Investigations for Ulcer of the Lower Extremity

7

Satyendra K. Tiwary

7.1 Introduction

Ulceration of the lower limb in aging population with comorbidity associated has always been a challenge in philosophy of wound management. Acute ulcers have a predictable outcome with rapid progression or regression and outcome achieved within 3–4 weeks. The etiology of the lower limb ulcerations varies from different geographic regions in world. In Western societies, most chronic lower limb ulcers are due to vascular diseases, whereas in developing countries, trauma, infections, malignancies, and poorly controlled diabetes remain the most common causes of chronic lower limb ulceration [1–3]. The treatment of chronic lower limb ulcers requires multidisciplinary approach [4, 5]. Multifactorial etiology and interdisciplinary approach in management are key factors determining outcome [5].

7.2 Epidemiology

Chronic leg ulcer (CLU) also termed as chronic lower limb ulcer is a chronic wound of the leg that shows no tendency to heal after 3 months of appropriate treatment or is not still fully healed at 12 months [6]. Ulcers can be defined as wounds with full thickness depth and a slow healing tendency [7]. Investigations are aimed for correct diagnosis to avoid inappropriate treatment that may delay wound healing, cause deterioration of wound, or harm the patient to detect underlying cause and work up the needful intervention. Complete history and accurate assessment clinically are prerequisites before any investigations. Chronic leg ulcers affect 0.6–3 % of those aged over 60 years, increasing over to 5 % of those aged over 80 years [8].

S.K. Tiwary
Department of General surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, 221005 Uttar Pradesh, India
e-mail: drsktiwary1@gmail.com

A.K. Khanna, S.K. Tiwary (eds.), *Ulcers of the Lower Extremity*,
DOI 10.1007/978-81-322-2635-2_7

Principles and philosophy of investigations are governed by history and examination to detect cause, direct treatment, and predict prognosis. Both modalities, invasive and noninvasive, are essential according to assessment after history and examination. Almost 70 % of leg ulcers have a venous etiology; approximately 20–25 % are due to arterial insufficiency; and some of these have a mixed vascular etiology. The remaining causes include infection, malignancy, vasculitis, lymphedema, pyoderma gangrenosum, and other conditions [9].

Lower extremity ulcers are always assessed systematically with skin assessment, vascular assessment, ulcer assessment, and limb assessment with assessment of the patient as a whole for comorbidities and risk factors associated [10]. After complete history and physical examination, mostly diagnosis of lower limb ulcer is almost settled, but the role of investigations is always integral for etiology, treatment, follow-up, and prevention. In acute ulcers, investigations are directed toward the detection of risk factors and comorbidities, but isolation of microbes and antibiotic sensitivity are key investigations. In chronic ulcers, almost 95–98 % are caused by venous, arterial, or diabetic and other less common causes are to be detected mostly by invasive investigation with biopsy.

7.3 Investigations

Lower extremity ulcers are investigated after proper clinical history and examination. Both types of investigations, diagnostic and prognostic, are essential for proper management.

7.4 Diagnostic and Prognostic Investigations

First-line routine biochemical and hematological laboratory investigations included are essential as they are sometimes diagnostic but always of prognostic significance. Blood investigations like complete blood count, erythrocyte sedimentation rate, lipid profile, renal function tests, and liver function tests are prognostic, while blood sugar is diagnostic.

Screening tests for vasculitis are urine analysis for proteinuria, hematuria, cylindruria, antinuclear antibodies (ANA), rheumatoid factor (RA), complement C4, circulating immune complexes, paraproteins, immunoglobulin fractions, antineutrophil cytoplasmic antibodies, serological tests and cultures for underlying infections, and finally routine and immunohistopathology of skin biopsies [11]. These battery of tests have both diagnostic and prognostic significances.

Clotting disorders are screened by activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), factor V Leiden mutation, factor II (thrombin) mutation, antithrombin III, protein C and protein S, and lupus anticardiolipin [11].

For majority of ulcers falling in venous category, color Duplex ultrasound is the standard investigation serving diagnostic purpose by evaluating venous obstruction and assessing location and extent of reflux in venous obstruction. Plethysmography and venography are supplementary investigations for planning surgery. Plain X-ray, CT, and MRI are used in selective ulcers to rule out osteomyelitis and malignancy.

Microbe isolation by culture and sensitivity is very specific and always performed once there is a possibility of infection. Ulcer biopsy is done for final tissue diagnosis once there is a possibility of malignancy. Gene variant analysis in venous ulcer is evolving as a prognostic tool for healing and prevention of ulcer well in advance once high risk is detected.

7.5 Noninvasive and Invasive Investigations

Diagnostic tests are first line followed by prognostic tests. Based on history and clinical examination, selected tests should be performed according to requirement. In chronic ulcers almost up to 98 % are due to venous, arterial, or diabetic, so the primary and most important investigations in chronic ulcers are vascular assessment. ABPI and Duplex ultrasound are two key investigations in all cases of ischemic ulcers. If an ulcer is recurring, etiology is unclear, and all invasive and noninvasive studies have been preformed, a biopsy is essential to establish a diagnosis and to further understand the etiology of the disease. As always, management of chronic wounds can be improved by understanding the true etiology and therefore treating the underlying problem.

Assess the vascular supply to the site of ulceration so that the likelihood of satisfactory wound healing may be estimated. Several methods of determining the adequacy of the pedal circulation are available, e.g., ABI, Xenon-133 clearance, and transcutaneous oxygen tension.

A. *Ankle-brachial pressure index* (*ABI/ABPI*): (Figs. 7.1 and 7.2) Most basic and essential tool of noninvasive investigations is ankle-brachial pressure index (ABPI) for accurate assessment of arterial perfusion. Ischemic ulcers should always be assessed by ABI. It is done by using a handheld Doppler ultrasound and sphygmomanometer. The results are used to determine the likelihood of arterial insufficiency and can be used to guide the management plan. It should be done after 10 min of rest and cuff placed proximal to the malleoli. ABI values below 0.9 are considered as evidence of peripheral arterial occlusive disease [12].

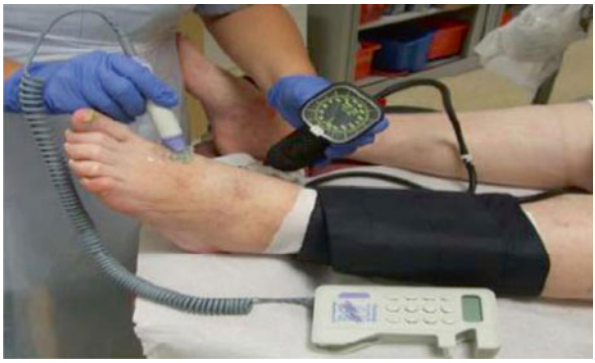

Fig. 7.1 Handheld Doppler for measurement of systolic pressure for ABPI

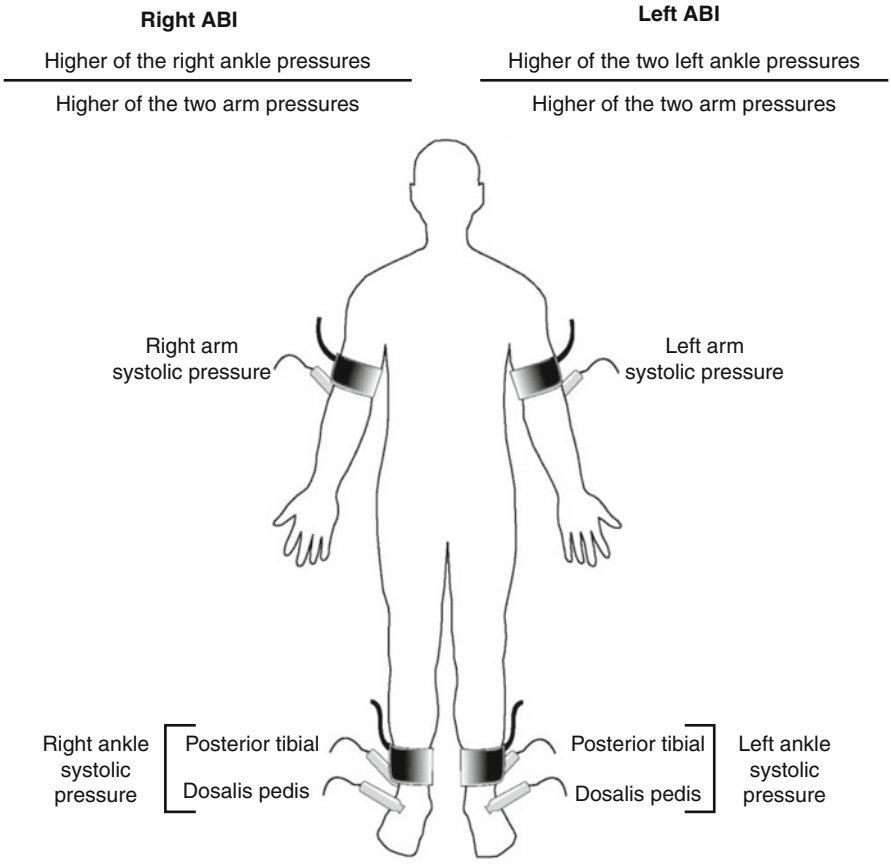

Fig. 7.2 ABPI

When Doppler tests indicate arterial insufficiency, arterial Duplex ultrasonography will (noninvasively) provide accurate anatomic and hemodynamic information on the site and extent of the arterial disease. When indicated, further detailed anatomic information for treatment planning can be obtained from magnetic resonance angiography, computer tomographic angiography, or digital subtraction angiography [12].

Ankle-brachial indices (ABIs) and toe digital pressures with pulse volume recordings can provide good clues to the perfusion of the foot. Findings are also predictive of wound healing, although they may be misleading in patients with diabetes and calcified noncompressible arteries. An ankle pressure greater than 55 mmHg suggests adequate leg perfusion. Research suggests that venous ulcers require a higher ABI for healing than arterial ulcers. The diagnosis of critical limb ischemia is supported by either an ankle systolic pressure of 50 mmHg or less or digital pressures less than 30 mmHg. Limitation of ABI is calcified atherosclerotic arteries mostly in diabetics giving higher false value of ABI due reduced compressibility caused by calcification.

B. *Xenon-133 clearance* to measure blood flow can help estimate the chance of wound healing. A rate of 2.6 mL/100 g is believed to be adequate for healing.
C. *Transcutaneous oxygen tension* may be measured; however, a wide discrepancy exists with the minimal level below which wound healing does not occur. Most agree that a pressure of 30–35 mmHg is sufficient for healing of more than 90 % of wounds.

7.5.1 Leg Ulcer Measurement Tools (LUMT)

Accurate and regular measurement of the wound is important to give an objective assessment of the effectiveness of the current management plan. The leg ulcer measurement tool (LUMT) is a validated tool that has been developed to quantify leg ulcer assessment and can be used to track change in wound status over time. Various methods have been used to document an ulcer of the lower extremity (Fig. 7.3). They can be broadly classified into the following:

(a) *Contact methods*
1. Ruler method
2. Graduated swab stick method
3. Alginate cast
4. Planimetry
5. Kundin gauge
6. Wound tracing

(b) *Noncontact methods*
1. Clinical photography
2. Stereophotogrammetry
3. Structured light techniques
4. Laser triangulation
5. Alfred/Medseed wound imaging system
6. Video image analysis
7. Magnetic resonance imaging

Fig. 7.3 Leg ulcer measurement tools

Role of ulcer measurement tools is to see progress of wound healing, and these are instrumental in monitoring of wound healing after different modes of interventions. Only limited role of ulcer measurement tools is well defined in experimental studies of chronic ulcers.

7.5.2 Doppler Duplex Scanning

Due to rapid, easy, and inexpensive assessment, Duplex ultrasound can be considered as a screening tool for detecting vascular pathology in chronic ulcers. Combination of ultrasound with Doppler detects the distribution and extent of vascular disease. It is regarded as the investigation of choice for the diagnosis of venous reflux, thrombosis, arterial obstruction, and aneurysms [13, 14]. Stenosis and occlusion can be clearly demonstrated and differentiated with adequate information about surface characteristics and plaque content to direct the treatment plan according to etiology and anatomical delineation of pathology of the vascular segment affected. In chronic cases or during follow-up, collateral pathways and flow dynamics are demonstrated leading to the identification of acute, chronic, or recurrent thrombosis. Duplex scan is an excellent tool to differentiate tumors, cysts, hematoma, clots, thrombus, and foreign body in vascular tree. Considering its excellent sensitivity and availability, it is integral in the diagnosis and follow-up with effect on treatment, progression of disease, or developments of new pathology. Duplex is superior in the detection of venous reflux with a sensitivity greater than 75 %, compared to approximately 40 % for descending venography. Neglen and Raju suggest that combining Duplex scanning with air plethysmography helps differentiate severe venous disease from mild venous disease [15].

7.5.3 Plethysmography

Considering the surgical options in chronic ulcers, plethysmography is used to detect and quantify arterial and venous diseases [16, 17]. Several types of plethysmography have been evolved such as air, photo, and strain gauge. Depending on the device used, pressure and flow measurements, waveform patterns, volume changes, amount of reflux, degree of venous obstruction, and the efficiency of calf muscle pumps can be calculated. All these parameters are important as the severity of the disease can be demonstrated to determine the impact of ulceration. So, these are good tests to assess treatment and progression of the disease. Venous pressure data are important in determining the need for surgical bypass or valve replacement. Quantitative data on venous obstruction, calf muscle pump ejection fraction, and reflux are provided by air plethysmography, whereas venous pressure studies assess the physiological importance of anatomic obstruction because the collaterals may or may not provide adequate compensation for an obstructed pathway. Dual significance with both diagnostic and prognostic efficacies is associated with plethysmography, but its use is limited mostly to academic and research purpose.

7.5.4 Computed Tomography (CT Arteriography and CT Venography) (Figs. 7.4 and 7.5)

In chronic ulcer, spiral CT angiography (CTA) is an accurate modality to assess the presence and extent of peripheral arterial disease (PAD). This investigation is done only if the active intervention is required to salvage the limb and improve the quality of life by reducing rate of limb loss and persistent pain. Modes of interventions are either endovascular, open surgery, or hybrid techniques. CT angiography has become popular due to rapid technical developments in terms of shorter acquisition times, thinner slices, higher spatial resolution, and improvement of multidetector computed tomographic scanners that enable the scanning of the whole vascular tree in a limited period with limited contrast [18]. CT venography (CTV) is also accurate to determine proximal venous obstruction, but as it is relatively static, one cannot evaluate reflux. CT can also detect aneurysms, AV fistulae, and any other pathologies like neoplasms and can determine the extent of disease and involvement of surrounding structures. Both CT arteriography and CT venography are considered as excellent tools for differential diagnosis to detect vascular lesion in chronic ulcers [19].

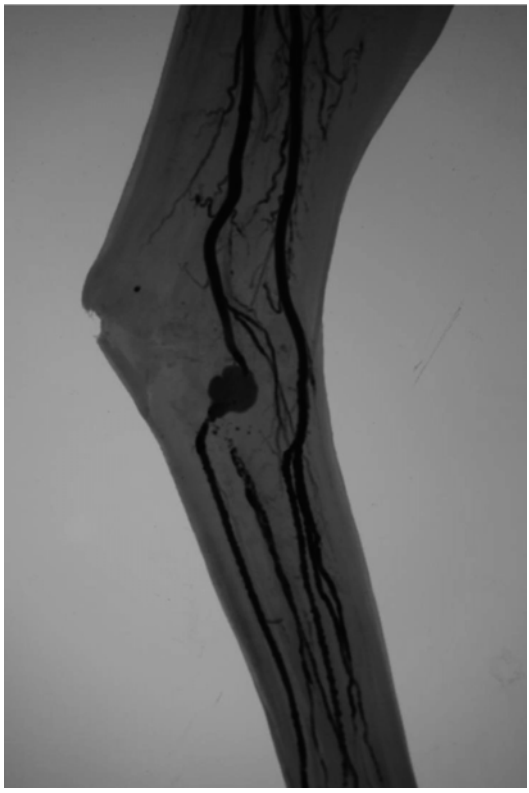

Fig. 7.4 CT angiography showing popliteal artery aneurysm. Patient presented with ulceration of foot

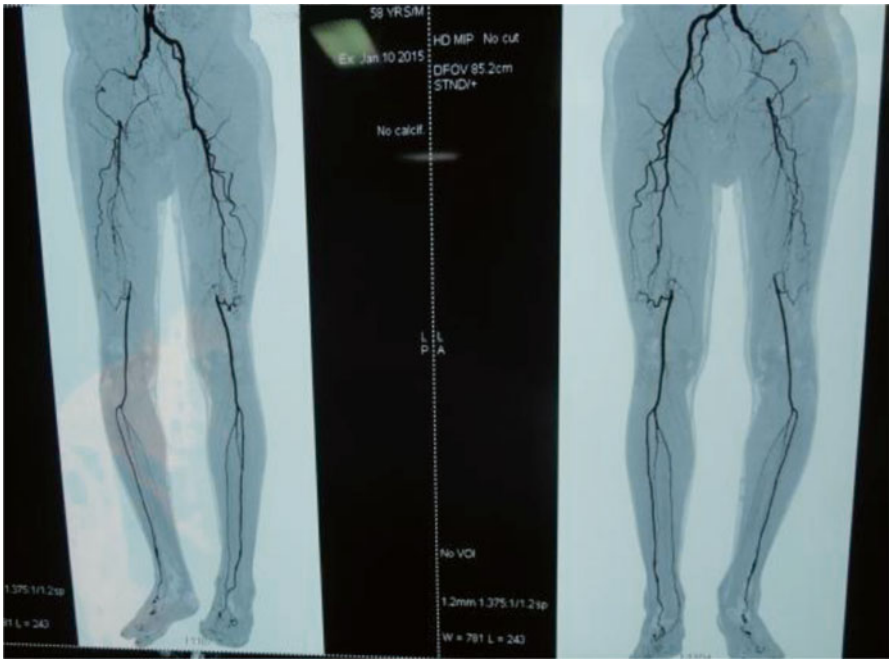

Fig. 7.5 CT angio showing bilateral femoral block at variable levels

7.5.4.1 Magnetic Resonance Imaging (MR Arteriography and MR Venography)

Despite limited availability and skilled interpretation of findings, magnetic resonance arteriography (MRA) equivalent to CT angiography is a noninvasive method to visualize the peripheral vasculature, to detect hemodynamically significant stenosis, and to distinguish focal from long-segment occlusive disease [20]. Advantage of its use is that it does not need any contrast, so the patients with deranged renal functions may undergo MRA. But the disadvantage is that it takes a much longer time to perform and also cannot be used in the patients on pacemakers and any other prosthesis. Further claustrophobia and the sounds produced during the procedure may not be palatable to the patients. MRA has the ability to define the pattern of the disease and help in planning for arterial access sites (retrograde or antegrade). More specifically, MRA has proven useful in detecting occult runoff channels, which can be used for distal bypass [21]. Ulcerations should be bright on T2-weighted imaging, with peripheral enhancement of the ulcer base. Magnetic resonance venography has a great accuracy in detecting proximal vein obstruction and may differentiate acute from chronic thrombosis [22]. Drawback of MRV is failure to demonstrate venous reflux. MRA and MRV can be easily compared to CTA and CTV for excellency in differential diagnosis tool for vascular lesions.

Magnetic resonance angiography (MRA) can also be useful when evaluating lower extremity disease. Yucel et al. found that MRA was 94 % accurate in

evaluating lower extremity vessels when compared to conventional angiography or surgery [23] Owen and coworkers found that MRA detected all runoff vessels when compared to conventional angiography and, in fact, was more sensitive than conventional arteriography for visualizing both runoff vessels and arterial stenosis [24].

7.5.4.2 Phlebography/Venography

Phlebography is usually not done because of the availability of noninvasive tests such as Duplex and relatively less invasive such as CT or MRI, but it can identify the location and extent of blood clots and enables the condition of the deep leg veins to be assessed. It is especially useful when there is a strong suspicion of deep vein thrombosis, after noninvasive tests have failed to identify the disease. Ascending venography also may be considered to obtain detailed anatomic information. This study can reveal axial channel patency, perforator incompetence, obstruction, and the presence of deep venous thrombosis. Phlebography can also be used to evaluate congenital vein problems and assess the function of the deep vein valves. Phlebography is a great method to demonstrate the extension of obstruction and all the collateral pathways. It can guide treatment and at the same time assess its effect. It is not used often, because it is painful, expensive, and time-consuming, exposes the patient to a fairly high dose of radiation, and can cause phlebitis, tissue damage, and the formation of deep vein thrombosis in a healthy leg. It is reserved for evaluating limbs that may need deep vein reconstruction or to open proximal vein obstruction [25].

7.5.4.3 Percutaneous Arteriography

It is integral to do angiography when visualization of the vessels of the lower extremities is desired. A femoral runoff study is the study of choice. It reveals the filling of leg vessels down to the ankle. The plantar arch also may be visualized if the location of the wound is distal enough to warrant it. This study is invaluable to both the plastic surgeon when providing coverage and to the vascular surgeon if revascularization is also performed.

Contrast arteriography is the gold standard for the evaluation of arterial disease. But again as being the invasive procedure, it is less used until unless a therapeutic procedure is planned. It can be done usually by transfemoral route (Seldinger's technique), but in the higher blocks, direct lumbar puncture into the aorta had been used in the past. It demonstrates the arterial tree in its entirety, to readily delineate the site of arterial stenosis and occlusion. It is indicated for select patients, who will undergo revascularization to reestablish perfusion. In addition to providing valuable anatomic information, pressure measurements across the arterial stenosis can be obtained to gauge the hemodynamic severity of a lesion. More importantly, interventions can be done using balloons, stents, and other devices. In patients with vascular malformations, selective catheterization can be performed to obliterate the feeding vessels. Limited use of arteriography in clinical practice due to catastrophic complications is occurring sometimes. There are several complications being an invasive technique, such as hematoma, pseudoaneurysm, arteriovenous fistula formation, embolism, dissection, and renal failure. As the amount of contrast used is

high, it can lead to renal failure. Further patient may go into anaphylaxis because of reaction to the contrast [26].

7.5.4.4 Intravascular Ultrasound (IVUS)

This technique is not available commonly, but it can differentiate between the normal and abnormal vessel. It also determines plaque volume within the wall of the artery and the degree of stenosis. Small lesions like intimal flaps or tears are well visualized because of their high fibrous tissue content and the contrasting echoic properties of surrounding blood. Intramural thrombus appears as echogenic homogenous mass with varying image attenuation beyond the location. IVUS can also differentiate noncalcified vessels versus calcified because the latter appear as a bright image with dense acoustic shadowing because the ultrasound energy is reflected by calcified plaque [27, 28]. It is also used to identify proximal venous obstruction. It allows precise estimation of the stenosis and the diameter of the lumen so the correct balloons and stents are used in the best possible position.

7.5.4.5 Skin Biopsy/Ulcer Biopsy

Although an invasive investigation, biopsy of the ulcer including normal adjoining skin is the final armamentarium in diagnosing doubtful, suspicious, or indeterminate causes of chronic ulcers. In acute ulcers biopsy has limited role and usually not recommended. Most common use of skin biopsies is in ulcers of indeterminate cause and suspected neoplastic pathology. Chronic ulcer of long durations of many years like venous ulcers may change to malignancy [29]. Marjolin's ulcer is an example of a malignant transformation of long-standing ulcers.

Ulcer biopsy is important in making a correct diagnosis and to rule out malignancy as these ulcers are prone to malignant transformation [29]. This requires taking a deep wedge of tissue from the ulcer edge and can usually be performed under local anesthesia [30]. Chronic ulcers are sometimes biopsied for experimental protocols to obtain information regarding the wound bed or the wound edge to grow cells in vitro from nonhealing wound. Chronic wounds do not worsen overall after a biopsy of the wound edge and wound bed is performed. In the majority of the patients, the biopsy site heals up to the original wound edge. The biological underpinning for this occurrence is unclear. There is a paucity of studies investigating the effects of biopsies on the healing of chronic wounds. However, recent research on the molecular pathogenesis of nonhealing wounds has shown that there are phenotypic differences in the cells populating the wound edge and the surrounding skin. Keratinocytes at the edge of the wound show deregulation in cell differentiation and cell migration, resulting in a hyperproliferative epidermal edge that fails to reepithelialize the wound bed. However, cells in the periwound area retain the capacity to differentiate, migrate, and respond appropriately to wound healing signals. In other studies, phenotypic changes have been found between dermal fibroblasts cultured from chronic versus acute wounds. Dermal fibroblasts cultured from the edge of chronic wounds have decreased responsiveness to transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor in terms of collagen production and proliferation. The mechanism for decreased responsiveness to TGF-β1 may be the result of downregulation of TGF-β type II

receptor expression and under phosphorylation of key signaling proteins, such as Smad2/Smad3 and p48/44 MAPK. The expression of a downstream TGF-β inducible protein, βig-H3, has been found to be decreased in chronic wound fibroblasts and in the dermis of chronic nonhealing wounds. Thus, taking a biopsy of the wound edge may remove part of the nonhealing edge and abnormal cell populations and thus produce an acute injury capable of resetting the healing process. This sequence of events may lead to healing in a more timely manner.

There are no studies that focused primarily on the safety of performing wound biopsies in chronic wounds. However, several practice guidelines have been recommended performing wound biopsies for histological diagnosis or microbiologic testing in wounds that have not improved within 2–6 weeks of appropriate management. The Food and Drug Administration (FDA) recommends performing biopsies of the wound, when needed clinically, as an objective tool to exclude neoplastic, immune-mediated, or primary infectious disease. In addition, they suggest performing wound biopsies to diagnose wound infections and to guide treatment. In the future, we may use this information to guide us when to best perform wound biopsies. One might recommend that diagnostic or experimental biopsies be performed several weeks after resolution of the infection.

Wound healing rates at 3 and 4 weeks have been used as predictors of ultimate wound closure. A recent study published on healing rates of both venous and diabetic ulcers determined that a healing rate of 1.5 mm/week was predictive of wound closure at 12 weeks. The biopsy site heals up to the original wound edge. It is quite possible that, once stabilized (standard therapy with a run-in period), chronic wounds reach a size that corresponds to the underlying pathophysiological defects and are, therefore, as large as they can be, based on those intrinsic abnormalities. Certainly, more studies with a larger sample size and a more homogenous patient population are needed. However, the data presented in this report are consistent with our long clinical experience that biopsies are a safe procedure in patients with otherwise impaired healing. These findings should prove useful for clinicians and investigators.

Usually neoplastic skin ulceration is squamous cell carcinoma in the lower extremity, but one may get a basal cell carcinoma, melanoma, and the sarcomas rarely.

7.6 Microbial Isolation

A quantitative bacterial culture is more specific and should be performed once wound infection is suspected [31]. This is performed by curetting or biopsying the bed of the ulcer. The quantitative biopsy is the current gold standard for assessing the quality and quantity of microbial pathogens within the wound [31, 32]. Quantitative biopsies containing greater than 10^5 organisms per gram of tissue are considered significant, and systemic antibiotic therapy should be considered. If osteomyelitis is suspected, representative cultures need to be obtained from the bone or deepest tissue layers [8]. Chronic wounds contain complex polymicrobial communities of sessile organisms that have been underappreciated because the

limitations of standard culture techniques increased bacterial diversity with an average of 17 genera in each wound. Data from microbial community profiling of chronic wounds were compared with published sequenced analyses of bacteria from normal skin. Increased proportions of anaerobes, Gram-negative rods, and Gram-positive cocci were found in chronic wounds. In addition, chronic wounds had significantly lower populations of *Propionibacterium* compared with normal skin. Using epifluorescence microscopy, wound bacteria were visualized in highly organized thick confluent biofilms or as scattered individual bacterial cells. Fluorescent in situ hybridization allowed for the visualization of *Staphylococcus aureus* cells in a wound sample. Quorum-sensing molecules were measured by bioassay to evaluate signaling patterns among bacteria in the wounds. A range of autoinducer-2 activities was detected in the wound samples. Collectively, these data provide new insights into the identity, organization, and behavior of bacteria in chronic wounds. Such information may provide important clues to effective future strategies in wound healing.

7.7 Gene Variant Analysis

The clinical application of gene variant analysis and evaluation in patients with venous leg ulcers implies that the high-risk minority of patients could be identified in advance by means of a simple blood test that would act as a genetic screening device. Utility of gene variant analysis is to prevent chronic ulcers by modifications of lifestyle and minimize morbidity in high-risk populations [33].

Conclusion

Majority of the ulcers may be diagnosed clinically by thorough history and examination, but investigations are needed for unusual cases. Baseline investigations must be available for all cases, and in special type of cases, specific investigations are needed. Majority of the chronic ulcers are vascular in origin, so Duplex ultrasound is the choice of investigation in almost all type of chronic leg ulcers. In long-standing cases where the ulcer is not responding to the treatment, a biopsy must be performed. The biopsy should be performed from the margins of the ulcer and not from the center which may show only necrotic material. The ulcers respond best if correctly diagnosed.

References

1. Barclay KL, Granby T, Elton PJ. The prevalence of leg ulcers in Hospitals. Hosp Med. 1998;59(11):8.
2. London NJ, Donnely R. ABC of arterial and venous disease Ulcerated lower limb. Br Med J. 2000;320:1589–91.
3. Cleverland TJ, Gaines P. Stenting in peripheral vascular disease. Hosp Med. 1999;60:630–2.
4. Ruckley CV. Caring for patients with chronic leg ulcer. BMJ. 1998;316:407–8.
5. Rahman IA, Fadeyi A. Epidemiology, etiology, and treatment of chronic leg ulcer: experience with sixty patients. Ann Afr Med. 2010;9(1):1–4.

6. Kahle B, Hermanns HJ, Gallenkemper G. Evidence based treatment of chronic leg ulcers. Dtsch Arztebl Int. 2011;108(14):231–7.
7. van Gent WB, Wilschut ED, Wittens C. Management of venous ulcer disease. BMJ. 2010;341(7782):1092–6.
8. Rayner R, Carville J, Keaton J, Prentice J, Santamaria XN. Leg ulcers: atypical presentations and associated comorbidities. Wound Practice Res. 2009;17(4):168–85.
9. Moloney M, Grace P. Understanding the underlying causes of chronic leg ulceration. J Wound Care. 2004;13:215–8.
10. Dean S. Leg ulcers: causes and management. Aust Fam Physician. 2008;36(7):480–4.
11. Mekkes JR, Loots MM, van der Wal AC, Bos JD. Causes, investigation and treatment of leg ulceration. Br J Dermatol. 2003;148(3):388–401.
12. Lazarides MK, Giannoukas AD. The role of hemodynamic measurements in the managements in the management of venous and ischemic ulcers. Int J Lower Extrem Wounds. 2007;6:254–61.
13. Androulakis AE, Giannoukas AD, Labropoulos N, Katsamouris A, Nicolaides AN. The impact of duplex scanning on vascular practice. Int Angiol. 1996;15:283–90.
14. Mansour M, Labropoulos N. Vascular diagnosis. Philadelphia: Elsevier Saunders; 2005.
15. Neglén P, Raju S. A rational approach to detection of significant reflux with duplex Doppler scanning and air plethysmography. J Vasc Surg. 1993;17(3):590–5.
16. Needham T. Physiological testing of lower extremity arterial disease: segmental pressure, plethysmography, and velocity waveforms. In: Mansour M, Labropoulos N, editors. Vascular diagnosis. Philadelphia: Elsevier Saunders; 2005. p. 215–22.
17. Labropoulos N, Leon L, editors. Evaluation of chronic venous disease. In: Vascular diagnosis. Philadelphia: Elsevier Saunders; 2005. p. 447–62.
18. Met R, Bipat S, Legemate DA, Reekers JA, Koelemy MJ. Diagnostic performance of computed tomography angiography in peripheral arterial diseases; a systemic review and meta-analysis. JAMA. 2009;301:415–24.
19. Uhl JF, Gillot C. Embryology and three dimensional anatomy of the superficial venous system of the lower limbs. Phlebology. 2007;22:194–206.
20. Poschenrieder F, Hamer OW, Herold T, et al. Diagnostic accuracy of intraarterial and i.v. MR angiography for the detection of stenoses of the infrainguinal arteries. AJR Am J Roentgenol. 2009;192:117–21.
21. Morasch M, Collins J. The current role of MRA in planning interventions for lower extremity ischemia. In: Mansour M, Labropoulos N, editors. Vascular diagnosis. Philadelphia: Elsevier Saunders; 2005. p. 293–306.
22. Froelich JB, Prince MR, Greenfield LJ, Downing LJ, Shah NL, Wakefield TW. "Bull's -eye" sign on gadolinium enhanced magnetic resonance venography determines thrombus presence and age: a preliminary study. J Vasc Surg. 1997;26:809–16.
23. Yucel EK, Dumoulin CL, Waltman AC. MR angiography of lower-extremity arterial disease: preliminary experience. J Magn Reson Imaging. 1992;2(3):303–9.
24. Owen RS, Carpenter JP, Baum RA, et al. Magnetic resonance imaging of angiographically occult runoff vessels in peripheral arterial occlusive disease. N Engl J Med. 1992;326(24):1577–81.
25. Masuda EM, Kistner RL. Long term results of venous valve reconstruction: a four to twenty one year follow up. J Vasc Surg. 1994;19:391–403.
26. Ayerdi J, Hodgson K. Principles of arteriography. In: Rutherford R, editor. Vascular surgery. 6th ed. Philadelphia: Elsevier Saunders; 2005. p. 281–99.
27. Buckly CJ, Arko FR, Lee S, et al. Intravascular ultrasound scanning improves long-term patency of iliac lesions treated with balloon angioplasty and primary stenting. J Vasc Surg. 2002;35:316–23.
28. Neglen P, Hollis KC, Olivier J, Raju S. Stenting of the venous outflow in chronic venous disease: long-term stent-related outcome, clinical and hemodynamic result. J Vasc Surg. 2007;46:979–90.
29. Agale SV, Kulkarni DR, Valand AG, Zode RR, Grover S. Marjolin's ulcer—a diagnostic dilemma. J Assoc Physicians India. 2009;57(8):593–4.

30. Ghauri SK, Nyamekye IK. Leg ulceration: the importance of treating the underlying pathophysiology. Phlebology. 2010;25(supplement 1):42–51.
31. Siddiqui AR, Bernstein JM. Chronic wound infection: facts and controversies. Clin Dermatol. 2010;28(5):519–26.
32. Han A, Zenilman JM, Melendez JH, et al. The importance of a multi-faceted approach to characterizing the microbial flora of chronic wounds. Wound Repair Regen. 2011;19(5):532–41.
33. Singh AV, Subhashree L, Milani P, Gemmati D, Zamboni P. Interplay of iron metallobiology, metalloproteinases, and FXIII, and role of their gene variants in venous leg ulcer. Int J Low Extrem Wounds. 2010;9(4):166–79.