A Histopathologic Basis for Surgical Debridement to Promote Healing of Venous Ulcers

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BACKGROUND: Pathologic analysis of deep tissue obtained during debridement of venous ulcers is often unnoticed in its importance. We previously reported pathologic findings on 139 patients with venous ulcers. The objective of this study was to correlate the pathologic findings in venous ulcers with wound healing to establish a negative margin for debridement.

STUDY DESIGN: Consecutive patients with a lower extremity venous ulcer present for at least 4 weeks, presenting to a single wound healing center, were included. Wounds underwent aggressive surgical debridement beyond the subcutaneous level until judged to have a viable base. Specimens were scored based on cellularity, vascularity, collagen composition, inflammation, and dense fibrosis, with a highest possible score of 13. Healing was the primary outcome for analysis.

RESULTS: Of the 26 patients who met inclusion criteria, only 50% of them (13 patients) with a total of 18 venous ulcers underwent surgical debridement available for pathologic analysis. Mean ulcer area was 34.7 cm² at initial presentation, and 89% of patients had a continuous positive healing curve as measured by decreasing wound area (from 34.7 cm² to 14.3 cm²). However, specimens with dense fibrosis, decreased cellularity, mature collagen, and pathology score less than 10 were predominantly nonhealing ulcers.

CONCLUSIONS: Presence of dense fibrosis and high levels of mature collagen in deep tissue specimens are significant correlative factors in nonhealing of venous ulcers. We recommend deep debridement on all venous ulcers that are refractory to healing until the level of absence of dense fibrosis and mature collagen is reached to promote venous ulcer healing. (J Am Coll Surg 2012;215:751–757. © 2012 by the American College of Surgeons)
underlying infection when present with systemic or topical antibiotics, and cell therapy with biologic materials when refractory to compression therapy. Although compression therapy is the mainstay for reducing venous distention, much study has been undertaken to determine the impact of surgical correction of venous incompetence. Venous ablation has proven effective in the management of venous ulcer recurrence. However, even after venous closure, these patients often have a prolonged time to complete healing.

The role of surgical debridement in promoting venous ulcer healing is less clear. The basic rationale is to excise nonviable tissue in order to decrease the bacterial wound burden because there is a well documented correlation between the level of biofilm produced by bacteria and non-healing. Surgical debridement has been shown to accelerate wound healing in diabetic foot ulcers and may also stimulate wound contraction and epithelialization. A recent study demonstrated that keratinocytes at the non-healing edges of venous ulcers do not execute either activation or differentiation pathway, resulting in thick callus-like formation at the wound edge of a venous ulcer. However, the extent of debridement, ie, width and depth, has not been established besides the surgical dogma of a subjectively "clean, pink, viable and bleeding base." There are currently no objective standards to determine the extent of debridement that will result in a wound edge primed for optimal wound healing, a so-called, "negative margin," even though this is a widely accepted technique to remove impaired cells.

In a retrospective study of 139 venous ulcer patients undergoing initial surgical debridement, Golinko and colleagues demonstrated the presence of inflammation, infection, and fibrosis in deep tissue specimens after a grossly satisfactory sharp debridement. However, the correlation between these pathologic findings and healing has not been elucidated. The objective of this study was to describe the histopathologic findings of sharp surgical debridement of venous ulcers and their associations with healing outcomes to better establish the extent of the depth of debridement.

METHODS
An observational study of consecutive patients presenting to the New York University (NYU) Wound Healing Center between July 2009 and July 2010 was conducted under IRB-approved protocol 08-391. Criteria for inclusion were patients with at least 1 venous ulcer present for at least 4 weeks with no evidence of healing, with at least 2 documented entries in the previously described online wound electronic medical record (OWEMR). Healing and non-healing outcomes were documented at the end of the study period. Patients were treated in accordance with published treatment protocol, which includes debridement (surgical and enzymatic), compression therapy, infection control, and venous duplex ultrasound for diagnosis of correctable venous incompetence. Patients undergoing debridement were admitted to the adjoining university-based tertiary care hospital with a dedicated wound healing unit. Wounds initially underwent aggressive surgical debridement, under sterile conditions in the operating room, beyond the subcutaneous level until a clean, viable base was established clinically. During debridement, the specimen was labeled and identified as deep tissue according to the deepest level of debridement closest to the wound edge achieved (dermis, fascia, muscle, or tendon). All specimens were sent to the core pathology laboratory for processing; they were paraffin embedded, and stained with hematoxylin and eosin. All specimens were evaluated using a Carl Zeiss microscope (Standard 25 ICS).

All clinical data were entered into the OWEMR. The OWEMR is a secure point-of-care informatics system that successfully integrates relevant clinical data, quantitative measurements of wound closure rates, and digital photographs of wounds. It captures up to 137 patient variables, including demographics, medical history, laboratory values, vascular testing data, radiology data, wound characteristics and photographs, wound debridement data (pathology and culture results), and amputation data. Using the data extracted from the OWEMR, a review of surgical pathology of initial operative debridement of venous ulcer patients was performed.

Development of pathologic scoring system
Pathology specimens of the wound edge and base were reviewed by 2 independent pathologists. The specimens were scored using the following pathologic findings: cellularity: based on the presence of fibroblasts, fibrocytes, and macrophages; vascularity: determined by the degree of neovascular proliferation; collagen composition: based on the extent of collagen maturation, such that "mature collagen" is denoted by dense acellular eosinophilic discrete bundles of collagen fibers lying parallel to each other with few fibrocytes interspersed; inflammation as demonstrated by the number of inflammatory cells; and presence of dense fibrosis. Each pathologic finding was then scored according to the criteria as shown in Table 1. The individual scores from the 2 independent pathologist scores were averaged to create a single score for each specimen for analysis. This is a novel scoring system for the histopathology of venous ulcers, with a maximum combined score of 13 when all findings are taken together.
Table 1. Scoring System for Venous Ulcer Wound Base Specimens

<table>
<thead>
<tr>
<th>Pathology finding</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td>Cellularity (includes presence of fibroblasts, fibrocytes, and macrophages)</td>
<td></td>
</tr>
<tr>
<td>Mild (&lt;25 cells/HPF*)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate (25–100 cells/HPF)</td>
<td>2</td>
</tr>
<tr>
<td>Abundant/active (&gt;100 cells/HPF)</td>
<td>3</td>
</tr>
<tr>
<td>Vascular proliferation</td>
<td></td>
</tr>
<tr>
<td>Normal (&lt;3 capillaries/HPF)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate (3–10 capillaries/HPF)</td>
<td>2</td>
</tr>
<tr>
<td>Abundant (&gt;10 capillaries/HPF)</td>
<td>3</td>
</tr>
<tr>
<td>Collagen</td>
<td></td>
</tr>
<tr>
<td>Mature: dense, acellular, eosinophilic discrete bundles of collagen fibers lying parallel to each other with few fibrocytes interspersed. Fibrocytes are spindle-shaped cells with slender dark blue nuclei that lack nucleoli and scant cytoplasm that blends in with the collagen fibers.</td>
<td>1</td>
</tr>
<tr>
<td>Immature: small, eosinophilic, wavy indiscernible bands of collagen mixed with fibroblasts and macrophages. Fibroblasts are spindle-oval cells with moderate amount of cytoplasm, round-oval nuclei, and prominent nucleoli.</td>
<td>2</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
</tr>
<tr>
<td>None (&lt;5 inflammatory cells/HPF)</td>
<td>1</td>
</tr>
<tr>
<td>Low (5–100 inflammatory cells/HPF)</td>
<td>2</td>
</tr>
<tr>
<td>Abundant (&gt;100 inflammatory cells/HPF)</td>
<td>3</td>
</tr>
<tr>
<td>Pre-existing dense fibrosis (dense acellular mature collagen with minimal vascularity)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>1</td>
</tr>
<tr>
<td>Absent</td>
<td>2</td>
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*High power field (HPF) microscopic examination under 20× magnification.

Statistical methods

Healing, defined as 100% epithelialization with no drainage, was the outcome for our primary analysis. Healing outcomes were assessed at scheduled weekly outpatient visits over the course of a year after presentation. Descriptive and comparative analyses of demographic and clinical characteristics between the healed and nonhealed venous ulcer groups were performed using chi-square tests for categorical variables and t-test for continuous variables. All reported p values are 2 sided and p < 0.05 was considered statistically significant. Analyses were performed in SPSS version 19.0 (IBM SPSS Statistics, 2010).

RESULTS

Patient characteristics

Twenty-six consecutive patients with a total 37 wounds met inclusion criteria. Thirteen patients were not surgically debrided, 7 were enzymatically debrided, and 6 had local wound care that did not involve debridement, and therefore did not provide pathologic specimens for analysis. Thirteen patients with a total of 18 venous ulcers underwent sharp surgical debridement, with pathologic specimens available for review summarized in Table 2. Eighty-seven percent underwent surgical debridement within 1 month of initial presentation. Two patients initially refused surgery, but eventually underwent debridement due to clinically present infection. Of these patients, 10 (71%) were women and 3 (29%) were men. Eighty-nine percent of wounds were debrided more than once, with an average of 5.7 debridements per patient. There was no statistically significant difference in the number of debridements between healed and nonhealed wounds (chi-square test, p = 0.596). The average age at presentation was 63.1 years. The average albumin levels at the time of presentation were normal (3.74 g/dL), as was the white blood cell (WBC) count (mean 6.61 × 10^9 cells/L). The overall mean wound area at initial presentation was 34.7 cm^2, and 38% of patients had documented venous insufficiency. There was no statistically significant difference in baseline demographic and clinical characteristics between healed and nonhealed patients as shown in Table 2. However, none of the male patients healed.

Thirty-three percent of all wounds achieved complete healing, defined as 100% epithelialization with no drainage. All of the healed wounds were in the female patients only (p = 0.063). Eighty-nine percent of wounds had a continuous positive healing curve, as measured by decreasing wound area (from 34.7 cm^2 to 14.3 cm^2). Twelve patients (46%) were found to have venous reflux on noninvasive vascular testing, and 6 of these patients underwent venous closure before debridement; the remaining patients were evaluated for closure after debridement. There was no statistically significant difference between healed and nonhealed patients who underwent venous ablation (p = 0.765).

Bacteriology

Eighty-three percent of all deep debridement specimens had cultures positive for bacteria. In the healed group, methicillin-sensitive Staphylococcus aureus (MSSA), methicillin-resistant Staphylococcus aureus, and Pseudomonas spp were the most predominant bacterial species, as shown in Figure 1A. In the nonhealed group, the most predominant bacterial species were MSSA, Pseudomonas spp, and Enterococcus faecalis, as shown in Figure 1B. There was no statistically significant difference between the nonhealing and healing ulcer groups with regard to the presence of bacteria (p = 1.00).
Pathology

Moderate to abundant cellularity, immature collagen, and an overall pathology score >10 were predominant characteristics in healing ulcers, with 66%, 67%, and 61%, respectively, of all healing wounds with these features. Conversely, 83% and 72%, respectively, of nonhealing ulcers were characterized by the presence of dense fibrosis and mild to moderate inflammation (Fig. 2). Although all pa-

Table 2. Patient’s Baseline and Wound Characteristics According to Outcomes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Healing</th>
<th>Nonhealing</th>
</tr>
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<tbody>
<tr>
<td>Sex, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Female</td>
<td>71</td>
<td>53.8</td>
<td>46.2</td>
</tr>
<tr>
<td>Age, y</td>
<td>63.1 ± 15.28</td>
<td>60.5 ± 11.7</td>
<td>64.42 ± 17.1</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>3.74 ± 0.34</td>
<td>3.7 ± 0.45</td>
<td>3.76 ± 0.29</td>
</tr>
<tr>
<td>WBC count, 10⁹ cells/L</td>
<td>6.61 ± 1.92</td>
<td>6.95 ± 1.30</td>
<td>6.44 ± 2.2</td>
</tr>
<tr>
<td>Initial area, cm²</td>
<td>34.67 ± 52.18</td>
<td>8.15 ± 6.96</td>
<td>47.93 ± 60.09</td>
</tr>
<tr>
<td>Final area, cm²</td>
<td>14.28 ± 27.40</td>
<td>0</td>
<td>21.43 ± 31.5</td>
</tr>
<tr>
<td>Debrided more than once, %</td>
<td>89</td>
<td>83.3</td>
<td>91.7</td>
</tr>
<tr>
<td>Duration of wound, d</td>
<td>147.50 ± 111.5</td>
<td>127.50 ± 51.5</td>
<td>157.50 ± 133</td>
</tr>
<tr>
<td>Presence of venous insufficiency, %</td>
<td>38</td>
<td>33</td>
<td>42</td>
</tr>
<tr>
<td>Presence of bacteria, %</td>
<td>83</td>
<td>83</td>
<td>83</td>
</tr>
</tbody>
</table>

Data are reported as means ± standard deviation, unless otherwise specified.

Figure 1. (A) Bacteriology of healed venous ulcers demonstrating predominance of methicillin-sensitive Staphylococcus aureus (MSSA), methicillin-resistant Staphylococcus aureus (MRSA), and Pseudomonas species. (B) Bacteriology of nonhealed venous ulcers demonstrating predominance of MSSA, MRSA, and Pseudomonas species.
tients with healing rates <50% of original wound area had mild to moderate cellularity (<100 cells/high power field [HPF]), only 1 patient with <100 cells/HPF progressed to full healing (Fig. 3). Vascular proliferation did not correlate strongly with either nonhealing or healing ulcer phenotypes. None of the healing ulcers had the combination of mild to moderate cellularity and dense fibrosis; all patients with healing rates <50% did (Fig. 4). Some degree of fibrosis, which is denoted by an increase in the collagenous component of the tissue associated with a decrease in cellularity, was found on all deep tissue pathologic specimens.

DISCUSSION
In this study, we analyzed the pathology of chronic venous ulcers and compared pathologic phenotypes to wound healing outcomes. Venous ulcers with dense fibrosis, mild to moderate inflammation, decreased cellularity, and mature collagen had poorer healing outcomes compared with those without these characteristics. Several studies have shown that cytokine and growth factor production is preserved, even upregulated, in these chronic wounds; however, they often fail to heal.26,29-31 Although previous studies have demonstrated a hyperkeratotic epidermis as a barrier to wound healing, this is the first iteration to our knowledge of a histopathologic phenotype correlated to wound healing that can easily be used to determine surgically adequate margins of debridement.32

These results should motivate wound care practitioners to perform and send debridement specimens for histopathologic analysis. The advantage of performing hematoxylin and eosin stains is that the results can be made available to the surgeon during the debridement, while the patient is still available for further debridement as necessary to a base that is free of fibrosis and mature collagen, not unlike surgical resection of solid tumors to “clear margins.” Venous ulcers need their best chance to heal, and an inadequate debridement can set them up to fail.33,34 Conversely, too aggressive a debridement can create a much larger surface area and mechanically remove healthy tissue that has growth factors and cytokines necessary for wound healing. Therefore, the overall pathologic score can be used as means to determine if the recently debrided wound will have the best chance of healing. Achieving a score ≥10 will likely yield a positive healing curve.
One limitation of the application of this nonhealing phenotype is that it can provide information only on the margin of depth of debridement, not the width, given the nature of lower extremity venous disease. Venous stasis dermatitis and lipodermatosclerosis import a histopathologically abnormal pattern throughout the lower extremity skin, even that unaffected by ulceration, which makes it difficult to ascertain where the healing edge begins and devitalized tissue ends. In order to determine the wide margin of debridement, molecular markers such as c-myc and β-catenin, though more costly and less practical, may provide a useful alternative.

Although complete healing can generally be achieved in all patients with ulcers of less than 1 year duration, many do not heal rapidly and are allowed to progress past 1 year. Several reasons for the increased prevalence and chronicity of these wounds have been described, mainly that venous ulcers are not aggressively treated at an early stage per numerous treatment guidelines, or treatment modalities are used sequentially and not in tandem, or important comorbidities are not significantly addressed.

These chronic venous ulcers need every possible advantage in achieving a positive healing curve. Although venous reflux surgery and compression have been shown to reduce the rate of ulcer recurrence, patients first need to heal existing venous ulcers completely. Early indication of healing is essential because it has been shown that the percentage change in the wound over the first 4 weeks of treatments represents a predictive measure of complete healing by 24 weeks. In our own practice, we have found an immediate positive healing rate primarily in patients undergoing surgical debridement. Sharp debridement using curettage has been proven safe and effective, and it increases healing rates.

CONCLUSIONS

The role of early surgical debridement to the deepest level of healthy tissue should be to objectively remove all noncellular fibrotic tissue, thereby increasing healing rates.

REFERENCES