OPEN

Bacterial Fluorescence Imaging to Address Racial Inequities in Wound Infection Assessment

Charles A. Andersen, MD, MAPWCA; Gabriel Ayoola, PhD; Alton R. Johnson, Jr, DPM, CWSP; Jonathan Johnson, MD, MBA; Martha R. Kelso, RN, HBOT; Thomas E. Serena, MD, MAPWCA; and Alisha Oropallo, MD

Racial and ethnic minority populations experience significant outcome disparities across all medical specialties. Patients with dark skin tones in wound care experience more chronic wounds and poorer healing than White populations.[1,2] In turn, those with dark skin tones are also likely to have more severe pressure injuries and higher amputation and mortality rates.[3–5] Research has begun to unravel the complex factors underlying these disparities, including social determinants of health, lack of skin tone diversity in medical research, and unconscious provider biases.[6,7] Racial and ethnic minority populations may experience suboptimal care and outcomes because of language barriers, cultural differences,[8] medical mistrust, and lack of implicit trust.[9] Critically, addressing these disparities necessitates a comprehensive approach encompassing policy changes, cultural competency training for healthcare professionals, improved access to high-quality care, and community engagement aimed at building trust and meeting the distinct needs of racial and ethnic minority communities.

Wound infection mounts over time as bacterial loads accumulate, beginning with innocuous contamination that starts to impede healing processes upon reaching loads around 10^4 colony-forming units (CFUs) per gram.[10] Clinical infection is typically associated with bacterial loads above 10^5 or 10^6 CFU/g, confirmed via deep tissue or swab cultures; however, this is dependent on the species present as well as host factors. Accurately identifying bacterial infection, particularly in the early stages, is key to preventing deterioration and serious complications.[10] Chronic inhibitory bacterial load (CIBL) is a relatively new diagnostic term that describes the persistent presence of pathologic bacteria levels that prevent healing and increase infection risk, with or without symptoms typically associated with infection.[10] Clinicians frequently fail to recognize symptoms of infection or CIBL in individuals with dark skin.[11] This leads to misdiagnoses and delayed treatment, resulting in disproportionally poor outcomes for patients with dark skin tones.

Detecting wound infection using clinical symptoms, most notably visual indications, can be challenging in all skin tones, but more so in patients with dark skin. Why is equitable wound assessment such a challenge? Clinicians need to recognize the importance of approaching wound assessment differently in patients with dark skin tones.[12] Improving clinical signs and symptoms (CSSs) training alone is unlikely to address this disparity; however, integrating objective technologies has shown great promise. Bacterial fluorescence imaging enables clinicians to identify CIBL promptly and intervene early, ideally before infection.[10] It also reduces the need for systemic antibiotic therapy.[13]

In this article, the authors discuss how skin tone can complicate the identification of wound infection or CIBL through the lens of a recent research study by Johnson et al[11] calling attention to this issue. They also highlight bacterial fluorescence imaging as an emerging biotechnology to help bridge the gap between the current standard of care and improved wound assessment.

SKIN TONE INFLUENCES THE APPEARANCE OF CHRONIC WOUND INFECTION

Understanding how skin tone (ie, melanin content) influences the appearance of common signs of chronic wound infection or CIBL and how its misinterpretation can affect patient outcomes is crucial for advancing equitable healthcare. Although pathogenic bacterial loads above 10^4 CFU/g of tissue can set the stage for a more aggressive wound infection, even the highest bacterial loads in patients with a wound may fail to express recognizable clinical symptoms.[10] As a result, delay in healing, as indicated by poor wound area reduction after 4 weeks of treatment, is often the first sign that bacterial infection or CIBL may be present.[10]

A recently published study by Johnson et al[11] analyzed clinical assessment findings and quantitative bacterial

Charles A. Andersen, MD, MAPWCA, is Chief, Wound Care Service, Madigan Army Medical Center Joint Base Lewis-McChord, Tacoma, Washington, USA. Gabriel Ayoola, PhD, is Lecturer, Afroamerican and African Studies, University of Michigan, Ann Arbor, Michigan. Alton R. Johnson, Jr, DPM, CWSP, is Clinical Assistant Professor and Attending Physician, Department of Orthopedic Surgery-Foot and Ankle, University of Michigan Medical School, Ann Arbor. Jonathan Johnson, MD, MBA, is Founder and Surgical Director, Comprehensive Wound Care Services and Capital Aesthetic & Laser Center, Washington, District of Columbia. Martha R. Kelso, RN, HBOT, is Founder and Chief Executive Officer, Wound Care Plus LLC, Blue Springs, Missouri. Thomas E. Serena, MD, MAPWCA, is Founder and Chief Executive Officer, SerenaGroup Inc, Cambridge, Massachusetts. Alisha Oropallo, MD, is Professor of Surgery, Zucker School of Medicine, Hofstra University/Northwell Health, Hempstead, New York, and Director, Comprehensive Wound Healing Center and Hyperbarics, Northwell Health, Lake Success, New York. **Acknowledgments**: Drs Andersen, Johnson, and Serena have received remuneration from MolecuLight, Inc, for previous speaking engagements. The authors have disclosed no other financial relationships related to this article. Submitted July 11, 2023; accepted in revised form January 16, 2024.

culture data from 350 chronic wounds to assess the occurrence and potential drivers of racialized disparities in wound infection assessment.[11] The analysis revealed that infections, as identified by the presence of three or more CSSs, were reported less often among patients with Fitzpatrick scores of 5 or 6 despite there being no significant difference in the amount of wound bacteria present (10^6 CFU/g, on average) in those patients versus patients with lower Fitzpatrick scores (1–4). The Fitzpatrick scale is a tool commonly used to classify patients according to their skin tone in relation to their response to UV light exposure. In all patients, the sensitivity of CSSs was poor (15%), and in patients with dark skin (scores 5 and 6), the sensitivity was only 3%.

What caused this disparity in assessment? The study revealed that the frequency of detection of erythema, wound breakdown and enlargement, and delayed healing varied based on skin pigmentation: these symptoms were detected less frequently among patients with high skin pigmentation. These findings were statistically significant, or near statistically significant in the case of erythema; there were no significant differences for the other infection symptoms studied. The rationale for poor erythema detection is evident: melanin pigmentation can alter the perception of erythema by creating a different color appearance.[14] A darker or more purplish hue may be apparent, thus making it more difficult to accurately assess the extent and severity of erythema—the limited educational references for wound assessment on skin of color compound this diagnostic challenge. The rationale for less frequent diagnosis of wound breakdown and delayed healing needs to be clarified.

The current point-of-care approach to detecting infection or CIBL in wounds and subsequently indicating the need for intervention (including sampling) is mostly based on CSSs. This approach is counterintuitive because CSSs are often absent or muted at this stage and/or in this patient base, leading to missed timely intervention and inappropriate use of antimicrobials and antibiotics. This lack of adequate diagnostics puts patients with dark skin tones at a distinct disadvantage in terms of wound care. Because CSSs are less frequently detected in dark skin, infection diagnosis and treatment are delayed, contributing to disproportionally poor outcomes. Education campaigns are essential to enhance the accuracy of CSSs-based wound status assessment among patients with dark skin tones, thereby facilitating a positive transformation in their healthcare. These initiatives should involve the development of practical guidance by medical educators, professional wound care associations, and clinicians. In addition, collaboration with regulatory bodies such as the CMS and the US FDA is crucial to support education initiatives through medical policy. Another key step toward equitable wound care is the universal adoption of objective assessment measures.

BACTERIAL IMAGING AS AN OBJECTIVE AND EQUITABLE DIAGNOSTIC TOOL

The past decade has seen a surge in biotechnology advances that enable more objective assessment of patients with all skin colors. Technology to assess wound healing progress systematically and indiscriminately for all patients is key to mitigating disparities in current clinical assessment. To that end, implementing technologies that objectively measure changes in wound size and wound bacterial infection markers (eg, bacterial autofluorescence imaging, thermal imaging) can better inform treatment decisions.

Johnson et al[11] undertook a post hoc clinical trial analysis to assess the performance of bedside fluorescence imaging for identifying and mapping regions of high bacterial loads across patients with a range of skin pigmentation.[11] The imaging device (MolecuLight; MolecuLight, Inc) detects locations of bacteria at loads greater than 10^4 CFU/g by safely illuminating the wound with violet light. In turn, bacterial metabolites emit endogenous fluorescence signals registered by the device (red for most species or cyan/bright white with a greenish blue "halo" for *Pseudomonas* specifically). The device also has digital wound measurement capabilities. However, the original clinical trial was a single time point and did not investigate wound area changes over time.

Johnson et al[11] found that combining clinical assessment with bacterial fluorescence imaging significantly improved the detection of high bacterial loads in wounds across all skin pigmentations. The most significant benefit was observed in patients in the higher pigment group (12-fold improvement). Despite evidencing a clear trend toward enhanced detection sensitivity among all skin tone groups, the detection sensitivity varied between groups and remained the lowest among patients with high skin pigmentation. This, along with the inherent principles of fluorescence imaging, may suggest that melanin content could interfere with bacterial fluorescence signals by competitively absorbing violet light. However, more research is needed to understand this potential confounding factor.

Using the imaging device, the treating clinician accessed immediate information on the wound's bacterial status without requiring invasive measures (eg, wound sampling) and achieved a higher detection sensitivity than standard, CSSs-based infection assessment. Similarly, Andersen et al[15] reported the ability of bacterial autofluorescence imaging for instances of wound-associated cellulitis that would have otherwise gone clinically undetected; they used these insights to guide management up to resolution. Other studies have demonstrated that leveraging imaging insights frequently changes treatment plans. This includes alerting the clinician to the need for additional cleansing or debridement,[16,17] the need for antimicrobials (including dressings),[16] and facilitating more accurate and representative culture samples.[18]

The ability to redesign, on-the-spot, personalized and evidence-backed treatment plans is the crux of the clinical utility of bacterial fluorescence imaging. All patients, but especially those with dark skin tones, would benefit from incorporating objective, unbiased diagnostics. However, underprivileged populations such as racial minorities, older adults, or long-term-care residents are often the last to gain access to emerging healthcare technologies. This is especially true for those who receive care in resource-limited settings or who live in geographic clusters of poverty. Census data have shown that residential segregation of racial and ethnic minority patient populations limits their access to primary care physicians.[19] Further, these minority populations more frequently live in regions of concentrated poverty in the US than do White populations,[20] limiting their access to adequate healthcare or resources needed to maintain at-home care plans. In this context, there is immense value in implementing easily accessible, bedside technologies that improve early and proactive diagnoses of wound infection.

CLINICAL EXAMPLES OF WOUNDS ON PATIENTS OF COLOR

Figure 1 illustrates examples of wounds on a range of skin tones. Objective localization of bacterial regions (red or cyan/bright white with a greenish blue "halo") is clear from the accompanying fluorescence images, including the wounds on dark skin. This detection enhancement has proven advantageous even for the most experienced clinicians in terms of fluorescence image interpretation.[11]

Irrespective of racial bias, the issue of missing covert bacterial loads during unassisted clinical assessment is all too common among chronic wounds with a torpid evolution and atypical presentation.[10] Following are two case examples of patients with dark skin tones and complex wounds for whom fluorescence imaging findings provided insight that led to more effective treatments. Case 1 describes a patient for whom debridement was ineffective and oral antibiotics were needed, and case 2 describes a delay in a planned skin grafting procedure due to unsuspected *Pseudomonas*. Both patients provided consent for their case details and images to be published.

Case 1

A 24-year-old man with Fitzpatrick score of 6 and a medical history of incomplete paraplegia because of a spinal injury in 2017 presented to the wound clinic with an unstageable pressure injury to the sacrococcygeal region and right ischium (Figure 2). Upon initial clinical assessment, erythema and scarring surrounded the sacral injury; however, the investigator determined that the wound did not contain excessive bacteria levels. Following cleansing with sterile 0.9% sodium chloride, the wound was scanned with a fluorescence imaging device in search of covert areas containing high bacterial loads. Extensive red (bacterial) fluorescence was observed in and around the sacral injury, and targeted, sharp debridement was performed under fluorescence guidance. However, unable to debride further, the last fluorescence scan demonstrated persistent bacterial loads. As a result, the provider prescribed oral doxycycline to control the persistent bacterial contamination and applied antimicrobial dressings.

Following the initial assessment, the patient received care from a home health nurse twice a week and returned to the clinic weekly for reassessment, including fluorescence scans. Two weeks into treatment, the bacterial regions were vastly reduced, the erythema had resolved, and a slough formed over the healing wound. After

Figure 1. STANDARD (TOP) AND FLUORESCENCE (BOTTOM) IMAGES OF CHRONIC WOUNDS ACROSS A RANGE OF SKIN TONES (FITZPATRICK SCORES 1–6)

Red/yellow fluorescence indicates most bacterial species at loads >10^4 CFU/g (panels 1–4, 6), whereas cyan/bright white fluorescence signal with a greenish blue "halo" indicates *Pseudomonas aeruginosa* specifically (panel 5).

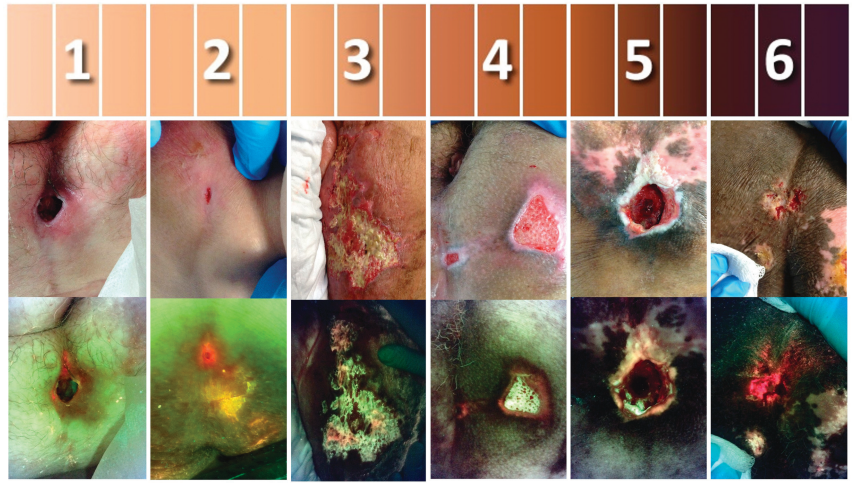
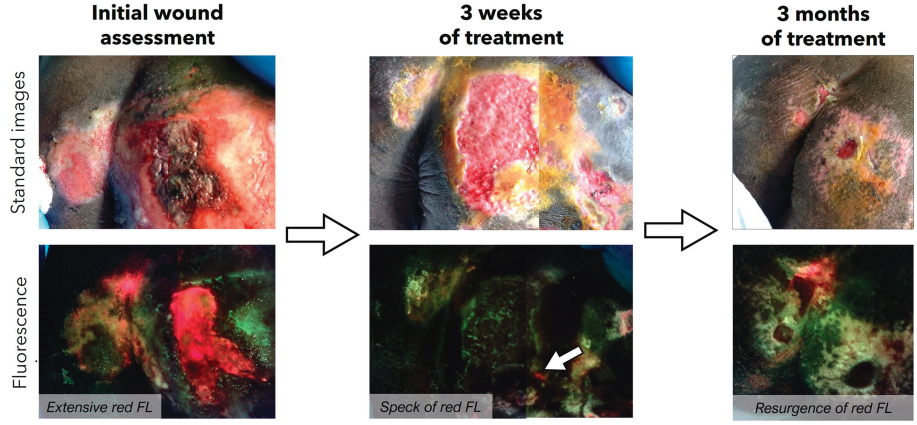


Figure 2. CASE 1



Abbreviation: FL, fluorescence.

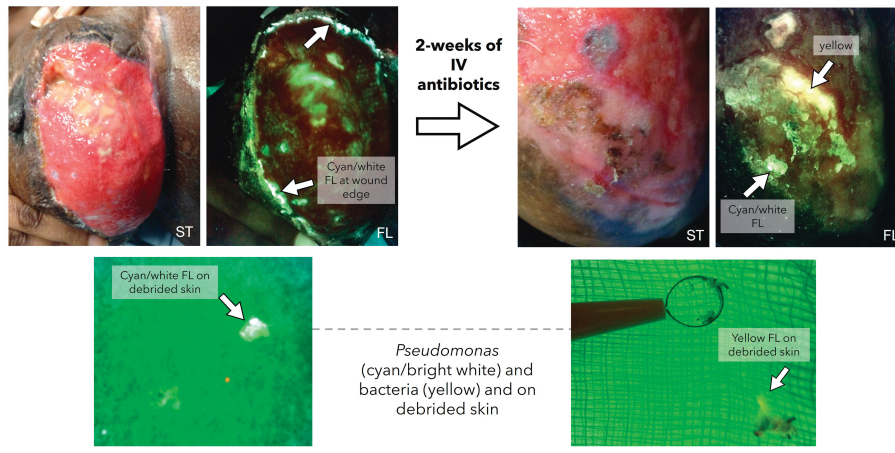
3 months of treatment, the wound continued to heal with the persistent removal of bacterial fluorescence near the sacrum. There was a resurgence of bacterial fluorescence at the 3-month visit, which is not uncommon in sacral pressure injuries due to urine and fecal matter. However, the ability to monitor this bacterial presence and address it through targeted hygiene offset this challenge.

Case 2

A 39-year-old woman with Fitzpatrick score of 5 presented with surgical site complications following a reduction mammoplasty (Figure 3). She was scheduled for a split-thickness skin graft at this visit, and upon clinical assessment, the wound's condition appeared suitable for grafting (no overt signs of infection). As per standard practice at the Madigan Army Medical Center wound clinic, the wound was scanned for bacterial fluorescence prior to the grafting procedure. Imaging revealed cyan fluorescence, appearing in this case as a bright white signal with a greenish blue "halo," indicative of *Pseudomonas aeruginosa* at >10^4 CFU/g, along the wound border, on the wound dressing, and on the debrided skin. *Pseudomonas* drastically reduces grafting success, and its presence is predictive of graft failure.[21] Although it can have a unique clinical presentation (odor, greenish tinge), *Pseudomonas* is clinically inapparent in up to 20% of wounds,[22] and yet likely to construct a tenacious biofilm that reduces antibiotic effectiveness. Therefore, the skin graft was postponed because of these imaging findings. The patient was treated with targeted debridement to disrupt the biofilm and IV antibiotics to clear the bacteria. Culture results from a sample collected from the fluorescence-positive region later confirmed the presence of *Pseudomonas* resistant to all oral antibiotics.

Upon examination after 2 weeks of IV antibiotics, the wound had decreased in size, and there were no CSSs of infection. Repeat fluorescence imaging still demonstrated *Pseudomonas* on the wound dressing and debrided skin and a yellow signal in the wound bed indicating subsurface bacterial loads. Because the bacteria were not resolved, the grafting was postponed again, and the patient continued to receive IV antibiotics. On follow-up after

Figure 3. CASE 2



Abbreviation: FL, fluorescence.

1 month of antibiotics, the wound size had significantly decreased, and grafting was not required.

CONCLUSIONS

The authors strongly believe that increasing access to technologies that narrow the assessment disparity, such as bacterial fluorescence imaging, can transform wound assessment for patients of color. Its visual nature transcends language barriers, aiding non-English speakers, and its portability facilitates use in remote areas and health campaigns. Proactive wound care enables early intervention, possibly reducing complications and improving population health. ●

REFERENCES

1. Bliss DZ, Gurvich O, Savik K, et al. Racial and ethnic disparities in the healing of pressure ulcers present at nursing home admission. Arch Gerontol Geriatr 2017;72:187-94.
2. Bauer K, Rock K, Nazzal M, Jones O, Qu W. Pressure ulcers in the United States' inpatient population from 2008 to 2012: results of a retrospective nationwide study. Ostomy Wound Manage 2016;62(11):30-8.
3. Brennan MB, Powell WR, Kaiksow F, et al. Association of race, ethnicity, and rurality with major leg amputation or death among Medicare beneficiaries hospitalized with diabetic foot ulcers. JAMA Netw Open 2022;5(4):e228399.
4. Traven SA, Synovec JD, Walton ZJ, Leddy LR, Suleiman LI, Gross CE. Notable racial and ethnic disparities persist in lower extremity amputations for critical limb ischemia and infection. J Am Acad Orthop Surg 2020;28(21):885-92.
5. Tan T-W, Shih C-D, Concha-Moore KC, et al. Disparities in outcomes of patients admitted with diabetic foot infections. PLoS One 2019;14(2):e0211481.
6. Riley J, Antza C, Kempegowda P, et al. Social deprivation and incident diabetes-related foot disease in patients with type 2 diabetes: a population-based cohort study. Diabetes Care 2021;44(3):731-9.
7. Oozageer Gunowa N. Skin tone bias and wound care: highlighting the current evidence and addressing the gaps in knowledge of dark skin tones. Wounds UK 2022;18(1):22-7.
8. Karliner LS, Jacobs EA, Chen AH, Mutha S. Do professional interpreters improve clinical care for patients with limited English proficiency? A systematic review of the literature. Health Serv Res 2007;42(2):727-54.
9. Armstrong K, Putt M, Halbert CH, et al. Prior experiences of racial discrimination and racial differences in health care system distrust. Med Care 2013;51(2):144-50.
10. Armstrong DG, Edmonds ME, Serena TE. Point-of-care fluorescence imaging reveals extent of bacterial load in diabetic foot ulcers. Int Wound J 2023;20(2):554-66.
11. Johnson J, Johnson AR Jr, Andersen CA, Kelso MR, Oropallo AR, Serena TE. Skin pigmentation impacts the clinical diagnosis of wound infection: imaging of bacterial burden to overcome diagnostic limitations. J Racial Ethn Health Disparities 2024;11(2):1045-55.
12. Dhoonmoon L, Fletcher J. Assessing skin tones in practice: results of an international survey. Wounds Int 2022;13(2):22-5.
13. 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.
14. Narla S, Heath CR, Alexis A, Silverberg JI. Racial disparities in dermatology. Arch Dermatol Res 2023;315(5):1215-23.
15. 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 2022;19(5):996-1008.
16. Jacob A, Jones LM, Abdo RJ, et al. Lights, fluorescence, action-Influencing wound treatment plans including debridement of bacteria and biofilms. Int Wound J 2023;20(8):3279-88.
17. 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(7):1601-9.
18. Serena TE, Snyder RJ, Bowler PG. Use of fluorescence imaging to optimize location of tissue sampling in hard-to-heal wounds. Front Cell Infect Microbiol 2023;12:1070311.
19. Bishaw A. Areas with Concentrated Poverty: 2006-2010. US Census Bureau; December 2011. https://www.census.gov/library/publications/2011/acs/acsbr10-17.html. Last accessed May 7, 2024.
20. Gaskin DJ, Dinwiddie GY, Chan KS, McCleary RR. Residential segregation and the availability of primary care physicians. Health Serv Res 2012;47(6):2353-76.
21. Soothill JS. Bacteriophage prevents destruction of skin grafts by *Pseudomonas aeruginosa*. Burns 1994;20(3):209-11.
22. 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.