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Key Concepts in Healing Venous Leg Ulcers

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ABSTRACT: VLUs represent 90% of lower extremity ulcers. They affect 1% of the general population and 2.2% of the Medicare population. That same incidence is seen in Europe where 1% of the population is affected. The incidence is 4 times as high in underdeveloped countries. Recent discoveries have helped better define the chronic nature of venous ulcer pathophysiology. Applying recently developed key concepts in a venous ulcer treatment plan may bring about improved healing outcomes. Important clinical considerations include the effective management of biofilm, control of protease levels, and the role of high-density ECM collagen in healing. For the practitioner, having a better understanding of pathophysiology and using a goal-directed treatment plan can be helpful in delivering quality outcomes for patients with VLUs. With the goal of improving outcomes for this patient population, this article provides awareness of key concepts directed at a modern pathophysiological approach for managing VLUs.

Introduction

VLUs are the most common ulcer of the lower extremity, representing nearly 90% of lower leg ulcers.[1,2] Prevalence of VLUs in the US population is 1%; when considering patients with ulcers and healed ulcers, this percentage increases to 1.8%.[1,2] In the United States, approximately 0.5% of patients who are privately insured have been reported to have VLUs, whereas the prevalence of VLUs in the Medicare population has been reported to be 2.2%.[2] There are over 6 million patients with VLUs in the United States.[3] In terms of cost, management of VLUs costs an estimated $14.9 to $17.4 billion dollars annually in the United States.[2,4] The reported average cost per patient of $5527 is anticipated to increase.[4]

In western Europe, the incidence of VLUs is similar to that of the United States at 1% of the population. Global estimation may be harder to compare, but data indicate a higher incidence in developing countries. Incidence rates of 4.5 per 1000 patient-years in India, 3.5 per 1000 patient-years in China, and 1.7 per 1000 patient-years in Brazil have been reported.[5]

In addition to the cost of treatment, patients with VLUs experience the economic loss of time off work and low quality of life scores.[2,6,7] Healing rates are often poor for VLUs. Healing can be protracted, with fewer than 60% healed by 12 weeks, and recurrence rates are high, with nearly 75% recurring within as little as 3 weeks.[8] Rates of venous ulceration tend to be higher and more commonly associated with older age, concomitant chronic venous insufficiency, female sex, obesity, a history of DVT or phlebitis, immobility, or a congenital absence of veins.[1,7,8]

Patients with VLUs will represent a significant portion of any wound care practice. For the practitioner, having a better understanding of pathophysiology and using a goal-directed treatment plan can be helpful in delivering quality outcomes for this population of patients. Awareness of key concepts directed at a modern pathophysiological approach can bring about improved outcomes for patients.

Keywords: etiology, matrix metalloproteinase, peripheral arterial disease, venous leg ulcer

Abbreviations: DVT, deep vein thrombosis; ECM, extracellular matrix; MMP, matrix metalloproteinase; ORC, oxidized regenerated cellulose; PAD, peripheral arterial disease; VLU, venous leg ulcer.

Etiology

Macro disease occurs in the dependent venous system in the legs. Whether the disease is congenital in origin or acquired after DVT, valvular incompetence results in further vein dilatation and thus further valve incompetence results. Commonly seen in the superficial saphenous system, venous hypertension can be transmitted to the deep system through the connecting perforator vessels. The calf muscle pump fails to move blood from the lower leg, and the result is venous hypertension. Venous hypertension can be demonstrated in greater than 84% of patients with ulceration.[9] The resultant venous hypertension in turn affects the microcirculation. It is at the microcirculation level that more molecular-based tissue damage and chronicity in this disease state are observed.[3,9] Dermal changes are seen, such as hemosiderin hyperpigmentation, lipodermatosclerosis, and ulceration of the skin.[3,9] The resultant microangiopathy with elongation and dilatation of the capillary beds leads to cyclical changes of capillary endothelial damage, widening of the interendothelial space, capillary cuffing, and pericapillary edema that increase vascular permeability and lead to accumulation of extravasated fluid. Leukocytes accumulate as do inflammatory macromolecules, leading to tissue damage and perpetuating ulceration.[3,9] The surrounding tissue is damaged by activation of increased amounts of cytokines, chemokines, MMPs, iron-free radicals, upregulated oxygen radicals, and nitrogen radical species.[3,9] The proinflammatory nature of venous ulceration should be carefully considered when treating these chronic wounds. Likewise, an understanding of how treatment will affect the proinflammatory nature of the wound should lead to improved healing. Treatment strategies addressing both the proinflammatory nature of the ulcer as well as improving the microcirculatory changes (ie, local tissue edema and extravasation) should be effective.

Pathophysiology of Venous Ulcers

VLUs are characterized and perpetuated by their inflammatory characteristics. While the process of venous hypertension leads to leaking capillaries and the release of inflammatory mediators and leukocytes, failure to heal and the presence of an open wound also leads to bacterial consequences. The concept of critical colonization has been replaced by the recognition that bacterial biofilms develop on the wound surface.[10] The constant presence of bacteria causes a chronic state of inflammation as the immune system reacts to but cannot clear the bacteria organized into a biofilm.[10] A recent meta-analysis identified that 78.2% of hard-to-heal wounds have biofilm whereas only 6% of acute wounds have biofilm.[11] The presence of biofilm produces MMPs as leukocytes respond to the presence of bacteria. That influx of inflammatory cells leads to an excess of protease activity that degrades tissue and prevents healing. Bacteria in and of themselves produce protease, which can be an indicator of virulence and likelihood of infection.[12]

Visual inspection and surface culture do not accurately identify bacterial biofilm nor estimate virulence and infectivity.[12] Therefore, using a validated point-of-care device to assess for invasion by common pathogenic bacteria would seem appropriate.[12,13] Identifying pathogenic bacteria can direct therapy so as to avoid infection and decrease the impact those bacteria have in attracting leukocytes that lead to protease excess. Additionally, bacterial proteases do participate in the breakdown of essential proteins and the ECM.[12,14] Protease activity supports the development and accumulation of biofilm while attracting leukocytes to the wound, elevating the MMP levels responsible for ECM destruction.[15] While there are no published data on wound-derived bacteria and the formation of biofilm, biofilm-forming bacteria have been shown to be more productive in developing biofilm in alkaline environments.[16]

The effect of MMPs on healing cannot be understated. When present in excess, leukocyte-produced MMPs delay or prevent wound healing. In one analysis, detection of an elevated MMP level by point-of-care testing was associated with impaired or failed healing of 90% of wounds.[14] Wounds that fail to heal will continue to fail as long as MMP levels are elevated.[14,15,17] To move wounds toward healing and closure, employing strategies to reduce MMP levels would seem effective.

Examinations of wounds treated with high-density ECM collagen (Endoform Collagen Dressing; Aroa BioSurgical) have demonstrated that MMP levels decrease over time. Comparisons of MMP levels with wound area have demonstrated that reducing MMPs brings about a correlating reduction in wound size (**Figure 1**).[18] The reduction in wound size lags behind MMP reduction by approximately 2 weeks. When MMP levels increase (**Figure 2**), the wound enlarges as healing stalls.[18] Elevated MMP levels break down the ECM as it is produced to heal the wound. Architecturally, the ECM provides the structure with which fibroblasts interact in signaling the next-step processes that then differentiate to other tissue functions.[10] Integrins in the ECM are central to this process. Healing is a dynamic process that relies on cellular signaling and reciprocal interactions between cells and the ECM.[10] As the cell attaches to the ECM via integrins, integrin signaling and resultant structural support work to direct gene expression, protein synthesis, actin organization, cell polarity, differentiation, proliferation, and cellular migration.[10] Cytoskeletal distortions cause changes that lead to differentiation. Without that structure, healing processes stall, and chronicity ensues.

Elevated MMP levels break down and degrade the collagen that structurally defines the ECM. Multiple MMPs can participate in degradation and result in a damaged ECM. Primary MMPs involved in the process are the collagenases MMP-1 and MMP-8 and the gelatinases MMP-2 and

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MMP-9.[18] Within structurally intact ECM, collagen degradation can be conceptualized as a stepwise process. MMP-1 and MMP-8, the collagenases, make the first cut in intact collagen by exposing collagen.[19] The collagenases unwind the triple helical structure of the collagen, exposing it to degradation into smaller pieces (peptide segments). MMP-2 and MMP-9, the gelatinases, then come in and degrade the exposed collagen into smaller and smaller peptide pieces.[19] Neutrophil elastase is also present in inflammatory wounds and is a potent serine proteinase that degrades tissue.[18]

Of note, MMP activity is pH senstive. Protease activity is increased in alkaline environments and reduced by acidic lower pH.[16] Consequently, the alkaline pH of hard-to-heal wounds may well promote protease activity.[16]

Goal-directed Treatment

Treatment planning starts with assessment of the patient and using data to predict response to treatment. Having a secure diagnosis is key to proceeding with confidence in the effectiveness of the proposed therapy. Venous duplex imaging of the veins and valves for confirmation of venous disease is the most common imaging confirmatory study and should be part of the initial evaluation. With a confirmed diagnosis, ulcer healing within 12 weeks should be the goal in treatment planning.

Using Margolis-Kantor data to assess patients at presentation can help frame the treatment plan and process (**Figure 3**).[16,20] According to a multicenter study from 2000, Margolis and Kantor found that VLUs with areas of less than 10 cm^2 that were present for less than 12 months in patients who did not have PAD had an 81% chance of healing by 24 weeks.[20] Conversely, ulcers with areas measuring greater than 10 cm^2, those present for 12 months or more, or those in patients with PAD had only a 22% chance of healing at 24 weeks.[20,21] The implication is that larger ulcers and those in patients with long-standing disease or concomitant PAD will be harder to heal. The response to treatment in the first 4 weeks, measured as a reduction in wound area, has been shown to be most relevant when predicting the likelihood to heal. Those wounds that responded to therapy and demonstrated a reduction in wound area by 30% or more were more likely to heal by 12 weeks.[20,22-24]

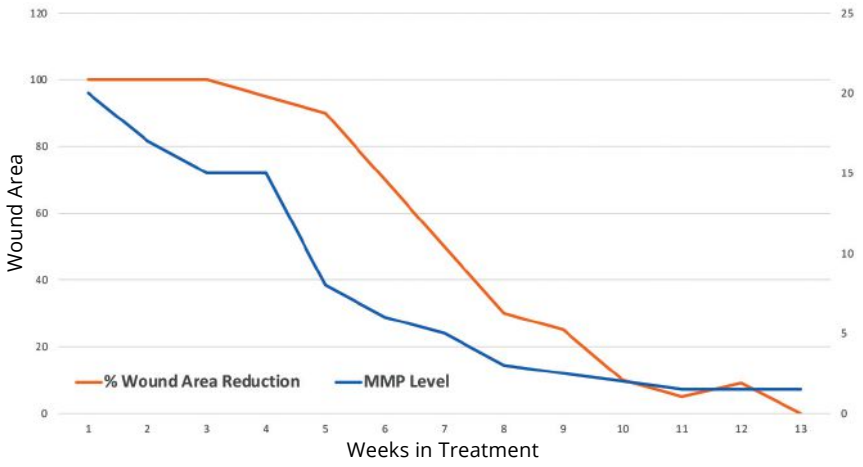


Figure 1. Wound size decreases follow as MMP levels fall.[18]
Abbreviation: MMP, matrix metalloproteinase.

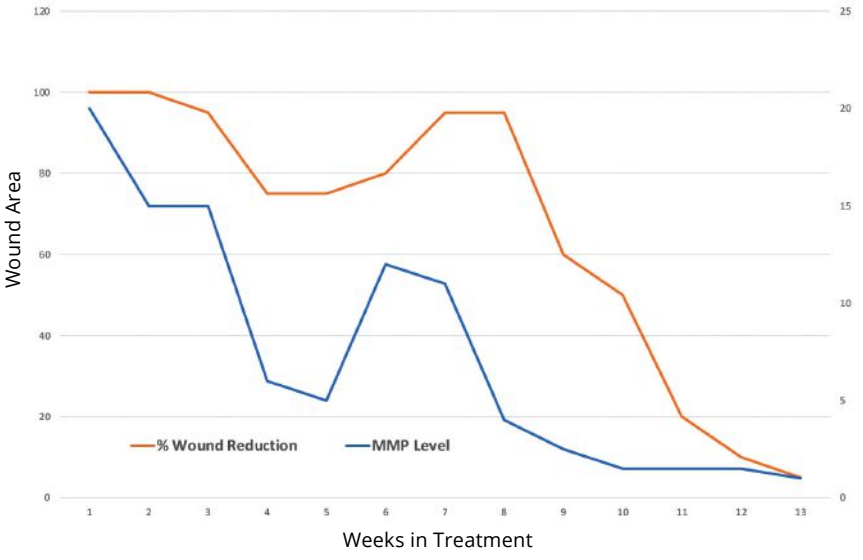
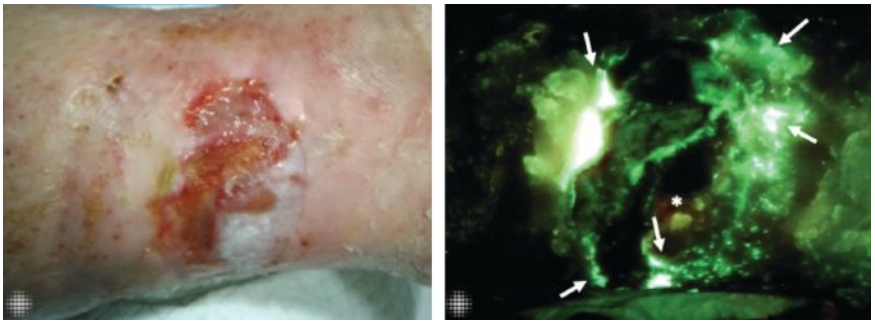


Figure 2. Wound healing stalls while MMP levels rise.[18]
Abbreviation: MMP, matrix metalloproteinase.

The need for treatment directed at both macrovascular and microvascular changes seems apparent given the current understanding of both venous hypertension in the saphenous vein system and the destructive environment created in the wound by biofilm and elevated MMP activity. While compression therapy will be addressed in greater detail later in this compendium, it is important to note the effect that compression has on the wound microenvironment. Not only does compression therapy address the edema caused by venous hypertension, but it also can lower the levels of MMPs and produce a dramatic effect on healing. Beidler et al reported on compression therapy and its effect on harmful MMP levels.[25] Results from their study confirmed the reduction

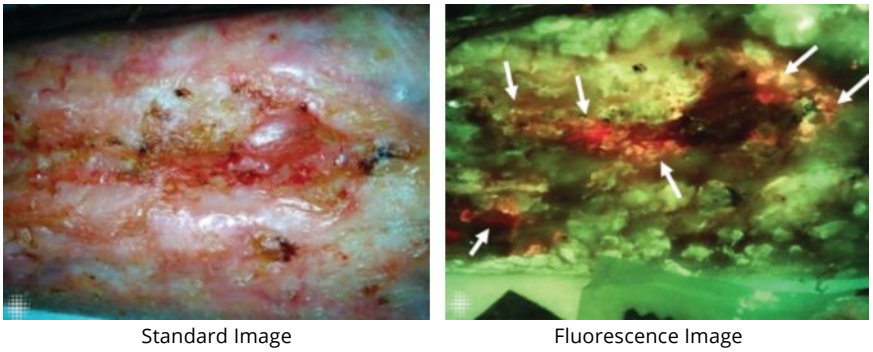
Positive Prognostic Factors	Negative Prognostic Factors
VLU: <10cm^2	**VLU:** ≥10cm^2
Duration: <12 mos	**Duration:** ≥12 mos
Absence of PAD: ABI >0.80	**Absence of PAD:** ABI <0.80
Closure from baseline at 4 wk: ≥30%	**Closure from baseline at 4 wk:** ≤30%

Figure 3. Predictors of healing for VLUs.
Abbreviations: ABI, ankle-brachial index; mos, month(s); PAD, peripheral arterial disease; VLU, venous leg ulcer; wk, week(s).



Standard Image Fluorescence Image

Figure 4. The cyan color (white arrows) on fluorescence imaging of this venous leg ulcer indicates the presence of *Pseudomonas* at the wound edge and in the periwound region. Red fluorescence is also present in the wound bed (asterisk), indicating presence of other bacterial species.



Standard Image Fluorescence Image

Figure 5. The presence of red fluorescence (white arrows) on fluorescence imaging of this venous leg ulcer indicates locations with bacterial loads above 10^4 CFU/g.
Abbreviation: CFU, colony-forming unit.

in MMP-1, -2, -3, -8, and -9 with adequate compression. All 5 MMPs are implicated in delayed wound healing when present in excess amounts.[25] As a result of lowering MMP levels, higher rates of wound healing were identified at 4 weeks.[25] Based on the reduction in MMP activity level, Beidler et al were able to identify good healers, average healers, and poor healers.[25]

Having an effective biofilm strategy is important in healing VLUs. While there are many topical treatments that can be applied to limit reformation of a biofilm, antibiotics are to be used only when invasive infection is present. Elevation in bacterial protease can also help identify infection risk.[12] The commonly used practice of debridement has been shown to improve wound healing.[26-28] While the role of debridement has been studied, recent focus has been on the role of debridement in managing biofilm. The use of bacterial autofluorescence imaging (MolecuLight i:X; MolecuLight Inc) during debridement has improved healing outcomes by identifying more complete removal of biofilm and bacteria (**Figures 4, 5**). Use of autofluorescence imaging to guide debridement has been shown to be helpful in more completely removing the bacterial load from the wound surface. In one randomized, controlled trial, the 12-week healing rates doubled from 22% in the standard of care group to 45% in the group that underwent autofluorescent-guided debridement.[29]

When applied appropriately to a hard-to-heal wound, collagen is an effective modulator of excess MMP activity, reversing the destructive effects of MMPs and thereby initiating wound healing (**Figure 1**). Broad-spectrum MMP buffering has been demonstrated with ECM collagen dressings. In multiple tests, ORC collagen dressings (Promogran Matrix; 3M) affected a more narrow spectrum of MMPs.[18,30] In vitro testing of ECM collagen dressings confirmed potent reduction in collagenases (MMP-1 and MMP-8), gelatinases (MMP-2 and MMP-9), and stromelysin (MMP-3).[18] ORC collagen had activity to lower the gelatinases (MMP-2 and MMP-9) while the collagenases and panel of proteases remained relatively unaffected.[18,30] Once MMP activity is lowered to a certain level, the wound progresses toward a healing trajectory for closure (**Figure 1**).[18,30] If MMP activity increases, a typical wound could experience stalled healing or enlarge (**Figure 2**).[18]

In addition, ECM collagen provides a provisional ECM that stimulates healing. Recent research has demonstrated that the remodeling of the provisional matrix releases a stem cell chemotactic factor, the May-Day protein. Macrophage-induced cleavage of decorin, via MMP-12, releases the chemotactic molecule May-Day, which in turn recruits cells to the site of damaged tissue.[31] The healing benefit of attracting stem cells to the wound site seems intuitive; however, further study will help clarify and define that benefit. Collagen fibril density has been demonstrated in vitro to impact differentiation of wound macrophages,

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converting them to resident macrophages and supporting fibroblast differentiation.[32] ECM collagen dressings have intact collagen and high fibril density. Compared with ORC collagen in treating VLUs, ECM collagen dressings have been reported to enhance and expedite healing (**Figure 6**).[33]

In a study conducted in a US Veterans Affairs hospital, early treatment of ulcers with ECM collagen resulted in improved wound healing outcomes. Using ECM collagen brought about a significantly greater number of wound closures while decreasing the number of advanced cellular or tissue-based products used.[5] The impact of utilizing key concepts in a goal-directed treatment plan can be illustrated in **Figure 7**. While Margolis-Kantor data would suggest this large VLU would be difficult to heal, biofilm management and MMP buffering were employed in combination with effective compression to elicit efficient healing.

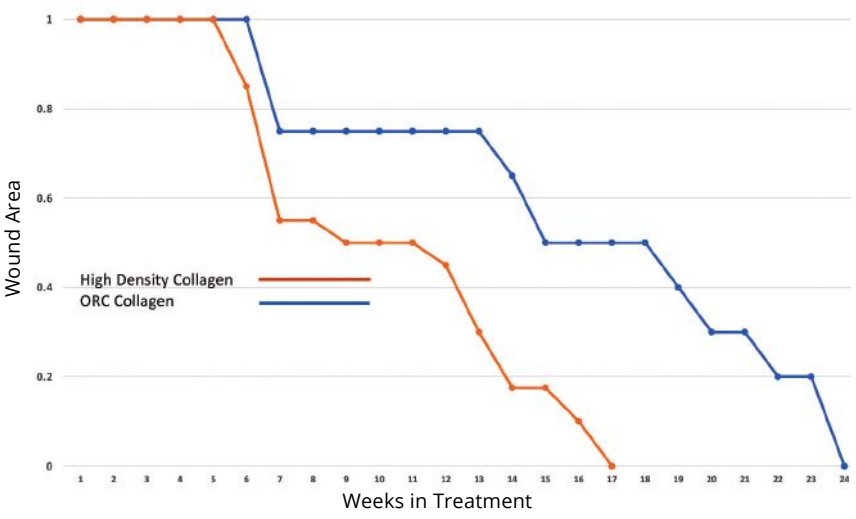

Figure 6. High-density extracellular matrix collagen heals ulcers in a shorter period of time. Abbreviation: ORC, oxidized regenerated cellulose.

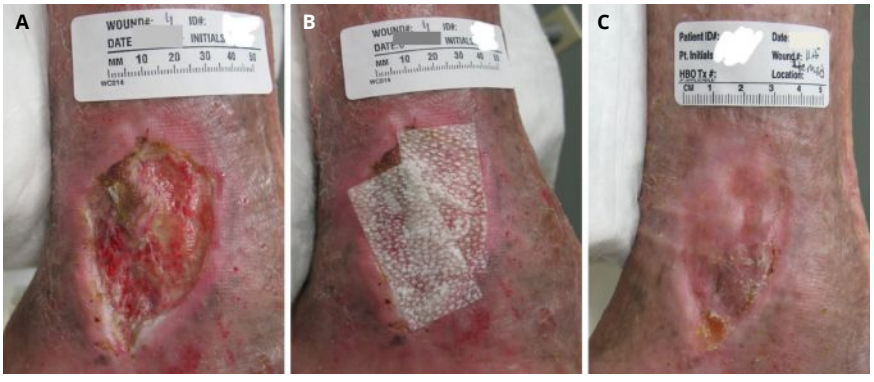

Figure 7. Venous leg ulcer present for more than 22 months that failed multiple advanced modality therapies; (A) on presentation, dimensions were 3.7 cm × 6.3 cm, and area was 23.31 cm^2. Large size and duration are negative predictive indicators for healing and have been associated with a 22% or less chance of healing by 24 weeks. (B) Application of high-density ECM collagen dressings. (C) Wound healed by 20 weeks with compression, attention to biofilm, and high-density ECM collagen dressings. Abbreviation: ECM, extracellular matrix.

Conclusion

The pathophysiology of VLUs has been more completely elucidated in recent years. Compression therapy has been used to treat the resultant edema and, in addition, demonstrated to lower MMP levels in this inflammatory, highly proteolytic ulcer.[25] The role of biofilm in causing inflammation in hard-to-heal wounds suggests the importance of dispersing this microbiome to foster improved healing. Elevated MMPs in a hard-to-heal VLU can be modulated and lowered by applying collagen to the wound. High-density collagen dressings with a broad spectrum of activity and intact collagen may be even more effective for balancing MMP levels and correcting the destruction of ECM. ECM restoration by supplying a provisional matrix can restart the repair process. An effective biofilm management strategy and MMP-modulating therapy may result in improved healing outcomes. Implementing these key concepts in conjunction with adequate leg compression, fluorescence-guided debridement, and early use of ECM high-density collagen dressings as a part of a goal-directed treatment plan may well improve healing outcomes, shorten time to heal, and reduce costs overall.

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