bacterial burden in wounds: results from the 350-patient fluorescence imaging assessment and guidance trial. *Adv Wound Care (New Rochelle)*. 2021;10(3):123-136. doi:10.1089/wound.2020.1272
- Raizman R, Little W, Smith AC. Rapid diagnosis of *Pseudomonas aeruginosa* in wounds with point-of-care fluorescence imaging. *Diagnostics (Basel)*. 2021;11(2):280. doi:10.3390/diagnostics11020280
- Jones LM, Dunham D, Rennie MY, et al. In vitro detection of porphyrin-producing wound pathogens with real-time bacterial fluorescence imaging. *Future Microbiol*. 2020;15(5):319-332. doi:10.2217/fmb-2019-0279
- Oropallo AR, Andersen C, Abdo R, et al. Guidelines for point-of-care fluorescence imaging for detection of wound bacterial burden based on Delphi consensus. *Diagnostics (Basel)*. 2021;11(7):1219. doi:10.3390/diagnostics11071219
- Wounds International (2016) International Consensus Update 2016 International Wound Infection Institute (IWII) Wound Infection in Clinical Practice: Principles of best practice. Available from: <https://www.woundsinternational.com/resources/details/wound-infection-in-clinical-practice-principles-of-best-practice>. Accessed June 22, 2022.

29. Rennie MY, Dunham D, Lindvere-Teene L, Raizman R, Hill R, Linden R. Understanding real-time fluorescence signals from bacteria and wound tissues observed with the MolecuLight i:X (TM). *Diagnostics (Basel)*. 2019;9(1):22. doi:[10.3390/diagnostics9010022](https://doi.org/10.3390/diagnostics9010022)
30. Rennie MY, Lindvere-Teene L, Tapang K, Linden R. Point-of-care fluorescence imaging predicts the presence of pathogenic bacteria in wounds: a clinical study. *J Wound Care*. 2017;26(8):452-460. doi:[10.12968/jowc.2017.26.8.452](https://doi.org/10.12968/jowc.2017.26.8.452)
31. Sheskin DJ. *Handbook of Parametric and Nonparametric Statistical Procedures*. 5th ed. Boca Raton: Chapman and Hall/CRC; 2011.
32. Andersen CA, McLeod K, Steffan R. Diagnosis and treatment of the invasive extension of bacteria (cellulitis) from chronic wounds utilising point-of-care fluorescence imaging. *Int Wound J*. 2021;1-13:996-1008. doi:[10.1111/iwj.13696](https://doi.org/10.1111/iwj.13696)
33. Lipsky BA, Hoey C. Topical antimicrobial therapy for treating chronic wounds. *Clin Infect Dis*. 2009;49(10):1541-1549. doi:[10.1086/644732](https://doi.org/10.1086/644732)
34. Heinzelmann M, Scott M, Lam T. Factors predisposing to bacterial invasion and infection. *Am J Surg*. 2002;183:179-190.
35. Hurlow JJ, Humphreys GJ, Bowling FL, McBain AJ. Diabetic foot infection: a critical complication. *Int Wound J*. 2018;15(5):814-821. doi:[10.1111/iwj.12932](https://doi.org/10.1111/iwj.12932)
36. Bjarnsholt T, Kirketerp-Møller K, Jensen P, et al. Why chronic wounds will not heal: a novel hypothesis. *Wound Repair Regen*. 2008;16(1):2-10. doi:[10.1111/j.1524-475X.2007.00283.x](https://doi.org/10.1111/j.1524-475X.2007.00283.x)
37. Sandy-Hodgetts K, Andersen CA, Al-Jalodi O, Serena L, Teimouri C, Serena TE. Uncovering the high prevalence of bacterial burden in surgical site wounds with point-of-care fluorescence imaging. *Int Wound J*. 2021;1-11:1438-1448. doi:[10.1111/iwj.13737](https://doi.org/10.1111/iwj.13737)
38. Conte MS, Bradbury AW, Kolh P, et al. Global vascular guidelines on the management of chronic limb-threatening ischemia. *J Vasc Surg*. 2019;69:3 S-125 S e140. doi:[10.1016/j.jvs.2019.02.016](https://doi.org/10.1016/j.jvs.2019.02.016)
39. Hurley CM, McClusky P, Sugruie RM, Clover JA, Kelly JE. Efficacy of a bacterial fluorescence imaging device in an outpatient wound care clinic: a pilot study. *J Wound Care*. 2019;28(7):438-443. doi:[10.12968/jowc.2019.28.7.438](https://doi.org/10.12968/jowc.2019.28.7.438)
40. Serena T, Harrell K, Serena L, Yaakov R. Real-time bacterial fluorescence imaging accurately identifies wounds with moderate-to-heavy bacterial burden. *J Wound Care*. 2019;28(6):346-357. doi:[10.12968/jowc.2019.28.6.346](https://doi.org/10.12968/jowc.2019.28.6.346)
41. Hill R, Woo K. A prospective multisite observational study incorporating bacterial fluorescence information into the UPPER/LOWER wound infection checklists. *Wounds*. 2020;32(11):299-308.
42. Haryanto H, Arisandi D, Suriadi S, et al. Relationship between maceration and wound healing on diabetic foot ulcers in Indonesia: a prospective study. *Int Wound J*. 2017;14(3):516-522. doi:[10.1111/iwj.12638](https://doi.org/10.1111/iwj.12638)
43. Raizman R, Dunham D, Lindvere-Teene L, et al. Use of a bacterial fluorescence imaging device: wound measurement, bacterial detection and targeted debridement. *J Wound Care*. 2019;28(12):824-834. doi:[10.12968/jowc.2019.28.12.824](https://doi.org/10.12968/jowc.2019.28.12.824)
44. Pouget C, Dunyach-Remy C, Pantel A, Schuldiner S, Sotto A, Lavigne JP. Biofilms in diabetic foot ulcers: significance and clinical relevance. *Microorganisms*. 2020;8(10):1580. doi:[10.3390/microorganisms8101580](https://doi.org/10.3390/microorganisms8101580)
45. Wilcox JR, Carter MJ, Covington S. Frequency of debridements and time to heal: a retrospective cohort study of 312 744 wounds. *JAMA Dermatol*. 2013;149(9):1050-1058. doi:[10.1001/jamadermatol.2013.4960](https://doi.org/10.1001/jamadermatol.2013.4960)
46. Moelleken M, Jockenhöfer F, Benson S, Dissemmond J. Prospective clinical study on the efficacy of bacterial removal with mechanical debridement in and around chronic leg ulcers assessed with fluorescence imaging. *Int Wound J*. 2020;17(4):1011-1018. doi:[10.1111/iwj.13345](https://doi.org/10.1111/iwj.13345)
47. Price N. Routine fluorescence imaging to detect wound bacteria reduces antibiotic use and antimicrobial dressing expenditure while improving healing rates: retrospective analysis of 229 foot ulcers. *Diagnostics (Basel)*. 2020;10(11):927. doi:[10.3390/diagnostics10110927](https://doi.org/10.3390/diagnostics10110927)
48. Hill R, Rennie MY, Douglas J. Using bacterial fluorescence imaging and antimicrobial stewardship to guide wound management practices: a case series. *Ostomy Wound Manage*. 2018;64(8):18-28.
49. Rahma S, Woods J, Nixon JE, Brown S, Russell DA. The use of point-of-care bacterial autofluorescence imaging in the Management of Diabetic Foot Ulcers: a pilot randomised controlled trial. *Diabetes Care*. 2022;45:1601-1609. doi:[10.2337/dc21-2218](https://doi.org/10.2337/dc21-2218)
50. Adanna Okeahialam N, Thakar R, Sultan AH. The clinical progression and wound healing rate of dehiscent perineal tears healing by secondary intention: a prospective observational study. *Eur J Obstet Gynecol Reprod Biol*. 2022;274:191-196. doi:[10.1016/j.ejogrb.2022.05.035](https://doi.org/10.1016/j.ejogrb.2022.05.035)

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