

# The safety of punch biopsies on hard-to-heal wounds: a large multicentre clinical trial

**Objective:** Punch biopsy is a simple and effective diagnostic technique used in hard-to-heal wound management. Histologic examination can rule out cancer in the wound bed or diagnose vasculitis in a hard-to-heal or suspicious ulcer. A biopsy can determine the level of bacteria in an ulcer when infection is suspected. Despite its use, health professionals practicing in wound clinics hesitate to perform punch biopsies. The reasons vary from the invasive nature of the procedure to a fear of complications.

**Methods:** A multicentre clinical trial evaluated the addition of fluorescence imaging to clinical examination in determining bacterial burden in hard-to-heal wounds. The protocol required a 6mm punch biopsy of any area (up to three) suspected of having moderate-to-high bacteria levels either on clinical examination or fluorescence image. If clinical examination and fluorescence imaging both did not indicate bacteria, the health professional took a biopsy of the centre of the ulcer. The biopsies were performed under local anaesthesia after cleansing the ulcer bed with sterile normal saline. Haemostasis was achieved with direct pressure and the occasional topical clotting

agent. All of the patients were followed for 30 days to monitor for adverse events.

**Results:** A total of 350 patients with wounds (diabetic foot ulcer n=138; venous leg ulcer n=106; pressure ulcer n=22; surgical site infection n=60; other n=24), enrolled by 20 investigators at 14 sites in the US, underwent a total of 412 punch biopsies. Haemostasis was achieved in all 412 biopsies. No biopsy sites required cautery or suture ligation to control bleeding. No subjects returned to the clinic secondary to bleeding. A patient developed an infection three days post-biopsy that could have been due to the punch biopsy. However, the patient was HIV-positive and the quantitative biopsy was positive for infection, biopsy bacterial load of  $10^6$  colony forming units (CFU)/g.

**Conclusion:** This trial used a 6mm punch to obtain tissue for culture, histology and additional biomarker research. In daily wound care practice, a smaller 3mm punch suffices. Adverse events were rare despite the larger biopsy. Punch biopsies are a safe procedure for obtaining tissue for histologic or microbiologic analysis.

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fluorescence imaging • hard-to-heal wounds • infection • microbiology • MolecuLight • tissue culture

A colleague once said, 'Think like an internist and act like a surgeon.' The practice of wound care incorporates providers from nearly every specialty of medicine as well as advanced health professionals. As such, they come to the wound clinic with a wide range of surgical skills. Some hesitate to debride aggressively or perform invasive procedures such as punch biopsies. The reasons cited range from apprehension over performing invasive procedures to fear of complications. In our experience, when performing clinical trials, we have encountered resistance by clinicians to performing the punch biopsies required by the protocol. Surprised by the opposition to perform a basic wound clinic procedure, we decided to evaluate the safety associated with punch biopsies in the outpatient wound clinic.

Punch biopsy is a useful diagnostic technique in the outpatient wound centre. Histologic examination of the tissue can rule out cancer in the wound bed or diagnose vasculitis in a hard-to-heal or suspicious ulcer. A punch biopsy also permits quantitative analysis of the bacterial burden in a wound where infection is suspected. The technique is relatively simple. After obtaining informed consent from the patient, the clinician prepares for the biopsy by placing an antiseptic, gauze, the punch biopsy instrument (Fig 1), forceps and scissors on a bedside stand. Clean technique is observed. The area of interest is prepped with antiseptic. Typically, 1% or 2% lidocaine, with or without epinephrine, is injected into the area as an anaesthetic. Most clinicians avoid using epinephrine in the digits, but in other parts of the body it hastens the anaesthetic effect of the lidocaine and decreases bleeding. For rare patients who report an allergy to lidocaine, tetracaine can be substituted.<sup>1</sup> Topical anaesthetics can substitute for injectable anaesthetic in patients with reduced sensation or neuropathy. Unless there is a suspicious area in the wound bed, the margin of the wound including a portion of intact skin is chosen for biopsy.<sup>2</sup> A 2–3mm punch biopsy

\*Thomas E. Serena,<sup>1</sup> MD, CEO, Medical Director\*; Windy Cole,<sup>2</sup> DPM, Director of Wound Care Research; Stacey Coe,<sup>2</sup> CCRC, Clinical Research Coordinator; Khristina Harrell,<sup>1</sup> RN, Research Nurse; Laura Serena,<sup>1</sup> RN, Research Nurse; Raphael Yaakov,<sup>1</sup> MS, Project Lead; Monique Y Rennie,<sup>3</sup> PhD, Scientific Affairs and Communications Manager

Corresponding author email: serena@serenagroups.com

<sup>1</sup> SerenaGroup Research Foundation, Cambridge, MA, US. <sup>2</sup> Kent State University, Cleveland, OH, US. <sup>3</sup> MolecuLight Inc., Toronto, ON, Canada.

**Fig 1.** A 6mm punch biopsy**Fig 2.** Central punch biopsy with a 6mm punch

instrument suffices in most cases. Holding the punch perpendicular to the skin (Fig 2), it is pressed into the wound with a circular motion. The specimen will separate from the surrounding tissues in most cases. Using forceps, the biopsy is gently grasped and the base cut with scissors. The specimen placed in the appropriate container: formalin for histology, and culture medium for microbiology. Direct pressure achieves haemostasis in the majority of cases. Occasionally, silver nitrate cauterization or suturing is needed.<sup>3</sup> A dressing suited to the wound is applied. In most patients the biopsy site heals in one week.<sup>3</sup>

The indications for punch biopsy include any suspicious lesion or wound that has failed to heal despite appropriate care.<sup>2</sup> In addition, punch biopsies allow quantitative analysis of the bacterial burden in the wound. In the wound clinic, contraindications to punch biopsy are encountered infrequently. These would include an area in which damage to an underlying artery or nerve might result. Patients taking anticoagulants can undergo punch biopsy.

A study examining the complications of 100 punch biopsies in an inpatient dermatology service reported a complication rate of 29% with 93% of the complications related to infection at the site of the biopsy.<sup>4</sup> The investigators biopsied intact skin lesions in this series. They also reported a higher infection rate with biopsies taken from inpatients as opposed to outpatients ( $p < 0.001$ ).<sup>4</sup> A PubMed search failed to reveal any studies addressing the complications of punch biopsies in chronic wounds. In the author's experience, the complication rate for wound bed biopsies is far below that of biopsies of intact skin.

## Method

A recent multicentre clinical trial on hard-to-heal wounds compared clinical assessment for the signs and symptoms of infection to point-of-care imaging of regions with moderate-to-heavy bacterial loads using a handheld fluorescence imaging device and quantitative tissue cultures (ClinicalTrials.gov Identifier: NCT03270904).<sup>5</sup> Before beginning the trial, all the investigators obtained ethics committee approval through the central institutional review board (Veritas IRB). The approval included the performance of up to three 6mm punch biopsies of the wound bed. All subjects were informed of the study procedures, including biopsies of their wound, and signed the IRB-approved informed consent form before undergoing any study-specific procedures. Subjects have provided written consent to publish case details.

Patients with hard-to-heal wounds drawn from 14 outpatient clinical research sites in the US and recruited by 20 investigators) underwent three assessments of bacterial burden: clinical signs and symptoms (CSS), fluorescence imaging (MolecuLight i:X, MolecuLight, Canada) and quantitative tissue culture biopsy. If the investigator suspected an area of the wound had moderate-to-heavy levels of bacteria using CSS or fluorescence imaging (FL), a 6mm biopsy was obtained from the region(s) of concern. If CSS did not indicate concerning levels of bacteria, the health professional took a single control biopsy in the centre of the ulcer. The biopsies were performed under local anaesthesia after cleansing the ulcer bed as described previously. The biopsies were taken for both bacterial counts and histology. Haemostasis was achieved with direct pressure and the occasional topical

clotting agent. Patients were followed for 30 days after the biopsies to monitor for adverse events.

## Results

A total of 350 patients with a variety of wounds (diabetic foot ulcers (DFU)  $n=138$ ; venous leg ulcer  $n=106$ ; pressure ulcer  $n=22$ ; surgical site infection  $n=60$ ; other wound  $n=24$ ) were recruited, and 412 biopsies were taken, all of which achieved haemostasis without difficulty. Multiple biopsies were taken in 60 patients.

An investigator used silver nitrate cautery on a DFU and several investigators employed haemostatic dressings or granules (OMNI-STAT, Omni-stat Medical Inc., US). No biopsy sites required suture ligation to control bleeding. Bleeding was measured as the need for haemostasis beyond simply applying pressure. There was no difference between patients on anticoagulation and those not on these medications. No subjects returned to the clinic secondary to bleeding complications. No adverse events related to bleeding occurred during the trial.

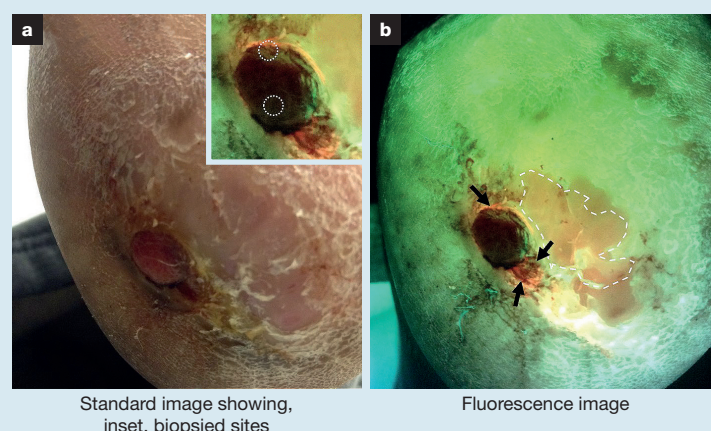
No allergic reactions or adverse events occurred secondary to the anaesthetics. All of the biopsy sites healed by secondary intention. No wound healing complications were reported. At three days post-biopsy, one patient developed a wound infection. The patient exhibited hallmarks of bacteria on the fluorescence image of their DFU before the biopsy being taken, and reported bacterial load from the biopsy was  $10^6$  colony forming units (CFU)/g (Fig 3). The investigator could not rule out the biopsy site as the source of infection and recorded the event as a possibly related non-serious adverse event. In addition, the patient has a history of HIV. The patient recovered completely with a combination of topical and oral antibiotics. Adverse events related to the punch biopsies in this multicentre clinical trial revealed a negligible incidence of complications (0.24%) with only one infection reported out of the 412 biopsies.

## Discussion

The results from this large multicentre clinical trial demonstrate a low incidence of adverse events related to the 6mm punch biopsies used for tissue culture in the study. In fact, 60 patients underwent multiple biopsies without complication. Concerns about bleeding, even for patients taking anticoagulants, proved unjustified.

The single infectious complication resolved with outpatient antimicrobial therapy. The incidence of infection in this study was much lower than reported in the dermatology literature.<sup>4-6</sup> However, dermatologists

**Fig 3.** A non-serious adverse event was reported in this patient with a diabetic foot ulcer when a wound infection developed three days post biopsy. At the time of biopsy, the wound (right heel ulcer, 0.5x0.4cm) had been present for more than six months and the patient exhibited no clinical signs and symptoms of infection based on the IWII 2016 international guidelines<sup>7</sup> (a) However, red fluorescence was evident on the MolecuLight image of the wound (b) both within the wound bed as well as in the peri-wound region (arrows). Moreover, blush red (white outline—indicative of subsurface bacteria—was present in surrounding tissues.<sup>8</sup> Biopsies were taken from the centre of the wound and at the periwound in a region positive for red fluorescence. Total bacterial load in the biopsied region of red fluorescence was  $2.7 \times 10^6$  CFU/g, primarily from *Finexgoldia magna* and *Peptoniphilus* species



usually biopsy intact skin and often close the biopsy sites with sutures. In this study, all the biopsies were taken from open wounds that were left open to heal by secondary intention. This may account for the observed differences in infectious complications.

## Limitations

A limitation of the study is the failure to look at healing rates after the biopsies. Although not part of the original protocol, weekly measurements for four weeks after the biopsies would have allowed the authors to determine if the punch biopsies influenced the rate of subsequent healing. A retrospective look at 30-day healing rates is planned.

## Conclusion

Health professionals must possess a minimal degree of surgical skill in order to competently function in the wound care centre. A requisite procedural skill, punch biopsy, is a simple safe diagnostic tool. On the basis of the results of this study, fear of complications would appear to be unfounded. **JWC**

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