Case Report

Fluorescent Imaging as a Component of Diagnosing Pyoderma Gangrenosum: A Case Report

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ABSTRACT

A 64-year-old White woman was admitted to the hospital with complaint of progressive right hip ulceration at the wound site following a total right hip arthroplasty. Initial history and physical examination gave a leading differential diagnosis of pyoderma gangrenosum. Until recently, the exclusion of infection for pyoderma gangrenosum has been largely clinical and supported by cultures/biopsies demonstrating the absence of infection. The MolecuLight *i:X* (MolecuLight, Toronto, Ontario, Canada) is a novel bedside fluorescent imaging device capable of determining the bacterial burden within a wound in real time. Fluorescent imaging excluded infection at the initial visit, and debridement was avoided. Subsequently, pathergy was avoided as well. The patient was started on topical clobetasol with hypochlorous acid-soaked dressings. She also received 80 mg daily of prednisone and high-dose vitamin D_3 (10,000 IU). Recovery was complicated by a deep tunnel along the incisional line at 3 months postdiagnosis, which required slowing of the prednisone taper and the addition of colchicine. Repeat cultures grew *Parvimonas*, *Pseudomonas*, and *Streptococcus* species. Appropriate antibiotics were given. The patient was transitioned from prednisone to adalimumab and started on negative-pressure wound therapy. Negative-pressure wound therapy was discontinued at 5 months, and the wound resolved at 6 months.

KEYWORDS: adalimumab, fluorescent imaging, hypochlorous acid, negative-pressure wound therapy, pyoderma gangrenosum, vitamin D

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INTRODUCTION

Pyoderma gangrenosum (PG) is an uncommon autoinflammatory neutrophilic dermatosis that presents a diagnostic dilemma and historically was a diagnosis of exclusion. However, with recent Delphi consensus of PG diagnostic criteria among international experts, an advised and validated set of criteria now exists. Chief among the criteria for diagnosis is histopathology demonstrating neutrophilic infiltration at the edge of an ulcer, in addition to the exclusion of bacterial infection. Further, a thorough and accurate patient clinical history to determine other potential causes of similar pathologic presentations, such as necrotizing fasciitis, is necessary for the diagnosis. Although described in the wound care literature, there is a paucity of data to evaluate or document the utility of bacterial fluorescence imaging to aid in PG diagnosis.

CASE REPORT

A 64-year-old White woman was admitted to the hospital with the diagnosis of progressively worsening right hip/groin ulceration in the periwound region 10 days following right total hip arthroplasty. Her medical history was notable for hypothyroidism, hypertriglyceridemia, moderate obesity, and a remote history of pancreatitis.

Physical examination revealed a well-demarcated ulcerated plaque with a raised pink/violaceous scalloped border and fibrinous base involving the right upper thigh that was causing the patient significant pain (Figure 1). Laboratory evaluation revealed anemia (hemoglobin, 7.9 g/dL), mild leukocytosis (white blood cells, 11,600/μL), and markedly elevated C-reactive protein (298 mg/l) and erythrocyte sedimentation rate (111 mm/hr). Peripheral blood smear and serum/urine immunofixation showed acute-phase reactive processes without monoclonal proteins. Imaging studies (ultrasound and noncontrast computed tomography of the hip and femur) demonstrated no subcutaneous air and no evidence of fluid or air at the hip arthroplasty site. No deep venous thrombus

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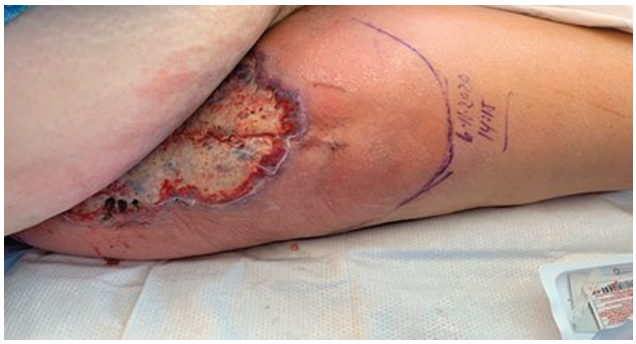
Figure 1. INITIAL PRESENTATION TO HOSPITAL FOR ADMISSION FOR GROIN AND RIGHT THIGH WOUND, 10 DAYS AFTER RIGHT HIP ARTHROPLASTY

Note the longitudinal surgical incision from the anterior approach for hip arthroplasty. The skin marker outlines initial extent of clinically suspected cellulitis, subsequently identified to be inflammatory changes.



was identified. Overall, the tests performed were nondiagnostic. Without evidence of hip arthroplasty infection, seroma, or hematoma, the leading diagnosis was soft-tissue infection.

Bedside fluorescent imaging with 405-nm-wavelength light (MolecuLight *i:X*, MolecuLight, Toronto, Ontario, Canada) did not reveal red or cyan fluorescence (Figure 2), colors associated with bacterial burden. Histopathology from the wound edge demonstrated focal ulceration, papillary dermal edema, and mixed inflammation rich in neutrophils (Figure 3). Grocott methenamine silver, periodic acid-Schiff, and Fite stains showed no evidence of microorganisms. Providers did not perform a quantitative biopsy at the initial consult, but aerobic, anaerobic, and fungal tissue cultures gathered with the Levine technique demonstrated no growth. Based on these results and a multiteam collaborative discussion, PG was favored as the final clinicopathologic diagnosis.

Although the patient was initially started on broad-spectrum antibiotics (cefepime, metronidazole, and vancomycin), these were discontinued shortly following a negative infectious workup. At hospital discharge, she was started on topical clobetasol ointment with hypochlorous acid (HOCL; Vashe; Urgo Medical, Fort Worth, Texas)-soaked gauze dressings changed twice daily to maintain wound pH approximating 5.5. In addition, she received 80 mg of prednisone daily with high-dose vitamin D_3 (10,000 IU)/calcium/omeprazole daily for prolonged steroid therapy prophylaxis.

Other than the right hip arthroplasty, the patient had no known underlying trigger or inciting factors for the PG; a recent colonoscopy did not show evidence of inflammatory bowel disease. The patient also had negative antinuclear antibodies and normal serum/urine protein electrophoresis and immunofixation studies.

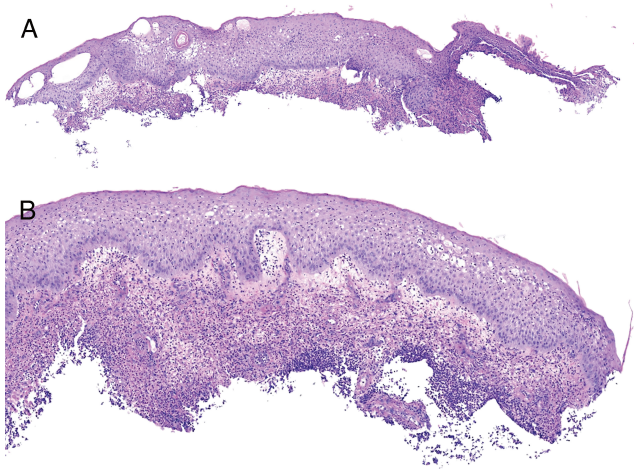
The outpatient clinical course was complicated by a deep tunnel along the incisional line, which necessitated slower steroid taper and the addition of colchicine (Figure 4). A reculture was performed with a tissue swab 2 months after initial presentation, and this second set of cultures grew *Parvimonas*, *Pseudomonas*, and *Streptococcus* species. The patient received appropriate, tailored antibiotic treatment to treat the identified organisms; repeat fluorescent

Figure 2. FLUORESCENCE IMAGING

A, Fluorescence imaging device with darkening drape attachment to obtain the dark conditions needed for fluorescence imaging. B, Positive fluorescence imaging showing red and cyan, which suggests high bacterial loads and infection present. This image provides a comparison for the case patient.

Figure 3. HISTOPATHOLOGY

Histopathology demonstrates a dense, undermining superficial dermal neutrophilic infiltrate with significant neutrophilic epitheliotropism and spongiform pustule formation in the epidermis. There is associated papillary dermal edema. There was no evidence of vasculitis. Special stains for infectious organisms including Gram, Fite, and periodic acid-Schiff were all negative. A, Magnification ×20; B, magnification ×40.



images were not obtained at this time because the device was not available. Three months after the initial presentation, the patient was started on adalimumab as a steroid-sparing agent, and the prednisone was tapered successfully. Because of a nonhealing soft-tissue tract along the original incisional line developed (Figure 4), topical clobetasol ointment with HOCL-soaked gauze dressings was discontinued, and negative-pressure wound therapy was applied (VAC; KCl/3M, St Paul, Minnesota) with Zorflex (L&R Healthcare USA Inc, Milwaukee, Wisconsin)-activated carbon cloth as an interface. At each NPWT change (three times per week), the wound was washed for 20 to 30 minutes with HOCL solution to decrease wound pH. The patient was later switched to high-flow fenestrated ovine foregut (Endoform; AROA Biosurgery, Auckland, New Zealand) as an interface under NPWT, and the HOCL soaks were continued three times weekly. At 5 months after initial presentation, NPWT was discontinued, and the patient was transitioned to Endoform antimicrobial daily dressing changes with HOCL soaks until closure approximately 1 month later (Figure 5).

Ethics

Written informed consent was provided by the subject of this case to publish the case details and associated images. Adalimumab, discussed in this case, is not currently approved for the treatment of PG, and its use in this case is experimental. MolecuLight *i:X* has been approved by the FDA for use in detecting bacteria within wounds. Multiple university dermatology staff were involved in the evaluation, treatment, and follow-up of this patient to maintain the highest standard of care.

DISCUSSION

To the best of the authors' knowledge, no previous peer-reviewed literature has used fluorescent images to demonstrate the absence of identifiable fluorescence characteristics of soft-tissue infection in the setting of acute, untreated PG or to assist in diagnosing PG. Although PG has historically been a diagnosis of exclusion, criteria have been proposed and validated following Delphi

Figure 4. DURING TREATMENT

A, Development of undermining and nonhealing tract 4 months into therapy. B, Wound packed with activated carbon cloth as an interface.

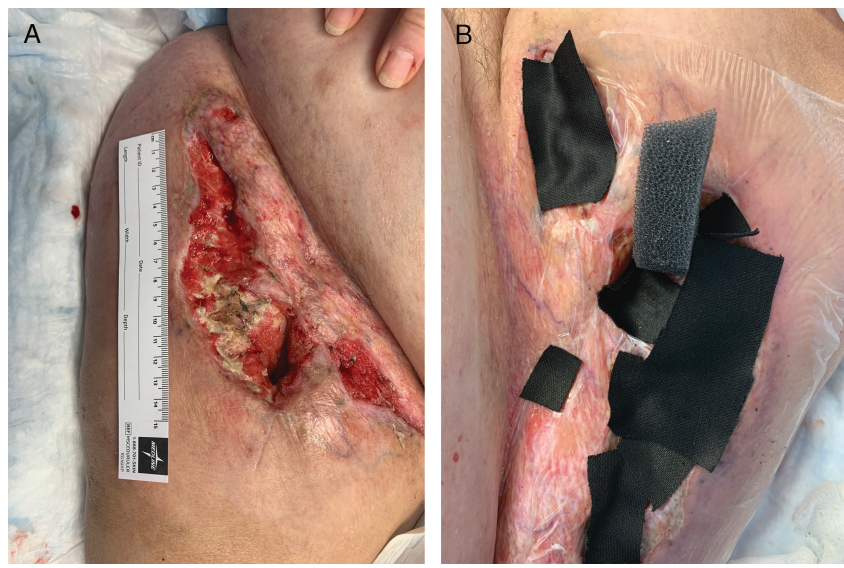
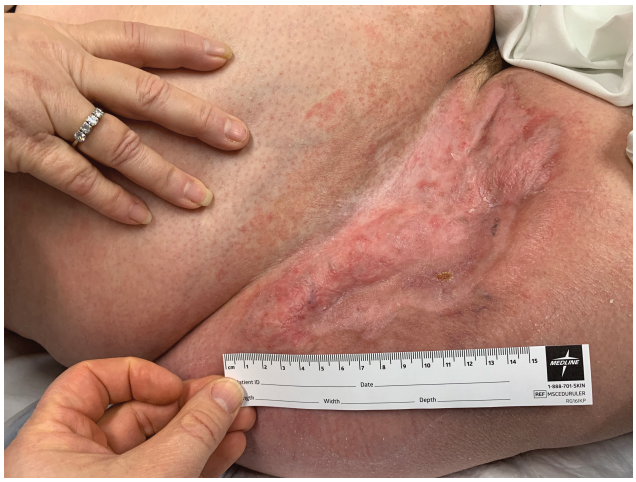


Figure 5. FULLY EPITHELIALIZED RIGHT GROIN WOUND 6 MONTHS AFTER INITIAL DIAGNOSIS



consensus to serve as guidelines for clinicians in the diagnosis of PG, ulcerative subtype. Under consensus guidelines, a patient with histopathology of an ulcer edge demonstrating an infiltrate of neutrophils, in addition to four of eight minor criteria, may be diagnosed with the condition with a specificity and sensitivity of 90% and 86%, respectively.[1] The eight criteria are as follows: (1) exclusion of infection; (2) pathergy; (3) history of inflammatory bowel disease or inflammatory arthritis; (4) history of papule, pustule, or vesicle ulcerating within 4 days of appearing; (5) peripheral erythema, undermining border, and tenderness at ulceration site; (6) multiple ulcerations, at least one on an anterior lower leg; (7) cribriform or "wrinkled paper" scar(s) at healed ulcer sites; and (8) decreased ulcer size within 1 month of initiating immunosuppressive medication(s).[1]

Although the criteria place an unprecedented emphasis on the benefits and necessity of histopathologic review specifically (over predominantly clinical features historically), patients require thorough workup to exclude underlying infection, evaluation of medical history, clinical characteristics and presentation, progression of the lesion, and response to treatment.[1–3]

Fluorescence imaging devices have been described in the wound care literature as unique point-of-care tools, predicting moderate to high bacterial loads in wounds with high accuracy.[4–6] Pathogens such as *Pseudomonas*, *Klebsiella*, *Staphylococcus*, and *Proteus* species commonly discovered in chronic wound beds may be detected by their signature red or cyan fluorescence under the violet light emitted by such devices with positive predictive values averaging 95%.[7,8] Fluorescence imaging was found to increase diagnostic accuracy of wound infection by a factor of 2.2 (29.43% to 65.14%) when compared with detection using clinical signs and symptoms alone.[7] Detection of such signatures provides immediate information regarding bacterial location and load (>10^4 colony-forming units/g), without requiring invasive studies or culture-associated delays.[7]

In this patient, the distinct absence of identifiable bacteria on the initial bedside fluorescence image was not consistent with a potential clinical diagnosis of erysipelas, cellulitis, or necrotizing fasciitis (ie, a surgical emergency requiring debridement and potentially adjunct hyperbaric oxygen surgery). The image raised the distinct possibility of noninfectious, inflammatory etiologies such as PG and helped avoid surgical intervention. Although fluorescent imaging is valuable in guiding diagnosis at the bedside, it neither negated nor superseded the need for biopsy as recommended under the Delphi consensus. Biopsy remains important for confirmation of the absence of infection because the penetration depth of fluorescence imaging is only ~1.5 mm.[7]

Use of NPWT, as with this patient, has several benefits to wound care, including dermal microdeformation and interstitial edema management, control and stabilization of dermal drainage (lymphorrhea), and direct wound stimulation, resulting in enhanced angiogenesis. Although NPWT is not the standard of care for PG because of concern for potential pathergy development with the trauma of dressing changes, there are situations in which its use is appropriate, such as PG complicated by infection, or wounds that have stalled or are resistant to first-line therapies. The decision to use NPWT in PG must be made by balancing the concerns for pathergy against the benefits of the therapy itself.

Several case studies and series have demonstrated success in treating PG ulcers using NPWT.[9–11] The negative pressure provided by vacuum-assisted closure devices counters natural tension on the wound from surrounding skin, bringing the edges of the wound closer together.[12] In addition, the subatmospheric pressure "pulls" fluid and stagnant material from the wound bed, keeping the area clean and reducing the risk of colonization and wound infection while maintaining a moist wound environment. This antimicrobial effect is additive to the occlusive seal of VAC devices, which reduces contamination of the wound bed.[12]

Microdeformation of individual cells within the wound promotes cell proliferation and migration, angiogenesis, and subsequent wound healing by providing constant tension on the cells themselves, reinforcing the microscaffold on which new cells will grow.[12,13] Further, there is evidence that fluid removal using this method can reduce compression on the microvasculature within the wound, increasing perfusion to the wound itself. Localized hypoxia and shear stress generated by NPWT promote the release of vascular endothelial growth factor and other endothelial signaling molecules, promoting

angiogenesis in wounds as well.[12,13] Tension on the individual cells created by the negative pressure mechanically induces capillary sprouts.[13] Together, these mechanisms greatly increase the capacity of the vasculature to perfuse the wound bed and promote more rapid healing.

High-dose vitamin D (10,000 IU) used in this case elicits a vasoprotective effect that is critical to wound healing and has a direct impact on endothelial cell function. Although vitamin D is most recognized for its role in calcium homeostasis, vitamin D deficiency has also been linked to vascular disease.[14] This link is attributed to interactions with endothelial cells through the vitamin D receptor, leading to vasodilatory, antioxidant, angiogenic, and anti-inflammatory effects.[14] Downstream signaling effects of the vitamin D receptor have an up-regulatory effect on endothelial nitric oxide (eNO) synthase, which produces eNO, a potent vasodilator. Loss of this regulatory input from vitamin D shifts the balance among signaling molecules toward the vasoconstrictors, precipitating vascular disease. Further, eNO has proangiogenic effects, such as endothelial migration and proliferation. Up-regulation of eNO by vitamin D would enhance this angiogenic effect.[14] In addition, eNO serves as an antioxidant, reducing oxidative stress and subsequently improving wound healing.[14,15]

Although to the authors' knowledge no studies have investigated vitamin D supplementation in patients with PG, published evidence has demonstrated a positive effect on diabetic ulcer healing in murine models.[16] This finding is notable because patients who are vitamin D deficient have a significantly increased risk of diabetic ulcers (odds ratio, 3.22).[17] High-dose vitamin D supplementation provides a low-cost, low-risk therapy with the potential for significant benefit to the patient with a chronic wound. Further study into the effects of vitamin D on wound healing are certainly warranted, especially considering recent studies examining the complex relationships between vitamin D, vitamin D receptors, and microRNA, as well as the multifaceted mechanism of wound healing among the three.[18]

Wound pH has become a focus of attention in the past several years. Chronic wounds have a higher pH of 7.15 to 9.0 as compared with the dermal acid mantle of 5.5.[19] Decreasing wound pH using HOCL washes to approximate the normal dermal range, as was performed in this case, may decrease biofilm, increase angiogenesis, and improve progression to wound closure.[19–21]

Anti-tumor necrosis factor (TNF) biologics, such as adalimumab, have shown great promise in treating a wide variety of autoimmune-related disorders, including PG.[22] The inflammatory cytokine TNF-α is associated with inflammatory bowel disease, which itself is often clinically associated with PG. In addition, TNF-α is responsible for the up-regulation of interleukin ligand 8 (IL-8), a neutrophil chemotactic agent that is also strongly associated with PG.[22] Inhibition of TNF-α using biologics such as adalimumab enables long-term control of systemic inflammation and resolution of chronic inflammatory ulcers that are characteristic of PG.[22] Evidence suggests that biologics targeting other proinflammatory cytokines such as IL-17 and IL-23 may also benefit PG treatment; however, adalimumab has had demonstrable efficacy in case studies and small trials, and dosing regimens are available.[22–25] Adalimumab serves as a viable option for recalcitrant disease after initial therapy with disease-modifying antirheumatic drugs and steroids, either as a monotherapy or combination therapy with any of the other immunomodulating drugs. In this case, the addition of adalimumab hastened the healing of the patient's ulcer and enabled faster tapering of prednisone.

CONCLUSIONS

Prompt diagnosis of PG is critical because it may be associated with underlying malignancy and systemic illness. To the authors' knowledge, device-assisted fluorescent imaging to assess wound bacterial burden has not been described in the setting of PG and may serve as an additional point-of-care tool in the clinician's armamentarium to diagnose PG accurately. As a result, the overwhelming healthcare costs that would have resulted from debridement of potential necrotizing fasciitis and morbidity associated with PG may be reduced. Using device-assisted fluorescent imaging on wounds that are clinically suspected to be necrotizing fasciitis can help to confirm that diagnosis or, as in this case, avoid unnecessary operative debridement of PG wounds, which could result in pathergy if PG is not accurately diagnosed. In addition, the ability to detect infection at each subsequent wound care visit may accelerate healing times and promote antibiotic stewardship.[26] Rapid healing and early identification will also help prevent negative outcomes associated with chronic wounds and skin infections such as lymphedema, which itself can further impair the wound healing process.[27] Further study is warranted with regard to fluorescent imaging for PG.

Limitations

The depth of penetration for fluorescence imaging is ~1.5 mm, leaving the opportunity for deeper infections to be missed at bedside if no surface bacteria are present.[7] In addition, not all bacteria fluoresce because fluorescence is dependent on porphyrin production.[27] However, 88% of the most common wound pathogens do fluoresce, and many chronic wound infections are polymicrobial, increasing the chances for detection.[27,28] Further, fungi do not fluoresce, so a fungal infection would not be seen on fluorescence imaging.[27] When the patient in this case study presented with tunneling and other signs of infection,

repeat images using fluorescence were not obtained. Although the clinical diagnosis and cultures pointed to infection, positive images would have been helpful to contrast with the initial presentation. ●

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